BUREAU FOR CHEMICAL SUBSTANCES

SUBSTANCE EVALUATION CONCLUSION

as required by REACH Article 48

and

EVALUATION REPORT

for

Cyclohexanone

EC No 203-631-1

CAS No 108-94-1

Evaluating Member State(s): Poland

Dated: 26 June 2017

Template Version 2.1
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Evaluating Member State Competent Authority

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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. CONCERN(S) SUBJECT TO EVALUATION

Cyclohexanone was originally selected for substance evaluation in order to clarify concerns about:

- suspected CMR,
- wide dispersive use,
- exposure of workers,
- high (aggregated) tonnage.

No additional concerns were identified during the substance evaluation.

2. OVERVIEW OF OTHER PROCESSES / EU LEGISLATION

A compliance check decision issued on 20 December 2012.

3. CONCLUSION OF SUBSTANCE EVALUATION

Table 1

CONCLUSION OF SUBSTANCE EVALUATION Conclusions	Tick box
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

4.1.1. Harmonised Classification and Labelling

The aim of the evaluation was to clarify if cyclohexanone is a potential CMR substance. Cyclohexanone has been reported to be positive in some mutanic/genotoxic assays. There are available in vitro and in vivo studies involving Ames test in bacteria, gene mutations in mammalian cells (Chinese hamster ovary, mouse lymphoma, human fibroblasts, lymphocytes), chromosome aberrations in rats or recessive lethal assay in Drosophila melanogaster. The results reported for the mutagenicity/genotoxicity are conflicting: both negative and positive effects were observed for the same endpoints (e.g. Ames test is positive in some of in vitro tests, as well as in in vivo micronucleus test in mice bone marrow. According to Guidance on the Application of the CLP Criteria Version 4.1 – June

2015, classification in Category 2 may be based on positive results of at least one in vivo valid mammalian somatic cell mutagenicity test, indicating mutagenic effects in somatic cells. A Category 2 mutagen classification may also be based on positive results of at least one in vivo valid mammalian somatic cell genotoxicity test, supported by positive in vitro mutagenicity results. In vitro results can only lead to a Category 2 mutagen classification in a case where there is support by chemical structure activity relationship to known germ cell mutagens. In the case where there are also negative or equivocal data, a weight of evidence approach using expert judgement has to be applied.

Following analysis the eMSCA considered that the available information is sufficient for the classification of cyclohexanone as Muta. Cat. 2.

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

-

4.1.3. Restriction

-

4.1.4. Other EU-wide regulatory risk management measures

-

5. CURRENTLY NO FOLLOW-UP FORESEEN AT EU LEVEL

5.1. No need for regulatory follow-up at EU level

Not applicable.

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

There is a need for regulatory follow-up at EU level: the eMSCA proposes to classify the substance as Muta 2.
During the evaluation process particular emphasis was put on the concerns listed in the Justification document for the selection of the candidate CoRAP substance. The information on toxicity submitted by the registrant(s) is considered as relevant. The evaluation of the data available in CSR as well as in additional literature sources led to the conclusion that cyclohexanone fulfills classification criteria as Muta Cat. 2.

The available information is sufficient and reliable to clarify the initial concerns. There is no need for new studies and information under this substance evaluation.

Part B. Substance evaluation

7. EVALUATION REPORT

7.1. Overview of the substance evaluation performed

Cyclohexanone was originally selected for substance evaluation in order to clarify concerns about:

- suspected CMR

- wide dispersive use,

- exposure of workers,

- high (aggregated) tonnage.

During the evaluation it is concluded that there is a need for harmonised classification and labelling as Muta Cat. 2.

Table 4

EVALUATED ENDPOINTS	
Endpoint evaluated	**Outcome/conclusion**
Suspected CMR	M properties confirmed
Exposure of workers	Concerns not confirmed
High (aggregated) tonnage	Concerns not confirmed

7.2. Procedure

The updated Community rolling action plan (CoRAP) was published on the ECHA website on 22 March 2016.

On 20 December 2012 the registrant(s) of cyclohexanone with tonnage band of 1000 tonnes or more per year was addressed a compliance check (CCH) decision by ECHA [2] (decision number:CCH-D-0000002577-67-04/F) requesting the following information:

- Risk characterisation for worker inhalation;
- Risk characterisation for worker dermal route;
- Environmental exposure assessment and risk characterisation and subsequent demonstration that the risk to the environment can be considered to be adequately controlled;
- Risk characterisation for physicochemical properties of the substance;
- Information on specifications of protective gloves

The deadline for submitting the information requested in the above CCH decision was 20 December 2013. The Registrant submitted an updated dossier with requested information on 19 August 2014.

[2] Available on ECHA website, http://echa.europa.eu.

The substance evaluation was performed based on the updated registration dossier (IUCLID file) and Chemical Safety Report (CSR) as well as on the the basis of additional information available in scientific databases and publications.

All the information was assessed regarding reliability for evaluation of the main grounds of concern. The particular emphasis was placed on the possible CMR properties of cyclohexanone. Other aspects as physical and chemical properties have been checked and described in general in this report.

The results of the evaluation are documented in this report. Available information is enough to clarify the initial concerns. Thus no further information on human health is requested under this substance evaluation.

7.3. Identity of the substance

Table 5

SUBSTANCE IDENTITY	
Public name:	Cyclohexanone
EC number:	203-631-1
CAS number:	108-94-1
Index number in Annex VI of the CLP Regulation:	606-010-00-7
Molecular formula:	C6H10O
Molecular weight range:	98.143
Synonyms:	Anon Anon, pure Anone Cyclohexanon Cyclohexanone Cyclohexanone (7CI, 8CI, 9CI) Cyclohexylketon Cyklohexanone Hexanon Hytrol Ketohexamethylen Nadone Oxocyclohexan P2K KA Pimelic ketone Pimelin ketone Pimelinketon Sexton

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

Structural formula:

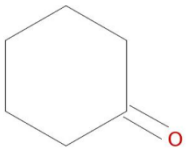

Table 6

Constituent			
Constituents	**Typical concentration**	**Concentration range**	**Remarks**
cyclohexanone EC no.: 203-631-1	> 99.8 % (w/w)		
Impurity			
Constituents	**Typical concentration**	**Concentration range**	**Remarks**
2-methylcyclohexanone EC no.: 209-513-6	< 180.0 mg/kg		
cyclohexanol EC no.: 203-630-6	< 150.0 mg/kg		
water EC no.: 231-791-2	< 500.0 mg/kg		

7.4. Physico-chemical properties

Table 7

OVERVIEW OF PHYSICOCHEMICAL PROPERTIES	
Property	**Value**
Physical state at 20°C and 101.3 kPa	liquid
Vapour pressure	7 hPa at 30 ^{0}C
Water solubility	86 g/L at 20 ^{0}C
Partition coefficient n-octanol/water (Log Kow)	0.81
Flammability	Based on chemical structure pyrophoric properties are not to be expected.
Explosive properties	There are no chemical groups associated with explosive properties in the molecule.
Oxidising properties	No oxidising properties.
Granulometry	Not applicable.
Stability in organic solvents and identity of relevant degradation products	Not applicable.

Dissociation constant	Not applicable.

7.5. Manufacture and uses

7.5.1. Quantities

Table 8

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

This substance is used in the following products: coating products, inks and toners, adhesives and sealants, plant protection products, biocides (e.g. disinfectants, pest control products), fillers, putties, plasters, modelling clay and laboratory chemicals. This substance has an industrial use resulting in manufacture of another substance (use of intermediates).

This substance is used in the following areas: building & construction work, printing and recorded media reproduction and agriculture, forestry and fishing. This substance is used for the manufacture of: chemicals, machinery and vehicles and furniture.

This substance can be found in products with material based on: metal (e.g. cutlery, pots, toys, jewellery), wood (e.g. floors, furniture, toys), paper (e.g. tissues, feminine hygiene products, nappies, books, magazines, wallpaper) and plastic (e.g. food packaging and storage, toys, mobile phones).

Table 9

USES	Use(s)
Uses as intermediate	Uses as intermediate
Formulation	Formulation
Uses at industrial sites	Uses at industrial sites
Uses by professional workers	Uses by professional workers
Consumer Uses	-
Article service life	-

7.6. Classification and Labelling

7.6.1. Harmonised Classification (Annex VI of CLP)

Table 10

HARMONISED CLASSIFICATION ACCORDING TO ANNEX VI OF CLP REGULATION (REGULATION (EC) 1272/2008)							
Index No	**International Chemical Identification**	**EC No**	**CAS No**	**Classification**		**Spec. Conc. Limits, M-factors**	**Notes**
				Hazard Class and Category Code(s)	**Hazard statement code(s)**		
606-010-00-7	cyclohexanone	203-631-1	108-94-1	Flam. Liq. 3 Acute Tox. 4*	H226 H332	-	-

7.6.2. Self-classification

Self-classification notifications for cyclohexanone (EC 203-631-1) are available in the C&L Inventory (https://echa.europa.eu/information-on-chemicals/cl-inventory-database/-/discli/details/427).

In the following table the additional notified classification for cyclohexanone is given (dating of September 2016).

Table 11

Classification	
Hazard Class and Category Codes	**Hazard Statement Codes**
Skin Irrit. 2 Eye Irrit. 2 Eye Dam. 1 STOT SE 3 Acute Tox. 4 Acute Tox. 4	H315 H319 H318 H335 (Respiratory system) H302 H312

7.7. Environmental fate properties

This evaluation was targeted to human health concerns and did not consider environmental fate properties.

7.8. Environmental hazard assessment

This evaluation was targeted to human health concerns and did not consider environmental hazards.

7.9. Human Health hazard assessment

According to CLP requirements cyclohexanone is classified for human health as:

- Harmful if inhaled (Acute Tox. 4*) – H332

The additional hazard categories were identified by notifiers:

- Causes skin irritation (Skin Irrit. 2) – H315,
- Harmful if swallowed (Acute Tox. 4) – H302
- Harmful in contact with skin (Acute Tox. 4) – H312
- Causes serious eye irritation (Eye Irrit. 2) – H319,
- Causes serious eye damage (Eye Dam. 1) – H318
- Specific target organ toxicity — single exposure (STOT SE 3) – H335

7.9.1. Toxicokinetics

Cyclohexanone is readily absorbed following oral, inhalation and dermal exposure. Cyclohexanone is mainly metabolized to the glucuronic conjugate of cyclohexanol and cyclohexanediols and excreted in urine or bile.

7.9.2. Acute toxicity and Corrosion/Irritation

Not evaluated.

7.9.3. Sensitisation

Not evaluated.

7.9.4. Repeated dose toxicity

Not evaluated.

7.9.5. Mutagenicity

The data submitted by the registrant(s) are summarised below:

No	Method	GLP	Results	Reference
1	Key study: Ames test/S. typhimurium TA 1535, TA1537, TA 98 and TA 100, E. coli WP2 uvr A 10-5000 ug/plate (Standard Plate Test); 10 - 1000 µg/plate PreIncubation Test)	Yes	Negative results with and without metabolic activation	Study of 1999
2	Key study: mammalian cell gene mutation assay (gene mutation) Chinese hamster Ovary 3.8, 7.7, 15.3, 30.6, 61.3, 122.5, 245.0, 490.0, 980.0 µg/mL	Yes	Negative results with and without metabolic activation	Study of 2012
3	Supporting study: mammalian cell gene mutation assay (gene mutation) mouse lymphoma L5178Y cells 312.5, 625, 1250,	Not specified	Negative results with and without metabolic activation	Study of 1988

	2500, 5000 µg/ml			
4	DNA damage and repair assay, unscheduled DNA synthesis in mammalian cells in vitro (DNA damage and/or repair human fibroblasts up to 9.48 mg/ml of culture medium	Not specified	Negative results with and without metabolic activation	Study of 1980
5	micronucleus assay (chromosome aberration), rat/inhalation-vapour 50 and 400 ppm	Not specified	Negative	Study of 1980
6	dominant lethal assay (chromosome aberration) rat/ inhalation-vapour 50 and 400 ppm	Not specified	Negative	Study of 1980

The additional publically available data

No	**Method**	**GLP**	**Results**	**Reference**
7	Ames test/Salmonella typhimurium strains TA1535, TA1537, TA98 2.9–2900 ug/plate EFSA opinion (2016): A preliminary assay was performed with the four strains using only one concentration level (3 µmol/plate). This assay gave uncertain results. In addition, strains TA98 and TA100 were exposed to 0.03 – 30 µmol/plate. The validity of the study	No data	Negative results with and without metabolic activation	Primary reference : Florin, I. et al. Toxicology, 15(3), 219-232, (1980) Secondary reference: OECD/SIDS. Screening Information Data Set (SIDS) of OECD High Production Volume Chemicals Programme, (1994)

	cannot be evaluated.			
8	Ames test/Salmonella typhimurium strains TA1535, TA1537, TA98, TA100 Only an abstract is available. No reporting with respect to metabolic activation. The validity of the study cannot be evaluated because of lack of experimental information.	No data	Positive results without metabolic activation	Primary reference : Massoud, A. et al. Mutation Research, 74(3), 174, (1980) Secondary reference: OECD/SIDS. Screening Information Data Set (SIDS) of OECD High Production Volume Chemicals Programme, (1994)
9	Ames test/Salmonella typhimurium strains TA98, TA100, TA1535, TA1537 33–10000 µg/plate EFSA opinion(2016): The highest level tested was the highest of either 10000 µg/plate, limit of solubility or maximal non-toxic concentration. The test was run twice. Both rat and hamster liver S9 were used. The test is considered valid.	No data	Negative with and without metabolic activation	Primary reference : Haworth S, Lawlor T, Mortelmans K, Speck W and Zeiger E, 1983. Salmonella mutagenicity test results for 250 chemicals. Environmental Mutagenesis 5(Suppl. 1), 3–142. Secondary reference: Flavouring Group Evaluation 51, Revision 2 (FGE.51Rev2): Consideration of alicyclic ketones and secondary alcohols and related esters evaluated by JECFA (59th meeting) structurally related to alicyclic ketones secondary alcohols and related esters in FGE.09Rev6 (2015) EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids (CEF), EFSA Journal 2016;14(1):4286
10	E. coli, polA assay The concentrations were not specified	No data	DNA damage occurred	Primary reference : Rosenkranz, H. S. and Leifer, Z. Chemical Mutagens. Principles and Methods for their Detection, 6, 109, (1980) Secondary reference: OECD/SIDS. Screening Information Data Set (SIDS) of OECD High Production

				Volume Chemicals Programme, (1994)
11	Gene mutation / Chinese hamster ovary cells 7.5 µl/ml Only an abstract is available with limited experimental information. The validity of the study cannot be evaluated	No data	No genotoxic effects with and without metabolic activation	Primary reference : Aaron, C. S. et al. Environmental Mutagenesis, 7 Suppl.3, 60-61, (1985) Secondary reference: OECD/SIDS. Screening Information Data Set (SIDS) of OECD High Production Volume Chemicals Programme, (1994)
12	Cytogenetic Assay for Chromosomal Aberrations/Human lymphocytes Concentration: 0.1–10 mM Study not reliable because of inadequate methods and reporting	No data	Chromosomal aberrations in human lymphocytes with or without metabolic activation.The results of the study are inconclusive due to little experimental details. Gaps, but no increase in breaks, were observed without any dose response relationship. There was no information with respect to cytotoxicity or presence of a control group.	Primary reference : Collin, V. P. Diabetes, 19(4), 215-221, (1971) Secondary reference: OECD/SIDS. Screening Information Data Set (SIDS) of OECD High Production Volume Chemicals Programme, (1994)
13	Cytogenetic Assay for Chromosomal Aberrations/Human lymphocytes 0, 0.005–0.1 µg/mL Study not reliable because of inadequate methods and reporting	No data	The yield of chromosome aberrations (single fragments) showed a 2.2 - 4 fold increase compared with the spontaneous frequency of aberrations Human lymphocytes from 15 donors were used; this resulted in a great fluctuation in the background aberration rate. As essential information is missing on the methods and results (incubation time, positive control, metabolic activation, gaps and breaks), this study cannot be used in the evaluation of genotoxicity.	Primary reference : Dyshlovoi, V. D. et al. Gigiena i Sanitariya (Hygiene and Sanitary), 46(5), 76-77, (1981) Secondary reference: OECD/SIDS. Screening Information Data Set (SIDS) of OECD High Production Volume Chemicals Programme, (1994)

14	A set of Ames tests with Salmonella strains TA98, TA100, TA1535 and TA1537) and a study with mouse lymphoma cells (L5178Y; tk+/-), including cloning efficiency and colony sizing provided convincingly negative results. The tests were carried out with and without metabolic activation at cyclohexanone levels up to 10000 µg/plate in the Ames tests and up to 5000 µg/mL in the mouse lymphoma assay. EFSA (2016): the tests by NTP are reliable.	No data	Negative results for genotoxicity with and without metabolic activation	National Toxicology Program. Technical Report Series, 2007) http://ntp.niehs.nih.gov/testing/status/agents/ts-10064-x.html
15	Unscheduled DNA synthesis/Human fibroblasts Exposures for 3 hours at concentrations up to 9.48 mg/mL.	No data	Negative results for genotoxicity with and without metabolic activation	Primary reference : Pevocco, P. et al. Toxicology Letters, 16(1-2), 69-76, (1983) Secondary reference: OECD/SIDS. Screening Information Data Set (SIDS) of OECD High Production Volume Chemicals Programme, (1994)*
16	Sex-Linked Recessive Lethal Assay/ Drosophila melanogaster 200-1600 mg/m3	No data	Negative effects of genotoxicity	Primary reference : McGregor, D. B. National Technical Information Service (PB number), PB-83-127571, (1980) Secondary reference: OECD/SIDS. Screening Information Data Set (SIDS) of OECD High Production Volume Chemicals Programme, (1994)
17	Phenocopies of tumor mutations/Drosophila melanogaster Exposure of male fruit flies to 0.1 mL	No data	No effects	Primary reference : Goncharova, R. I. Tsitologiya i Genetika (Cytology and Genetics), 137-142, (1970) Secondary reference:

	cyclohexanone/100 mL for 3 days. Article in Russian. Only an abstract available in English. The validity of this study cannot be assessed.			OECD/SIDS. Screening Information Data Set (SIDS) of OECD High Production Volume Chemicals Programme, (1994)
18	Sex-Linked Recessive Lethal Assay/Drosophila melanogaster The concentrations were not specified	Yes	Negative result	OECD/SIDS. Screening Information Data Set (SIDS) of OECD High Production Volume Chemicals Programme, (1994)
19	Forward mutation assay/L51784 th+/tk- mouse lymphoma cells Concentrations up to 5000 ug/mL	No data	No significant reductions in survival or increases in mutant fractions occurred with and without metabolic activation	Primary reference : McGregor, D. B. et al. Environmental and Molecular Mutagenesis, 12, 85-154, (1988) Secondary reference: OECD/SIDS. Screening Information Data Set (SIDS) of OECD High Production Volume Chemicals Programme, (1994)
20	Dominant Lethal Assay/mouse 5 d/7 h/d 200 or1600 mg/m3 Exposure to cyclohexanone vapors of 50 or 400 ppm for 5 days.	No data	No effects	Primary reference : McGregor, D. B. National Technical Information Service (PB number), PB83-127571, (1980) Secondary reference: OECD/SIDS. Screening Information Data Set (SIDS) of OECD High Production Volume Chemicals Programme, (1994)
21	In vivo Cytogenetic Assay; Chromosome Aberrations in Bone Marrow/rat 1-5 d 7 h/d 200 or 1600 mg/m3 Inhalation exposure to vapors of 50 or 400 ppm for 1 or 5 days.	No data	Negative result	Primary reference : McGregor, D. B. National Technical Information Service (PB number), PB83-127571, (1980) Secondary reference: OECD/SIDS. Screening Information Data Set (SIDS) of OECD High Production Volume Chemicals Programme, (1994)

22	Cytogenetic Assay In vivo; Chromosome Aberrations in Bone Marrow/rat 100-1000 mg/kg bw Observation time : 6, 24, 48 h The fact that the most marked effects occurred after 6 hours and the lack of a control group limit the meaningfulness of the experiments.	No data	Chromosome aberrations were induced at all doses and time intervals. Incidence of abnormalities increased with dose and decreased with time. They consisted of chromatid gaps and break, centric fusions, centrometric attenuation, chromatid exchanges and polyploidy. The changes were most apparent after 6 hours and weakest after 48 hours.	Primary reference : De Hondt, H. A. et al. Egyptian Journal of Genetics and Cytology, 12(1), 31-40, (1983) Secondary reference: OECD/SIDS. Screening Information Data Set (SIDS) of OECD High Production Volume Chemicals Programme, (1994)
23	In vivo micronucleus test/mice bone marrow Mice/300, 600, 1200 mg/kg/24 h	Yes	Increase of the micronucleated polychromatic erythrocytes at 1200 mg/kg	Kim S. at al. Journal of Life Science, 24(7), 804-811, (2014)
24	In vitro micronucleus test in bovine peripheral lymphocytes 2 or 28 hours 0.1, 0.5, 1.0, 5.0 and 10 mmol.l^{-1}	No data	Very slight increase in micronucleus frequency in the cultures at the lowest concentration	E. Piesova, K. Sivikova, J. Dianovsky, B. Holeckova. Folia Veterinaria 47(3), 161-163, (2003)
25	In vitro comet assay in human epidermal skin models Concentration up to 1600 µg/cm^2/3h	No data /project supported by the EU Reference Laboratory on Alternatives to Animal Testing	Negative	A.A. Reus et al. Mutagenesis 28(6), 709-720, (2013).

* Study included in CSR

Summary of the available information on mutagenicity of cyclohexanone:

- in silico model:

no alerts for genotoxicity/carcinogenicity in (DEREK Expert system for the prediction of toxicity).

- in vitro tests:

Ames test: among five study results (one presented in the registration dossier (No 1/GLP) and four found in the available databases (No 7, 8, 9 and 14), performed using different strains of Salmonella), four gave the negative effects with and without metabolic activation. The results of one study (No 8) gave positive results without metabolic activation. Study No 7 and 8 were considered of poor quality.

Gene mutation/chromosome aberration tests: the negative results were obtained in the three studies from the registration report (No 2/GLP and 3). Two other tests gave positive results and one was negative, but all of them were considered inadequate (no dose-response relationship in positive test, limited experimental information).

DNA damage (UDS/human fibroblasts) test: results of the studies No 4 (registration dossier) and No 15 showed negative results with and without metabolic activation.

Comet assay: the results of study No 24 indicated no genotoxic potential of cyclohexanone.

- in vivo tests:

In the registration dossier the results of two tests: micronucleus assay and dominant lethal assay performed following inhalation exposure of rats to cyclohexanone was shown to be negative (No 5 and 6). This effect was confirmed in dominant lethal assay in mice exposed via inhalation to cyclohexanone (No 20) and in one chromosome aberration test in rats bone marrow (No 21). The other one chromosome aberration test in rats bone marrow gave the positive result however the meaningfulness of this experiment was limited (No 22).

In the analysis of various in vivo test results, the study of Kim et al (No 23/GLP) seems to be decisive. The aim of the study was to screen the cytogenetic damage that results in micronuclei formation. In this study no specific symptoms in animals orally exposed to cyclohexanone were observed. Cyclohexanone did not inhibit bone marrow cell proliferation in all the treated groups, though it did initiate micronuclei induction.

The available in vitro and in vivo data were analysed for the different endpoints: gene mutations or chromosome aberrations. According to the information provided by the registrant(s), cyclohexanone is not mutagenic in Salmonella typhimurium and Escherichia coli in the presence or absence of metabolic activation with concentrations ranging from 10 - 5000 µg/plate and in Chinese hamster ovary cells with concentrations ranging from 3.8 - 980.0 µg/mL (key studies in CSR). The results of another in vitro study - mammalian cell gene mutation assay (with and without metabolic activation) - indicate no mutagenicity) with concentrations ranging from 312.5 - 5000 µg/ml (supporting study). The results of in vivo genotoxicity studies: micronucleus assay (rats) and dominant lethal assay (supporting studies in CSR) were negative via the inhalation route (7h/d for 5 days and 50 and 400 ppm respectively for these assays).

The assessment of the literature data relating to mutagenicity of cyclohexanone revealed ambiguous results. Cyclohexanone was not mutagenic in an Ames test considered to be valid. Cyclohexanone was negative in recessive lethal assay on Drosophila melanogaster, dominant lethal assay on mouse and unscheduled DNA synthesis test on human fibroblasts. Negative and positive results were obtained in several other in vitro bacterial tests. Positive results were reported in in vivo cytogenetic assays for chromosomal aberration and in a micronucleus test (mice). Cyclohexanone induced chromosomal aberrations and increased in chromosomal damage in cultured human leucocytes, however the validity of these studies was poor due to lack of experimental information. Abnormalities were also observed in in vivo chromosome aberrations in bone marrow assay.

Cyclohexanone was also investigated in the frame of the assessment of genotoxicity of substances migrating from polycarbonate replacement baby bottles to identify chemicals of high concern (Martens et al. (2016). According to the authors, the data did not allow to conclude on genotoxic potential of cyclohexanone, however, in silico predictions and the results of Vitotox test did not indicate such potential.

Cyclohexanone was one of the subjects of research conducted within the framework of the project whose purpose was to verify the intra- and inter-laboratory reproducibility related to genotoxic properties investigated in in vitro comet assay in human epidermal skin models (Reus et al., 2013). The results supported the intra- and inter-laboratory reproducibility of the assay. In the report of this study cyclohexanone was considered to be negative overall in terms of genotoxic potential.
Cyclohexanone exhibited no genotoxic alert in the Expert system for the prediction of toxicity (Derek) and it is not included in the EURL ECVAM Genotoxicity and Carcinogenicity Consolidated Database of Ames Positive Chemicals (https://eurl-ecvam.jrc.ec.europa.eu/databases/genotoxicity-carcinogenicity-db)

Conclusion:

The database on mutagenicity of cyclohexanone is relatively wide. It consists of in vitro and in vivo studies, but the available information is controversial. More data indicates no mutagenic activity of the substance as well as the prediction of genotoxicity performed using in silico models. Some studies showed possible mutagenic potential, however the quality of part of them is poor due to limited experimental information or lack of dose response relationship in the obtained results. All in vitro tests performed according to GLP requirements or considered as adequate by EFSA were negative. One in vivo micronucleus test fulfilling GLP rules was positive. A micronucleus assay is recognized as one of the most successful and reliable assay for genotoxic substances.

According to Guidance on the Application of the CLP Criteria Version 4.1 – June 2015, classification in Category 2 may be based on positive results of at least one in vivo valid mammalian somatic cell mutagenicity test, indicating mutagenic effects in somatic cells. A Category 2 mutagen classification may also be based on positive results of at least one in vivo valid mammalian somatic cell genotoxicity test, supported by positive in vitro mutagenicity results. In vitro results can only lead to a Category 2 mutagen classification in a case where there is support by chemical structure activity relationship to known germ cell mutagens. In the case where there are also negative or equivocal data, a weight of evidence approach using expert judgement has to be applied.

Following analysis the eMSCA considered that the available information issufficient for classification of cyclohexanone as Muta. Cat. 2.

7.9.6. Carcinogenicity

Cyclohexanone was tested for carcinogenicity following its oral administration (in drinking water) to rats and mice (report from CSR, also described in publicly available data base - Lijinski and Kovatch (1986)). Animals were exposed to doses of 0, 3300 and 6500 ppm (male and female rats), 0, 6500 and 13000 ppm (male mice) and 0, 6500, 13000 and 25000 ppm (female mice), for 104 days (continuously)).

Survival and weight gain at all the lowest cyclohexanone dose, in both genders and both species, were similar to those of control group. Weight gain was decreased at all higher doses. Most of the neoplasms in the treated groups did not differ in number from the control group.

The incidences of adrenal cortex adenomas were higher in the lowest dose group of male rats and decreased with the higher dose of cyclohexanone.

The incidence of lymphomas increased only in female mice in the lowest dose group, but it was within the historical control range. At the dose of 6500 ppm of cyclohexanone male mice showed an increase of incidence of hepatocellular adenomas and carcinomas, while in male mice treated with 13000 ppm dose the number of these neoplasms was decreased. The incidence of lymphomas in exposed male mice and hepatocellular neoplasms in exposed female mice did not differ from control.

Based on these results it was concluded that the background incidence of tumors was high and in the absence of a dose-response relationship the substance is of marginal carcinogenic potential. IARC considered that cyclohexanone is not classifiable as to its carcinogenicity to humans (IARC, 1989). The concern has been clarified and no further information is requested.

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

The registrant(s) submitted the results of four studies regarding toxicity for reproduction and developmental toxicity.

In a two-generation reproduction toxicity study the parental generation of rats (P) was exposed via respiratory system to cyclohexanone at the concentration of 1030, 2040 and 4100 mg/m^3 (6h/d, 5 (males) and 7 (females) d/week). The total duration of exposure of P generation was about 4 months. The first progeny generation (F1) was further exposed to 1020, 2030 and 5700 mg/m^3. The total duration of exposure of F1 generation was about 8 months. The examination of parental generation revealed no changes in particular in the sexual organs. The offspring of P generation (F1) were not different from the controls in any of the exposed groups.

In F1 generation no effects on fertility were observed in the low and middle concentration group. In the 5700 mg/m^3 group male fertility calculated as males which were paired with fertile females was less than in the control. Mating indices in this group were less compared to control. The mean number of progeny born viable by 5700 mg/m^3 females was not statistically reduced. No treatment-related changes were observed in progeny. Microscopic examination of the reproductive organs from 5700 mg/m^3 parent animals revealed no evidence of treatment-related effects.

In two inhalatory studies, rats and mice were exposed after mating from 6 to 19 and 6 to 17 days of gestation, respectively for 6h/d to 1280, 2744 and 5520 mg/m^3 of cyclohexanone for the rats and 5520 mg/m^3 for the mice.

In case of rats the body of the females was reduced at the highest concentrations. The clinical observation showed lacrimation, nasal discharge, vaginal discharge, reduced reaction and lethargy. In the foetuses the reduction of weight, delays in ossification of the skull, breastbone and forelimbs were observed. There were no malformations.

In mice the clinical symptoms were similar as in rats. Maternal toxicity included a decrease of mean body weight and uterus weight, mean number of viable foetuses and their weights and increase in the number of resorption. No external malformation were reported for the offspring.

The prenatal developmental toxicity study on rabbits following oral exposure to cyclohexanone did not indicate effects in dams (except reduction of body weight) or in foetuses.

Taking into account the study results, it can be concluded that cyclohexanone administered by the inhalatory route at exposure level to about 2744 mg/m^3 was not considered maternally toxic, embryo or teratogenic. At the highest exposure levels (above 5000

mg/m^3) some effects indicating the maternal toxicity were observed. In offspring no changes were observed except delayed ossification or reduced body weight.

Some additional results described in IARC monograph (1989) indicated that following dietary administration (800 mg/kg/d) of cyclohexanone to mice (8-12 d of gestation) no treatment-related maternal or developmental effects were observed. In a similar study, mice were administered orally to 2200 mg/kg/d of cyclohexanone. The results indicated serious maternal toxicity leading to death of more than 21% of females. The only effects seen in the offspring of surviving females included reduced body weight. The i.p. injection of female mice for 25 days (dose of cyclohexanone: 50 mg/kg/d) did not affect fertility of mice.

The available information indicates that cyclohexanone administered at high doses can cause maternal toxicity. The effects observed in offspring included decreased body weight and delayed ossification.

According to CLP guidelines, if some effects include the incidence of spontaneous defects in the foetus, small changes in the proportions of common foetal variants such as are observed in skeletal examinations, or in foetal weights, or small differences in postnatal developmental assessments, these alterations are considered to be of low or minimal toxicological significances and classification may not necessarily be the outcome.

The concern has been clarified and no further information is requested. Based on the above it is concluded that cyclohexanone is not classifiable as to its reprotoxic potential.

7.9.8. Hazard assessment of physico-chemical properties

Not relevant for this evaluation.

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

According to Section R.8.4 of the REACH Guidance on Information Requirements and Chemical Safety Assessment (ECHA, 2012), DNEL for the leading health effect needs to be derived for every relevant human population and every relevant route, duration and frequency of exposure, if feasible.

The lead registrant uses the EU IOEL value (40 mg/m^3) as a DNEL that protects workers from long-term systemic effects caused during inhalation exposure to cyclohexanone and the EU IOEL short term value (80 mg/m^3) as a DNEL that protects workers from acute systemic effects caused during inhalation exposure to cyclohexanone. According to eMSCA the lead registrant is allowed to use an IOEL as a DNEL for the same exposure route and duration.

The lead registrant has calculated DNELs values (both equal to 4 mg/kg bw) that protect workers from long-term and acute systemic effects caused during dermal exposure to cyclohexanone. The point of departure was taken from a repeated dose i. v. infusion study (NOAEL 100 mg/kg bw and day) in rats. The resulting DNEL (4 mg/kg bw) is more than 200-fold below the dermal LD_{50} value in rabbits after occlusive exposure and more than 100-fold below the oral NOAEL in chronic drinking water studies in rats and mice. Taking this into account and the conservative selection of the key study (infusion study) eMSCA accepted provided values.

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

According to Guidance on the Application of the CLP Criteria Version 4.1 – June 2015, classification in Muta Category 2 may be based on positive results of at least one in vivo

valid mammalian somatic cell mutagenicity test, indicating mutagenic effects in somatic cells. A Category 2 mutagen classification may also be based on positive results of at least one in vivo valid mammalian somatic cell genotoxicity test, supported by positive in vitro mutagenicity results. In vitro results can only lead to a Category 2 mutagen classification in a case where there is support by chemical structure activity relationship to known germ cell mutagens. In the case where there are also negative or equivocal data, a weight of evidence approach using expert judgement has to be applied.

Following analysis the eMSCA considered that the available information is sufficient for classification cyclohexanone as Muta. Cat. 2.

7.10. Assessment of endocrine disrupting (ED) properties

Not evaluated.

7.11. PBT and VPVB assessment

Not evaluated.

7.12. Exposure assessment

7.12.1. Human health

Worker

Cyclohexanone is a skin irritant classified for local dermal irritation. It causes severe damage to eyes. Therefore, all workers who come in contact with the substance shall use Personal Protective Equipment (PPE), such as goggles, chemical resistant gloves and protective clothing in order to protect eyes and skin. Local exhaust ventilations should be placed at the potential emission sources.

Consumer

It was clarified that cyclohexanone is not used as such by consumers. The consumer uses included in the registration dossiers as "identified uses" are not valid anymore. To date several registration dossiers have been updated. Therefore based on the information given by the Cyclohexanone Consortium the additional concern was not identified. However, the latest available version of the dissemination site summarising the registration data still includes the entry in question.

7.12.2. Environment

Not evaluated.

7.12.3. Not evaluated. Combined exposure assessment

Not evaluated.

7.13. Risk characterisation

For quantitative risk characterization of cyclohexanone, data from inhalation and dermal exposure were compared with the derived long-term systemic dermal and inhalation DNELs, respectively. The exposure assessment was made based on the estimations given in the CSRs.

Calculation of long-term exposure values was done for systemic effects of the inhalative and the dermal exposure.

Calculation of the risk characterization ratio (RCR) was done based on the indicative occupational exposure limit of the EU (EU IOEL) taken as the DNEL for the inhalative route (40 mg/m3). For the dermal route, the calculation was based on the DNEL value of 4 mg/kg bw.

The risk for workers in both industrial and professional indentified uses of the substance appears to be controlled taking into account RMM an OCs proposed by the registrant(s).

7.14. References

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7.15. Abbreviations

CLP – Classification, Labelling and Packaging
CoRAP – Community Rolling Action Plan
CSR – Chemical Safety Report
DMEL - Derived Minimal Effect Level
DNEL – Derived No Effect Level
EPA – Environmental Protection Agency
LOAEL – Lowest Adverse Observed Effect Level
LOAEC - Lowest Adverse Observed Effect Concentration
MSCA – Member State Competent Authority
NOAEC - No Observed Adverse Effect Concentration
NOAEL – No Observed Adverse Effect Level
PBT – Persistent, Bioaccumulative, Toxic
SVHC – Substance of Very High Concern
vPvB – very Persistent, very Bioaccumulative