SUBSTANCE EVALUATION CONCLUSION

as required by REACH Article 48

and

EVALUATION REPORT

for

Acetone Oxime

EC No 204-820-1

CAS No 127-06-0

Evaluating Member State: Austria

Dated: 6 November 2017

Evaluating Member State Competent Authority

Austrian MSCA:
Austrian Federal Ministry of Agriculture, Forestry, Environment and Water Management
Stubenring 1
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Year of evaluation in CoRAP: 2016

Member State concluded the evaluation without any further need to ask more information from the registrants under Article 46(1) decision.

Further information on registered substances here:

http://echa.europa.eu/web/guest/information-on-chemicals/registered-substances

DISCLAIMER

This document has been prepared by the evaluating Member State as a part of the substance evaluation process under the REACH Regulation (EC) No 1907/2006. The information and views set out in this document are those of the author and do not necessarily reflect the position or opinion of the European Chemicals Agency or other Member States. The Agency does not guarantee the accuracy of the information included in the document. Neither the Agency nor the evaluating Member State nor any person acting on either of their behalves may be held liable for the use which may be made of the information contained therein. Statements made or information contained in the document are without prejudice to any further regulatory work that the Agency or Member States may initiate at a later stage.

Foreword

Substance evaluation is an evaluation process under REACH Regulation (EC) No. 1907/2006. Under this process the Member States perform the evaluation and ECHA secretariat coordinates the work. The Community rolling action plan (CoRAP) of substances subject to evaluation, is updated and published annually on the ECHA web site[1].

Substance evaluation is a concern driven process, which aims to clarify whether a substance constitutes a risk to human health or the environment. Member States evaluate assigned substances in the CoRAP with the objective to clarify the potential concern and, if necessary, to request further information from the registrant(s) concerning the substance. If the evaluating Member State concludes that no further information needs to be requested, the substance evaluation is completed. If additional information is required, this is sought by the evaluating Member State. The evaluating Member State then draws conclusions on how to use the existing and obtained information for the safe use of the substance.

This Conclusion document, as required by Article 48 of the REACH Regulation, provides the final outcome of the Substance Evaluation carried out by the evaluating Member State. The document consists of two parts i.e. A) the conclusion and B) the evaluation report. In the conclusion part A, the evaluating Member State considers how the information on the substance can be used for the purposes of regulatory risk management such as identification of substances of very high concern (SVHC), restriction and/or classification and labelling. In the evaluation report part B the document provides explanation how the evaluating Member State assessed and drew the conclusions from the information available.

With this Conclusion document the substance evaluation process is finished and the Commission, the Registrant(s) of the substance and the Competent Authorities of the other Member States are informed of the considerations of the evaluating Member State. In case the evaluating Member State proposes further regulatory risk management measures, this document shall not be considered initiating those other measures or processes. Further analyses may need to be performed which may change the proposed regulatory measures in this document. Since this document only reflects the views of the evaluating Member State, it does not preclude other Member States or the European Commission from initiating regulatory risk management measures which they deem appropriate.

[1] http://echa.europa.eu/regulations/reach/evaluation/substance-evaluation/community-rolling-action-plan

Contents

Part A. Conclusion

1. CONCERNS SUBJECT TO EVALUATION

Acetone oxime was originally selected for substance evaluation in order to clarify the following concerns:

- Human Health: Suspected CMR (carcinogenic, mutagenic)
- Human Health: Suspected sensitiser
- Exposure of workers
- Wide dispersive use
- Exposure of environment
- High RCR

For more information, please refer to the according sections of Part B or to the Justification document prepared prior to this assessment.

https://echa.europa.eu/documents/10162/6bf7da2f-155e-4789-b8cb-8a65b04fa73b

2. OVERVIEW OF OTHER PROCESSES / EU LEGISLATION

No existing relevant EU legislation has been identified for acetone oxime.

Based on the substance evaluation of the structurally similar substance butanone oxime performed by the German competent authority in 2013 (closed in March 2014), the German competent authority identified the need for a harmonised classification of butanone oxime as carcinogenic: Carc. 1B H350: May cause cancer.

3. CONCLUSION OF SUBSTANCE EVALUATION

The evaluation of the available information on the substance has led the evaluating Member State to the following conclusions, as summarised in the table below.

Table 3-1: Conclusion of substance evaluation

Conclusions	
Need for follow-up regulatory action at EU level	X
Harmonised Classification and Labelling	X
Identification as SVHC (authorisation)	
Restrictions	
Other EU-wide measures	
No need for regulatory follow-up action at EU level	

4. FOLLOW-UP AT EU LEVEL

4.1. Need for follow-up regulatory action at EU level

Harmonised Classification and Labelling

Referring to the self-classifications of the Registrant(s), different classifications are available. Referring to the endpoint carcinogenicity, some registrants consider acetone oxime to be Carc. 2. Whereas, others do not provide any classification for this end point. Therefore, a harmonisation of classification is considered necessary to ensure equal classification of the substance throughout industry sectors.

The eMSCA concludes that it is justified to apply butanone oxime as source substance for read-across for the endpoint carcinogenicity based on structural similarities, similar properties and toxicokinetics of both substances. The German competent authority identified the need for a harmonised classification of butanone oxime as carcinogenic: Carc. 1B, H350: May cause cancer.

Depending on the final opinion of Risk Assessment Committee (RAC), the harmonised classification on carcinogenicity for butanone oxime should also apply to acetone oxime. At this stage, based on the available data, the eMSCA considers acetone oxime to be Carc. 1B, H350: May cause cancer.

Occupational risk

The registrants derived DNELs for workers, long term, systemic for the inhalation and dermal route (effects on the hematopoietic system, repeated dose toxicity).

DMELs to describe the likelihood of risks to workers concerning the carcinogenic potential of acetone oxime were not derived. Based on the calculations of the eMSCA, this effect is predicted to be the most critical one leading to the lowest hazard reference levels for risk characterisation, that need to be taken into account.

The current exposure predictions calculated by the registrants are not considered acceptable by the eMSCA, as they exceed the DMELs derived by the eMSCA. Some elements of the exposure prediction might have an overestimating and others an underestimating impact on the predictions of risk characterisation ratios (RCR). Nevertheless, based on the available data, it cannot be ruled out that real exposure levels exceed the DMELs.

Therefore, further risk management measures shall be considered by the eMSCA, if the carcinogenicity of the substance is confirmed by RAC.

4.1.1. Harmonised Classification and Labelling

Acetone oxime has currently no harmonised classification according to Regulation (EC) 1272/2008. The outcome of the evaluation performed leads to the need for a new entry in CLP-Annex VI for acetone oxime.

The eMSCA concludes that butanone oxime can be applied as source substance for read-across for the endpoint carcinogenicity based on structural similarities, similar properties and toxicokinetics. Depending on the final opinion of RAC, the harmonised classification on carcinogenicity for butanone oxime should also apply to acetone oxime. At this stage, based on the available data, the eMSCA proposes: Carc. 1B H350: May cause cancer.

The eMSCA proposes the following classification and labelling:

Table 4.1.1-1 Classification and labelling proposal of acetone oxime

Classification and Labelling	Pictograms, Signal word
Acute Tox. 4 H312: Harmful in contact with skin	GHS05, GHS07, GHS08 Danger
Eye Dam. 1 H318: Causes serious eye damage	
Skin Sens. 1B H317: May cause an allergic skin reaction	
STOT SE 3 H336: May cause drowsiness or dizziness	
STOT RE 2 H373: May cause damage to blood system through prolonged or repeated exposure	
Carc. 1B H350: May cause cancer	

Acute toxicity
For acute dermal toxicity in rabbits the LD50 was determined to be >1000 mg/kg; based on the range finding study a dose of 2000 mg/kg caused 100% mortality (unpublished study report, 1991b). Therefore, acetone oxime meets the criteria for classification and labelling as Acute Tox.4, H312: Harmful in contact with skin.

Eye damage
Severe eye lesions detected in an eye irritation study (unpublished study report, 1990b) justifies the classification and labelling of acetone oxime as Eye dam. 1, H318: Causes serious eye damage.

Skin sensitisation
Based on a positive guinea pig maximisation test (GPMT; unpublished study report, 1990c), supporting evidence from butanone oxime and the known hydrolysis of acetone oxime to the sensitizing hydroxylamine a classification for skin sensitisation is warranted. In the GPMT a skin sensitisation response of ≥30 % at >1.0% intradermal induction dose

was observed, therefore acetone oxime meets the criteria for classification in subcategory 1B, H317: May cause an allergic skin reaction.

Specific target organ toxicity- single exposure
After oral and dermal single exposure ataxia, hypoactivity and lethargy were reported at higher dose levels in two species, rat and rabbit (unpublished study report, 1991a; unpublished study report, 1991b; supported by unpublished study report, 1989a). The analogue substance butanone oxime is proposed to meet the classification for specific target organ toxicity after single exposure based on its narcotic effects in rats and rabbits after acute oral, inhalation and dermal as well as after repeated oral exposure. Because of the structural similarities to acetone oxime including the common functional oxime group and based on the available experimental and read-across information there is sufficient evidence that acetone oxime meets the criteria for the classification of STOT SE 3, H336: May cause drowsiness or dizziness.

Specific target organ toxicity- repeated exposure
Acetone oxime affects the hematopoietic system and associated organs and caused adverse effects after repeated exposure to 50 mg/kg bw/day (unpublished study report, 1991c). A classification as STOT RE 2 could therefore be warranted for classification for target organ toxicity through repeated exposure (STOT RE 2), H373 May cause damage to blood system through prolonged or repeated exposure.

Carcinogenicity
For carcinogenicity read-across to butanone oxime is used for classification and labelling. In combined reliable and well documented chronic toxicity/carcinogenicity studies in rats and mice exposed by inhalation to butanone oxime sufficient evidence of animal carcinogenicity (liver tumor formation) in two species was demonstrated (Newton et al., 2001; Germany, 2014). Supporting information from the 90 day repeated dose toxicity study (unpublished study report, 1991c) with acetone oxime as well as from published non-guideline experimental studies for carcinogenicity indicated that acetone oxime has also a carcinogenic potential. Therefore, acetone oxime could be proposed to be classified as Carc. 1B H350: May cause cancer.

4.1.2. Identification as a substance of very high concern, SVHC (first step towards authorisation)

Not applicable at this stage.

4.1.3. Restriction

Not applicable at this stage.

4.1.4. Other EU-wide regulatory risk management measures

Not applicable at this stage.

5. CURRENTLY NO FOLLOW-UP FORESEEN AT EU LEVEL

n.a.

6. TENTATIVE PLAN FOR FOLLOW-UP ACTIONS (IF NECESSARY)

Table 6-1: Proposed follow-up actions

Follow-up action	Date for intention	Actor
CLH dossier	To be decided	Member State Austria

Part B. Substance evaluation

7. EVALUATION REPORT

7.1. Overview of the substance evaluation performed

Acetone oxime was originally selected for substance evaluation in order to clarify the following concerns:

- Human Health: Suspected CMR (carcinogenic, mutagenic)
- Human Health: Suspected sensitiser
- Exposure of workers
- Wide dispersive use
- Exposure of environment
- High RCR

For more information, please refer to the according sections or to the Justification document prepared prior to this assessment.

https://echa.europa.eu/documents/10162/6bf7da2f-155e-4789-b8cb-8a65b04fa73b

Table 7-1: Summary of hazard assessment performed

Endpoint evaluated	Outcome/conclusion/classification
Acute toxicity: oral	No classification proposed
Acute toxicity: inhalation	No classification proposed
Acute toxicity: dermal	Classification proposal: Acute Tox. 4, H312: Harmful in contact with skin (1000 < ATE ≤ 2000 mg/kg bw)
Narcotic effect	Classification proposal: STOT SE 3, H336: May cause drowsiness or dizziness
Skin irritation	No classification proposed
Eye damage	Classification proposal: Irreversible effects on the eye category 1, H318: Causes serious eye damage
Sensitisation	Classification proposal: Skin sensitizer sub-category 1B, H317: May cause an allergic skin reaction
Repeated dose toxicity	Classification proposal: Specific target organ toxicity through repeated exposure (STOT RE 2), H373: May cause damage to blood system through prolonged or repeated exposure. Borderline case
Mutagenicity	No classification proposed
Carcinogenicity	Classification proposed:

Endpoint evaluated	Outcome/conclusion/classification
	Category 1B carcinogen, H350: May cause cancer
Reproductive toxicity	No classification proposed
Aquatic toxicity	No classification proposed

Summary of risk assessment

Selection of critical hazard reference levels for risk assessment

For risk characterisation the registrants provided calculated DNELs from animals studies either performed with acetone oxime or with an analogue substance. DNELs were derived for workers, long term, systemic effects (effects on the hematopoietic system, repeated dose toxicity) for the inhalation and dermal route.

However most notably none of the registrants provided hazard reference levels, DMELs, to describe the likelihood of risks to workers concerning the carcinogenic potential of acetone oxime. Based on the calculations of the eMSCA, this effect is predicted to be the most relevant one leading to the lowest hazard reference levels for risk characterisation, that need to be taken into account.

The eMSCA calculated a $DMEL_{worker,inhalation,liver\ tumours}$ of 11.57 µg/m^3 for a cancer risk level of 10^{-5} that could be seen as indicative tolerable risk level for workers. This value is approximately 30 times lower than the DNELs for the inhalation route used by the registrants for risk characterisation.

For the dermal exposure route the $DMEL_{worker,dermal,liver\ tumours}$ is 1.65 µg/kg bw/d for a cancer risk level of 10^{-5}. This value is approximately 30 and 60 times lower than the DNELs for the dermal route used by the registrants for risk characterisation.

Exposure- and risk assessment

The current exposure predictions calculated by the registrants are not considered safe when the substance is considered carcinogenic and by using the DMELs derived by the eMSCA. Some elements of the exposure prediction might have an overestimating and others an underestimating impact on the predictions. Nevertheless, based on the available data, it cannot be ruled out that real exposure levels at workplaces of some/all uses/tasks exceed the DMELs under real conditions.

7.2. Procedure

Evaluation of acetone oxime was launched in March 2016. The Registrant(s) of acetone oxime were contacted before start of evaluation and asked to support the evaluation by providing the original studies used for the individual registrations. The Registrant(s) provided these studies. In a first step, the performed evaluation of acetone oxime was not targeted and covered all sections of the chemical safety assessment. In a second step, evaluation was focused on the areas of concern- identified prior and during evaluation. Studies provided by the Registrant(s), publicly available studies/data, studies for source substances like butanone oxime, QSARs and exposure modelling tools were used by the eMSCA for assessment and conclusion. Based on the available data in total,

no need for new data was identified. They were considered to be sufficient for clarifying the identified concerns indicated in the previous section and concluding on them. Evaluation of acetone oxime was closed in March 2017.

This document containing a conclusion document (Part A) and a substance evaluation report (Part B) was finalized in November 2017. It is intended for the public summarizing key elements and outcome of the evaluation. Confidential information is anonymized or not presented.

7.3. Identity of the substance

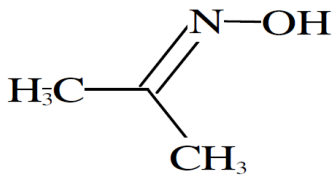
Acetone oxime

Table 7.3-1: Substance identity of acetone oxime

Public name:	acetone oxime
EC number:	204-820-1
CAS number:	127-06-0
Molecular formula:	C3H7NO
Molecular weight range [g/mol]:	73.09
Synonyms:	---

Type of substance ☒Mono-constituent ☐ Multi-constituent ☐ UVCB

Structural formula of acetone oxime



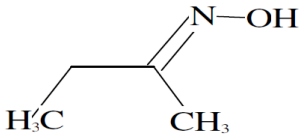
Main read-across substance: butanone oxime (see also Annex I)

Table 7.3-2: Substance identity of read-across substance butanone oxime

SUBSTANCE IDENTITY of read-across substance butanone oxime	
Public name:	butanone oxime
EC number:	202-496-6
CAS number:	96-29-7
Molecular formula:	C_4H_9NO
Molecular weight range [g/mol]:	87.12
Synonyms	• methyl ethyl ketone oxime (MEKO), • butan-2-one oxime

Trade names:	• Antioxidant B, • antiskinning agent KL-841, • Durham CA111, • Exkin 2, • MEKO • SKINO

Structural formula of butanone oxime



7.4. Physico-chemical properties

Table 7.4-1 Data available on ECHA's dissemination web site based on registration data (last review: July 2017)

OVERVIEW OF PHYSICOCHEMICAL PROPERTIES	
Property	**Value**
Physical state at 20°C and 101.3 kPa	White solid
Melting point	60°C at 101.3 kPa (measured, registration data)
Boiling point	The boiling temperature of acetone oxime has been determined to be 134 ± 1 °C at 99.2 kPa (measured, registration data)
Vapour pressure	242 Pa at 25°C (measured, registration data) 164 Pa at 25°C (calculated with QSAR, registration data)
Water solubility	Very soluble in water The water solubility of acetone oxime has been determined to be in the range of 30.3 to 32.7 % w/w at 20.0 ± 0.5 °C. (measured, registration data)
Partition coefficient n-octanol/water (Log Kow)	0.077 The test was carried out at approximately neutral pH (ca. 7) with the test item in its non-ionized form (pKa: 12.42 at 24.9°C). (measured value, registration data)
Flammability	Highly flammable (measured, registration data)
Explosive properties	Not expected. There are no chemical groups associated with explosive properties present in the molecule.
Oxidising properties	Not expected. The substance is incapable of reacting exothermically with combustible materials, on the basis of the chemical structure.

Granulometry	Substance is not used in granular form. It is placed on the market in massive form for example as a large solid block of solidified melt. (Statement given in registration data)
Dissociation constant	The pKa of the substance is 12.42 at 24.9°C. (measured, registration data)

Comment of the eMSCA on properties relevant for risk assessment:

Data on granulometry (dustiness) of manufactured acetone oxime were/are not provided by the Registrant(s) in the registration dossiers, as the substance is sold in massive form for example as large solid blocks of solidified melt based on Registrant(s) statements. Referring to the volatilisation potential of solid substances, dustiness (distribution of particles in air) and gaseous release need to be distinguished.

Based on the opinion of the eMSCA, potential present or future sale of acetone oxime as powder cannot be ruled out fully. Referring to available safety data sheets, granular powders are also available and used in the EU. Potential dust exposure might be relevant for industrial uses of the substance as manufactured in some cases, although not expected and considered by the Registrant(s) at this stage. Nevertheless, referring to the use of acetone oxime dispersed in matrices like paints, the expectation of no relevant dustiness is shared as well by the eMSCA for exposure to these products.

The potential for gaseous release is moderate based on the measured and predicted vapour pressures (242 and 164 Pa at 25°C). The order of magnitude is also in the range of the vapour pressure of the structurally similar substance butanone oxime (1070 Pa at 20°C, ECHA dissemination site), which is applied as source substance for read-across.

Based on registration and publicly available data, water solubility is in the range of 300g/L and thus to be very high in comparison to many other organic substances.

The order of magnitude of the low logKow is in line with the expectations and estimations based on the structure (low MW, presence of polar elements) and high water solubility of acetone oxime.

7.5. Manufacture and uses

7.5.1. Quantities

Table 7.5.1-1: Aggregated tonnage

AGGREGATED TONNAGE (PER YEAR)				
☐ 1 – 10 t	☐ 10 – 100 t	☒ 100 – 1000 t	☐ 1000- 10,000 t	☐ 10,000-50,000 t
☐ 50,000 – 100,000 t	☐ 100,000 – 500,000 t	☐ 500,000 – 1000,000 t	☐ > 1000,000 t	☐ Confidential

7.5.2. Overview of uses

Table 7.5.2-1: Uses identified in registration dossiers (July 2017)

USES	
Uses as intermediate	Use as an intermediate for manufacture of silicon sealants Use as an intermediate
Formulation	Formulation in preparations, (re-) packaging and distribution
Uses at industrial sites	Use of coatings / printing inks Uses in laboratory
Uses by professional workers	Use of coatings / printing inks Uses in laboratory
Consumer Uses	Consumer uses were not registered
Article service life	no

Acetone oxime is used as anti-skinning agent for the preparation of coatings/printing inks. Inhalation and dermal exposure are expected to be the most relevant routes of exposure for industrial and professional users manufacturing and using coatings/printing inks. Tasks like brush, roller (PROC 10) and industrial spraying (PROC 7) applications are covered among others for example, which reveal higher potential for human exposure.

Acetone oxime is also used as intermediate for the manufacture of other substances/products. Intermediate use of oximes covers mainly manufacture of oxime silanes, which are applied as cross-linkers for silicon sealants. During the curing process in the presence of atmospheric moisture acetone oxime is released and evaporated. Inhalation and dermal exposure are relevant for industrial and professional users manufacturing and during the curing process of silicon sealants. The latter scenario based on the hydrolysis of oxime silanes is not covered by the registration, as release of acetone oxime is led back to decomposition of these oxime silanes and therefore covered by their registrations under REACH.

Consumer uses were not registered, but exposure of the general public is also expected to be possible via use of paints/printing inks and silicon sealants in non-industrial settings.

For the full list of use descriptors covered by the individual registered uses, please see ECHA's dissemination site:

https://echa.europa.eu/registration-dossier/-/registered-dossier/5744

7.6. Classification and Labelling

Please find current information on classification in C&L Inventory database on ECHA web site. The inventory includes both harmonised classification when available and the notified self-classifications.

http://echa.europa.eu/web/guest/information-on-chemicals/cl-inventory-database.

7.6.1. Harmonised Classification (Annex VI of CLP)

The substance has no harmonized classification according to CLP Regulation (Regulation (EC) 1272/2008).

7.6.2. Self-classification

- In the registration(s):

Registrant A:

Flam. Solid 1, H228

Acute Tox. 4, H312

Eye Damage 1, H318

Skin Sens. 1B , H317

Carc. 2, H351

Registrant B:

Flam. Solid 1, H228

Eye Damage 1, H318

STOT RE 2, H373 (red blood cells)

- The following hazard classes are in addition notified among the aggregated self-classifications in the C&L Inventory:

Skin Sens 1, H317

Flam. Solid 2, H228

7.7. Environmental fate properties

7.7.1. Degradation

Hydrolysis

The principle of the manufacturing process of acetone oxime is based on the condensation of acetone (CAS no. 67-64-1) and hydroxylamine (CAS no. 7803-49-8). Water is formed as by-product and removed for purification and enrichment of acetone oxime. As the condensation process is reversible, addition of water/hydrolysis results as well in the release of the initial raw materials acetone and hydroxylamine (or derivatives like salts based on the media).

The Registrant(s) provided data on hydrolysis using OECD QSAR Application Toolbox version 3.0. The half-life of acetone oxime was predicted to be 18 days at neutral pH (~pH 7). The hydrolysis products at acidic, basic and neutral pH, were predicted to be acetone and hydroxylamine.

Referring to ECHA's dissemination site, hydrolysis data for butanone oxime (at 0.01 M) at pH 4, 7, and 9 and at three different temperatures (20, 35 and 50 °C) are publicly available.

At pH 4, butanone oxime was hydrolytically unstable and hydrolysed "immediately", regardless of the temperature (t1/2 < 0.3 minutes). At pH 7, the hydrolysis reaction was much slower; 14% hydrolysis at 20 °C was obtained after 4 days and 44% hydrolysis was obtained at 50 °C after 7 days. At pH 9, no measurable hydrolysis under any of the test conditions was observed. The hydrolysis plateaued at 40-50% by days 4 to 7. The hydrolysis products are methyl ethyl ketone and a hydroxylamine salt.

A degree of 14% hydrolysis after 4 days (measured for butanone oxime at pH 7) corresponds to a half life of 18.5 days at neutral pH (20°C). This value is similar to the QSAR prediction for acetone oxime (18 days at pH 7) performed by the Registrant(s).

Referring to the available data for the structurally similar substance butanone oxime, it is concluded that acetone oxime is highly hydrolytically instable under acidic conditions (pH 4), stable under basic conditions (pH 9) and has a half life of 18 days neutral pH (pH 7). Acetone and hydroxylamine (potentially as salt) are expected as hydrolysis products.

Biodegradation

According to a recent ready biodegradability study according to OECD Guideline 301 D acetone oxime is not readily biodegradable (unpublished study report, 2012a): 9.1% of the substance was biodegraded after 28 days (O_2 consumption). In another biodegradation study also according to OECD Guideline 301 D acetone oxime was also not readily biodegradable (unpublished study report, 2012b): 0% of the substance was biodegraded after 28 days (O_2 consumption).

It is concluded that acetone oxime is not readily biodegradable.

7.7.2. Environmental distribution

According to EPIsuite KOCWIN v.2.00 the estimated Koc via Molecular Connectivity Index is 60.7 L/kg while this value is 1.27 L/kg using an estimate via a an estimated log Kow of 0.12. It is concluded that the potential for adsorption to soil is low.

Regarding volatilisation an estimation with HENRYWIN (v.3.2) is resulting in a value of 0.79 Pa.m^3/mol at 25 °C (using bond method). Volatility from the aqueous phase is low due to the significant affinity of acetone omime for the aqueous phase (water solubility: 330g/L).

Due to the high water solubility and the low adsorption potential to soil it can be expected that the major fractions of acetone oxime are found in the water phase or air (e.g. drying of wet coatings) according to the covered uses and expected release patterns.

7.7.3. Bioaccumulation

Reliable measured bioconcentration fish data are not available for acetone oxime. Nevertheless, the very low log Kow value of 0.077 (according to OECD Guideline 107) or 0.2 according to Jaroš T at al., 2007 indicate a low bioaccumulation potential. Also according to EPISUITE BCFBAF (v3.01) the estimated BCF value is low with 3.162 L/kg wet weight (based on a log Kow of 0.12).

These findings are supported by data from the potential read-across substance butanone oxime (unpublished study report, 1982): The measured BCF range for the 0.2 mg/L exposure was 2.8-5.8. The measured BCF range for the 2.0 mg/L exposure was 0.5-0.6.

It is concluded that the bioaccumulation potential of acetone oxime is low.

7.8. Environmental hazard assessment

7.8.1. Aquatic compartment (including sediment)

7.8.1.1. Fish

In a short-term toxicity study with fathead minnows (*Pimephales promelas,* similar method to OECD Guideline 203) an 96h-EC_{50} value of 558 mg/l (meas. arithm. mean) was obtained for acetone oxime (unpublished study report, 1990).

Another registrant provided also a study with *Oryzias latipes* on the analogue substance butanone oxime according to OECD 203 (unpublished study report, 1998a): A 96 h-LC_{50} of > 100 mg/L (nominal) was obtained.

A 14-day study following OECD 304 methods with juvenile medaka (Oryzias latipes) in a flow-through system with the analogue substance butanone oxime is available (unpublished study report, 1998b). The 14-day LC_{50}, based on mortality and NOEC, based on growth, Koc are calculated to be >100 mg/L and 50 mg/L, respectively. Nevertheless this test is not adequate for assessment of chronic fish toxicity and no proper and valid read-across justification was provided by the registrant, who used this study. Differences in algae toxicity indicate a limited usability of butananone oxime for assessment of aquatic toxicity of acetone oxime.

It is concluded, that there is no acute hazard for fish.

No reliable long-term toxicity data are available for the toxicity of acetone oxime to fish. Nevertheless, as no concerns were identified indicating potential long term-term toxicity to fish corresponding to potential risk for the environment, this test was not requested.

No data for assessment of potential endocrine effects in fish are available.

7.8.1.2. Aquatic invertebrates

An acute immobilisation test on Daphnia magna was performed with acetone oxime in accordance with OECD 202 (unpublished study report, 2012c). An 48h-EC_{50} of 544.34 mg/L based on mobility (measured geometric mean concentration) was obtained.

In another short term invertebrate study with *Daphnia magna* an 48h-EC_{50} of ca. 201 mg/L (nominal) was gained (unpublished study report, 1998c).

No reliable long-term toxicity data are available for the toxicity of acetone oxime to invertebrates. An OECD 211 with *Daphnia magna i*s provided by one registrant with the analogue substance, butanone oxime: The 21-day NOEC, based on reproduction, was >100 mg/L (nominal). No proper and valid read-across justification was provided by the registrant, who used this study rendering the use of the study for environmental hazard assessment of acetone oxime as not usable – particularly as algae toxicity seems to be different for the two substances (unpublished study report, 1998d).

It is concluded, that there is no acute hazard for aquatic invertebrates.

No reliable long-term toxicity data are available for the toxicity of acetone oxime to aquatic invertebrates. Nevertheless, as no concerns were identified indicating potential long term-term tocxicity to invertebrates corresponding to potential risk for the environment, this test was not requested under substance evaluation.

7.8.1.3. Algae and aquatic plants

In an OECD 201 test with *Pseudokirchneriella subcapitata* an 72h-EC_{50} value of 252.92 mg/L (based on growth rate, nominal) and a NOEC of 50 mg/L (based on growth rate, nominal) were gained for acetone oxime (unpublished study report, 2012d).

A study (unpublished study report, 1998e) provided by another registrant is using another algae study with *Selenastrum capricornutum* with the analogue substance butanone oxime is showing higher toxicity: the 72h-EC_{50} based on growth rate was 11.8 mg/L (nominal) and the respective 72h-NOEC was 2.56 mg/L (nominal). The difference in toxicity is indicating that read-across for aquatic toxicity from butanone oxime to acetone oxime is not adequate.

It is concluded, that there is no acute or chronic hazard for algae.

7.8.1.4. Sediment organisms

No data are avaible on sediment organisms.

7.8.1.5. Other aquatic organisms

No data are avaible on other aquatic organisms.

7.8.2. Terrestrial compartment

No data on terrestrial soil macroorganisms, terrestrial arthropods, terrestrial plants or soil microorganisms are available.

7.8.3. Microbiological activity in sewage treatment systems

No respiration inhibition test is available. Nevertheless, data from an OECD Guideline 301 D test can be used (unpublished study report, 2012a): In the toxicity control containing the test item and the reference item sodium benzoate a mean of 38.2 % biodegradation was recorded within 14 days and 41% biodegradation after 28 days. It can be therefore that acetone oxime was not inhibitory at the applied concentration level of 2.5 mg/L.

This is confirmed by another OECD Guideline 301 D test (unpublished study report, 2012b), in which the toxicity control attained 26% degradation after 14 and 28 days and therefore confirming that the test item was not toxic to the sewage treatment micro-organisms used in the test at the applied concentration level of 2.5 mg/L (source substance used sodium benzoate).

One registrant provided also a study with *Pseudomonas putida* with the potential analogue substance butanone oxime (unpublished study report, 1988): The gained 17hr-EC_{10} and 17hr-EC_{50} values were 177 and 281 mg/L, respectively.

Another registrant used additionally the potential read-across substance Wasox-VMAC2 (2-Propanone, oxime, reaction products with ethenyltrimethoxysilane and trichloroethenylsilane), resulting in an 3h-EC_{50} of > 198.96 mg/L. Nevertheless no proper and valid read-across explanation was provided for a substance containing also other substances.

It is concluded that at 2.5 mg/L no toxicity to microorganisms in sewage treatment systems occurs.

7.8.4. PNEC derivation and other hazard conclusions

Table 7.8.4-1: PNEC derivation and other hazard conclusions

Hazard assessment conclusion for the environment compartment	Hazard conclusion	Remarks/Justification
Freshwater	PNEC aqua (freshwater): 0.253 mg/L	Assessment factor: 1000 used on the most sensitive LC_{50}/EC_{50} value (algae)
Marine water	PNEC aqua (marine water): 0.0253 mg/L	Assessment factor: 10000 used on the most sensitive freshwater LC_{50}/EC_{50} value (algae)
Intermittent releases to water	PNEC aqua (intermittent releases): 2.53 mg/L	Assessment factor: 100 used on the most sensitive LC_{50}/EC_{50} value (algae)
Sediments (freshwater)	PNEC sediment (freshwater): 0.943 mg/kg sediment dw	Equilibrium partioning using a Koc value of 1.27
Sediments (marine water)	PNEC sediment (marine water): 0.094 mg/kg sediment dw	Equilibrium partioning using a Koc value of 1.27
Sewage treatment plant	PNEC STP: 0.25 mg/L	Assessment factor: 10 used on the concentration on which no toxicity was seen in OECD 301 D tests (2.5 mg/L)
Soil	PNEC soil: 0.04 mg/kg soil dw	Equilibrium partioning using a Koc value of 1.27 and a Henry´s law constant of 0.79
Air	No hazard identified	
Secondary poisoning	Potential for Bioaccumulation considered to be very low	

7.8.5. Conclusions for classification and labelling

No classification for aquatic toxicity is warranted for acetone oxime according to CLP Regulation (EC) No. 1272/2008.

7.9. Human Health hazard assessment

7.9.1. Toxicokinetics

For the toxicokinetics of acetone oxime several in vitro metabolism studies were available. The registrants depicted 2 experimental in-vitro studies (rated as Klimisch 2) in their Chemical safety reports (CSRs). However available data do not satisfy basic toxicokinetic parameters such as percentages of absorption by the oral, inhalation and dermal uptake route, tissue distribution, bioavailability, AUC, C_{max}, T_{max}, clearance or half-life. According to Regulation (EC) No 1907/2006 the assessment of the toxicokinetic (TK) behaviour of the substance should be performed to the extent that can be derived from the relevant available information. Therefore, also data on physico-chemical properties, QSAR and supporting information from an analogue substance were used.

Absorption

Properties of a substance that affect absorption include octanol/water partition coefficient (log Kow) and water solubility. The log Kow value of acetone oxime is 0.077 and in the range of -1 to 4 that is proposed to be favourable for absorption (ECHA, 2014). In addition the high water solubility of 303 to 327 g/L and the low molecular weight of 73.09 g/mol further facilitate absorption. Also the high pKa value of 12.42 indicates that the molecule is not ionized upon oral, dermal or inhalation exposure and contact with biological tissues. According to the estimations based on the Danish QSAR Database[2] oral absorption is likely. The bioavailability score (Lipinski's Rule-of-five score) is zero, which indicates that the substance may be bioavailable. Absorption from the gastrointestinal tract for a 1 mg and a 100 mg dose was estimated to be 90% with this QSAR tool. Oral absorption of another oxime (analogue substance, see section Annex I), butanone oxime (MEKO) was 100% after administration of a single dose (gavage) and primarily converted to CO_2 after 24 hours of dosing (Germany, 2014[3]). For the evaluation of acetone oxime 100% oral absorption was assumed.

Toxicokinetic studies with dermal application are not available. In an acute dermal toxicity study with acetone oxime in rabbits at 1000 mg/kg changes in blood parameters and mortality at a dose of 2000 mg/kg was observed (unpublished study report, 1991b). Based on the low molecular mass and a log Kow of acetone oxime that is within the range of -1 to 4 the default value of 100% skin absorption is applied according to ECHA (2014). Experimental evidence from butanone oxime indicates dermal absorption of 2.7 mg/kg bw and 270 mg/kg bw butanone oxime after 72 hour exposure of 13% and 26%, respectively (Germany, 2014). Also inhalative absorption can be anticipated. For both routes the default values for route-to-route extrapolation according to ECHA (2014) were applied (cf. chapter 7.9.9).

Distribution

Once the substance is absorbed, it is expected to be distributed via the blood to the liver and other tissues (cf. acute toxicity and repeated dose studies, chapters 7.9.2 and 7.9.4). There is evidence that acetone oxime and/or the metabolites are present in the blood and liver based on toxicological effects seen in these organs.

Systemic effects including organ weight and histopathological changes in rats at 50 and 250 mg/kg bw/day indicate further bioavailability and distribution of acetone oxime, and/or metabolites in a 90-day repeated dose study (see Chapters 7.9.4).

[2] http://qsardb.food.dtu.dk/db/index.html
[3] https://echa.europa.eu/documents/10162/f6670512-1c38-470a-94f4-17d32f3f86b9

Metabolism

The Chemical Safety Reports (CSRs) from the REACH registrants quoted two studies on toxicokinetic: Völkel et al. (1999) and Kohl et al. (1992). Findings from other studies, Kreis et al (2000), Caro et al. (2001), Andrae et al. (1999) were summarized by the evaluating member state. Also the OECD toolbox v.3.3[4] was used to gain additional information on metabolites and the rate of adsorption. Table 7.9.1-1 summarises the single dose toxicokinetic experiments.

Table 7.9.1-1. Studies on metabolism

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

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

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

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

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

No information of skin metabolism was available. Therefore the skin simulator of the OECD QSAR Toolbox V3.3.5[6] that mimics the metabolism of chemicals in the skin compartment was used. No skin metabolites were identified with the OECD tool. The rat liver S9 metabolism simulator indicated oxidation of acetone oxime. The proposed structure is given in Figure 7.9.1-1.

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

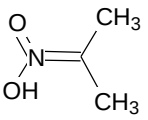


Figure 7.9.1-1: Predicted oxidation of Acetone oxime to propane 2-nitronic acid, OECD Toolbox

The hydrolysis products at acidic, basic and neutral pH, were predicted to be acetone and hydroxylamine (see Figure 7.9.1-2) according to the Hydrolysis Simulator of the OECD Toolbox (see section 7.7.1). Taking a study for butanone oxime into account, hydrolysis is also expected to be fast under acidic conditions (pH 4), to be significantly slower at neutral pH (half life of 18.5 days at pH 7 and 20°C) and stable under basic conditions (pH 9). However, according to NTP (1999) hydrolysis of ketoximes in vivo is probably enzymatic and not simply a reaction of the oxime with water; for example, aqueous exposure solutions are quite stable. Also Bergström et al. (2008) stated that the hydrolysis may occur both enzymatically and non-enzymatically. Haas-Jobelius et al. (1991) showed no hydrolyses in incubation experiments with 200 µM Acetone oxime for up to 90 min with primary hepatocytes and Chinese hamster cells (V79) as well as in control incubations without cells. However the duration was too short at the assumed neutral pH value for hydrolysis.

Figure 7.9.1-2: Predicted hydrolytic metabolites (acetone and hydroxylamine) in aqueous solution, OECD Toolbox

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

Excretion

Acetone oxime and P2-N were excreted in urine with comparable concentrations (0.1 – 0.4 mM) during 76 hours in rats (Kohl et al. 1992). According to data from an analogue oxime, 2- butanone oxime single oral doses were extensively converted to CO_2; excretion of other residues was primarily via urine (Germany, 2014).

Conclusion:

No toxicokinetic study according to OECD guideline was available for acetone oxime. Based on physical chemical properties, QSAR estimates and information from an

analogue oxime (MEKO) high oral absorption is estimated. Also dermal and inhalative absorption is likely. Once the substance is absorbed, it is expected to be distributed via the blood to the liver and other tissues based on the findings from other toxicological studies (cf. section 7.9.2; 7.9.4).

In vitro and in vivo metabolism studies showed that acetone oxime is converted in liver tissue of rats, mice and humans to P2-N (propane 2-nitronate) most likely by activation of cytochrome P 450 enzymes. Amounts of P2-N and 2-nitropropane (2-NP) were reported to be small in in vitro and in vivo studies. In vitro experiments with mice and rats liver microsomes and human hepatocytes indicate that acetone oxime is metabolized to the corresponding nitronate at rates 50% of those observed with butane oxime oxidation (Völkel et al. 1999). However, 2-NP can also undergo cellular reduction to acetone oxime.

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

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

Acetone oxime and its metabolites are shown to be excreted via urine in rats.

7.9.2. Acute toxicity and Corrosion/Irritation

Acute toxicity: oral

For acute oral toxicity, results from one experimental study with acetone oxime in rats were submitted by one registrant. The results of the experimental studies performed with acetone oxime on acute toxicity after oral administrations are summarized in the following Table 7.9.2-1 (key studies and reliability scores are also indicated).

Table 7.9.2-1. Acute oral toxicity study

Study/Method	**Results**	**Remarks/Reference**
Sprague-Dawley CD rats 5/sex/group Dose levels: 0, 300, 1000 and 3000 mg/kg For haematology: satellite groups (5/sex/300 and 3000 mg/kg) Test substance: acetone oxime Vehicle: distilled water Oral: gavage Controls: distilled water	LD50 >3000 mg/kg High dose group: ataxia immediately after dosing on day 1 and hypoactivity up to 4 days after dosing; decreased food consumption; no abnormal reflexes or other indications of neurologic impairment; 1 male died at day 2; Mid dose group: ataxia and hypoactivity in one animal and decreased food consumption in another animal Dose related reduced body weights (bw) and bw gains	**unpublished study report (1991a)** No appendices were submitted by the registrant; therefore the purity of acetone oxime is not specified. GLP Klimisch 2 **Key study** In a range finding study 2/2 animals died at 5000 mg/kg. Neurological examination revealed ataxia immediately

Study/Method	Results	Remarks/Reference
Duration: 14 days Similar to OECD 401	(reversible from day 7 in the low dose group, only) Dose related methemoglobinemia (day 1, 300 and 3000 mg/kg dose satellite group) and anemia Gross post-mortem examination: increased significant absolute and relative spleen weights (males and females); microscopic examination revealed increased extramedullary haematopoiesis and pigments in reticuloendothelial cells compared to controls (generally without a clear dose relationship).	after dosing that can be interpreted as sighs of transient narcosis.
Mouse m/f, 10 animals per dose group Test substance: acetone oxime Duration: 20 days Vehicle: 2% starch solution	LD50 values of >3000 mg/kg bw The study aimed to investigate the antispasmodic activity of oximes.	Friedman et al. (1977) Controls were included, however full documentation of the used method is lacking Test substance administration was not reported in detail, based on the vehicle the oral route was assumed. Klimisch 3 Supportive study

Acute toxicity: inhalation

According to the REACH Regulation in addition to the oral route (8.5.1), for substances other than gases, the information mentioned under 8.5.2 to 8.5.3 shall be provided for at least one other route. The choice for the second route will depend on the nature of the substance and the likely route of human exposure. The registrants provided information on the oral and dermal, but not the inhalative route based on physico-chemical properties (physical state, vapour pressure) and exposure considerations.

In addition to exposure considerations waiving for acute inhalation toxicity should be considered for low volatility substances, which are defined as having vapour pressures $<1 \times 10^{-5}$kPa for indoor uses, and $<1 \times 10^{-4}$kPa for outdoor uses (ECHA, 2016). However, acetone oxime has a vapour pressure of 242 Pa at 25°C and uses compatible also with likely inhalative exposure. The analogue substance butanone oxime was not classified for acute inhalation toxicity (Germany, 2014).

Acute toxicity: dermal

For acute dermal toxicity three experimental studies in rats and rabbits were summarized in the table 7.9.2-2 leading to different self-classification by the two registrants for this endpoint.

Table 7.9.2-2. Acute dermal toxicity studies

Study/Method	**Results**	**Remarks/Reference**
CRL:(WI) rats 5/m/f/dose Test material: Acetone oxime (99.6%) Dose level: 2000 mg/kg applied to approximately 10% area of the total body surface Contact time: 24- hour Vehicle: water Duration: 14 days OECD 402	LD50 >2000 mg/kg No clinical signs were observed after the treatment with the test item or during the 14-day observation period. No effects on bw and no test item related findings of the macroscopic examination.	**unpublished study report (2012a)** GLP Klimisch 1 **Key study** Please see further experimental information at https://echa.europa.eu/registration-dossier/-/registered-dossier/5744/7/3/4/?documentUUID=9528072c-0bd4-490f-9e0c-9da5ffd9aff7
Test guideline not stated Rat, m/f 1/sex per dose Dose level: 100, 300, 1000 mg/kg Test substance: Acetone oxime Contact time: 24- hour Vehicle: water Duration: 14 days Test guideline not stated	LD50 >1000 mg/kg Clinical signs: Lethargy in test animals at all dose groups; Body weight gain: dose dependent decrease in males during the observation period No macroscopic abnormalities in the post mortem examination.	unpublished study report (1989a) Strain not specified Method description und documentation incomplete Purity of test substance not reported No information concerning GLP Klimisch 3 Supportive study
Rabbit (New Zealand White) 5/sex/dose Test material: Acetone oxime Dose levels range finding study include 1000 and 2000 mg/kg	LD50 >1000 mg/kg Main test: Several animals in the high dose group (1000 mg/kg) were hypoactive, had fecal staining and exhibited a dark coloration to the eye (iris) at 24 hours and/or on day 2.	**unpublished study report (1991b)** GLP Klimisch 2 **Key study** Purity of the test material was not specified.

Study/Method	Results	Remarks/Reference
Dose levels main test: 0, 100, 500, 1000 mg/kg Contact time: 24- hour Vehicle: water Type of coverage: occlusive Study duration: 15 days Similar to OECD Guideline 402	Two animals showed poor food consumption. Low and mid dose group: single animals showed fecal staining. These effects were reversible at day 4. Haematology: dose-related methemoglobinemia on day 1 and anaemia on day 1 and 5. Effects on most of the haematology parameters in the high dose group. Organ weights and body weights were unaffected. Neurological examination at day 1, 7 and 15 gave no unusual observation. Gross post-mortem observation revealed no treatment related abnormalities. Microscopic examination revealed myeloid and erythroid hypercellularity of the femoral bone marrow in 4 of 10 mid-dose animals and in all 10 high-dose animals. Range-finding test: 2/2 animals died at 2000 mg/kg and 1/2 at 1000 mg/kg.	No details/methods of the neurological examination were reported.

Reliable LD50 values for classification of acetone oxime were derived from rats and rabbits, indicating that rabbits were more susceptible to effects caused by acetone oxime by the dermal route. In rats a LD50 >2000 mg/kg (limit test, GLP study) was obtained; no treatment related clinical signs or effects were observed (unpublished study report, 2012a). In the GLP study with rabbits (unpublished study report, 1991b) the LD50 was determined to be >1000 mg/kg; based on the range finding study a dose of 2000 mg/kg caused mortality in the two animals tested. Clinical signs were evident in several animals in the high dose group (hypoactive, fecal staining, dark coloration of the eye) and in a few animals in the mid and low dose groups (fecal staining). Concerning the observed toxic effects, methemoglobinemia and anaemia were observed. Acetone oxime meets therefore the criteria for classification as Acute Tox.4, H312 according to Regulation (EC) No. 1272/2008 (1000 < ATE ≤ 2000).

After oral and dermal administration ataxia, hypoactivity and lethargy were reported at higher dose levels in two species (rats and rabbits) (unpublished study report, 1991a; unpublished study report , 1991b; supported by unpublished study report, 1989a). While not details on the neurological examinations were available ataxia was reported as treatment related effects after oral administration in rats. No other specific studies that address neurotoxicity were available. However Derelanko and Rusch (2008) claimed that

narcosis has been found consistently with low molecular oximes such as acetone oxime. Data on acetone oxime were unpublished (Derelanko and Rusch, 2008).

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

The analogue substance butanone oxime is proposed by the MSCA DE to meet the classification for specific target organ toxicity after single exposure based on its narcotic effects in rats and rabbits after acute oral, inhalation and dermal as well as after repeated oral exposure. Neurotoxicity following acute and subchronic exposure was studied in rats including a Functional Observational Battery, assessment of motor activity, and neuropathology evaluations (Schulze and Derelanko, 1993).Oral single doses of ≥300 mg/kg bw butanone oxime administered by gavage were found to produce transient and reversible changes in neurobehavioral function (changes in gait and aerial righting reflex) consistent with CNS depression, but no evidence of cumulative neurotoxicity was detected (Schulze and Derelanko, 1993; Germany, 2014). After subchronic exposure transient treatment-related changes in ease of cage removal, ease of handling, and in posture, gait, and aerial righting were observed at the 400 mg/kg/day (Schulze and Derelanko, 1993), however in rabbits (dams) effects occurred at much lower dose levels (≥ 40 mg/kg bw/d, cf. Section 7.9.7, Derelanko et al. 2003). In the acute inhalation toxicity study a strong but transient narcotic effect occurred in both sexes at 4.83 mg/L/4h during the exposure (unpublished study report, 1984[7]; Germany, 2014).

The structural similarities to acetone oxime including the common functional oxime group may justify the consideration of such a classification for acetone oxime as well. The mechanism is not available or known for acetone oxime. Though the available data for acetone oxime on this endpoint is limited and effect levels for narcosis might be higher compared to butanone oxime there is concern that acetone oxime can elicit transient narcotic effects as evidenced by decreased activity, ataxia or lethargy in laboratory animals after single exposure. Evidence at any dose level for narcotic effects could support classification with Category 3 according to ECHA (2015). Based on the available information there is sufficient evidence that acetone oxime meets the criteria for the classification of STOT SE 3, H336: May cause drowsiness or dizziness according to Regulation (EC) No. 1272/2008 (for further details of the read-across please see Annex I).

Acute toxicity: other routes

Intraperitoneal injection was chosen as exposure route for two studies, one in rat (Wistar, m/f) (Mirvish et al. 1982) and one in mouse (Dultz et al. 1957). Mirvish et al. (1982) determined a LD50 of 840 mg/kg bw, the study duration was 7 days. In mice a higher LD50 value of 4000 mg/kg bw using six animals per group and different dose levels was reported by Dultz et al. (1957).

Conclusion:

The available data for acute oral toxicity in rats are acceptable and do not indicate a concern for acute toxicity. No classification for the oral route is required. For acute

[7] https://echa.europa.eu/de/registration-dossier/-/registered-dossier/14908/7/3/3

dermal toxicity in rabbits the LD50 was determined to be >1000 mg/kg; based on the range finding study a dose of 2000 mg/kg caused mortality in the two animals tested. Therefore acetone oxime meets the criteria for classification and labelling as Acute Tox.4, H312: Harmful in contact with skin. The acute toxicity studies as well as the read-across to butanone oxime also justify a classification for specific organ toxicity (single exposure) category 3 (STOT SE 3), H336: May cause drowsiness or dizziness according to Regulation (EC) No. 1272/2008.

SEV has verified the concern that there is a need to harmonize the classification for these endpoints according to Regulation (EC) No. 1272/2008.

Skin irritation

The registrants provided a study on skin irritation; the results are summarised in the following Table 7.9.2-3:

Table 7.9.2-3 Studies on skin irritation

Study/Method	**Results**	**Remarks/Reference**
6 rabbits (New Zealand White) 4 female (f) and 2 male (m) Coverage: occlusive (clipped) Test material: Acetone oxime Exposure period 24 hours 0.5 g of test item was applied at the skin beneath a gauze patch. This patch was affixed to the application site. similar to OECD Guideline 404 (Acute Dermal Irritation / Corrosion)	The effects were recorded in accordance with the DRAIZE scores. Erythema scores (wet): Mean score 1.25 (maximum 2) time point: 27/48/72 h Edema scores (wet): Mean score 0.5 (maximum 2) time point: 27/48/72 h Slight irritating effects were fully reversible at day 5.	**unpublished study report (1990a)** GLP Klimisch 2 key study Based on the results no classification for skin irritation is proposed. Longer exposure period than stated in the OECD guideline (4 hours) Negative and positive controls reported

According to the GLP study (unpublished study report, 1990a) no classification for skin irritation is proposed. The criteria for skin irritation – Category 2 where at least 4 out of 6 rabbits have to show a mean score per animal of ≥ 2.3 ≤ 4.0 for erythema/eschar or for oedema is not met. Effects were fully reversible at day 5.

Eye irritation

Please see the compilation in Table 7.9.2-4 concerning the study on eye irritation (submitted by the registrants).

Table 7.9.2-4. Studies on eye irritation

Study/Method	Results	Remarks/ Reference
3 female (f) and 3 male (m) rabbits (New Zealand White) Test item: Acetone oxime 0.1 g or 0.1 ml volume of the test substance was applied with no vehicle Duration: 21 days similar to OECD Guideline 405	Irreversible effects on the eye based on corneal damage that were not reversible within 21 days. Also conjunctival irritation and iridial changes or damage were observed.	**unpublished study report (1990b)** Klimisch 2 Key study GLP The effects were recorded in accordance with the DRAIZE scores. Results of eye rinsed animals after 24 hours (after application) are not shown.

Conclusion:

The available information is reliable and acceptable for the evaluation of skin and eye irritation and corrosion. According to the experimental results in rabbits (GLP study, unpublished study report, 1990a) no classification for skin irritation is proposed.

However, acetone oxime produced severe eye lesions in New Zealand rabbits (GLP study, unpublished study report, 1990b). While the classification criteria corneal opacity ≥ 3 and/or iritis> 1, 5 are not met, (only 1 animal showed a score for corneal opacity of 3 and for iris damage of 2 on day 3; all other scores were ≤2 for corneal opacity and ≤1 for iris) the scores for corneal ulceration were 4 in three animals at day 1 and in one animal at day 2. In addition to ulceration also pannus was observed in two animals, in one animal these effects continued till study termination. Necrosis of the conjunctivae was observed in 6/6 animals at 24 hours till day 7 after exposure. Based on these severe eye lesions acetone oxime meets the criteria for classification and labelling as 'irreversible effects on the eye' Category 1, (Eye dam. 1) H318: Causes serious eye damage according to Regulation (EC) No. 1272/2008.

7.9.3. Sensitisation

A different self-classification by the two registrants for this endpoint strengthened the need for an in-depth evaluation of the available data in the course of the substance evaluation.

For the evaluation of this endpoint a guinea pig maximisation tests (GPMT) (key study), a mouse ear swelling test (supporting study), a read-across (GPMT with butane oxime) and a LLNA (key study) are included in the registration data. No human data on the sensitising potential of acetone oxime are available. Animal studies are documented in Table 7.9.3-1.

Table 7.9.3-1: Animal studies on the sensitizing property of acetone oxime

Study/Method	Results	Reference /Remarks
Guinea Pig Maximisation Test (OECD Guideline 406) guinea pig, Dunkin-Hartley, f induction: intradermal and epicutaneous challenge: epicutaneous, occlusive Concentration: Induction: Intradermal: 5% in distilled water Topical induction: 100% (solid material, moistened with 0.9% saline). Challenge: 100% (solid material, moistened with 0.9% saline).	Sensitizing Results: 24h after challenge: 6/15 (40%) 48h after challenge: 5/15 Neg. control: 24h after challenge: 0/5 48h after challenge: 0/5 pos. control: 24h after challenge: 15/15 48h after challenge: 15/15	**unpublished study report (1990c)** Klimisch 2 (reliable with restriction) Key study GLP Test material: acetone oxime
Mouse ear swelling test Mouse, Balb/c, f Induction: epicutaneous, open; 35% w/v (days 1, 2, 3, 4 and 7) Challenge: epicutaneous, open, 17.5% w/v (day 14, 21)	Not sensitizing No reaction in 10 dosed and 5 negative control animals	unpublished study report (1989b) Klimisch 4(not assignable) Test material: acetone oxime
LLNA (OECD Guideline 429; EU Method B.42) mouse (CBA) female	not sensitising Negative control (AOO):SI 1.0 Positive control (25 (w/v) % HCA in AOO): SI 10.7 Acetone oxime: 50 (w/v) % in AOO: SI 1.3 25 (w/v) % in AOO: SI 1.7 10 (w/v) % in AOO: SI 1.6	**unpublished study report (2013)** Klimisch 1 (reliable without restriction) Key study GLP Experimental result Test material: acetone oxime, purity: 99.6%

guinea pig, Hartley, f guinea pig maximisation test induction: intradermal and epicutaneous challenge: epicutaneous, occlusive Concentration: <u>Induction:</u> Intradermal: 3% in propylene glycol and 1% in FCA Topical induction (day 7): undiluted substance, occluded patch <u>Challenge</u> (day 21): 50% (in propylene glycol), occluded patch.	Sensitizing Test results: 24h after challenge: 9/10 48h after challenge: 8/10 Neg. control: 24h after challenge: 0/10 48h after challenge: 0/10 Pos. control: 24h after challenge: 10/10 48h after challenge: 10/10	unpublished study report (1983) Klimisch 1 (reliable without restriction) Supporting study GLP Test material: butanone oxime (liquid)

A GPMT (GLP study, according EU method) with acetone oxime shows a clear response in 6/15 treated guinea pigs (equates to 40%) 24h after challenge (unpublished study report, 1990c).

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

In 2012 a LLNA (according to GLP) was conducted according to EU test method B.42 (unpublished study report, 2013). 4 animals/group were exposed to vehicle Acetone: olive oil (4:1) (neg. control), 25% HCA (pos. control) and acetone oxime (50%, 25%, 10% (w/w) in AOO). The test substance showed no irritating property or systemic toxicity. The appearance of the lymph nodes was normal in treated groups and negative control group. The observed stimulation index values were 1.3, 1.7 and 1.6 at concentrations of 50, 25 and 10 (w/v) % acetone oxime, respectively. Using this method acetone oxime showed no sensitizing property.

Butanone oxime (CAS 96-29-7, EC 202-496-6), an analogue substance, is used by the registrants as read-across substance for several endpoints, also for sensitisation. A GPMT with butanone oxime (unpublished study report, 1983) presented by the registrants shows a strong sensitizing potential. For butanone oxime also a positive Bühler test and a positive mouse ear swelling test are available (see dissemination site[8]). A negative LLNA with butanone oxime has been disregarded by the registrants as it is inconsistent with the positive results of the above mentioned studies. Based on these data butanone oxime is classified as Skin Sens 1B according to Germany (2014).

Butanone oxime as well as acetone oxime are negative in the LLNA but positive in the GPMT. Maybe these conflicting results are due to basic differences between these tests. LLNA measures lymphocyte proliferation after topical application of the test substance Induction phase). The GPMT is an adjuvant-type test in which the acquisition of sensitisation is potentiated by the use of Freund's Complete Adjuvant (FCA) and in which both intradermal and topical exposure are used during the induction phase. The Buehler test on the other side is a non-adjuvant method involving for the induction phase topical application only. But both tests assess the elicitation phase (ECHA, 2016). In terms of accuracy, sensitivity, specificity, and positive and negative predictivity, the performance of the LLNA was found to be similar to that of the GPMT. One limitation of the LLNA is the variability of the results due to vehicle chosen (ECHA, 2016)). For both substances – acetone oxime and butanone oxime - AOO (4:1 v/v) was chosen as vehicle in the LLNA.

In general oximes can readily be hydrolysed to the corresponding ketones or aldehydes, which are chemically reactive electrophilic compounds and can react with nucleophilic groups in macromolecules in the skin, thereby producing complete antigens and inducing contact allergy (Nilsson et al., 2005). This hydrolysis may occur both enzymatically and non-enzymatically (Bergström, 2008). As a second product of this reaction hydroxylamines will be released. Acetone oxime itself will be hydrolysed to acetone and hydroxylamine, a known sensitizer. The tendency to degradation by hydrolysis will be in the same order of magnitude like for the analogue butanone oxime (the rate of reaction increasing in acidic conditions). For butanone oxime stability in water has been experimentally determined (see dissemination database - hydrolysis, environmental fate) resulting in a half-live of <0.3min at pH4, >7d at pH7 and <14d at pH9. The pH level of the skin is acidic, ranging from pH4 to pH7, with a natural level below pH5 (Lambers, 2006).

Based on a positive GPMT, supporting evidence from butanone oxime and the known hydrolysis of acetone oxime to the sensitizing hydroxylamine a classification for skin sensitisation is warranted. In the GPMT a skin sensitisation response of ≥30 % at >1.0 % intradermal induction dose was observed, therefore acetone oxime meets the criteria for classification in subcategory 1B.

Conclusion:

There is sufficient information available for evaluation of skin sensitisation of acetone oxime.

SEV has verified the concern that there is a need to harmonize the classification for this endpoint according to Regulation (EC) No. 1272/2008. A classification as Skin Sens 1B is indicated by the use of animal data, information on hydrolysis products and supporting evidence from butanone oxime, a structural analogon.

[8] https://echa.europa.eu/registration-dossier/-/registered-dossier/14908?p_auth=MhqE5Q0Z

7.9.4. Repeated dose toxicity

For repeated dose toxicity results from an experimental study (cf. Table 7.9.4-1) with acetone oxime in rats were submitted by one registrant. For this endpoint the two registrants proposed different self-classifications.

Table 7.9.4-1. Study on repeated dose toxicity

Study/Method	Results	Remarks/ Reference
rat (Sprague-Dawley) male/female 25/sex/dose 5/sex/dose and 10/sex/dose were sacrificed after 45 days and 90 days, respectively. Test material: Acetone oxime Dose levels 0, 10, 50, 250 mg/kg bw/d Administration route: gavage Vehicle: water Study duration: 90 days followed by a 30 day recovery period equivalent or similar to OECD Guideline 408 (Repeated Dose 90-Day Oral Toxicity in Rodents)	NOAEL: 10 mg/kg bw/d Effects indicative of anemia: =10 mg/kg bw/d (f, only at day 45): Blood: statistically significant: ↓ haemoglobin (-9%), haematocrit and RBC ≥50 mg/kg bw/d (m/f) at 45 and 90 days: blood: elevated methaemoglobin, regenerative anaemia, compensatory reticulocytosis, erythrocytic morphology consistent with polychromia and occasional Howell-Jolly bodies; Spleen: ↑↑ absolute and relative weight (high dose not reversible, 30 -50% still increased compared to control after recovery) ≥ 50 mg/kg bw/d (m only) at 45 and 90 days: reversible thrombocytosis, ↓ Cholesterol (30% compared to control), ↓ total protein and albumin = 250 mg/kg bw/d (m) at 45 and 90 days: Liver: ↑ absolute and/or relative weights (in males not reversible >10%), at 90-days ↑relative heart weight = 250 mg/kg bw/d (m/f) at 45 and 90 days: reversible leucocytosis, ↑ bilirubin, ↑ ALP (90 d only), ↑ A/G ratio	unpublished study report **(1991c)** Klimisch 2 (reliable with restrictions) key study GLP Urine analysis lacking Dosing volume was not adjusted to the same volume for the dose levels No functional observations Extramedullary hematopoiesis in the liver and spleen (m/f) with increasing severity from 50 to 250 mg/kg and from 45 to 90 days. See further experimental information at https://echa.europa.eu/registration-dossier/-/registered-dossier/5744/7/6/2/?documentUUID=14c6691e-0f94-40eb-b99f-ae177c7b2b99

Study/Method	Results	Remarks/ Reference
	No mortalities and no significant clinical signs reported.	

In a GLP compliant 90-day study (unpublished study report, 1991c) in rats at 10, 50 and 250 mg/kg bw/day, dose-related statistically significant methemoglobinemia, anemia and erythrocyte morphology changes were observed in mid- and high-dose animals. Therefore it can be concluded that acetone oxime causes damage to mature erythrocytes in the peripheral blood, resulting in alterations in the measured erythrocyte-related parameters at the haematological examination. As a compensatory reaction reticulocytosis, hypercellualrity in the bone marrow and haematopoiesis in the spleen and liver were observed with an increase in incidence and severity with time and dose. Haemoglobin was slightly but not statistically significantly decreased at 90-day study termination in male and female rats, in contrast to the 45 days finding. At this sampling point haemoglobin values were 10.1% and 13% statistically significantly decreased in males and females, respectively.

Treatment-related changes of the liver and spleen were consistent with elevated liver weights in high dose males and spleen weights in mid- and high-dose group. Differences in mean absolute and relative spleen weights were dose related and generally statistically significant. Weights at 50 mg/kg were approximately twice those of control animals, while spleen weights for high dose animals were approximately 3 to 4 times increased compared to control. At termination of the recovery period, spleen weight for 50 mg/kg were comparable to control values but for the high dose animal's weights remained 30% to 50% higher than control values (unpublished study report, 1991c).

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

Clear liver cell foci were present in almost all high dose animals with slight to severe/high grading at 90 day. Also basophilic cell foci in all animals at the high dose were observed ranging from minimal to moderate. Foci of cellular alteration in the liver were already observed at 50 and 250 mg/kg bw/d at the interim sacrifice at day 45. The clear cell foci, composed of hepatocytes with clear finely granular cytoplasm, varied considerable in size and sometimes coalesced to form large areas of alternation. Basophilic cell foci consisted of more discrete alterations which were composed of hepatocytes with round central nuclei with prominent nuclear chromatin and basophilic staining cytoplasm. Cellular atypia, increased mitoses or compression were absent. Slight to moderate cytoplasmic vacuolization (characterized by intracytoplasmic accumulation of clear vacuoles resembling lipid) and slight bile duct proliferation was observed in males at the high dose level. The proliferating bile ducts were often in close association with macrophages containing hemosiderin-like pigment in the portal areas of the liver (unpublished study report, 1991c). Alterations in the spleen included dose dependant increases in extramedullary hematopoiesis, pigmentation and congestion of the red pulp. Capsular fibrosis was overserved in 1 male and 1 female at 250 mg/kg (high dose group) (unpublished study report, 1991c).

One registration provided additional experimental information (OECD Guideline 408, GLP study), on a similar substances, methyl isobutyl ketoxime (MIBKO) that resulted in a NOAEL of 15 mg/kg bw/day for 90 days oral exposure in rats (corrected for molecular weight for acetone oxime 9.52 mg/kg bw/day).

Conclusion:

For repeated dose toxicity the available information is acceptable and sufficient for evaluation.

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

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

The observed methemoglobinemia does not result in lethality but exposure to acetone oxime results in signs of damage to the erythrocytes, haemolysis and anaemia. According to ECHA (2015)[9] the formation of methaemoglobin shall be classified accordingly either in STOT-SE or STOT-RE and is warranted if any consistent and significant adverse changes in haematology is observed at the guidance values for category 2: oral (rat): 10 < C ≤ 100 mg/kg bw/d (Annex I, Part 3 of the CLP Regulation). The assessment shall take into consideration not only significant changes in a single organ or biological system but also generalised changes of a less severe nature involving several organs according to CLP Annex I, 3.9.1.4 (ECHA, 2015).

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

This combination of effects on the hematopoietic system and associated organs demonstrates an adverse effect after repeated exposure (90 days) to 50 mg/kg bw/day. A classification as STOT RE 2 could therefore be warranted. SEV has verified the concern that there may be a need to harmonize the classification for this endpoint. Based on the available data, acetone oxime does fulfil the criteria for classification for target organ toxicity through repeated exposure (STOT RE 2), H373 May cause damage to blood system through prolonged or repeated exposure according to Regulation (EC) No. 1272/2008.

[9] ECHA (2015): Guidance on the Application of the CLP Criteria Version 4.1 – June 2015

7.9.5. Mutagenicity

Acetone oxime has been evaluated in a battery of genotoxicity studies comprising of in vitro gene mutation assays in bacterial cells; in vitro gene mutation assays in mammalian cells, in vitro unscheduled DNA synthesis assays, in vitro comet assay as well as in vivo DNA and RNA adduct formation and the SMART assay. No in vitro cytogenicity/micronucleus study was provided by the registrant with acetone oxime; therefore both registrants provided information from read-across (different analogues). A summary of the standard information requirements including results and reliability scores is shown in Table 7.9.5-1 (key studies are also indicated). No information concerning in vitro cytogenicity study in mammalian cells or in vitro micronucleus study with acetone oxime (Annex VIII of Regulation (EC) No. 1907/2006) was provided by the registrants.

Table 7.9.5-1. Summary of genotoxicity studies

Test system / Study	Concentration range or dose levels tested	Results		Reference/ Remarks
In vitro tests:				
S. typhimurium (strains TA 1535, TA 97, TA 98 and TA 100) *In vitro* gene mutation assay, bacterial reverse mutation test Test material: Acetone oxime Standard NTP study protocol, preincubation method Similar to OECD 471	Main test - S9: 0, 100, 333, 1000, 3333, 10000 µg/plate. Main test + S9 (10% and 30% of male SD rat and Syrian hamster, respectively): 0, 100, 333, 1000, 3333, 10000 µg/plate. Negative and positive controls included	**+ S9** –	**- S9** –	**NTP (2002)** GLP Klimisch 2 Key study -maximum test concentration for soluble non-cytotoxic substances of 5 mg/plate exceeded -only 4 strains tested -no detailed study report was available, however NTP is regarded as reliable information source
S. typhimurium (strains TA 1535, TA 1537, TA 1538, TA 98 and TA 100) *In vitro* gene mutation assay, bacterial reverse mutation test Test material: Acetone oxime Spot assay (=suspension method) and plate assay, Reference for used method: Maron and Ames (1983). Revised methods for the *Salmonella* mutagenicity test. Mutation Research, 113, 173-215	Plate assay: TA100 and *TA 98*: Test - S9/+S9: *TA 100* and *TA 98*: 0.25 – 2.5 µg/plate. Plate assay: *TA 100* and *TA 1535*: Test + S9: 2 – 8 mg/plate. Spot assay: *TA 1535*, *TA 1537*, *TA 1538*, *TA 98* and *TA 100*: Test +/- S9 Authors reported high volatility No information about controls included	**+ S9** –	**- S9** –	Mirvish et al. (1998) No GLP Klimisch 3 Supportive study - strains slightly different from OECD 471 -results on purity not reported -no detailed study report, not all test concentrations were documented -maximum OECD recommended test concentration of 5 mg/plate exceeded for *TA 100* and *TA 1535*

S. typhimurium (strains TA 2637, TA 98 and TA 100), E. coli WP2 uvrA/*pKM101* *In vitro* gene mutation assay bacterial reverse mutation test Test material: Acetone oxime preincubation method (37°C, 20 min)	-/+ S9 no concentrations nor controls reported/included	**+ S9** –	**- S9** –	Araki et al. (1986) No GLP Klimisch 3 Supportive study -no detailed study report was available -no information on purity, controls
mammalian cell gene mutation assay (gene mutation) mouse lymphoma L5178Y cells Test material: Acetone oxime, purity 99.6% 3 and 24-hour treatment with/without metabolic activation Phenotypic expression period 3 days Former OECD Guideline 476 Current OECD 490	negative -Test + S9, 3 h treatment (duplicate): 5000; 3750; 2500; 1250; 625 and 312.5 µg/mL -Test –S9, 3 h treatment: 5000; 3750; 2500; 1250; 625 and 312.5 µg/mL -Test –S9, 24 h treatment: 5000; 3750; 2500; 1250; 625 and 312.5 µg/mL: Acceptability criteria cloning efficiency for solvent and untreated control not met (<65%). Positive control – S9: 4-Nitroquinoline-N-oxide Positive control + S9: Cyclophosphamide Solvent (DMSO) and untreated controls	**+S9** –	**- S9** –	**unpublished study report (2012)** GLP Klimisch 2 Key study -cytotoxicity was determined by relative survival, not by relative total growth (RTG) as recommended by the OECD guideline; thus relative cell growth during treatment and expression was not considered; -top dose selection not in line with new recommendations; -acceptability criteria for 3 assays met. More study details: https://echa.europa.eu/registration-dossier/-/registered-dossier/5744/7/7/2/?documentUUID=44c59269-fdee-4cda-bb61-6cb05fef5d48

mammalian cell gene mutation assay (gene mutation) Chinese hamster lung fibroblasts (V79) Test material: Acetone oxime Purity: 98% Treatment period: 3 h Concentration of 6-thioguanine: 11 µg/ml similar to OECD 476	0 (solvent), 0.23, 0.45, 0.5 mM acetone oxime (-S9) Solvent DMSO (1% v/v) Positive controls valid Top dose was chosen based on 20% RS (relative survival) Positive response with: Isopropyl hydroxylamine	**+ S9** No	**- S9** –	Haas-Jobelius et al. (1991) no GLP Klimisch 3 Supportive study Only 1 out of 5 acceptability criteria (selection of top dose) was sufficiently documented in the study. -spontaneous mutant frequency of the control not in the recommended range of $5x10^{-6}$ -no metabolic activation used -no OECD recommended source substance used.
Test system / Study	**Concentration range or dose levels tested**	**Results**		**Reference/ Remarks**
Structural and numerical chromosome aberrations from analogues				
In vitro chromosome aberration test Primary human lymphocytes Test item: Wasox-MMAC2; Reaction mass of propan-2-one-O,O'-(methoxymethylsilandiyl)dioxime; propan-2-one-O-(dimethoxymethylsilyl)oxime; propan-2-one-O,O',O''-(methylsilantriyl)trioxime (CAS 797751-44-1) 3 and 20-hour treatment with/without metabolic activation OECD Guideline 473	Test - 5% S9, 3 h treatment, 5.000, 1.670, 0.560, and 0.185 µL/mL Test - 5% S9, 20 h treatment, 5.000, 1.670, 0.560, and 0.185 µL/mL: The highest test substance concentration was not analysed for chromosome aberrations (Mitotic Index (MI) of 7%, due to a very high cytotoxicity which impeded scoring. At 1670 µg/L MI of 45%) Test + 5% S9, 3 h treatment, 5.000, 1.670, 0.560, and 0.185 µL/mL Positive controls: Methylmethanesulfonate –S9, Cyclophosphamide +S9	**+ S9** –	**- S9** –	unpublished study report (2005a) Klimisch 2 GLP Read-across, supportive -100 instead of 300 metaphases were investigated per concentration -the report did not state that also for the 3 h incubation period 1.5 cell cycles occurred. -no information on by-products or impurities of the test item

Test system / Study	Concentration range or dose levels tested	Results		Reference/ Remarks
In vitro chromosome aberration test Primary human lymphocytes Test item: Wasox-VMAC2, UVCB, Reaction mass of acetone O,O'-[methoxy(vinyl)silanediyl]oxime; acetone O,O',O''-(vinylsilanetriyl)oxime and acetone O-[dimethoxy(vinyl)silyl]oxime (CAS 797751-33-0) 3 and 20-hour treatment with/without metabolic activation OECD Guideline 473	Test - 5% S9, 3 h treatment, 5.000, 1.670, 0.560, and 0.185 µL/mL Test - 5% S9, 20 h treatment, 5.000, 1.670, 0.560, and 0.185 µL/mL: The two highest test substance concentrations were not analysed (MI of 5% and 39%). The other doses caused test substance concentrations related numerical and structural chromosome aberrations (multiple chromatid breaks, fragments or interchanges). Test + 5% S9, 3 h treatment, 5.000, 1.670, 0.560, and 0.185 µL/mL Positive controls: Methylmethanesulfonate –S9, Cyclophosphamide +S9	**+ S9** - -	**- S9** – +	unpublished study report (2005b) Klimisch 2 GLP Read-across, supportive -200 instead of 300 metaphases were investigated per concentration -the reported did not state that also for the 3 h incubation period 1.5 cell cycles occurred. -no information on by-products or impurities of the test item
In vivo tests				
chromosome aberration assay rat (Sprague-Dawley) male/female Test item: butanone oxime oral: gavage Vehicle: water similar to EPA OPPTS 870.5385 (In Vivo Mammalian Cytogenetic Tests: Bone Marrow Chromosomal Analysis)	Dose levels 300, 600 and 1200 mg/kg bw Test results: toxicity: yes; vehicle controls valid, positive controls valid. In a chromosome aberration assay in male and female Sprague-Dawley rats no significant increase in chromosomal aberrations in the bone marrow was found after single oral doses by gavage of up to 1200 mg/kg bw butanone oxime (Germany, 2014).	Negative		**unpublished study report (1990d)** Klimisch 2 Read-across Key study -original study was not submitted by the registrant; study was evaluated by Germany (2014)

Test system / Study	Concentration range or dose levels tested	Results	Reference/ Remarks
Mammalian Erythrocyte Micronucleus Test Mouse, strain Crl:NMRI BR 5 m/f per dose; high dose and control 10 m/f Test item: Wasox-VMAC2, Reaction mass of acetone O,O'-[methoxy(vinyl)silane diyl]oxime; acetone O,O',O''-(vinylsilanetriyl)oxime and acetone O-[dimethoxy(vinyl)silyl] oxime Sampling 24 and 48 hours after treatment. OECD 474	Single dose of 1000, 1500, and 2000 mg/kg bw Vehicle: corn oil The dose volume was uniformly 10 mL per kg body mass. No cytotoxicity in the bone marrow was noted (PCE/NCE ratio not effected) at 2000 mg/kg bw (highest dose tested according to the guideline) Positive control: 40 mg/kg bw Cyclophosphamide	Negative	unpublished study report (2007) GLP Klimisch 2 Read-across, supportive -no information on by-products and impurities

Acetone oxime does not produce gene mutations in studies with prokaryotic cells *in vitro*, (NTP 2002, GLP, supported by two other publications; Mirvish et al., 1998 and Araki et al., 1986); either in the presence or absence of a mammalian metabolic activation system. In GLP compliant gene mutations assays suitable to detect not only gene mutations, but also to some extent the induction of structural chromosomal mutations acetone oxime produced negative results with and without metabolic activation (unpublished study report, 2012, supported by Haas-Jobelius et al., 1991). No adequate tests with acetone oxime for structural chromosome aberrations/clastogenicity were available. According to ECHA (2016) non-testing methods such as read-across approaches, may also provide information on the mutagenic potential of a substance.

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

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

Moreover in vitro testing data of two additional substances provided by one registrant were used in a weight of evidence approach. The analogue substances Wasox-MMAC2 and Wasox-VMAC2 undergo rapid hydrolysis in aqueous media to acetone oxime and reactive methyl or vinyl substituted silanetriols. The methyl or vinyl silanetriols can condense to form substituted silanols, disilanols and higher molecular weight siloxanes. OECD (2009) concluded that the mammalian toxicity profile of butanone oxime is similar to that seen for the MEKO based methyl and vinly substituted oximino silanes methyltris(methylethylketoxime)silane and vinyltris(methylethylketoxime)silane (trifunctional oxime silanes; containing three methylethylketoxime groups that also hydrolyse upon contact with water/moisture to MEKO and reactive methyl or vinyl substituted silanetriols).

Therefore it is reasonable to consider data generated for WASOX-MMAC2 and WASOX-VMAC2 for acetone oxime, though the substances are UVCBs and contain either one, two or three acetone oxime groups (mono-, di- and trifunctional oxime silanes). WASOX-MMAC2 and WASOX-VMAC2 release acetone oxime during hydrolyses also reactive methyl or vinyl substituted silanetriols and condensed silanol material are formed, that may contribute to the overall toxicity (please see Annex I). Therefore the read-across for these two UVCB substances is only used in support of other lines of evidence.

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

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

Indicator tests (detecting putative DNA lesions):

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

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

In ovine seminal vesicle (OSV) cells that lack cytochrome P450 enzymes but express phenol sulfotransferase acetone oxime did not induce DNA repair or any detectable DNA modification (DX1, 8-aminodGuo, 8–oxodGuo) in OSV cells or in cultured rat hepatocytes according to Kreis et al. (1998). Also Haas-Jobelius et al. (1991) found no induction of

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

DNA repair (test protocol partly in line with OECD 482, full reporting lacking) in primary rat hepatocytes and V79 cells including positive and negative controls.

An in-vitro alkaline comet assay using cultured human lympoblastoid cell line TK6 and acetone oxime concentrations from 625 to 10000 µM including solvent (DMSO) and positive (etoposide) controls the test compound did not induce a statistically significant increase in tail intensity (unpublished study report, 2016; no GLP). The comet assay can detect single and double strand breaks in eukaryotic cells, however an international test guideline only exists for in-vivo, therefore this information is considered as supportive study.

Hussain et al (1990) investigated DNA and RNA adduct formation of acetone oxime and 2-nitropropane (2-NP) in-vivo in male SD and male F344 rats (single animals, 300-390 g and 180-190 g, gavage and i.p administration, respectively). The used vehicle was 4:1 water-Emulphor 620. Liver DNA and RNA were analysed after 6 hour following administration. Detection of 8-hydroxyguanine (8-oxoguanine, 8-OH-G) levels in liver DNA and RNA were increased compared to control and showed a similar pattern in both species. No significant strain differences were observed for 8-OH-G. Quantitative results in SD rats showed that 8-hydroxy-2'-deoxyguanosine (8-OH-dG) formation after i.p. administration of 2-NP was in the same range as measured after oral administration of acetone oxime. However acetone oxime was administered at an approximately 3 times higher dose. The amount of detected DNA modifications (8-OH-dG, unknown modified deoxynucleoside DX1) was approximately half compared to 2-NP and around one third of the RNA modifications (8-OH-GR, RX1, RX2) caused by 2-NP in F344 rats after i.p. administration. In summary the main DNA and RNA modifications were 8-hydroxy-2'-deoxyguanosine and 8-hydroxyguanine; the DNA modification was around 3 times elevated compared to control and in RNA 6 times higher. The unknown RNA modification RX2/GR was 7 to 9 times higher compared to control after i.p. or oral administration in F344 and SD rats, respectively.

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

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

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

Conclusion:

Sufficient information is available for the evaluation of germ cell mutagenicity of acetone oxime. For mutagenicity assessment of acetone oxime there are available adequate in-

vitro and in-vivo experimental data and non-testing information (read-across). Read-across from the analogue butanone oxime was accepted based on structural, metabolic (hydrolyses, minor oxidation to corresponding nitronate) and toxicological similarities (cf. Annex I read-across justification). Also information from two methyl or/and vinyl substituted oxime silanes containing one, two or three acetone oxime groups, released upon contact with water, are considered.

Acetone oxime did not induce reverse mutations in *Salmonella typhimurium* strains or *Escherichia coli*. In mammalian in-vitro systems acetone oxime did not cause gene mutations in mouse lymphoma cells or Chinese hamster fibroblasts. Information from analogue substances (butanone oxime and methyl or/and vinyl substituted oxime silanes) cover clastogenicity/aneuploidy. Butanone oxime did not induce chromosome aberrations in cultured Chinese hamster ovary cells. The only evidence from standard in-vitro tests that acetone oxime could cause chromosome aberration is based on a positive result with methyl/vinyl substituted oxime silane as test substance (that hydrolysis rapidly to acetone oxime). The substance induced structural and numerical damage to chromatids/chromosomes in peripheral human lymphocytes. Weather this difference is associated with the methyl/vinyl silane portion of Wasox-VMAC2 is unclear because the result with the methyl substituted analogue (same study design and laboratory) was negative. Furthermore in another in-vivo experiment with *Drosophila* (SMART assay) acetone oxime showed a dose related increase in wings spots in non-transgenic and transgenic flies indicative for genotoxicity. However this assay has no international harmonisation or validation.

Key in-vivo studies have been carried out with read-across substances. The studies have been conducted according to guidelines and GLP criteria. A mammalian erythrocyte micronucleus test in-vivo with Wasox-VMAC2 did not produce relevant increases of the numbers of micronuclei in polychromatic erythrocytes. In a further second in-vivo study (chromosome aberration assay) carried out with butanone oxime in rats no significantly increase chromosomal aberrations in the bone marrow occurred.

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

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

In summary the eMSCA concludes, that based on the available information and the read-across to butanone oxime no classification for germ cell mutagenicity according to the CLP Regulation (EC) 1272/2008 is proposed.

7.9.6. Carcinogenicity

Experimental evidence for carcinogenicity submitted by the registrants is provided in Table 7.9.6-1. The self-classification of the registrants for this endpoint differed, one registrant used read-across to butanone oxime.

Table 7.9.6-1. Studies on carcinogenicity with acetone oxime

Study/Method	Results	Remarks/ Reference
rat (MRC-wistar) male/female	LOAEL (carcinogenicity): <= 1000 ppm	Mirvish et al. (1982) No GLP

Study/Method	Results	Remarks/ Reference
15/16 m/f (40 weeks) Test material: Acetone oxime oral: drinking water, 5 days/ week Dose level 1000 mg/L water, Total dose/rat: 7 g/male rat, 6.2 g/ female rat Study duration: 18 months	Incidence of liver tumours in male rats was 80% at week 93 (statistically different to 0% in the control); in females 17% incidence by week 111). Tumours were characterised as hepatocellular adenomas mostly 1-4 cm diameter; These were composed of circumscribed masses of cells, having abundant cytoplasm and small, round nuclei; In 1 male with focal malignant degeneration. 2 males had in addition haemangiomas.	Klimisch 3 supportive study Purity: not given Control group of 23/20 m/f rats were started 8 months apart because this group served also as controls for another trial. Limited study documentation Average daily doses for male and female were 25.4 mg/kg and 24.6 mg/kg bw/d, respectively (Carcinogenic Potency Database[11])
rat liver foci model male MRC-Wistar and Wistar rats; Up to 10 animals/strain Acetone oxime 1000 ppm in drinking water single diethylnitrosamine (DEN) i.p. treatment (200mg/kg bw) 2 weeks after DEN: test substance administration for 8 weeks 3 weeks after DEN: partial hepatectomy	Significantly higher frequency of hyperplastic liver nodules (HLN) compared to control. Authors suggested that Acetone oxime may be a liver promotor (Mirvish et al. 1988).	Mirvish et al. (1988) No GLP Klimisch 3 Supportive study Purity: not given

There is concern regarding the carcinogenic potential of this substance based on the following information sources:

Read-across from the structural analogue substance butanone oxime

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

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

two animal species (cf. Table 7.9.6-2).

Table 7.9.6-2. Studies on carcinogenicity with butanone oxime

Study/Method	Results	Remarks/ Reference
rat (F344) male/female Test substance: butanone oxime 0, 15, 75, 374 ppm Purity: 99.9% inhalation: vapour, 6h/d, 5d/week Duration: 26 months interim sacrifice at 3, 12 and 18 months; similar to OECD TG 453	Positive: Liver tumours 0, 15, 75, 374 ppm, equivalent to 54, 270, 1346 mg/m^3, males: liver carcinomas 0/50, 0/51, 1/51, 12/51; statistically significant at 374 ppm males: liver adenomas 0/50, 2/51, 5/51, 18/51; statistically significant at 75 and 374 ppm males: fibroadenomas in mammary gland (2/50, 2/50, 4/50, 9/50; statistically significant at 374 ppm 0, 15, 75, 374 ppm, females: liver adenomas 0/50, 0/50, 2/50, 4/51; not statistically significant females: fibroadenomas in mammary gland 10/50, 7/50, 9/50, 17/50; not statistically significant	Newton et al. (2001) Germany (2014) Klimisch 2 key study At study termination testes weight was elevated by 82% compared to control without microscopic findings. $LOAEC_{sys, m}$ = 15 ppm (54 mg/m^3) for liver tumour development (Germany, 2014)
CD-1 mice male/female Test substance: butanone oxime Purity: 99.9% inhalation: vapour, 6h/d, 5d/week Duration 18 months, interim sacrifice at 12 months; similar to OECD TG 453	Positive: Liver tumours Carcinomas in males at 374 ppm (1346 mg/m^3); and adenomas in all test groups, ≥ 15 ppm (≥54 mg/m^3); decrease in latency for liver carcinomas at 374 ppm 0, 15, 76, 374 ppm, males: liver carcinomas 2/50, 2/50, 1/50, 10/50; statistically significant at 374 ppm males: liver adenomas 4/50, 11/50, 10/50, 11/50, not statistically significant, but within historical control range females liver adenomas 0/50, 0/50, 1/50, 3/50; not statistically significant	Newton et al. (2001) Germany (2014) Klimisch 2 Key study $LOAEC_{sys, m}$ = 15 ppm (54 mg/m^3) for liver tumour development (Germany 2014)

The combined chronic toxicity/carcinogenicity studies in rats and mice (similar to OECD TG 453) have demonstrated that butanone oxime causes liver tumours (adenomas and carcinomas) in both species at all tested exposure concentrations. Statistically significant increases in incidence were observed at 270 and 1346 mg/m^3 for liver adenomas in male rats and at 1346 mg/m^3 for liver carcinomas in male rats and mice. An increased incidence of liver adenomas occurred also in female rats and mice at 270 and 1346 mg/m^3, but was not statistically significant. A dose-response relationship for tumour induction in the liver of rats and mice was observed in both sexes. The incidence of fibroadenomas in the mammary gland was also significantly increased in male rats at 1346 mg/m^3 (Germany, 2014). Therefore Germany proposed in comparison to the given criteria for the CLP Regulation that butanone oxime fulfils the criteria for Category 1B carcinogen.

Based on a preliminary exposure estimation relevant exposure routes for professionals are inhalation and the dermal route. Therefor inhalation exposure, which was the administration route of the carcinogenicity study with the analogue substance butanone oxime, is also relevant for acetone oxime. In addition the vapour pressure of acetone oxime (estimated: 242 Pa at 25°C) is comparable to butanone oxime (two values available: 1070 Pa at 20°C and 140 Pa at 20°C; While for the first value the exact method is not known ("equivalent or similar to OECD Guideline 104") the second value is cited in NTP and according to Germany (2014) also in studies of the US EPA and of the Canadian Environment. Therefore it could be assumed that this value should also be valid according to Germany (2014) (for details of the read-across please see Annex I).

Histopathological findings from the 90-day study

Hepatocellular changes were more severe in treated male rats than in female rats in the 90-day study (unpublished study report, 1991c) indicating more pronounced effects in males. Foci of cellular alteration are common in rodent studies greater than duration of twelve months and may be seen in short duration toxicity studies following exposure to certain chemicals (Thoolen et al., 2010). Clear liver cell foci were present in almost all high dose animals with slight to sever grading, also minimal clear cell foci were detected in the mid dose group after 90 days (cf. section 7.9.4). The used rat strain was Sprague-Dawley (unpublished study report, 1991) that does not have a high incidence rate for this lesion compared to F344 rats. Also basophilic cell foci were detected in all animals in the highest dose group. The observed foci of cellular alteration were more frequently observed in the males (also in the high dose group) compared to females. Dose dependant onset of foci of cellular alteration was already observed at day 45 (unpublished study report, 1991c).

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

Supportive evidence from non-guideline studies/investigations

Mirvish et al. (1982) investigated the carcinogenic potential of acetone oxime according to a non-guideline non-GLP compliant study. Acetone oxime was administered to male and female MRC Wistar rats in a dose of 1000 mg/L drinking water during 18 months. For males only, the liver tumour incidence of 80% was significantly higher compared to control. All these tumours had benign histologic criteria despite occasional differences in nuclear size, except in one male rat in which focal malignant degeneration was noted. Three rats (including 2 males) had liver haemangiomas in addition to the adenomas. Though the study has major deficiencies the finding concerning the carcinogenic property of acetone oxime cannot be neglected. From this study Gold et al. (1989) calculated a TD50 of 12.1 mg/kg bw/day (male rat).

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

QSAR information

The QSAR prediction from the QSAR Toolbox V3.3.5 gave the endpoint specific structural alert Category: Oncologic primary classification C-Nitroso and Oxime Type for acetone oxime. However, no supporting mechanistic chemistry is available for this profiler. The profiler was developed by the Laboratory of Mathematical Chemistry (LMC) solely to mimic the structural classes of known/potential carcinogens covered in version 7.0 of the United States Environmental Protection Agency's (US EPA) OncoLogic Cancer Expert System for predicting carcinogenic potential[12].

Conclusion:

To assess the carcinogenicity of acetone oxime no guideline and GLP compliant carcinogenicity study was available. Classification of a substance as a carcinogen is based on consideration of the strength of the evidence of available data for classification with considerations of all other relevant information (weight of evidence) being taken into account as appropriate (ECHA, 2016). In absence of robust and reliable experimental carcinogenicity information for acetone oxime results from the analogue substance butanone oxime are also considered. The read-across approach is used as one line of evidence in a weight-of-evidence argumentation. Butanone oxime is currently legally classified for carcinogenicity as carcinogen category 2 according to Regulation (EC) 1272/2008. Based on the available carcinogenicity data in rats and mice butanone oxime may meet the classification criteria for carcinogenicity category 1 B. Germany (2014) proposed category 1B and intended the submission of a CLH-Report[13].

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

[12] https://www.oecd.org/chemicalsafety/risk-assessment/genetic%20toxicity.pdf
[13] https://echa.europa.eu/registry-of-submitted-harmonised-classification-and-labelling-intentions?diss=true&search_criteria_ecnumber=202-496-6&search_criteria_casnumber=96-29-7&search_criteria_name=Butanone+oxime

to reactive intermediates and radical formation might play a role. Also it is likely that males are more vulnerable based on higher incidences of foci of cellular alteration in the liver, higher incidences of benign and malignant liver tumours as well as DNA adduct formation.

SEV has verified the concern that there is a need to harmonize the classification for this endpoint according to Regulation (EC) No. 1272/2008. Depending on the final opinion of RAC, the eMSCA concludes that a harmonised classification on this endpoint for butanone oxime should also apply to acetone oxime.

7.9.7. Toxicity to reproduction (effects on fertility and developmental toxicity)

No experimental studies with acetone oxime on reproduction are available. According to the unpublished study report (1991c) the gross post-mortem as well as the histopathologic examinations of the mammary gland (right inguinal with skin), ovaries, uterus and testes (with epididymis) showed no treatment-related effects compared to control (cf. section 7.9.4).

According to ECHA (2016) for reproductive toxicity, a grouping and category approach and weight of evidence adaptation are options for non-animal approaches for the time being to adapt the information requirements for reproductive toxicity. Therefore experimental data on butanone oxime and acetaldehyde oxime (AAO) are used. For read-across justification please see Annex I. The information provided by the registrants and in Germany (2014) is summarised in Tables 7.9.7-1 and 7.9.7-2.

Table 7.9.7-1: Study on fertility

Study/Method	Results	Remarks/ Reference
rat (Sprague-Dawley) male/female 28/28 m/f/dose level Test substance: AAO (purity>49.1%), 50% aqueous liquid Oral gavage (2 ml/kg bw) vehicle: water Dose level 0 (control), 5, 15 or 50 mg/kg bw Study duration: 10 week prior mating until postnatal day 21 OECD 415 (One-Generation Reproduction Toxicity Study)	LOAEL (parental toxicity): 5 mg/kg bw/day based on histological effects in the spleen (m) (hemosiderosis and extramedullary hematopoiesis) NOAEL (reproductive toxicity and F1 generation): 50 mg/kg bw/day (based on no effects on reproductive parameters, implantation losses, post implantation survival index, live birth index and pub mortality). All females except 1 in the highest dose group mated. The 5 mg/kg group had a statistically significant smaller litter size, but values were within the historical control and no effects were observed at higher doses.	Rusch et al. (2009) OECD (2006) No information on GLP Klimisch 2 Key study Control group included Read-across Study documentation sufficient, however no primary data available. Hematology was evaluated in this study.

Table 7.9.7-2: Studies on developmental toxicity

Study/Method	Results	Remarks/ Reference
rat (Sprague-Dawley) Test substance: butanone oxime (>99%) oral: gavage, exposure: GD 6-15 (daily), duration: until sacrifice on GD20 0, 60, 200 or 600 mg/kg bw/day; Vehicle: water OECD 414 (Prenatal Development Toxicity Study)	Main study: NOAEL: 600 mg/kg bw/d for developmental toxicity, based on the parameters evaluated during caesarean section including the number of corpora lutea, implantation sites, viable foetuses, resorptions, foetal sex ratios, and foetal body weights; no treatment-related foetal malformations; no visceral or skeletal malformations (Germany, 2014) LOAEL: 60 mg/kg bw/d for maternal toxicity (toxicity to the haematopoietic system) based on spleen enlargement (Germany 2014)	Derelanko et al. (2003) and Germany (2014) Klimisch 2 Key study Control group included Read-across
Rabbit (New Zealand White) Test substance: butanone oxime (>99%) oral: gavage, exposure: GD 6-18 (daily), duration: until sacrifice on GD 29 Main study: 0, 8, 14, 24 or 40 mg/kg bw/day; Vehicle: water OECD 414	Main study LOAEL: 40 mg/kg bw/d for developmental toxicity, based on abortions in 3/10 adult females in pregnant rabbits (Germany 2014) NOAEL: 24 mg/kg bw/d for developmental toxicity, based on any treatment-related gestational effects, malformations or developmental variations (↓ mean number of viable foetuses of 5.3, but fell in the historic control range of 4.6-9.1); not noted at 20 mg/kg bw/d in the preliminary study (Germany, 2014) LOAEL: 10 mg/kg bw/d for maternal toxicity (toxicity to the haematopoietic system) based on signs of anaemia in the dams (Germany, 2014)	Derelanko et al. (2003) and Germany (2014) Klimisch 2 Key study Control group included Read-across <u>Preliminary study</u> (dose range-finding study) 80 mg/kg bw/d: mortality in 5/5 dams between GD 8-10 40 mg/kg bw/d: mortality in 2/5 on GD 10 or 11, 1 dam aborted on GD 20, 2/5 survived to scheduled sacrifice on GD 29

Conclusion:

No experimental studies with acetone oxime on reproduction are available. According to the examination of reproductive organs in the 90-day study (unpublished study report, 1991c) no treatment-related effects compared to control on reproductive organs occurred. The reproductive toxicity assessment of acetone oxime is based on read-across data from the structurally related substances butanone oxime and acetaldehyde oxime (cf. Table 7.9.7-1 and 7.9.7-2). For the read-across justification please see Annex I.

The developmental toxicity of butanone oxime (MEKO) was investigated in rats and rabbits according to OECD 414 (prenatal developmental toxicity study). No treatment-related gestational effects, malformations or developmental variations were observed in the rats. In rabbits excessive maternal mortality and abortions at the 40 mg/kg dose level was evident, thus only 6 rabbits produced litters. The severe maternal toxicity and limited number of litters precluded a full assessment of developmental toxicity at 40 mg/kg. Dams showed neurological effects, e.g. decreased activity, wobbly gait, at this dose level and higher. Nevertheless MEKO did not appear to be teratogenic to the rabbit at this dose level (Germany, 2014). No reproduction or developmental toxicity was reported for AAO in a one-generation study up to dose levels of 50 mg AAO/kg bw/day. The one-generation reproductive toxicity study showed no evidence for effects on fertility (Rusch et al. 2009). In addition, no changes in testicular weight or microscopic pathology of the testes or ovaries of rats were observed in a sub-chronic study tested up to levels of 112.5 mg AAO/kg bw/day (OECD, 2006). The NOAEL for developmental toxicity is considered to be >50 mg AAO/kg bw/day. Available information for effects on reproduction in rats and rabbits on structurally similar substances, does not indicate a concern for reproductive or developmental toxicity for acetone oxime.

7.9.8. Hazard assessment of physico-chemical properties

No need for clarification of potentially relevant concerns was identified.

7.9.9. Selection of the critical DNEL(s)/DMEL(s) and/or qualitative/semi-quantitative descriptors for critical health effects

The aspects considered for the derivation of the DNEL and DMEL follow the REACH Guidance on Information Requirements and Chemical Safety Assessment, Chapter R.8: Characterisation of dose [concentration]–response for human health (ECHA, 2012). Different DNELs have been derived for workers by the registrants. The $DNEL_{s\,worker,inhalation,long\text{-}term,systemic\ effects}$ were calculated with 0.35 mg/m³ and 0.33571 mg/m³; The $DNEL_{s\,worker,dermal,long\text{-}term,systemic\ effects}$ were 0.0476 mg/kg and 0.1 mg/kg according to the CSRs. No DMELs were provided by the registrants.

Acetone oxime is produced in Europe, formulated into mixtures, used at industrial sites and by professionals. DNELs and DMELs for the general population are not necessary based on the currently registered uses covering industrial and professional uses only. Considering its physico-chemical properties and industrial use, exposures to acetone oxime at the workplace occur primarily by the inhalation and dermal route (cf. section 7.12). The derivation of a DNEL and DMEL for workers for the inhalation and dermal route is based on data from studies with experimental animals.

The human health assessment indicates that major concerns associated with short-term exposure to acetone oxime are acute dermal toxicity, irreversible effects on the eye, and skin sensitisation:

The LD50 in an acute dermal toxicity study with rabbits was determined to be >1000 mg/kg; based on the range finding study a dose of 2000 mg/kg caused 100% mortality in the animals tested. Clinical signs were evident in several animals in the high dose group (hypoactive, fecal staining, dark coloration of the eye) and in a few animals in the mid and low dose groups (fecal staining). Concerning adverse effects, methemoglobinemia and anemia were observed (unpublished study report, 1991c).

Acetone oxime produced severe and irreversible eye lesions in New Zealand rabbits (unpublished study report, 1990b).

Skin sensitisation of acetone oxime was investigated in a GPMT that showed a clear response in 6/15 treated guinea pigs (equates to 40%) 24h after challenge. Acetone oxime is a moderate skin sensitiser (unpublished study report, 1990c).

No dose-response data are available for the eye effects. Also for skin sensitisation no quantitative dose descriptor is available. Therefore a quantitative risk characterisation is neither possible for eye irritation or for skin sensitisation. The available data allow a qualitative risk characterisation for eye irritation and skin sensitisation. However the local effects can be considered as covered by the critical DMEL (see below).

Sub-chronic exposure of acetone oxime is associated with effects on the blood, liver and spleen. Also there is evidence that acetone oxime induces carcinogenicity. Thus critical health effects associated with occupational exposure to acetone oxime are systemic effects on the hematopoietic system as well as carcinogenicity (cf. Table 7.9.9-1, 7.9.9-2). The mode of action for carcinogenicity for acetone oxime is not established. Though metabolic activation and reactive intermediates as well as radical formation might play a role the data including the read-across experimental carcinogenicity studies with butanone oxime do not allow to exclude a non-threshold mode of action. Also in the combined chronic/carcinogenicity study no NOAEC for liver tumour formation could be established (Newton et al. 2001; Germany, 2014).

According to the ECHA (2012) guidance, if it is not clear, the assumption of a non-threshold mode of action would be the prudent choice.

Consequently, four types of thresholds are required to characterise the risk to acetone oxime exposure at the workplace

- A long-term systemic DNEL (inhalation) for protection of effects on the hematopoietic system

- A long-term systemic DNEL (dermal) for protection of effects on the hematopoietic system

- A DMEL (derived minimal effect level) for assessment of the cancer risks associated with inhalation acetone oxime exposure.

- A DMEL for assessment of the cancer risks associated with dermal acetone oxime exposure.

Table 7.9.9-1 Critical DNELs for acetone oxime

CRITICAL DNELS					
Endpoint of concern	**Type of effect**	**Critical study**	**Corrected dose descriptor(s) (e.g. NOAEL, NOAEC)**	**DNEL**	**Justification/ Remarks**
Repeated dose toxicity *Inhalation exposure*	Effects on the hemato-poietic system	90 d repeated oral dose study in rats(Unpublished study report, 1991c) NOAEL 10 mg/kg bw/d	NOAEC 8.8 mg/m^3 (corrected for different exposure conditions and respiratory volumes according to ECHA (2012): respiratory volume rat, route-to-route extrapolation and respiratory volume for workers) =NOAEL*(1/0.38 m^3/kg/d)*(1/2)*(6.7 m^3/10 m^3)	0.353 mg/m^3	No experimental data on repeated inhalation exposure is available. Intraspecies AF_{worker} 5 AF for interspecies differences: 2.5 AF for duration extrapolation: 2 Overall AF: 25
Repeated dose toxicity *Dermal exposure*			Dermal NOAEL 10 mg/kg bw/d (based on the default assumption that dermal absorption will not be higher than oral absorption)	0.1 mg/kg bw/d	No experimental data on repeated dermal exposure is available. Interspecies AF 10 Intraspecies AF_{worker} 5 AF for duration extrapolation 2 Overall AF: 100

The $DNEL_{worker–inhalation–long-term\ systemic\ effects}$ was based on the 90-day oral repeated dose toxicity study with acetone oxime. Acetone oxime caused damage to mature erythrocytes in the peripheral blood, resulting in alterations in blood parameters. A dose-related statistically significant methemoglobinemia, anemia and erythrocyte morphology changes as well as adverse effects on the spleen were observed at 50 and 250 mg/kg bw/day leading to a NOAEL of 10 mg/kg bw/d in rats (unpublished study report, 1991c). This dose descriptor was corrected for route-to-route extrapolation and for respiratory volume rat and worker (cf. ECHA, 2012). Route-to-route extrapolation has a higher degree of uncertainty. In absence of substance specific absorption data the default values (50%

oral and 100% for inhalation) were applied. The correct starting point for DNEL derivation was 8.8 mg/m^3. Assessment factors (AF) to cover the uncertainties for intra- and interspecies differences as well as for the extrapolation from subchronic to chronic exposure lead to an overall AF of 25. The DNEL$_{worker,inhalation,long-term,systemic\ effects}$ was calculated with 0.353 mg/m^3. The DNEL of the analogue substance butanone oxime for worker, exposure route inhalation and long-term systemic effects was 0.7 mg/ m^3 (Germany, 2014). For this DNEL no route-to-route extrapolation was necessary (based on available animal inhalation studies). The two DNELs are comparable (cf. also Annex I read-across justification).

The DNEL$_{worker,\ dermal,\ long-term,\ systemic\ effects}$ was also based on the 90 day repeated dose toxicity study with acetone oxime (unpublished study report, 1991c). Based on the assumption that dermal absorption will not be higher than oral absorption (ECHA, 2012), no correction for the oral-to-dermal extrapolation was applied. Also no experimental dermal absorption data are available. Thus the correct dose descriptor as a point of departure for DNEL derivation was the NOAEL of 10 mg/kg bw/d. The assessment factors accounting for inter-, intraspecies differences and exposure duration were 10, 5 and 2 leading to an overall AF of 100 and a DNEL of 0.1 mg/kg bw/d. The DNEL of the analogue substance butanone oxime for worker, dermal exposure route, long-term systemic effects was also 0.1 mg/ kg bw/d (Germany, 2014).

Table 7.9.9-2 Critical DMELs for acetone oxime

CRITICAL DMELS					
Endpoint of concern	**Type of effect**	**Critical study**	**Corrected dose descriptor(s) (e.g. NOAEL, NOAEC)**	**DMEL**	**Justification/ Remarks**
Carcinogenicity *Inhalation exposure*	tumour development in the liver	LOAEC = 15 ppm (54 mg/m^3) for butanone oxime,; Newton et al. (2001) LOAEC for acetone oxime = 12.58 ppm	T25 value was chosen as starting point (cf. ECHA, 2012). The dose for 10% incidence of liver adenomas in male rats was experimentally determined as 75 ppm (270 mg/m^3) for butanone oxime. The calculated T25 value was 675 mg/m^3 (linear extrapolation, butanone oxime), corrected for molecular mass 567 mg/m^3. After the correction for differences in human and experimental exposure conditions, respiratory volumes and occupational lifetime conditions an overall factor of 1.02 was applied leading to a starting point of 578.34 mg/ m^3	11.57 µg/m^3 (1:100 000, linear)	Read-across butanone oxime; To account for the usage of alternative data (read-across) an AF of 2 was applied. High to low dose risk extrapolation factor 25.000
Carcinogenicity *Dermal exposure*			Please see above; DMEL $_{worker,\ inhalation,liver\ tumours}$ was used for the calculation.	1.65 µg/kg bw/d	DMEL $_{worker,\ inhalation,liver\ tumours}$ was multiplied by 10 m^3 (wRV) and divided by 70 kg bw

For the derivation of the DMEL$_{worker,\ inhalation,liver\ tumours}$ the carcinogenicity study of Newton et al. (2001) with the analogue substance butanone oxime (rats, inhalation route, 26 months duration) was used. The quantitative endpoint LOAEC from the read-across from butanone oxime is corrected for the difference in molecular mass of both compounds.

The correction factor is: 73.09/87.122 = 0.84. Thus, the LOAEC for acetone oxime is calculated as follows: $LOAEC_{acetone\ oxime} = LOAEC_{butanone\ oxime} \times 0.84$.

According to a study summary by Germany (2014) butanone oxime causes tumours in the liver (adenomas and carcinomas) in rats and mice at all tested exposure concentrations. Statistically significant increases in incidence were observed at the both highest concentrations for liver adenomas in male rats and at the highest concentration for liver carcinomas in male rats and mice. An increased incidence of liver adenomas occurred also in female rats and mice at the both highest concentrations, but reached no statistically significance. A statistically significant increased incidence of mammary gland fibroadenomas was also observed in male rats at the highest concentration (Germany, 2014).

According to Newton et al. (2001) and the evaluation by Germany (2014) the incidence of liver adenomas was significantly increased in male rats at a concentration of 75 ppm (270 mg/m^3). Incidence of liver adenomas in the control group was 0/50 and in the 75 ppm group 5/51, which is equivalent to a 10% increase in liver tumour formation. For the calculation of the DMEL the T25 value (25% of test animals show occurrence of liver adenomas) was used as starting point. As the dose for 10% incidence of liver adenomas in male rats was experimentally determined as 75 ppm (270 mg/m^3), T25 value for developing hepatocellular carcinomas and liver adenomas in male rats was calculated as 675 mg/m^3 (Germany, 2014). After adjustment for differences in experimental and human/worker exposure conditions (6 h/8 h), respiratory volumes (6.7 m^3/10 m^3) and occupational lifetime conditions (52 w/48 w; 75 y/40 y) an overall factor of 1.02 was applied. The correction for molecular mass (0.84) leads to a starting point of 578.34 mg/m^3. To account for the uncertainty that no reliable carcinogenicity study with acetone oxime is available and read-across to butanone oxime was applied an additional assessment factor of 2 is appropriate. Although there is no tolerable risk level for carcinogens in the EU, cancer risk levels of 10^{-5} could be seen as tolerable risk level for workers (ECHA, 2012). Therefore the high to low dose risk extrapolation factor relating to a 10^{-5} risk of 25000 was applied. The $DMEL_{worker,inhalation,liver\ tumours}$ was calculated with 11.57 µg/m^3.

The $DMEL_{worker,dermal,liver\ tumours}$ was based on the $DMEL_{worker,inhalation,liver\ tumours}$ of 11.57 µg/m^3 and the adjustment of the respiratory rate of 10 m^3 for light activity and a default body weight of 70 kg. The $DMEL_{worker,dermal,liver\ tumours}$ is 1.65 µg/kg bw/d. These DMELs are a factor 2 lower compared to butanone oxime based on the AF for the quality of the whole database for the endpoint carcinogenicity.

For workers the Carcinogen and Mutagens Directive 2004/37/EC requires compliance with objectives to prevent exposure, substitution of dangerous chemicals by less dangerous alternatives and if not technically possible, to minimise exposure.

7.9.10. Conclusions of the human health hazard assessment and related classification and labelling

A summary of the human health hazard assessment and resulting proposed classification and labelling is compiled in Table 7.9.10-1.

Table 7.9.10-1. Conclusions for classification or non classification

Endpoint	Route, Species	Results and reference	Classification
Acute toxicity	Oral, rat	LD50 >2000 mg/kg (limit test) (unpublished study report, 2012a).	No classification proposed

Endpoint	**Route, Species**	**Results and reference**	**Classification**
Acute toxicity	Inhalation	No experimental data with acetone oxime available	No classification proposed
Acute toxicity	Dermal, rabbit	LD50 >1000 mg/kg (unpublished study report, 1991b)	Classification proposal: Acute Tox. 4, H312: Harmful in contact with skin (1000 < ATE ≤ 2000 mg/kg bw)
Narcotic effect	Oral, rats Dermal, rabbit	Ataxia, hypoactivity and lethargy were reported at higher dose levels in two species (rats and rabbits) Read-across to butanone oxime: Transient and reversible changes in neurobehavioral function consistent with CNS depression (Germany, 2014)	Classification proposal: STOT SE 3, H336: May cause drowsiness or dizziness
Skin irritation	Dermal, rabbit	Slightly irritating to skin (unpublished study report, 1990a)	No classification proposed
Eye irritation	Eye, rabbit	Severe irreversible eye lesions (unpublished study report, 1990b)	Classification proposal: Irreversible effects on the eye category 1, H318: Causes serious eye damage
Sensitisation	Dermal, guinea pig	Skin sensitizer In the GPMT a skin sensitisation response of ≥30 % at >1.0 % intradermal induction dose was observed (unpublished study report, 1990c)	Classification proposal: Skin sensitizer sub-category 1B, H317: May cause an allergic skin reaction
Repeated dose toxicity	Oral rat	NOAEL: 10 mg/kg bw/d (unpublished study report, 1991c) Haemolytic anemia was the main toxic effect corresponding to decreased red blood cell parameters and increased breakdown products of haemoglobin, increased pigmentation and extramedullary hematopoiesis in spleen and liver. Reduction of haemoglobin ≥10% at 45 days in both sexes, reduction of RBC of 14% in females and of 11% in males at study termination at 50 mg/kg bw/d, together with significant increase of haemosiderosis in the spleen accompanied by increased organ weight by approximately 100% compared to control in this dose group. Liver toxicity (more pronounced in males)	Classification proposal: Specific target organ toxicity through repeated exposure (STOT RE 2), H373: May cause damage to blood system through prolonged or repeated exposure.

Endpoint	Route, Species	Results and reference	Classification
Mutagenicity	Various test designs (in vitro, in vivo)	Negative in standard mutagenicity/genotoxicity tests Read-across to butanone oxime for chromosome aberration	No classification proposed
Carcinogenicity	Inhalation, rat, mouse	Read-across to butanone oxime: LOAEC = 15 ppm (54 mg/m^3) for liver tumour development (Newton et al. 2001, Germany, 2014) Supporting information: Histopathological findings in the 90 day repeated dose toxicity study (unpublished study report, 1991c) Incidence of liver tumours in male rats at a LOAEL ≤1000 ppm (Mirvish et al. 1982) Significantly higher frequency of hyperplastic liver nodules (HLN) compared to control (Mirvish et al. 1988) QSAR alert for carcinogenicity (OECD Toolbox)	Classification proposed: Category 1B carcinogen, H350: May cause cancer
Reproductive toxicity	Oral, rat, rabbit	Acetone oxime: no indication of adverse effects in reproductive organs in unpublished study report (1991c). Read-across AAO, OECD 415: $LOAEL_{rat}$ (parental toxicity): 5 mg/kg bw/day based on histological effects in the spleen (m) (hemosiderosis and extramedullary hematopoiesis) NOAEL (reproductive toxicity and F1 generation): 50 mg/kg bw/day, highest dose tested (Rusch et al.,2009; OECD 2006) Read-across butanone oxime, OECD 414: $LOAEL_{rat}$: 60 mg/kg bw/d for maternal toxicity $NOAEL_{rat}$: 600 mg/kg bw/d for developmental toxicity $LOAEL_{rabbit}$: 10 mg/kg bw/d for maternal toxicity $NOAEL_{rabbit}$: 24 mg/kg bw/d for developmental toxicity Derelanko et al. (2003) and Germany (2014) Available information for effects on reproduction in rats and rabbits does not indicate a concern for reproductive or developmental toxicity.	No classification proposed

7.10. Assessment of endocrine disrupting (ED) properties

7.10.1. Endocrine disruption – Environment

For potential endocrine effects in the environment no data are available, but no indications for endocrine disruption were noticed so far.

7.10.2. Endocrine disruption - Human health

There is no concern for endocrine disrupting properties from the available information for acetone oxime.

7.10.3. Conclusion on endocrine disrupting properties (combined/separate)

For potential endocrine effects in the environment no data are available, but no indications for endocrine disruption were noticed so far. Based on the human health hazard evaluation there is no evidence for endocrine disrupting properties.

7.11. PBT and VPVB assessment

The assessment of the PBT/vPvB properties resulted in the conclusion that the P/vP criterion is potentially fulfilled as the substance is not readily biodegradable. The B/vB criterion is not fulfilled as the bioaccumulation potential is considered to be very low. Based on the upcoming RAC opinion on butanone oxime and the applied read-across to acetone oxime for carcinogenic cat. 1B the T-criterion is likely to be met.

Overall, it can be concluded the acetone oxime is not a PBT or vPvB substance.

7.12. Exposure assessment

Referring to section 7.5.2, acetone oxime is used as anti-skinning agent for the preparation of coating/printing inks and as intermediate for the manufacture of silicone sealants, which release acetone oxime during curing. Only industrial and professional uses were registered. Consumer uses have not been identified and therefore, not assessed by the registrants.

Environmental exposure is expected to be not relevant based on the reviewed registration data.

The following relevant observations have been made referring to the human exposure estimation performed by the registrants:

ECETOC TRA version 3 was used by the Registrant(s) for predicting human exposure of industrial workers and professionals. ECETOC TRA is a Tier 1-exposure estimation tool and expected to be conservative in general, if applied correctly and uses are covered.

Referring to the calculations of the registrants, acetone oxime was considered as solid revealing low dustiness. As acetone oxime is distributed as soild block or incorporated/dispersed homogeneously into viscous matrices like paints, the descritption of acetone oxime by low dustiness is comprehensible. Nevertheless, as the vapour pressure of acetone oxime is moderate (242 Pa at 25°C), consideration of gaseous release needs to be taken into account as well in addition to the potential of dust formation. Gaseous release might be more relevant than exposure to particles in the identified uses.

Considering the vapour pressure of acetone oxime (by considering substance as liquid in ECETOC TRA), significantly higher air concentrations are predicted for the same scenarios than given in the registration dossiers. Assuming acetone oxime as liquid in ECETOC TRA is justified and does not lead to a falsified result, as the selection of solid or liquid state triggers only, if inhalation exposure to particles based on dustiness or vapours based on vapour pressure is estimated. Based on the results of both options "solid: low dustiness" and "liquid: vp: 242 Pa at 25°C", gaseous release is indicated to be the more relevant route than formation of dust.

As gaseous release was not considered in the ECETOC TRA predictions of the registrants, the calculated exposure levels are expected to be not sufficiently conservative and to underestimate potentially real situations referring to this issue.

In addition, it needs to be highlighted that according to publicly available safety data sheets, acetone oxime is also available and used as granular powder in the EU. Therefore, the current situation and the appropriateness of the exposure assessment performed by the registrants is not fully clear.

7.13. Risk characterisation

Hazard assessment: Selection of critical hazard reference levels

For risk characterisation the registrants provided calculated DNELs from animals studies either performed with acetone oxime or with an analogue substance. DNELs were derived for workers, long term, systemic effects (effects on the hemato-poietic system, repeated dose toxicity) for the inhalation and dermal route.

0.35 mg/m³ and 0.33571 mg/m³ were indicated as $DNEL_{worker,inhalation,long\text{-}term,systemic\ effects}$ in the registration data. Whereas 0.0476 mg/kg and 0.1 mg/kg were given for $DNEL_{worker,dermal,long\text{-}term,systemic\ effects}$.

However most notably none of the registrants provided hazard reference levels, DMELs, to describe the likelihood of risks to workers concerning the carcinogenic potential of acetone oxime. Based on the calculations of the eMSCA, this effect is predicted to be the most relevant one leading to the lowest hazard reference levels for risk characterisation, that need to be taken into account.

The eMSCA calculated a $DMEL_{worker,inhalation,liver\ tumours}$ of 11.57 µg/m^3 for a cancer risk level of 10^{-5} that could be seen as indicative tolerable risk level for workers. This value is approximately 30 times lower than the DNELs for the inhalation route used by the registrants for risk characterisation.

For the dermal exposure route the $DMEL_{worker,dermal,liver\ tumours}$ is 1.65 µg/kg bw/d for a cancer risk level of 10^{-5}. This value is approximately 30 and 60 times lower than the DNELs for the dermal route used by the registrants for risk characterisation.

Please see chapter 7.9.9 for the derivation of these levels.

Exposure assessment and risk characterisation

Using the significantly lower DMELs of the eMSCA and the exposure estimations of the Registrant(s), the eMSCA identified unacceptable risk for human health for most scenarios.

As the Registrants used the lower DNELs and unacceptable risk was not identified by them for any of the registered uses and corresponding exposure scenarios, it is comprehensible, that there was no need for the registrants to reconsider and refine the exposure estimations- if possible- by using potententially higher Tier-exposure estimation tools and more realistic input parameters.

According to the frequently, common approach for exposure assessments by registrants, if no need for refinement is identified as risk is calculated to be acceptable, parameters are kept high/maximum/low/minimum by the registrants for having no or limited requirements for the users related to the use. For example, assuming concentration to be up to 100% (maximum), duration of use to be up to 8 hours/day (maximum, full work day) and no use of installed RMM and PPE (minimum), although concentration and duration might be lower in practice, RMM and PPE are used at work place. This approach is allowed and reasonable for some cases, as the user can use the whole range of flexibility, but on the contrary, this leads to conservative and potentially overestimating predictions for some/all uses and/or users.

As the eMSCA has not more information/details on the uses of the downstream users (industrial workers and professionals), such refinements of the exposure scenarios as required in this case leading to more realistic and potentially lower exposure levels cannot be performed by the eMSCA. Nevertheless, as the DMELs are significantly lower than the DNELs, it is not for sure, if Tier 1 or Tier 2 exposure estimations tools using more realistic/less conservative parameters would predict sufficiently low (and still realistic) exposure levels demonstrating acceptable risk under realistic conditions.

In addition, if it's not possible to demonstrate acceptable risk based on the currently used data, there are still the options to install/oblige better RMM and to consider this for the calculations or to demonstrate acceptable risk with monitoring data/studies and to provide them in the registration data.

Nevertheless, referring to the previous section on exposure assessment, a potential of underestimating exposure was also identified in the ECETOC TRA predictions performed by the Registrant(s).

Conclusion:

Harmonised Classification and Labelling

Acetone oxime has currently no harmonised classification according to Regulation (EC) 1272/2008.

Referring to the self-classifications of the Registrant(s), different classifications are available. Referring to the endpoint carcinogenicity, some registrants consider acetone oxime to be Carc. 2. Whereas, others do not provide any classification for this end point. Therefore, a harmonisation of classification is considered necessary to ensure equal classification of the substance throughout industry sectors.

The eMSCA concludes that it is justified to apply butanone oxime as source substance for read-across for the endpoint carcinogenicity based on structural similarities, similar properties and toxicokinetics of both substances. The German competent authority identified the need for a harmonised classification of butanone oxime as carcinogenic: Carc. 1B, H350: May cause cancer.

Depending on the final opinion of RAC, the harmonised classification on carcinogenicity for butanone oxime should also apply to acetone oxime. At this stage, based on the available data, the eMSCA considers acetone oxime to be Carc. 1B, H350: May cause cancer.

Occupational risk

The registrants derived DNELs for workers, long term, systemic for the inhalation and dermal route (effects on the hematopoietic system, repeated dose toxicity).

DMELs to describe the likelihood of risks to workers concerning the carcinogenic potential of acetone oxime were not derived. Based on the calculations of the eMSCA, this effect is

predicted to be the most critical one leading to the lowest hazard reference levels for risk characterisation, that need to be taken into account.

The current exposure predictions calculated by the registrants are not considered acceptable by the eMSCA, as they exceed the DMELs derived by the eMSCA. Some elements of the exposure prediction might have an overestimating and others an underestimating impact on the predictions of risk characterisation ratios (RCR). Nevertheless, based on the available data, it cannot be ruled out that real exposure levels exceed the DMELs.

Therefore, further risk management measures shall be considered by the eMSCA, if the carcinogenicity of the substance is confirmed by RAC.

Environmental risk

Unacceptable risk for the environment is not expected based on the reviewed registration data.

7.14. References

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7.15. Abbreviations

AAO	Acetaldehyde oxime
AF	Assessment factor
ALP	Alkaline phosphatase
AOO	Acetone:olive oil
AF	assessment factor
CHO	Chinese Hamster Ovary
CSR	Chemical Safety Report
d	day
DEN	diethylnitrosamine
DMSO	Dimethyl sulfoxide
DNEL	derived no effect level
ECHA	European Chemcial Agency
eMSCA	Evaluating memberstate competent authority
ESR	Electron Spin Resonance
FCA	Freund's Complete Adjuvant
GD	Gestational Day
GPMT	guinea pig maximisation test
HCA	hexyl cinnamic aldehyde (CAS No 101-86-0)
HGST	human glutathione S-transferase
i.p.	intraperitoneal
Kow	partition coefficient octanol/water
LOAEL	Lowest Observed Adverse Effect Level
MEK	Methyl Ethyl Ketone
MEKO	Methyl Ethyl Ketoxime
MEST	mouse ear swelling test
MI	Mitotic Index
m/f	male/female
NADPH	Nicotinamide adenine dinucleotide phosphate
NCE	normochromatic erythrocytes
NOAEL	No Observed Adverse Effect Level

NO	Nitric oxide
NO_2^-	Nitrogen dioxide
2-NP	2-Nitropropane
NTP	National Toxicology Program (https://ntp.niehs.nih.gov/)
n.r.	not reported
OSV	ovine seminal vesicle
OECD	Organisation for Economic Co-operation and Development
PCE	polychromatic erythrocytes
P2-N	Propane 2-nitronate
RAC	ECHA Risk Assessment Commiittee
RDT	repeated dose toxicity
RTG	relative total growth
RS	relative survival
RBC	Red blood cells
SCE	sister chromatid exchange
SD rat	Sprague Dawley rat
SMART	somatic mutation and recombination test
TD50	Tumorogenic dose (TD) which would induce tumors in half the test animals at the end of a standard lifespan for the species
TK	Toxicokinetic
UVCB	chemical substances of unknown or variable composition
wRV	worker respiratory volume

ANNEX I: Read-across justification

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

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

Table I-1: Studies used for read-across

Endpoint	Source Substance	Study type and reference
Carcinogenicity	Butanone oxime	Key study Newton et al. (2001). A chronic inhalation toxicity/oncogenicity study of methyl ethyl ketoxime in rats and mice.
Mutagenicity	Butanone oxime	NTP (1999). Technical Report on the Toxicity Studies of Methyl Ethyl Ketoxime
	Butanone oxime	Key study Unpublished study report (1990). Acute In Vivo Cytogenetics Assay in Rats.
	Reaction mass of propan-2-one-O,O'-(methoxymethylsilandiyl)dioxime; propan-2-one-O-(dimethoxymethylsilyl)oxime; propan-2-one-O,O',O''-(methylsilantriyl)trioxime WASOX MMAC2, CAS 797751-44-1	Supportive study Unpublished study report (2005a). Wasox-MMAC2: In vitro mammalian cytogenetic study (chromosome analysis)
	Reaction mass of acetone O,O'-[methoxy(vinyl)silanediyl]oxime; acetone O,O',O''-(vinylsilanetriyl)oxime and acetone O-[dimethoxy(vinyl)silyl]oxime WASOX VMAC2, CAS 797751-33-0	Supportive study Unpublished study report (2005b). Wasox-VMAC2: In vitro mammalian cytogenetic study (chromosome analysis)
	WASOX VMAC2, CAS 797751-33-0	Supportive study Unpublished study report (2007). Wasox-VMAC2: Micronucleus Test with Mice.

Narcotic effect	Butanone oxime	Key study Schulze and Derelanko (1993) Assessing the Neurotoxic Potential of Methyl Ethyl Ketoxime in Rats
		Key study TL2 (1984). Acute inhalation toxicity study of MEKO. Germany (2014)
		Key study Derelanko et al. (2003) Developmental toxicity studies of methyl ethyl ketoxime (MEKO) in rats and rabbits
Reproductive toxicity	Butanone oxime (MEKO)	Key study Derelanko et al. (2003) Developmental toxicity studies of methyl ethyl ketoxime (MEKO) in rats and rabbits.
	Acetaldehyde oxime (AAO)	Key study Rusch et al. (2009) Comparative reprotoxicity of three oximes.

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

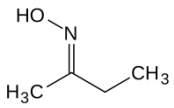
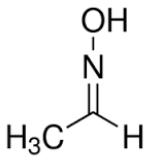
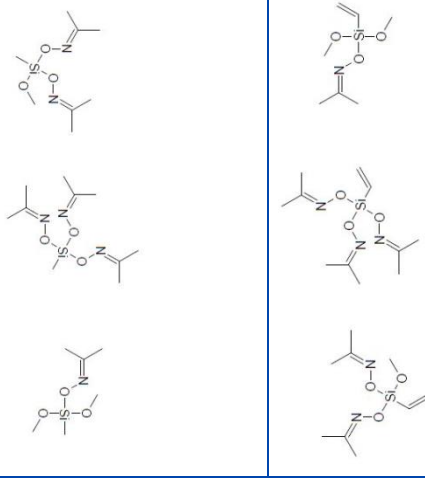
According to the ECHA (2008) Guidance "Guidance on information requirements and chemical safety assessment, Chapter R.6: QSARs and grouping of chemicals, the used data needs to be assessed for its adequacy. Therefore, the available experimental data have been evaluated for adequacy according to Chapter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

For a detailed evaluation of the available data depicted in Table I-1 please refer to the respective endpoint(s) in this document (chapter 7.9.5, chapter 7.9.6, and chapter 7.9.7). The experimental studies for the analogue approach have been analysed for adequacy and reliability and are classified with Klimisch score 1 or 2.

Identity and characterisation of the source substances

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

Table I-2: Chemical identity of the source substances

SUBSTANCE IDENTITY				
Public name:	Butanone oxime	Acetaldehyde oxime	Reaction mass of propan-2-one-O,O'-(methoxymethylsilanediyl)dioxime; propan-2-one-O-(dimethoxymethylsilyl)oxime; propan-2-one-O,O',O''-(methylsilantriyl)trioxime	Reaction mass of acetone O,O'-[methoxy(vinyl)silanediyl]oxime; acetone O,O',O''-(vinylsilanetriyl)oxime and acetone O-[dimethoxy(vinyl)silyl]oxime
IUPAC name	(2E)-N-Hydroxy-2-butanimin (Chemspider, 2017)[14] Butan-2-one oxime (Germany, 2014)	acetaldehyde oxime	n.r.	n.r.
EC number:	202-496-6	203-479-6	460-110-3	458-680-3
CAS number:	96-29-7	107-29-9	797751-33-0	797751-44-1
Molecular formula:	C_4H_9NO	C_2H_5NO	n.r. (multiconstituent substance)	n.r. (multiconstituent substance)
Molecular weight range [g/mol]:	87.122 g/mol	73.09 g/mol	n.r. (multiconstituent substance)	n.r. (multiconstituent substance)
Synonyms:	MEKO, methylethyl ketoxime, 2-butanone oxime	Acetaldoxime, aldoxime, ethanal oxime ethylidenehydroxy-lamine	WASOX MMAC2	WASOX VMAC2
Chemical structure				

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

SUBSTANCE IDENTITY				
Purity	>99%*	99.5% (marketed as a 50% aqueous solution)**	n.r. (UVCB)	n.r. (UVCB)

* Germany (2014), **OECD (2006), n.r. (not reported)

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

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

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

In the present read-across butanone oxime and acetone oxime have a common functional group (oxime group, imine group) and both are ketoximes. Butanone oxime has one additional carbon atom compared to acetone oxime. For the endpoint reproduction acetaldehyde oxime, also belonging to the low molecular weight oximes is added to build a read-across. Acetaldehyde oxime (AAO) is an aldoxime (oxime derived from an aldehyde) and has only one methyl group in addition to the oxime (imine) group.

For mutagenicity WASOX MMAC2 and WASOX VMAC2, which are multicomponent substances containing one, two or three acetone oxime groups with the difference being that they are also methyl or vinyl substituted on the silicon atom are used to support the data requirements. The substances undergo rapid hydrolysis (DT50 <1 hour) in aqueous solution to acetone oxime and reactive methyl or vinyl substituted silanetriols. The methyl or vinyl silanetriols can condense to from substituted silanols, disilanols and higher condensed siloxanes. However it is unclear at which concentrations these chemical reactions take place and no details on the chain of chemical reactions or conditions was given by the registrant(s). Therefore it is not clear if the continuous condensation reaction produces only higher molecular weight siloxanes or if also other silanols are present. OECD (2009) concluded that the mammalian toxicity profile of butanone oxime is similar to that seen for the methyl and vinly substituted oximino silanes containing three methylethylketoxime groups (that also hydrolyse rapidly in water to MEKO and reactive methyl or vinyl substituted silanetriols). However, though WASOX MMAC2 and WASOX VMAC2 release acetone oxime during hydrolyses also methyl or vinyl substituted silanetriols and condensed silanol material is formed, that may contribute to the overall toxicity.

Acute and repeated oral and dermal toxicity studies in rats with methyl/vinyl-MEKO-silane and methyl/vinyl-methyl isobutyl ketoxime-silane indicate that difunctional oxime silanes, containing both a methyl and a vinyl group caused degeneration of the seminiferous tubules of the testes. The testicular toxicity appears to be associated with the methyl/vinyl silane portion and not the oxime group of the oxime silane molecules (Derelanko and Rusch, 2008). Stable silanetriols have been found to reversibly inhibit the acetylcholinesterase activity at a 100 µM concentration in-vitro (Blunder et al., 2011) indicating biologically activity of this moiety. Therefore the read-across for these two substances is only used in support of other lines of evidence. However, the negative in vivo micronucleus test conducted with WASOX-VMAC2 can be used in the read across for

the endpoint mutagenicity as it can be concluded that neither the oxime nor the silanol moieties produced micronuclei in this system.

A stepwise approach for applying read-across is set out in Chapter R.6 section 6.2.3 "Guidance on a stepwise procedure to perform the analogue approach" (ECHA, 2008). The outcome of these step wise approach to perform the read-across from butanone oxime to acetone oxime for the endpoints mutagenicity, carcinogenicity and reproductive toxicity is provided in this Annex. For the endpoint mutagenicity the information requirement for structural and numerical chromosome aberrations for butanone oxime was used for read-across to acetone oxime supported by the read-across from WASOX MMAC2 and WASOX VMAC2 to acetone oxime. For the endpoint reproduction an additional read-across from acetaldehyde oxime to acetone oxime is provided. The values obtained from the source substances were used in a way that the prediction constitutes a worst case (no underestimation of the effects that would be observed in a study with the target substance if it were to be conducted).

Bias that may influences the prediction

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

Because acetone oxime is the tautomeric form of 2–nitrosopropane, a reduction product of 2-nitropropane, another possible similar compound for the endpoint mutagenicity/carcinogenicity is 2-nitropropane, a genotoxic hepatocarcinogen in rats (NTP, 2000)[15]. While propane 2-nitronate was experimentally determined in vivo in urine in rats as well as in in-vitro liver microsome studies also with human hepatocytes (Kohl et al. 2002, Völkel et al. 1999), the amounts were relatively small. Standard mutagenicity tests for acetone oxime do not indicate a genotoxic potential. Please see also the following chapter "Hypothesis for the analogue approach".

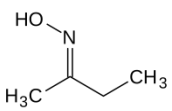
For the endpoint reproduction also experimental evidence from another oxime, methyl isobutyl ketoxime following the OECD 415 guideline in rats (Rusch et al. 2009) is available. Methyl isobutyl ketoxime may also be eligible for read-across based on structural similarities to acetone oxime. However, this would not change the final conclusion on this endpoint because for this substance as well as for butanone oxime and AAO the NOAELs for reproductive toxicity were the highest doses tested in the corresponding studies on reproductive toxicity (cf. section 7.9.7). The $NOAEL_{reproduction\ and\ F1\ generation}$ for methyl isobutyl ketoxime was the highest dose of 100 mg/kg/day tested (Rusch et al., 2009).

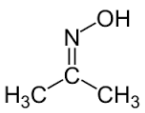
Hypothesis for the analogue approach

1 Butanone oxime used as source substance

Endpoint: Carcinogenicity

Butanone oxime displays a high structural similarity to acetone oxime (see Figure I-1).





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

Figure I-1: Chemical structures of butanone oxime and acetone oxime

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

No species-specific mode of action for butanone oxime carcinogenesis was identified. Butanone oxime and acetone oxime can hydrolyse to butane and acetone, respectively as well as to possibly the common metabolite hydroxylamine. NTP (1999) stated as possible second hydrolyses product for butanone oxime hydroxylamine. Also both substances can be converted to a minor degree to butane-2 nitronate and acetone-2 nitronate, respectively. The involvement of reactive metabolites/oxygen and/or nitrosating species in the aetiology of the observed effects that may lead to carcinogenicity however remains unclear.

The QSAR prediction from the QSAR Toolbox V3.3.5 gave the same endpoint specific structural alert for the source and the target compound: Category: Oncologic primary classification C-Nitroso and Oxime Type (cf. section 7.9.6).

Endpoint: Mutagenicity

Please refer for structural similarities and metabolism of the source chemical butanone oxime and the target acetone oxime to the section above. Butanone oxime and acetone oxime did not induce gene mutations in bacterial reverse mutation assays (cf. chapter 7.9.5). Based on in vitro and in vivo mutagenicity data, Germany (2014) concluded that there was no evidence of germ cell mutagenicity of butanone oxime in standard mutagenicity or genotoxicity tests. Also results from standard mutagenicity or genotoxicity tests on acetone oxime were negative. However both substances produced RNA adduct formation, for acetone oxime also DNA modifications in rats (in vivo) were shown (cf. Table I-3, chapter 7.9.5).

The QSAR prediction from the QSAR Toolbox V3.3.5[16] gave no general mechanistic structural alert for DNA binding for the source and the target compound. DNA binding is one mechanism well linked to carcinogenicity and genotoxicity. However an endpoint specific structural alert for in vivo mutagenicity (micronucleus): H acceptor-path3-H acceptor for both substances was identified indicating that possibly the chemical can interact with DNA and/or proteins via non-covalent binding, such as DNA intercalation or groove-binding.

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

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

a product of the metabolic detoxification (reduction) of the nitronate (Kohl et al. 2002, Völkel et al. 1999 and Haas-Jobelius et al. 1991).

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

In addition to butanone oxime also WASOX MMAC2 and WASOX VMAC2 were considered as source substances for the read-across. Both substances releases one, two or three moles of acetone oxime and one mole of reactive methyl or vinyl substituted silanetriol. Due to rapid abiotic transformation, only low systemic exposure to parent compounds WASOX MMAC2 and WASOX VMAC2 is likely to occur. From the hydrolysis study[17] (preliminary test according to EU Method C.7 and GLP) with WASOX MMAC2 and WASOX VMAC2 the half-lives of the 3 main components of the test substances at 25 °C and at pH 4, pH 7 and pH 9 were shorter than 1 hour in each case. Therefore the experimental data on these source substances can be used to predict effects also caused by acetone oxime. The condensed high molecular weight silanetriols is not considered to be very biologically active, however it is unclear if methyl and/or vinyl substituted silanetriol contribute to toxicological effects. Experimental in vivo data in rats showed that testicular toxicity appears to be associated with the methyl/vinyl silane portion and not the oxime group (Derelanko and Rusch 2008). Stable silanetriols have been found to reversible inhibit the acetylcholinesterase activity at a 100 µM concentration in-vitro (Blunder et al., 2011) indicating biologically activity of the moiety. Therefore this read-across is used as supportive information.

Endpoint: Transient Narcosis

Please refer for structural similarities and metabolism of the source chemical butanone oxime and the target acetone oxime to the section above.
According to literature transient narcosis is a common effect in laboratory animals for low molecular weight oxime compounds (Derelanko and Rusch 2008). For butanone oxime narcotic effects are described in single/acute and repeated dose/sub-chronic exposure situations, for acetone oxime effects are reported only in acute toxicity studies:
After oral administration in SD rats at 1000 mg/kg and 3000 mg/kg ataxia after dosing and hypoactivity were reported (Unpublished study report, 1991a). Hypoactivity was also observed in the high dose group at 1000 mg/kg in rabbits after acute dermal exposure (Unpublished study report, 1991b), but this could also be possibly attributed to the compromised health status of the animals. In a supportive study in rats in all dosed animals (100, 300 and 1000 mg/kg lethargy was reported (Unpublished study report, 1989a).

The data base for butanone oxime is more extensive and the reported dose levels for transient narcotic effects on an acute basis are lower compared to acetone oxime. Oral single doses of ≥300 mg/kg bw butanone oxime were found to produce transient and reversible changes in neurobehavioral function consistent with CNS depression, but no evidence of cumulative neurotoxicity was detected (neurotoxicity study, Schulze and Derelanko, 1993; Germany, 2014). After subchronic exposure transient treatment-related changes in ease of cage removal, ease of handling, and in posture, gait, and aerial righting were observed at the 400 mg/kg/day (Schulze and Derelanko, 1993). The highest dose tested in the repeated dose toxicity study with acetone oxime was 250 mg/kg/d, no clinical signs indicative of narcosis were reported (Unpublished study report, 1991c).

In the developmental study in rabbits (dams) with butanone oxime decreased activity and wobbly gait occurred at much lower dose levels at ≥ 40 mg/kg bw/d (Derelanko et al. 2003). In the acute inhalation toxicity study with butanone oxime a strong but

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

transient narcotic effect occurred in both sexes at 4.83 mg/L/4h during the exposure (unpublished study report, 1984). Narcosis was also observed after acute inhalation of AAO in an acute toxicity study in rats (OECD, 2006). No developmental or acute inhalation toxicity study with acetone oxime is available.

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

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

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

Endpoint: Toxicity to reproduction (developmental toxicity)

Acetaldehyde oxime (AAO) and butanone oxime display a high structural similarity to acetone oxime (see Figure I-2).

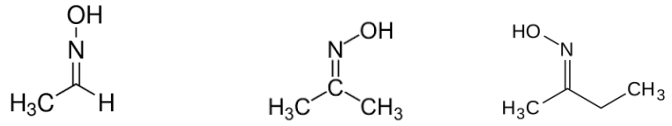


Figure I-2: Chemical structures of acetaldehyde oxime, acetone oxime and butanone oxime

The regular pattern concerns the R2 on the imine group, in case of acetaldehyde and hydrogen, for acetone oxime a methyl and for butanone oxime an ethyl group. Acetaldehyde oxime has two isomers. No effects on reproductive organs for butanone oxime (Germany, 2014), acetone oxime (Derelanko et al., 2003) or acetaldehyde oxime (Rusch et al., 2009) were identified in oral repeated dose toxicity studies. No inhalation repeated dose studies are available for acetone oxime and acetaldehyde oxime. The only finding on reproductive organs in the combined inhalation chronic toxicity/carcinogenicity study with butanone oxime was enlarged testes in male rats at 75 and 374 ppm (270 and 1346 mg/m^3), which did, however, not correlate with any microscopic findings. No effects on reproductive parameters or on parental reproductive organ histology were observed in a two-generation toxicity study similar to OECD 416 (oral route) in rats (Germany, 2014).

It can be assumed that hydrolysis will play a role in the metabolism of the source and the target compounds leading to a proposed common metabolite hydroxylamine but also to different metabolites. For example acetaldehyde oxime (AAO), when administered to the whole animal, is rapidly hydrolyzed and the carbon portion of the molecule undergoes further oxidation in the tricarboxylic acid (TCA) cycle. Both alpha and beta isomers of AAO as well as 4 metabolites were found in urine of animals given 375 mg/kg of ^{14}C-AAO (OECD, 2006)[18]. Other metabolic pathways/reactions may be relevant for the other compounds. Butanone oxime biotransformation appears to be complex (Germany, 2014). Though differences in the metabolic fate of the source substances and the target cannot

[18] http://webnet.oecd.org/hpv/ui/SIDS_Details.aspx?id=481ab9a1-4066-499a-b350-3230a976f212

be excluded it is assumed that common/comparable metabolites responsible for the observed toxicity are formed. While the oxime group is thought to be responsible to target the erythrocyte resulting in haemolytic anemia with compensatory responses no reproductive effects were identified in the two studies on fertility/development performed with acetaldehyde oxime and butanone oxime (Rusch et al., 2009; OECD, 2006; Germany, 2014).

Purity/impurities

The purity of the analogue substance butanone oxime is according to Table I-2 information very high (above 99%). Also the purity of AAO is 99.5% (with 0.1% impurity of acetaldehyde). Impurities are not likely to influence the overall toxicity. WASOX MMAC2 and WASOX VMAC2 are multicomponent mixtures; no information on impurities was available.

Chemical property similarity

AAO, acetone oxime and butanone oxime are low molecular weight compounds with a shared oxime group. They all have a high water solubility, low partition coefficient octanol/water (Kow), are stable at higher pH values and have moderate to high vapour pressures (cf. Table I-3).

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

WASOX MMAC2 and WASOX VMAC2 are multicomponent mixtures that hydrolyse fast (DT50 <1 hour) to acetone oxime and reactive methyl or vinyl substitutes silanetriol. Therefore the physical chemical properties, Kow and vapour pressure could not be measured according to the information provided by the registrants, data waiving was used for water solubility.

Based on these physico-chemical properties compiled in Table I-3 and resulting behaviour of the analogues, it is justified that AAO and butanone oxime are appropriate source materials for read-across.

Mammalian toxicological data

As depicted in Table I-4 AAO, butanone oxime, acetone oxime, WASOX MMAC2 and WASOX VMAC2 have some similar toxicological patterns with regard to mammalian toxicological endpoints.

Concerning local effects AAO, acetone oxime and butanone oxime were severe eye irritants and slight irritating effects on skin were observed in animal studies. For acute dermal toxicity it seemed that rabbit was more sensitive compared to rats for acetone oxime and butanone oxime. The acute dermal LD50 value in rats was >2000 mg/kg bw (Unpublished study report, 2012) compared to an LD50 in rabbits that was <2000 mg/kg bw (range >1000 <2000 mg/kg bw) (Unpublished study report, 1991b) indicating that rabbit is more susceptible to effects of acetone oxime. While more data are available for butanone oxime from single dose studies in rats and from repeated dose studies in rabbits, rabbits appear more sensitive than rats to the acute toxic effects of butanone oxime (Germany, 2014). The data from the other analogues do not allow drawing a conclusion on species differences.

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

In repeated or chronic dose studies the determined effect values are in the same range for all the analogue substances if exposure duration is taken into consideration. The target is the hematopoietic system consistent with haemolytic anemia, methemoglobin

formation and compensatory responses such as reticulocytosis, extramedullary hematopoiesis, splenic and hepatic hemosiderin pigment accumulation and increased spleen and liver weights.

Mutagenicity studies showed mixed results for AAO and WASOX VMAC2 in in-vitro systems. In vivo indicator studies concerning DNA and RNA adduct formation in rat liver were available with acetone oxime and butanone oxime. For butanone oxime no DNA modifications were detected. In liver RNA from butanone oxime exposed rats, a dose, sex and time-dependent formation of 8-aminoguanosine and 8-oxoguanosine was observed (Germany, 2014). The main identified DNA and RNA lesion in male and female rats were 8-hydroxy-2'-deoxyguanosine and 8-hydroxyguanine. Also a higher DNA adduct formation in males compared to females occurred (Hussain et al., 1190; Guo et al., 1990). A mammalian erythrocyte micronucleus test in-vivo with Wasox-VMAC2 did not produce relevant increases of the numbers of micronuclei in polychromatic erythrocytes. In a further second in-vivo chromosome aberration assay with butanone oxime in rats no significantly increased chromosomal aberrations in the bone marrow occurred. Based on standard information mutagenicity and genotoxicity test AAO, acetone oxime and butanone oxime are not expected to induce directly heritable mutations in mammals.

The available experimental studies on reproduction performed with AAO and butanone oxime indicate no effects on fertility and development (cf. Table I-4).

Table I-3: Data matrix for the analogue read-across: physico-chemical properties

Substances	Acetaldehyde oxime[1]	Acetone oxime	Butanone oxime[2]	Wasox-MMAC2[3]	Wasox-VMAC2[3]
Read-across	Source chemical	Target chemical	Source chemical	Source chemical	Source chemical
State of the substance at 20°C and 101.3 kPa	liquid	White solid	liquid	n.r	n.r
Melting point	46.5°C (α-form) 12°C (β-form)	60°C (measured)	-29.5°C (measured data)	35 ºC (measured data)	-5 ºC (acetoneO,O'-[methoxy(vinyl)silanediyl]oxime); 12.9 ºC (acetoneO,O',O''-(vinylsilanetriyl)oxime) -25.6 ºC (acetoneO-[dimethoxy(vinyl)silyl]oxime), (estimated values)
Boiling point	114°C	134°C*	>152°C (at 1013kPa)	Decomposition before boiling at about 190°C. The decomposed test substance boiled from about 205°C on.	Decomposed before and during boiling (205 ºC). Decomposed substance boiled from 220 ºC
Relative density	0.966 at 20°C	1.06 at 20°C*	0.92 at 20°C*	1.01 at 20ºC	1.02 at 20ºC
Vapour pressure	870 Pa at 20°C	242 Pa at 25°C (measured) 164 Pa at 25°C (estimated)	1070 Pa at 20°C 140 Pa at 20 °C	not possible to determine the vapour pressure	not possible to determine the vapour pressure
Dissociation constant pKa:	n.r.	12.42 at 24.9°C	12.45 at 25°C (measured data)	n.r.	n.r.
Water solubility	>10 g/L	Very soluble in water	100000 mg/L at 25°C and pH 7 (measured data)	Not applicable since the substance is hydrolytically unstable (half-life <12h)	Not applicable since the substance is hydrolytically unstable (half-life <12h)

Partition coefficient octanol/ water	-0.13 (measured value)	0.077	0.63 at 25°C (estimated)	Experimental determination not possible due to hydrolytically unstable (half-life <12 h)	Experimental determination not possible due to hydrolytically unstable (half-life <12 h)
Hydrolysis	Experimental result: pH 4.0, 7.0 and 9.0 (at 50°C). The percentage hydrolysis of AAO was found to be less than 10% under all test conditions AAO may become hydrolytically unstable at lower pH values (AAO decomposes by aqueous HCl into acetaldehyde and hydroxylamine)	Half-life of 18 days at neutral pH (QSAR estimation). Acetone and hydroxylamine (potentially as salt) are expected as hydrolysis products.	Experimental result: Half-life = 18.5 days at 20°C. The hydrolysis products are methyl ethyl ketone and a hydroxylamine salt*	Experimental result: DT50 <1 hour at 25 °C (EU Method C.7, GLP study)**	Experimental result: DT50 <1 hour at 25 °C (EU Method C.7, GLP study)***
Biodegradation	Experimental result: Readily biodegradable (OECD 301B)	Experimental results: Not readily biodegradable (OECD 301D, GLP study)	Experimental results: Not readily biodegradable; Inherently biodegradable*	Experimental results: Not readily biodegradable (OECD 301B, GLP study)	Experimental results: Not readily biodegradable (OECD 301B, GLP study)

Information source: [1]OECD 2006, [2]Germany 2014, [3]Chemcial safety reports (CSR) on acetone oxime *https://echa.europa.eu/registration-dossier/-/registered-dossier/14908/1
** https://echa.europa.eu/registration-dossier/-/registered-dossier/17570/5/2/3, ***https://echa.europa.eu/de/registration-dossier/-/registered-dossier/2278/5/2/3

Table I-4: Data matrix for the analogue read across: mammalian toxicity

Substances	Acetaldehyde oxime[1]	Acetone oxime	Butanone oxime[2]	Wasox-MMAC2[3, **]	Wasox-VMAC2[3, ***]
Read-across	Source chemical	Target chemical	Source chemical	Source chemical, supportive	Source chemical, supportive
Acute Toxicity: Oral	LD50 740 mg /kg (rats, OECD 401, no GLP)	LD50 >3000 mg/kg (rats, GLP) Ataxia	LD50 = 2326 mg/kg (male rats, OECD 401) LD50 >900 mg/kg (m/f rats, acute neurotoxicity study) LD50 = 160 mg/kg (rabbits, OECD 414)	Experimental results: LD50 >2000 mg/L (rat, female) (OECD 423, GLP study)	Experimental results: LD50 >2000 mg/L (rat, female) (OECD 423, GLP study)
Acute Toxicity: Inhalation	LC50 8.8 mg/L (vapour, 4-h exposure, rats, ~OECD 403, no GLP) Signs of narcosis (OECD, 2006)	No data	LC50 assumed to be higher than 13.2 mg/L/4h (likely >20 mg/L/4h, vapour, rats, in-house protocol) Signs of narcosis	No data	No data
Acute Toxicity: Dermal	LD50 >1000 mg/kg (rabbit, 16 CFR 1500.40, no GLP) LD50 >1000 mg/kg (rats, method n.r, GLP study)	LD50 >2000 mg/kg (rats, OECD 402, GLP) LD50 >1000 mg/kg, (rabbit, ~OECD 402, GLP study, hypoactivity)	LD50 >1000 mg/kg (rabbit, OECD 402) LD50 = 1848 mg/kg bw (rabbit, EPA OTS 798.1100)	LD50 >2000mg/L (rat, male/female) (OECD 402, GLP study).	LD50 >2000mg/L (rat, male/female) (OECD 402, GLP study).
Skin irritation	AAO is mildly irritating to the skin (Draize method according to 16 CFR 1500.41, no GLP)	Slight skin irritant (~OECD 404, GLP study)	Slight skin irritant (no specified test method) No irritating (OECD 404)	Non-irritant (OECD 404, GLP study)	Non-irritant (OECD 404, GLP study)
Eye irritation	moderately to severely irritating to the eye (CFR 1500.42)	Irreversible effects on the eye (GLP study, ~OECD 405)	Irreversible effects on the eye (OECD 405)	Non-irritant (rabbits) (OECD 405, GLP study)	Non-irritant (rabbits) (OECD 405, GLP study)
Skin Sensitization	Negative GPMT study (OECD 406, GLP study).	Positive GPMT study(OECD 406, GLP study)	Positive GPMT study (OECD 406, GLP) Negative in LLNA in mice (OECD 429)	No skin sensitizer (Local Lymph Node Assay, OECD 429, GLP study).	No skin sensitizer (Local Lymph Node Assay, OECD 429, GLP study).

Substances	Acetaldehyde oxime[1]	Acetone oxime	Butanone oxime[2]	Wasox-MMAC2[3, **]	Wasox-VMAC2[3, ***]
		Negative in LLNA in mice (OECD 429, GLP study)			
Repeated Dose Toxicity	LOAEL = 5 mg/kg bw/day (from the one generation reproduction study OECD 415) LOAEL = 12.5 mg/kg bw/day from a 13-week repeated-dose study in rats exposed to 12.5, 37.5 and 112.5 mg AAO/kg bw/day by gavage; Target: hematopoietic system (decreased hematocrit, hemoglobin, erythrocyte counts), increased total bilirubin, blood urea nitrogen, plasma and erythrocyte cholinesterase levels; increased spleen, thyroid, heart and liver weights. Microscopic examination revealed extramedullary hematopoiesis and increased pigment in the spleen and liver (OECD, 2006). Liver weights were elevated only in males at the mid-dose level, for both sexes in the high dose group.	NOAEL = 10 mg(kg bw/d (rat, OECD 408) target: hematopoietic system; anemia, elevated methemoglobin level, regenerative anaemia, compensatory reticulocytosis, erythrocytic morphology consistent with polychromia and occasional Howell-Jolly bodies; effects in the spleen and liver (extramedullary hematopoiesis, increased organ weights). Elevated liver weights in high dose males and spleen weights in mid- and high dose group. Hepatocellular changes were more severe in treated male rats than in female rats.	LOAEL = 10 mg/kg bw/d (rat, rabbit, mouse) target: hematopoietic system in rats, rabbits, and mice; neurobehavioral effects in rats and rabbits; degeneration of the nasal olfactory epithelium in rats and mice; hyperplasia of the urinary bladder transitional epithelium in mice. The lowest oral LOAEL of 10 mg/kg bw/d, based on effects in the spleen and liver of adult rats observed in a two-generation reproduction study. In adult female rabbits signs of anemia at this dose in a range-finding developmental study (Germany, 2014)	28 day-NOEL = 20 mg/kg bw/day (rats, male/female) based on effects on the hematopoietic system, extramedullary, liver and spleen weight changes, haematopoiesis in the spleen and hypercellularity in the bone marrow (OECD 408, GLP study)	28day-NOEL = 20 mg/kg bw/day (rats, male/female) (haemolytic anemia). Test substance related alteration in liver and spleen weight; Histopathologically, extramedullary haematopoiesis in the spleens of all high dosed animals (200mg/kg/d) and most mid dosed animals at 63 mg/kg/d. Hypercellularity of the bone marrow occurred. (OECD 407, GLP study)

Substances	Acetaldehyde oxime[1]	Acetone oxime	Butanone oxime[2]	Wasox-MMAC2[3, **]	Wasox-VMAC2[3, ***]
Gene mutation in bacteria (in vitro)	In vitro mutagenicity data were mixed; positive results were seen with and without metabolic activation in one Ames and one mouse lymphoma test. All in vivo genotoxicity studies (sister chromatid exchange, UDS and mammalian cell transformation) were negative (OECD, 2006)	Negative (+/-S9) in *Salmonella typhimurium* TA97, TA98, TA100, TA1535. (OECD 471, GLP study)	Negative (+/-S9) in several standard bacterial strains (OECD 471)	Negative (+/-S9) in *Salmonella typhimuriumTA97a, TA98, TA100, TA102 and TA1535.* (OECD 471, GLP study)	Negative (+/-S9) in *Salmonella typhimuriumTA97a, TA98, TA100, TA102 and TA1535* (OECD 471, GLP study)
Chromosomal aberration (in-vitro)		Read-across (no data)	In cytogenetic tests no induction of SCE was observed up to cytotoxicity (500 µg/mL) in the absence of S9 or up to the assay limit (5000 µg/mL) in the presence of S9. No increase in chromosomal aberrations was observed in cultured CHO cells treated with up to 5000 µg/mL butanone oxime +/- S9.	Negative (+/-S9) in human lymphocytes. (OECD 473, GLP study)	Positive without metabolic activation with 20h treatment; negative (+/-S9; 3 h treatment) in human lymphocytes. (OECD 473, GLP study)
Mammalian gene mutation (in vitro)		Negative mammalian cell gene mutation assay in mouse lymphoma cells (OECD 476, GLP)	Mouse lymphoma study (OECD 476) found evidence of mutagenic activity in mouse lymphoma L5178Y cells in the absence of S9 activation but in the presence of cytotoxicity (growth inhibition of 50-92.5 % at doses of 2.8-6.5 µL/mL).	No data	No data
Indicator mutagenicity tests		-Negative in unscheduled DNA Synthesis (UDS) assays in V79 cell lines, OSV cells and rat primary hepatocytes -Negative in-vitro alkaline comet assay	-Negative in unscheduled DNA Synthesis (UDS) test in rat primary hepatocytes -No SCE induction (see above)	n.r.	n.r.

Substances	Acetaldehyde oxime[1]	Acetone oxime	Butanone oxime[2]	Wasox-MMAC2[3, **]	Wasox-VMAC2[3, ***]
Genetic Toxicity in vivo		-Detected DNA and RNA adducts in rat liver -Positive in the SMART assay (*Drosophila melanogaster*)	-*Drosophila melanogaster* sex-linked recessive lethal test (~OECD 477): negative -Chromosome aberration test (~OECD 475): negative -Mammalian Erythrocyte Micronucleus test (~OECD 474): negative -RNA and DNA adducts in liver (rat): RNA adducts detected	No data	Negative (Mammalian Erythrocyte Micronucleus Test, OECD Guideline 474, GLP Study).
Carcinogenicity	No data In the C&L inventory two notifiers suggested the classification Carc. 2 (H351)[19]	Read-across Supporting: LOAEL ≤ 1000 ppm Incidence of liver tumours (hepatocellular adenomas) in male rats was 80% at week 93 (statistically different to 0% in the control); (no Guideline, Klimisch 3)	**Positive** LOAEC = 15 ppm (54 mg/m^3) for tumour development, Tumours in the liver (adenomas and carcinomas) in rats and mice at all tested exposure concentrations. Statistically significant increases in incidence at the high concentration for liver adenomas in male rats and at the highest concentration for liver carcinomas in male rats and mice; Increased incidence of liver adenomas occurred also in female rats and mice in the high concentration group, but no statistically significance; Statistically significant increase of mammary gland fibroadenomas in male rats at the highest concentration. A NOAEC for carcinogenicity was	No data	No data

[19] https://echa.europa.eu/information-on-chemicals/cl-inventory-database/-/discli/details/59086, 2017-07-18

Substances	Acetaldehyde oxime[1]	Acetone oxime	Butanone oxime[2]	Wasox-MMAC2[3, **]	Wasox-VMAC2[3, ***]
			not derived for rats and mice (Germany, 2014).		
Toxicity to reproduction (oral)	NOAEL >50 mg/kg bw/day for reproduction toxicity (highest dose tested) No reproduction (fertility effects) or developmental toxicity was reported in a one-generation study up to dose levels of 50 mg AAO/kg bw/day (rats, OECD 415). No effects of testicular weight or microscopic changes of the testes/ ovaries of rats were observed in a 13 week sub-chronic study tested up to levels of 112.5 mg AAO/kg bw/day (OECD, 2006).	Supporting: In the 90d RDT study, no effects on reproductive organs or tissues up to the highest dose tested.	Oral route: NOAEL =200 mg/kg bw/d Two-generation toxicity study in rats; no reproductive toxicity was observed at 200 mg/kg bw/d, the highest dose studied (~OECD 416). Inhalation route: $LOEC_{sys}$ = 75 ppm Chronic toxicity/carcinogenicity study enlarged testes in male rats at 75 and 374 ppm (270 and 1346 mg/m^3), but no histopathological changes (Germany, 2014).	Supporting: In the 28d RDT study, no effects on reproductive organs or tissues up to the highest dose tested.	Supporting: In the 28d RDT study, no effects on reproductive organs or tissues up to the highest dose tested.
Developmental toxicity	NOAEL >50mg/kg bw/day (rats, OECD 415)	No data (read-across)	$NOAEL_{rat}$ =600 mg/kg bw/d $NOAEL_{rabbit}$ =24 mg/kg bw/d (OECD 414) No indications of developmental toxicity in absence of excessive maternal toxicity. Rabbits (dams): decreased activity, wobbly gait	No data	No data.

Information source: [1]OECD 2006, [2]Germany 2014, [3]Chemcial safety reports (CSR) on acetone oxime *https://echa.europa.eu/registration-dossier/-/registered-dossier/14908/1
** https://echa.europa.eu/registration-dossier/-/registered-dossier/17570/5/2/3, ***https://echa.europa.eu/de/registration-dossier/-/registered-dossier/2278/5/2/3