SUBSTANCE EVALUATION CONCLUSION

as required by REACH Article 48

and

EVALUATION REPORT

for

Methylcyclohexane

EC No 203-624-3

CAS No 108-87-2

Evaluating Member State(s): Finland

Dated: 08 April 2017

Evaluating Member State Competent Authority

Finnish Safety and Chemicals Agency

PL 66 (Opastinsilta 12 B), 00521 Helsinki, FINLAND

name.surname(at)tukes.fi

Year of evaluation in CoRAP: 2013

Before concluding the substance evaluation a Decision to request further information was issued on 19 December 2014.

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

Methylcyclohexane (MCH) was originally selected for substance evaluation in order to clarify concerns about:

- suspected PBT/vPvB

- wide dispersive use

- high aggregated use

- consumer use

During the evaluation an additional concern was identified related to the environmental exposure and effects assessment. The risk assessment was performed assuming that the substance is readily biodegradable. However, based on the evaluation of the eMSCA no reliable information was available on the ready biodegradability of MCH.

Based on a proposal for amendment submitted in accordance with REACH Article 52(1) a further additional concern was identified,

- concern whether workers are properly informed to use the right type of personal protective equipment (e.g. gloves) to protect themselves against exposure to chemicals.

2. OVERVIEW OF OTHER PROCESSES / EU LEGISLATION

No apparent other relevant processes at the time of writing.

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 1
CONCLUSION OF SUBSTANCE EVALUATION

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

4. FOLLOW-UP AT EU LEVEL

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

4.1.1. Harmonised Classification and Labelling

Not relevant.

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

Not relevant.

4.1.3. Restriction

Not relevant.

4.1.4. Other EU-wide regulatory risk management measures

Not relevant.

5. CURRENTLY NO FOLLOW-UP FORESEEN AT EU LEVEL

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

Table 2

REASON FOR REMOVED CONCERN The concern could be removed because	Tick box
Clarification of hazard properties/exposure	X
Actions by the Registrants to ensure safety, as reflected in the registration dossiers(e.g. change in supported uses, applied risk management measures, etc.)	

Based on the available information, it is not possible to conclude on P although the substance fulfils the P screening criterion as it is not readily biodegradable. For the T assessment, there are only screening level data available, which do not allow a direct comparison to the T criteria and therefore it is not possible to conclude on T. However, generation of new data for the P and T assessment is not considered necessary since during the evaluation it was concluded that the substance does not fulfil the B criterion and therefore the concern for PBT properties was removed. (For details see part B, especially chapters 7.7.3 and 7.11.).

Regarding the additional concerns on ready biodegradability and personal protective equipment, further information was requested in a Sev-decision (December 2014).

Regarding the additional concern related to ready biodegradability of the substance, new data from an OECD 301D ready biodegradation test was received and, based on all available information, it was concluded that the substance is not readily biodegradable. The Registrant up-dated the chemical risk assessment based on the conclusion that the substance is not biodegradable. Based on the risk characterization ratios (RCRs), there is no indication of risk.

Regarding the information request on personal protective equipment the Registrant updated his dossier (7.10.2015) by including in all exposure scenarios where gloves were recommended a footnote giving reference to a breakthrough test on one brand of gloves (Showa 720R Nitrile gloves).

The eMSCA considered at the time that the information submitted did not provide sufficiently detailed information as required by the Sev-decision for several reasons, most importantly because,

- there were no specific recommendations for gloves in the chemical safety report (CSR), neither were specific recommendations mentioned in IUCLID section 11 (guidance on safe use).

- the information submitted consisted of a reference to a breakthrough test report on a specific brand of gloves (Showa 720R), whereas the recommendation for suitable gloves should be general and specify, as a minimum, the glove material.

After communications with the Registrant, the Registrant made a further update of the dossier (02 June 2016) and modified the footnote by replacing the reference to a specific brand of gloves with general properties of gloves (1.1 mm Nitrile gauntlets or 0.9 mm Nitrile disposable gloves). In Section 11 of the IUCLID dossier a recommendation "to wear suitable gauntlets (1.1 mm thickness, nitrile rubber) and/or suitable gloves (0.9

mm thickness, nitrile rubber) if a risk assessment indicates this is necessary" was included. For further details see Annex 1.

In conclusion, the eMSCA considers that there is no need for regulatory follow-up at EU level.

The eMSCA notes, however, that no clear exposure scenario specific recommendations for gloves are given in the CSR. The eMSCA further notes that the tested/recommended gloves are quite thick (0.9 mm/1.1 mm) and it is not clear whether such thick gloves are suitable for all exposure scenarios (e.g. laboratory work). **The eMSCA recommends to include exposure scenario specific recommendations for protective gloves in the CSR.**

5.2. Other actions

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

Not relevant.

Part B. Substance evaluation

7. EVALUATION REPORT

7.1. Overview of the substance evaluation performed

Methylcyclohexane (MCH) was originally selected for substance evaluation in order to clarify concerns about,

- suspected PBT/vPvB

- wide dispersive use

- high aggregated use

- consumer use.

During the evaluation an additional concern was identified related to the environmental exposure and effects assessment. The risk assessment was performed assuming that the substance is readily biodegradable. However, based on the evaluation of the eMSCA no reliable information was available on the ready biodegradability of MCH.

Based on a proposal for amendment submitted in accordance with REACH Article 52(1) a further additional concern was identified,

- concern whether workers are properly informed to use the right type of personal protective equipment (e.g. gloves) to protect themselves.

Table 3

EVALUATED ENDPOINTS	
Endpoint evaluated	**Outcome/conclusion**
Persistence	*Not possible to conclude. MCH fulfils P screening criterion (MCH is not readily biodegradable). No need to generate further information on P because the substance does not fulfil B criterion.*
Bioaccumulation	*MCH does not meet criteria for bioaccumulation (B).*
Toxicity	*Not possible to conclude. No need to generate further information on T because the substance does not fulfil B criterion.*

7.2. Procedure

The substance was initially screened for the Community rolling action plan (CoRAP) for substance evaluation under REACH regulation as a potential PBT/vPvB with wide dispersive uses. The original scope of the evaluation was to investigate further the reasoning, rationale and applicability of the used category approach, read across and QSARs. In addition, the classification as compared to the classification of another member of the same category, isoheptane, should be evaluated.

In February 2013 the dossier was updated with significant changes. The original dossier was based on read-across to "C6-C7 n-alkanes, isoalkanes, cyclics, < 5 % n-hexane". In the up-dated dossier, read-across to that substance was not used. With the up-dated dossier new data on methylcyclohexane (MCH) was introduced including read across to

cyclohexane. In addition, the Registrant informed that they were conducting a new ready biodegradation study, the results of which were available only at the end of 2013.

Therefore the scope of the evaluation was modified with the consent of ECHA. It was agreed that the evaluation would include:

- the evaluation of PBT/vPvB properties based on the information available
- the evaluation of the relevance and validity of the read-across to cyclohexane
- the evaluation of the environmental hazard classification
- the evaluation of any other relevant concerns identified during the substance evaluation

The comparison to the classification of isoheptane was omitted as irrelevant.

During the evaluation it was concluded that the substance does not fulfil the B criterion and therefore the concern for PBT was removed.

During the evaluation an additional concern was identified related to ready biodegradability of the substance. The risk assessment was performed assuming that the substance is readily biodegradable. However, based on the evaluation of the eMSCA no reliable information was available on the ready biodegradability of MCH.

Based on a proposal for amendment submitted in accordance with REACH Article 52(1) a further additional concern was identified,

- concern whether workers are properly informed to use the right type of personal protective equipment (e.g. gloves) to protect themselves.

A Substance Evaluation Decision was sent to the Registrants of methylcyclohexane in December 2014[2], which requested the following information:

1) Ready biodegradability study - closed bottle test (Test method EU C.4-E/OECD 301D) with chemical analysis to verify the test substance concentration.

2) Documentation for the recommended personal protective equipment, i.e. gloves to be worn when handling the substance need to be specified clearly (Article 14(6), Annex I, 5.1.1. of the REACH Regulation

The deadline for submitting the requested information was 26 June 2015.

The registrant updated his dossier and included an OECD 301D ready biodegradability study on 25 June 2015. Regarding the information request on personal protective equipment, the registrant explained that testing was still on-going and assured that the missing information would be submitted by the end of September 2015. he Registrant updated his dossier 07 October 2015 and included in the exposure scenarios a footnote with a reference to a breakthrough test on MCH on a specific protective glove.

[2] Link to decision: http://www.echa.europa.eu/information-on-chemicals/evaluation/community-rolling-action-plan/corap-table?search_criteria_name=Methylcyclohexane&search_criteria_ecnumber=203-624-3&search_criteria=Methylcyclohexane

7.3. Identity of the substance

Table 4

SUBSTANCE IDENTITY	
Public name:	Methylcyclohexane
EC number:	203-624-3
CAS number:	108-87-2
Index number in Annex VI of the CLP Regulation:	601-018-00-7
Molecular formula:	C7H14
Molecular weight range:	98.1861
Synonyms:	-

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

Structural formula:

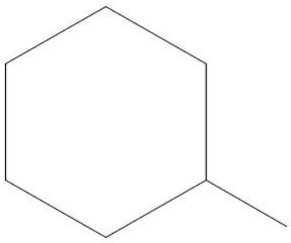


Table 5

Constituent			
Constituents	**Typical concentration**	**Concentration range**	**Remarks**
Methylcyclohexane 203-624-3	confidential	confidential	-

7.4. Physico-chemical properties

Table 6

OVERVIEW OF PHYSICOCHEMICAL PROPERTIES		
Property	**Value**	**Remarks**
Physical state at 20°C and 101.3 kPa	Liquid	Lide Handbook (2005)
Melting/freezing point	- 126.6 °C	Lide Handbook (2005)
Boiling point	100.9 °C	Lide Handbook (2005)
Vapour pressure	1) 6180 Pa at 25 °C 2) 5550 Pa at 25 °C 3) 6130 Pa at 25 °C	1) Lide Handbook (2005) 2) QSAR; mean VP of Antoinen & Grain method) 3) Exper. cited in Episuite
Surface tension	23.29 mN/m	Lide Handbook (2005) The substance is not surface active
Water solubility	1) 14 mg/l at 25 °C 2) 17.22 mg/l 3) 28.4 mg/l	1) Exper. Yalkowsky et al. 1992cited in Episuite 2) QSAR (WatSol from fragments (v1.01) 3) QSAR (WSKOW v.1.41) using logKow = 3.61
Partition coefficient n-octanol/water (log value)	1) 3.88 2) 3.61 3) 3.59	1) Lide handbook (2005) 2) Exper. Hansch et al. 1995 cited in Episuite 3) QSAR (KOWWIN v1.67)

7.5. Manufacture and uses

7.5.1. Quantities

Table 7

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 8

USES	Use(s)
Uses as intermediate	industrial
Formulation	industrial
Uses at industrial sites	Use as an Intermediate, uses in Coatings, use in Cleaning Agents, Lubricants and Polymer processing
Uses by professional workers	Uses in Coatings (solvent), Use in Cleaning Agents (solvent), Lubricants (solvent), Use in Agrochemicals, Polymer processing (solvent), Solvent in other applications
Consumer Uses	Uses in Coatings (solvent), Use in Cleaning Agents (solvent), Use in Agrochemicals (solvent), Solvent in other applications.
Article service life	-

7.6. Classification and Labelling

7.6.1. Harmonised Classification (Annex VI of CLP)

Table 9

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)**		
601-018-00-7		203-624-3	108-87-2	Flam.Liquid 2 Asp. Tox. 1 Skin Irrit.2 STOT Single Exp. 3 Aquatic chronic 2	H225 H304 H315 H336 H411	-	-

7.6.2. Self-classification

In the registration(s)*:*

In addition to the harmonised hazard classes, the following are included in the registration:

Aquatic Acute 1 (H400)

Aquatic Chronic 1 (H410)

The following hazard classes are in addition notified among the aggregated self-classifications in the C&L Inventory*:* no additional hazard classes.

7.7. Environmental fate properties

7.7.1. Degradation

7.7.1.1. Abiotic degradation

7.7.1.1.1. Hydrolysis

There are no functional groups in the molecular structure of MCH that are liable to hydrolysis. Therefore hydrolysis is not considered relevant.

7.7.1.1.2. Phototransformation/photolysis

7.7.1.1.2.1. Phototransformation in air

Due to its relatively high vapour pressure, methylcyclohexane has the potential to volatilize to air, where it is subject to atmospheric oxidation. Based on a QSAR calculation for methylcyclohexane using AOPWIN v1.92, the substance is susceptible to indirect photodegradation in air. The estimated half time for the reaction with OH-radicals is 37.9 hours (Table 10). Therefore, indirect photodegradation may be an important environmental fate process for this substance.

The predicted half-life in air is below the criterion for persistent organic pollutants (POP) (2 d) as defined in the Annex D of the Stockholm convention (Stockholm Convention, 2001) and therefore the substance is not expected to have long-range transport potential.

Table 10 Phototransformation in air

Method	Results by Registrant	Remarks by eMSCA
Calculation based on AOPWIN v1.92, Estimation Programs Interface Suite™ for Microsoft® Windows v 4.10. US EPA, United States Environmental Protection Agency, Washington, DC, USA. PHOTOCHEMICAL REACTION WITH OH RADICALS - Concentration of OH radicals: 0.5E+06 radicals/cm3 (approximate 24 hour-mean in Central Europe) - Degradation rate constant: 10.1676 E-12 cm3/molecule-sec - Temperature for which rate constant was calculated: 25 °C - Computer programme: AOPWIN v1.92	Half-life (DT50): 37.871 h (calculation based on a 24 h day)	eMSCA is in agreement with the summary by the Registrant. The same result was obtained by the eMSCA with US EPA Epi Suite vers 4.00, AOPWIN Vers. 1.92 using the same OH radical concentraton It is noted in the AOPWIN documentation that there is no universally accepted definition of model domain and that property estimates may be less accurate for compounds outside the Molecular Weight range of the training set compound and for compounds which have structural features not represented in the training set and for which no fragment coefficient was developed. However, the complete training sets for AOPWIN are not available. It is noted also that the current applicability of the methodology is best described by its accuracy in predicting available experimental values. In AOPWIN documentation, an experimental half-life (10.4×10^{-12} cm3/molecule-sec) for MCH is cited, which is close to the predicted value (10.2×10^{-12} cm3/molecule-sec).

7.7.1.1.2.2. Phototransformation in water

No information available.

7.7.1.1.2.3. Phototransformation in soil

No information available.

7.7.1.2. Biodegradation

7.7.1.2.1. Biodegradation in water

7.7.1.2.1.1. Estimated data

Estimation using BIOWIN models

The Registrant(s) used BIOWIN QSAR models for the estimation of biodegradability of MCH. The Registrant(s) included results of seven different EPISUITE 4.10 BIOWIN models. The overall prediction given by the EPISUITE software is that MCH is readily biodegradable. However, it should be noted that of the BIOWIN models, only BIOWIN models 5, 6, and 7 are considered applicable for MCH. This is because the molecular fragments of MCH (methyl, -CH2- [cyclic], -CH- [cyclic]) are included in the lists of fragments which are used for the prediction (i.e. for which a fragment coefficient have been calculated) in BIOWIN 5,6, and 7. In contrast, BIOWIN models 1, 2, 3, and 4 do not include coefficients for fragments relevant to MCH and therefore the prediction of degradability by BIOWIN 1, 2, 3, and 4 is based only on the molecular mass of the substance. Although molecular mass has significance for biodegradation of hydrocarbons, a prediction based on molecular mass only is not reliable as other factors such as ring structures are significant for biodegradability. Therefore, of the BIOWIN models, only BIOWIN 5, 6 and 7 can be used to estimate the biodegradability of MCH. The results of the "Ready Biodegradability prediction: YES or NO " given by the BIOWIN output, are thus not valid as BIOWIN 3 is needed for this prediction. Similarly, the screening criteria in the ECHA guidance (ECHA 2008a and ECHA 2008b) are not applicable as BIOWIN 2 and BIOWIN 3 models are necessary for these screening criteria.

Therefore, it is concluded that the use of BIOWIN models 1, 2, 3, and 4 for predicting ready biodegradability for MCH is not scientifically justified. A reliability score of 3 (not reliable) and purpose flag "disregarded" are assigned to the BIOWIN 1, 2, 3, and 4 estimation for MCH.

The results of the individual BIOWIN models which are deemed applicable for MCH, are presented in Table 11. The results for cyclohexane are included for comparison as cyclohexane has been used by the Registrant as a read across substance.

Table 11 Biowin results

	MCH	MCH	CH	CH
model	probability	prediction	probability	prediction
BIOWIN 5 (MITI Linear Biodeg Probability)	0.5315*	Readily biodegradable*	0.5801*	Readily biodegradable*
BIOWIN 6 (MITI Non-Linear Biodeg Probability)	0.6821	Readily biodegradable	0.8198	Readily biodegradable
BIOWIN 7 (Anaerobic Linear Biodeg Prob)	0.1959	Does not biodegrade fast	0.1160	Does not biodegrade fast

* According to ECHA (2008a) it is relevant to consider dropping use of predictions which are close to the borderline cut off between ready and not ready biodegradability. It has for example been proposed not using BioWIN 1, 2, 5, 4 or 6 model predictions with a biodegradability probability score between 0.4 and 0.6. (because the cut off point is 0.5). Such a strategy seems, according to an analysis done by RIVM on the SIDS data set included in OECD 2004, ENV/JM/TG/2004)26Rev, to increase the level of predictability (Rorije, 2005).
A Probability Greater Than or Equal to 0.5 indicates --> Biodegrades Fast
A Probability Less Than 0.5 indicates --> Does NOT Biodegrade Fast

Estimation using BIOHCWIN model

BIOHCWIN model has been developed for determining quantitative primary biodegradation half-lives for individual petroleum hydrocarbons. This model uses a fragment-based approach that is similar to several other biodegradation models, such as those within the Biodegradation Probability Program (BIOWIN) estimation program. The eMSCA concludes that the use of BIOHCWIN model for MCH is justified due to the following reasons:
-the model includes in its training set fragments relevant to MCH
-MCH only contains structural features that are represented by the training set compounds
-the number of instances of each of the fragments does not exceed the maximum for all training set compounds
-the molecular weight of MCH is within the range of the training set compounds used for the model (70.14 - 478.94)
The half-life of 7.31 d for MCH was obtained by BIOHCWIN (for comparison, the half-life for cyclohexane is 55.38 d).

Although the BIOHCWIN model is suitable for MCH, its relevance to the present assessment is limited because the BIOHCWIN model gives a primary biodegradation half-life estimate and data obtained with mixtures has been used in its training set (Howard et al. 2005). Since it is known that cometabolism affects MCH biodegradation, models which are based on experimental biodegradation data obtained with mixture studies are not reliable for evaluating ready biodegradability. Moreover, a positive result in a ready biodegradability test requires that the substance is ultimately degraded. Therefore, primary degradation data has only limited value for evaluating ready biodegradability.

7.7.1.2.2. Screening tests

In the registration dossier submitted for substance evaluation, one ready biodegradability test on MCH was included (OECD 301 D test; METI 1985). In addition, the Registrant(s) submitted five new ready biodegradability tests to the eMSCA: four tests during the substance evaluation period and one test afterwards, as a response to the substance evaluation decision. These have been conducted according to the guidelines OECD 301 F (Harlan 2012), OECD 310 (Harlan 2013), OECD 301 D (Fraunhofer 2013, Fraunhofer 2015), and OECD 310 (Fraunhofer 2013). In addition, the Registrant(s) have submitted a document (Knoell 2013) in which the ready biodegradability tests are discussed. Information from this document is taken into account in the evaluation of the tests by eMSCA. The available ready biodegradability tests are described below and summarized in Table 12.

Table 12: Ready biodegradability tests on MCH

No	Test method	Results	eMSCA Remarks	Reference
1	OECD 301 D (Closed Bottle Test, DOC removal), non-GLP	0 % degradation after 28 d	TS conc. 10 mg/l Inoculum: Activated sludge Reliability score: Not assignable (4) due to deficiencies in documentation and uncertainties related to TS bioavailability.	METI 1985
2	OECD 301 D (Closed Bottle Test, O_2 measured by electrode), non-GLP	0 % degradation after 28 days	TS conc. 3.2 mg/l (Corrected value, in the test report conc. has been miscalculated as 5.3 mg/l). 0.5 µl of TS injected through septum with a gas tight syringe, no headspace. According to Registrant: "no visible droplet during substance application". Inoculum: Mixture of two activated sludges, pond water and soil eluate. Degradation in toxicity control (21%) did not exceed 25%. According to test guideline, inhibition by test substance can be assumed. Reliability score: Not assignable (4) due to deficiencies in documentation and uncertainties related to TS bioavailability.	Fraunhofer 2013
3	OECD 310 (CO_2 in Sealed Vessel), non-GLP	No biodegra-dation detected	TS conc. 7.7 mg/l and 8.6 mg/l, (Corrected values, in the test report conc. has been miscalculated) TS injected into vessels with a gas tight syringe, headspace to liquid ratio 1:3 and 1:4, sealed vessels shaken once a day. Inoculum: Mixture of two activated sludges, pond water, and soil eluate. The mean amount of TIC present in the blank controls at the end of test exceeded 3 mg/l and therefore the validity criterion concerning TIC concentration (<3 mgC/L) was not fulfilled. 0% biodegradation was reported; however, due to high concentration of TIC in inoculum blanks it cannot be concluded from this test that biodegradation of test substance was 0 %) Reliability score: Not assignable (4) due to deficiencies in	Fraunhofer 2013

			documentation and uncertainties related to TS bioavailability.	
4	OECD 310 (CO_2 in Sealed Vessel), GLP	0 % degradation after 28 days	TS conc. 11.5 mg/l TS injected through a septum, Headspace to liquid ratio 1:2, Constant shaking 150 rpm, Inoculum: A mixed population of sewage sludge micro-organisms from the secondary treatment stage of a sewage treatment plant treating predominantly domestic sludge. Reliability score: Not assignable (4) due to deficiencies in documentation and uncertainties related to TS bioavailability.	Harlan 2013
5	OECD 301 F (Manometric Respirometry Test, BOD), GLP	0 % degradation after 28 days	TS conc. 10 mg/l, Sealed culture vessels used Inoculum: A mixed population of sewage sludge micro-organisms from the final treatment stage of a sewage treatment plant treating predominantly domestic sludge. Reliability score: Not assignable (4) due to deficiencies in documentation and uncertainties related to TS bioavailability.	Harlan 2012
6	OECD 301D (Closed Bottle Test), GLP	0% degradation after 28 days	TS conc. 2.45 mg/l, Inoculum: Secondary effluent from a sewage treatment plany (mainly fed with municipal wastewater). Reliability score: Not reliable (3) due to problems with procedural control.	Fraunhofer 2015

METI (1985) - ready biodegradability test according to OECD 301 D guideline

This study is a ready biodegradability test according to the guideline OECD 301 D (Closed bottle test). Degradation of test substance was 0 % after 28 days (based on DOC removal) and therefore MCH was not readily biodegradable. According to the Registrant, this test is not reliable and they mention that the reason for these low biodegradation results might be due to technical difficulties when testing such highly volatile and potentially toxic substances.

In informal discussions with the eMSCA the Registrant provided the following additional information:

-MCH exhibits a high biological oxygen demand of 3.42 mg O_2 per mg test item allowing only minimal test item volumes in the test vessels in order to maintain aerobic conditions. Therefore, the 10 mg/L test item concentration, applied in this test is considered too high.

-Due to limitations in the documentation the actual test performance was not fully

comprehensible. For example, the handling / test substance application was not explained in detail and assuming an "ordinary" test performance, high volatile losses can thus not be excluded during application and sampling.

-The test was not performed under GLP.

-There was no toxicity control available.

-Based on new data available to the Registrant with MCH, 10 mg/L is not inhibitory to microorganisms.

Some of these issues are discussed also in the report submitted in September 2013 (Knoell 2013)

In summary, the validity of this ready biodegradability test is questionable and cannot be verified due to poor documentation. Therefore**, a reliability score of 4 ("not assignable") is applied.**

Harlan (2012) - ready biodegradability test according to OECD 301 F guideline

This test report was not included in the original registration dossier (Feb 2013) submitted for substance evaluation. The report was submitted by the Registrant(s) to the eMSCA in September 2013. In the document by Knoell (2013) it is mentioned that this test (Harlan 2012) was chosen as an initial test, since for cyclohexane, this test design had been successfully used (EU, 2004). The method followed was designed to be compatible with the OECD Guideline 301 F "Ready biodegradability; Manometric Respirometry test". The test was performed in compliance with UK GLP standards and the study director's statement of GLP compliance is included in the report.

An initial test at a concentration of 100 mg/l was conducted but the toxicity control showed less than 25% biodegradation on day 14, and it was concluded that the test item was toxic to microorganisms at this concentration (Knoell 2013). Therefore, the study was repeated using a MCH concentration of 10 mg/l. Sewage treatment micro-organisms were used as inoculum. The test was conducted at 21±1°C. The degradation of MCH was assessed from the daily oxygen consumption values. Inoculum alone, reference control (aniline), and toxicity control (MCH + aniline) assays were also performed. Due to the volatility of MCH, the test item was added directly to the test vessels using a gas tight syringe. The test was conducted using a respirometer system which consists of a sample flask sealed by a sensor head/CO_2 trap immersed in a water bath. The samples were stirred for the duration of the test with a magnetically coupled stirrer. The CO_2 formed is absorbed into ethanolamine solution causing a net reduction in gas pressure within the sample flask. The pressure reduction triggers an electrolytic process generating oxygen and restoring the pressure. The data generated from the respirometer's memory was collected. The biodegradation was calculated as percentage of theoretical oxygen demand (for 100 mg/l of MCH, ThOD is 342 mgO_2/l)

MCH attained 0 % degradation after 28 days.

The validity criteria were fulfilled. BOD of the inoculum blank was 42.13 mg O_2/l after 28 days, pH in MCH vessels was 7.7-7.9 on Day 28, the difference between extremes of replicate BOD values was less than 20 %, the toxicity control attained 67 % degradation after 14 days, and in procedure control the reference substance attained 76 % degradation after 14 days.

General validity criteria of OECD 301: A test is considered valid if the difference of extremes of replicate values of the removal of the test chemical at the plateau, at the end of the test or at the end of the 10-d window, as appropriate, is less than 20% and if the percentage degradation of the reference compound has reached the pass levels by day 14. If in a toxicity test, containing both the test substance and a reference compound, less than 35% degradation (based on total DOC) or less than 25% (based on total ThOD or ThCO2) occurred within 14 days, the test substance can be assumed to be inhibitory.

Specific validity criteria for OECD 301 F: The oxygen uptake of the inoculum blank is normally 20-30 mg 0_2/l and should not be greater than 60 mg/l in 28 days. Values higher than 60 mg/l require critical examination of the data and experimental technique. If the pH value is outside the range 6-8.5 and the oxygen consumption by the test substance is less than 60%, the test should be repeated with a lower concentration of test substance.

The test report concluded that MCH attained 0% degradation after 28 days and therefore cannot be considered as readily biodegradable under the strict terms and conditions of OECD guideline 301 F.

Comments by eMSCA: In the report submitted by the Registrant(s) (Knoell 2013) it is mentioned that in this test (Harlan 2012) the substance was very likely not bioavailable to the microorganisms since it would very likely accumulate in the headspace of the test bottles. The eMSCA acknowledges that the substance is very volatile and that it is possible that in this test as well as in the other ready biodegradability tests (tests 1-5 in Table 12) the substance has not been sufficiently bioavailable for micro-organisms. However, no measured concentration data from liquid and gas phases have been provided to verify the assumed poor bioavailability. The study has been conducted according to GLP and validity criteria are fulfilled. It is also noted that specific caution was taken to avoid volatilisation, the test substance concentration was below water solubility, and no indications of oxygen depletion were observed in the test. In the test report there are no indications of poor bioavailability. Therefore, it cannot be overruled that the reason for not detecting biodegradation in the test is that the substance is not susceptible to biodegradation under the conditions of this test. It is also noted that the concentration 100 mg/l was toxic to microorganisms and therefore at this concentration the test substance seems to have been bioavailable as at that concentration it was able to cause toxicity to microorganisms. If MCH was not bioavailable in the test vessels at the concentration of 10 mg/l, then the same applies also to the toxicity control vessels in which the same test substance concentration was used. In that case the toxicity control would not be representative of effects of toxicity on biodegradation of MCH.

A reliability score of 4 "not assignable" is applied due to the suspected poor bioavailability of test substance which, however, is not verified by measurement data.

Harlan (2013) - ready biodegradability test according to OECD 310

This test report was not included in the original registration dossier (Feb 2013) submitted for substance evaluation. The report was submitted by the registrant(s) to the eMSCA in September 2013. It is mentioned (Knoell 2013) that this test was chosen after the OECD 301 F test (Harlan, 2013), in which no biodegradation was observed. In this test the following test adaptations were made (Knoell 2013):
-low test substance concentration was applied (11.5 mg/l) due to the expected toxicity tomicroorganisms
-limitation of volatility losses: in order to minimize substance losses during test substance application, MCH was injected by means of a gas tight syringe through a septum.

The method followed was designed to be compatible with the guidelines OECD 310 and ISO 14593. The test was performed in compliance with UK GLP standards and study director statement of GLP compliance is included in the report. The test was conducted in sealed vessels. Biodegradation of MCH was assessed by measuring the inorganic carbon present in the headspace of the vessels (determination of carbon dioxide produced). MCH concentration was 10 mgC/l. Sewage treatment micro-organisms were used as inoculum. The test was conducted at 20±1°C. Inoculum alone, reference control (sodium benzoate), and a toxicity control (MCH + sodium benzoate), were also performed.

Due to properties of MCH (poorly water soluble, volatile, non-viscous liquid), and following the recommendations of the ISO (1995), the test item was added directly to the test vessel using a high precision volumetric syringe. The test was conducted in 125 ml bottles each containing 107 ml of solution (headspace to liquid ratio 1:2). Incubation was done in

constant shaking at approximately 150 rpm. CO_2 production was determined by measuring the increase in the concentration of inorganic carbon in the headspace. Inorganic carbon was analysed after acidifying each test vessel and analyzing headspace samples using a TOC analyser. DOC analysis was conducted for inoculum control and procedure control vessels by TOC analyzer after filtration whereas it was mentioned that for the test item and toxicity control vessels DOC analysis was not possible due to the insoluble nature of the test item in water. The biodegradation was calculated by dividing the total inorganic carbon (after correction for endogenous IC production) by total organic carbon added to test vessels.

MCH attained 0% degradation after 28 days.

The validity criteria OECD 310 and ISO 14593 were fulfilled as the mean TIC of the control vessels was 0.11 mg/l on Day 28 (and thus <3 mgC/L and ≤ 15% of the TOC added initially as the test compound) and as percentage degradation in the procedure control was 76% after 14 days.

According to the OECD 310 guideline a test is considered valid if:
(a) the mean percentage degradation in vessels F_C containing the reference substance is >60% by
the 14th day of incubation; and

(b) the mean amount of TIC present in the blank controls F_B at the end of the test is >3mg C/L.

(It is assumed that there is an error in the test guideline and the criterion should be <3 mg C/L rather than >3mg C/L))

According to the ISO 14593 the test is considered valid if:

(a) the mean percentage degradation in the vessels Fc containing the reference compound is ≥ 60% on the 14^{th} day of incubation;

(b) the mean amount of TIC produced from the blank controls at the end of the test is ≤ 15% of the organic carbon added initially as the test compoumd

According to the test report the toxicity control attained 41 % degradation after 14 days and confirmed that the test item was not toxic to the micro-organisms under the conditions of the study.

It is noted that according to OECD 310 guideline, the evaluation of inhibition is based on the theoretical IC yield anticipated from only the reference component in the toxicity control (this is a different approach from OECD 301 where degradation in toxicity control is related to the combined amount of test substance and reference substance). In OECD 310, if, at day 28, the difference between *degradation percentage of reference substance in procedural control* and *calculated degradation percentage of reference substance in procedural control* is >25% of the degradation percentage in procedural control, it can be assumed that the test substance inhibited the activity of the inoculum. In the present test, toxicity control was not measured after day 14. Based on day 14 results there was no toxicity as the difference (-8.6%) did not exceed 25% (The calculated degradation of reference substance in toxicity control was higher (82%) than degradation of reference substance in procedural control (76%)).

The test report concluded that MCH attained 0 % degradation after 28 days and therefore cannot be considered as readily biodegradable.

Comments by eMSCA
In the report submitted by the Registrant(s) (Knoell 2013) it is mentioned that in this test (Harlan 2013) the substance was very likely not bioavailable to the microorganisms since it would very likely accumulate in the headspace of the test bottles. The eMSCA acknowledges that the substance is very volatile and that it is possible that in this test as well as in the other ready biodegradability tests (tests 1-5 in Table 12) the substance has not been sufficiently bioavailable for micro-organisms. However, no measured concentration data from liquid and gas phases have been provided to verify the assumed

poor bioavailability. The study has been conducted according to GLP and validity criteria are fulfilled. It is also noted that specific caution was taken to avoid volatilisation, the test substance concentration was below water solubility, and no indications of oxygen depletion were observed in the test. In the test report there are no indications of poor bioavailability. Therefore, it cannot be overruled that the reason for not detecting biodegradation in the test is that the substance is not susceptible to biodegradation under the conditions of this test.

It is also noted that the concentration 100 mg/l was toxic to microorganisms in OECD 301 F (see Harlan 2012) and therefore at this concentration test substance seems to have been bioavailable as at that concentration it was able to cause toxicity to microorganisms. If MCH was not bioavailable in the test vessels at the concentration used in this OECD 310 test (10 mgC/l), then the same applies also to the toxicity control vessels in which the same test substance concentration was used. In that case the toxicity control would not be representative of effects of toxicity on biodegradation of MCH.

A reliability score of 4 "not assignable" is applied due to the suspected poor bioavailability of test substance which, however, is not verified by measurement data.

Fraunhofer (2013) - ready biodegradability tests according to OECD 301 D and OECD 310 guidelines

This test report was not included in the original registration dossier (Feb 2013) submitted for substance evaluation. The report was submitted by the registrant(s) to the eMSCA in September 2013.

In the report by Knoell (2013) it is mentioned that "a comprehensive pre-testing program was initiated in order to evaluate how to overcome the technical difficulties and to judge on the best suited method and experimental conditions for the main test". The tests were not performed under GLP. However it is mentioned that the test facility is GLP certificated and the only discrepancies to GLP-regulations in this study are the lack of quality audit and archiving. A "Statement of accuracy" with signatures of the study director, as well as a statement of the Quality assurance unit, are included.

Test according to OECD 301 D
In this test the following test adaptations were made (Knoell 2013):
-low test substance concentration were used due to expected toxicity to microorganisms
-limitation of volatility losses: in order to minimize substance losses during test substance application, MCH was injected by means of a gas tight syringe through a septum. In situ measurements of dissolved oxygen by an oxygen electrode were used.
-mixed inoculum was used

The inoculum used was a mixture of two activated sludges, pond water and soil eluate. 900 ml of each of the two activated sludges, 100 ml of pond water, and 100 ml of soil eluate, were mixed. The test was conducted at 20±1°C. The degradation was assessed by dissolved oxygen analyses. Inoculum alone, reference control (sodium benzoate), and toxicity control (MCH + sodium benzoate) were also performed. 120 ml serum bottles were used. The bottles were completely full (no headspace). A volume of 0.5 µl of MCH was injected into the vessels using a gas tight 10 µl syringe. This corresponds to 3.2 mg/l. It is noted that in the test report there are miscalculations regarding the concentration of MCH and theoretical oxygen demand (test material and toxicity control). 3.2 mg/l is the corrected test substance concentration calculated by the eMSCA (using density and reported volume of test substance); this corrected value is used in the dossier update submitted by the Registrant in June 2014)

The biodegradation of MCH was 0 % after 28 days. The recalculation of the biodegradation results using corrected concentration and ThOD of test substance does not change the results because oxygen consumption in test substance assays was not higher than oxygen consumption in inoculum blank assays.

According to the test report, the validity criteria were fulfilled. The percentage degradation in the procedure control was 64 % after 7 days and varied from 50.0% to 61.2% in measurements after 14, 21, and 28 days with the exception of one vessel in which a value of 32.2% was observed after 14 days. Oxygen depletion in the inoculum blank did not exceed 1.5 mg dissolved oxygen during 28 days and the residual oxygen concentration did not fall below 0.5 mg/L.

According to the test report, in the toxicity control no inhibitory effects were seen as 34% biodegradation occurred within 14 days. However, it is noted that due to miscalculation the toxicity control results in the test report are not correct. In addition, in the dossier update (June 2014), in which corrected test substance concentration is used, there are still incorrect values for toxicity control (48 % biodegradation after 7 days). Calculation by the eMSCA from the raw data presented in test report/robust study summary indicate that biodegradation in toxicity control after 14 days was 21 %. According to the test guideline in such cases the substance can be assumed to be inhibitory. Similar reference substance concentrations were used in the toxicity control and procedural control and, therefore, similar oxygen consumption would be expected in both assays assuming no biodegradation of test substance and no inhibition by test substance. Oxygen consumption (corrected for the blank) in toxicity control after 14 days was 14 % lower than in the procedural control, based on one replicate. It is noted that in the procedural control in one of the two replicates on day 14 the oxygen content was significantly higher compared to any other bottle from day 7 onwards, suggesting some sampling/measurement problem and therefore this result is not used by the eMSCA (This deviating result is however not commented in the test report). Moreover, on days 7 and 21, oxygen consumption (mean of two replicate bottles) in the toxicity control was 8 % and 12 % lower, respectively, than in procedural control.

General validity criteria of OECD 301

Test is considered valid if the difference of extremes of replicate values of the removal of the test chemical at the plateau, at the end of the test or at the end of the 10-d window, as appropriate, is less than 20% and -if the percentage degradation of the reference compound has reached the pass levels by day 14.

If in a toxicity test, containing both the test substance and a reference compound, less than 35% degradation (based on total DOC) or less than 25% (based on total ThOD or ThCO2) occurred within 14 days, the test substance can be assumed to be inhibitory.

Specific validity criteria of OECD 301 D

Oxygen depletion in the inoculum blank should not exceed 1.5 mg dissolved oxygen/l after 28 days. Values higher than this require investigation of the experimental techniques. The residual concentration of oxygen in the test bottles should not fall below 0.5 mg/l at any time. Such low oxygen levels are acceptable only if the method of determining dissolved oxygen used is capable of measuring such levels accurately.

Comments by eMSCA on the OECD 301 D test by Fraunhofer (2013):
In the report submitted by the Registrant(s) (Knoell 2013) it is mentioned that in this test (Harlan 2013) the substance was very likely not bioavailable to the microorganisms since it would very likely accumulate in the headspace of the test bottles. In addition, the Registrant(s) is unsure whether the substance entered the test vessels during the preparation of the test systems as there was "no visible droplet during substance application". The eMSCA acknowledges that the substance is very volatile and that it is possible that in this test as well as in the other ready biodegradability tests (tests 1-5 in Table 12) the substance has not been sufficiently bioavailable for micro-organisms. However, no measured concentration data has been provided to verify the assumed poor bioavailability. Validity criteria were fulfilled, with the exception of the toxicity control result. It is also noted that specific caution was taken to avoid volatilisation, the test substance concentration was below water solubility, and no oxygen depletion was observed in the test. Therefore, it cannot be overruled that the reason for not detecting

biodegradation in the test is that the substance is not susceptible to biodegradation under the conditions of this test.

The miscalculations in the report do not prevent the use of the test for the evaluation. The degradation in the procedural control exceeded the pass levels according to the report indicating that if substantial biodegradation of test substance occurred it would be detectable with the experimental set-up used. In toxicity control biodegradation was below 25 % in 14 days, suggesting that some inhibition by test substance may have occurred. It is noted that oxygen consumption in the toxicity control was 8-14 % lower than in procedural control, as described above, and the concentration of reference substance was the same in both assays. In toxicity control the oxygen consumption corresponded to 54.4 % (mean of measurements on days 7, 14, and 21) of the theoretical oxygen consumption of the reference substance added, while in the procedural control the percentage was 61.3 %. It is also noted that in toxicity control theoretical oxygen demand of the added test substance (10.97 mgO_2/l) was higher than that of reference substance (7.52 mgO_2/l) and therefore the ThOD of reference substance accounts for 40.6 % of the combined ThOD of reference substance and test substance. Therefore, the chosen concentrations in the toxicity control were relatively stringent in terms of reaching the degradation level (25 % of ThOD) (for comparison, in the OECD 310 test (Fraunhofer 2013), the TOC of reference substance in the toxicity control was 73 % of the combined TOC). Moreover, if toxicity has occurred then the test substance has been at least partially bioavailable.

It is also noted that the concentration 100 mg/l was toxic to microorganisms in OECD 301 F (see Harlan 2012) and therefore at this concentration test substance seems to have been at least partially bioavailable as at that concentration it was able to cause toxicity to microorganisms. If MCH was not bioavailable in the test vessels at the concentration used in this OECD 301 D test (3.2 mg/l), then the same applies also to the toxicity control vessels in which the same test substance concentration was used. In that case the toxicity control would not be representative of effects of toxicity on biodegradation of MCH.

A reliability score of 4 is applied ("not assignable") for the following reasons
-the suspected poor bioavailability of test substance which, however, is not verified by measurement data
-deficiencies in documentation in test report (miscalculated values; contradictions between raw data and biodegradation percentages)
- although calculations have been corrected in robust study summary of updated dossier, biodegradation values for toxicity control is still not reproducible from the reported raw data
-the relatively low degradation in toxicity control (21%) indicating that inhibition by test substance may have influenced the result. According to the OECD 301 test guideline, with such toxicity control result, the test series should be repeated, using a lower concentration of test substance and/or a higher concentration of inoculum. In the present case, however, the test has not been repeated.

<u>Test according to OECD 310</u>

In this test the following test adaptations were made (Knoell 2013):
-low test substance concentration were used due to expected toxicity to microorganisms
-limitation of volatility losses: in order to minimize substance losses during test substance application, MCH was injected by means of a gas tight syringe through a septum. In situ measurements of dissolved oxygen by an oxygen electrode were used.
-mixed inoculum was used
-increase of bioavailability: headspace was reduced from 33% to 25% and 16.7%, and test vessels were shaken head first every working day for a few seconds to mix the gas phase and the liquid phase in order to increase the contact and bioavailability of the test item to the inoculum

The inoculum used was a mixture of two activated sludges, pond water and soil eluate. 900 ml of each of the two activated sludges, 100 ml of pond water, and 100 ml of soil eluate, were mixed. The test was conducted at 20°C. The degradation was assessed by determining the carbon dioxide produced via total inorganic carbon (TIC) measurements

after absorption to sodium hydroxide. Inoculum alone, reference control (sodium benzoate), and toxicity control (MCH + sodium benzoate) were also performed. 120 ml test vessels were used and 90 ml or 100 ml liquid volume was used, corresponding to 1:3 and 1:4 headspace to liquid ratios. A fixed volume of 1 µl test item was injected to the mineral medium. Two different test item concentrations were thus obtained (TS conc. 7.7 mg/l and 8.6 mg/l, corresponding to 6.6 mgTOC/l and 7.3 mgTOC/l. It is noted that these are corrected values calculated by the eMSCA (using density and reported volume of test substance)(in the test report there are miscalculations regarding the concentration of MCH in test material and toxicity control; however, in the dossier update submitted by the Registrant(s) in June 2014 corrected values are used). The biodegradation of MCH was 0 % after 28 days. The recalculation of the biodegradation results using corrected concentration of test substance does not change the results because TIC production in test substance assays was not higher than TIC production in inoculum blank assays.

The validity criteria concerning the procedure control was fulfilled. Percentage degradation in the procedural control was 78.7 % after 7 days, 76.3 % after 21 days, and 81.5 % after 28 days (values for day 14 are not used as they are not valid due to contamination by CO_2 from air according to the test report). However the OECD 310 validity criterion concerning TIC of the control vessels was not fulfilled as the TIC in the inoculum blank vessels (approx. 10 mg/l) exceeded the validity level (<3 mgC/l) (although TIC of the inoculum blanks was claimed to be below 3 mg/l in the test report). The validity criterion concerning TIC of ISO 14593 was however fulfilled as the TIC of the inoculum blanks did not increase during the test.

According to the OECD 310 guideline a test is considered valid if:
(a) the mean percentage degradation in vessels F_C containing the reference substance is >60% by
the 14th day of incubation; and
(b) the mean amount of TIC present in the blank controls F_B at the end of the test is >3mg C/L.

(Remark by eMSCA: It is assumed that there is an error in the test guideline and the criterion should be <3 mg C/L rather than >3mg C/L))

According to the ISO 14593 the test is considered valid if:

(a) the mean percentage degradation in the vessels Fc containing the reference compound is ≥ 60% on the 14^{th} day of incubation;
(b) the mean amount of TIC produced from the blank controls at the end of the test is ≤ 15% of the organic carbon added initially as the test compoumd

According to test report, the toxicity control attained 49 % degradation after 7 days incubation, indicating that MCH was non-toxic under the conditions of the study. It is noted that this percentage is based on wrong test substance concentration and therefore the correct percentage is higher (57 %). In the updated dossier submitted in June 2014 the corrected value is presented. In addition it is noted that according to OECD 310 guideline, the evaluation of inhibition is based on the theoretical IC yield anticipated from only the reference component in the toxicity control (this is a different approach form OECD 301 where degradation in toxicity control is related to the combined amount of test substance and reference substance). In OECD 310, if, at day 28, the difference between *degradation percentage of reference substance in procedural control* and *calculated degradation percentage of reference substance in procedural control* is >25% of the degradation percentage in procedural control, it can be assumed that the test substance inhibited the activity of the inoculum. In the present test, the calculated degradation of reference substance in toxicity control (corrected values) were 78.4%, 57.7%, and 75.0% at days 7, 21, and 28, respectively. No toxicity can be assumed as the difference (7.9%) at day 28 did not exceed 25%.

Comments by eMSCA on the OECD 310 test by Fraunhofer (2013):
In the report submitted by the Registrant(s) (Knoell 2013) it is mentioned that in this test (Harlan 2013) the substance was very likely not bioavailable to the microorganisms since it would very likely accumulate in the headspace of the test bottles. The eMSCA acknowledges that the substance is very volatile and that it is possible that in this test as

well as in the other ready biodegradability tests (tests 1-5 in Table 12 the substance has not been sufficiently bioavailable for micro-organisms. However, no measured concentration data from liquid and gas phases have been provided to verify the assumed poor bioavailability. Moreover, a miscalculation was identified in the report and one of the validity criteria (TIC production from inoculum blank) was not fulfilled (although it was claimed to be fulfilled in the report). The OECD 310 validity criterion concerning TIC in inoculum blank vessels is not fulfilled as TIC exceeded 3 mg/l. However, no additional TIC was produced during the test (ISO 14593 validity criterion was fulfilled). Despite the miscalculation, and the high TIC value in blank assays, it is considered that the biodegradation result can still be used in the evaluation. The procedure control, toxicity control, and the calculations by eMSCA indicated that if substantial biodegradation of test substance occurred it would be detectable with the experimental set-up used and therefore it can be concluded that MCH was not readily biodegradable in the present test. However, it is also noted that due to the high TIC of the inoculum, the detection limit for test substance biodegradation is relatively high because the deviation between TIC values of replicate inoculum bottles was at highest 2.19 mg/l which corresponds to 30-33% of TOC of the test substance added (6.6-7.3 mgTOC/l) is used)), which is relatively high compared to the pass level of the test (60% of TOC. As long as TIC production of test substance is lower than the variation in TIC production between inoculum blanks, degradation is not necessarily detectable. Therefore, from this test it cannot be concluded that biodegradation was 0 %.

It is also noted that specific caution was taken to avoid volatilisation, the test substance concentration was below water solubility, and no oxygen depletion was observed in the test. Therefore, it cannot be overruled that the reason for not detecting biodegradation in the test is that the substance is not susceptible to biodegradation under the conditions of this test.

It is also noted that the concentration 100 mg/l was toxic to microorganisms in OECD 301 F (see Harlan 2012) and therefore at this concentration test substance seems to have been bioavailable as at that concentration it was able to cause toxicity to microorganisms. If MCH was not bioavailable in the test vessels at the concentration used in this OECD 310 test (7.7-8.6 mg/l), then the same applies also to the toxicity control vessels in which the same test substance concentration was used. In that case the toxicity control would not be representative of effects of toxicity on biodegradation of MCH.

A reliability score of 4 is applied ("not assignable") for the following reasons:
-the suspected poor bioavailability of test substance which, however, is not verified by measurement data
-deficiencies in documentation in test report (miscalculated values; contradictions between reported raw data and biodegradation percentages) (calculations have been corrected in robust study summary in updated dossier)

Fraunhofer (2015) - Closed Bottle Test (OECD 301 D)

Fraunhofer 2015. Closed bottle test. Ready biodegradability of methylcyclohexane by secondary effluent. Fraunhofer Institute for Molecular Biology and Applied Ecology (IME). Schmallenberg, Germany. August 13th, 2015.

This test was submitted in order to fulfill the information requirement set by ECHA's decision on substance evaluation on MCH. The report was submitted by the Registrant(s) to the eMSCA on 7.10.2015. The test was performed under GLP.

The inoculum used was a secondary effluent from a sewage treatment plant mainly fed with municipal wastewater. The test was conducted at 20±1°C. The degradation was assessed by dissolved oxygen analyses. In addition, test substance concentrations were measured in the beginning and in the end of the study. Inoculum alone, reference control (sodium benzoate), and toxicity control (MCH + sodium benzoate) experiments were also performed. 120 ml serum bottles were used. Test item was applied using stock solutions prepared by stirring in a sealed vessel; after this equilibration phase the dissolved test item concentration was measured by chemical analysis.

Ultimate degradation was monitored by in situ measurements of dissolved oxygen by oxygen electrode which allowed measurements without opening the vessels or withdrawing a subsample (the sensor is injected through a septum).

To verify test item concentration in the test vessels, six vessels of the test item assays and six vessels from the toxicity control assays were sampled at test start and test end; the samples were taken with a syringe through the septum of the sealed vessels. Test item concentration was determined by GC/MS. The mean initial concentration of MCH in the test assays was 2.45 mg/litre mineral test medium and in the toxicity control assays 3.09 mg/litre mineral test medium. Mean concentration of MCH at the test termination was 1.71 mg/l in test item vessels and 2.48 mg/l in toxicity control vessels (70% and 78% of the initial level, respectively).
The reported biodegradation of MCH based on oxygen consumption was 0 % after 28 days.

Table 13) and the conclusion in the test report is, accordingly, that MCH must be considered not readily biodegradable under the chosen test conditions. The decrease in concentration of MCH was 30% during 28 days.

It is noted that the evaluating MSCA was not able to reproduce the exact degradation values from the reported raw data. It is not indicated whether the degradation in the test report was calculated using ThOD value based on mean concentration or values of replicate vessels. However, recalculation of the biodegradation results using, e.g., ThOD values from individual vessels would not change the conclusion on ready biodegradability because oxygen consumption in test substance assays was at highest 6.4% of the mean ThOD of test substance added, and there was no increasing trend in oxygen consumption, indicating that biodegradation was clearly below the pass level (60%).

According to the test report, the validity criteria were fulfilled. The evaluating MSCA agrees that the study fufils the validity criteria concerning the oxygen consumption in the inoculum blank and oxygen concentration in the test vessels. The validity criterion concerning the difference in replicate values is not fulfilled; however, this may be attributed to the low degradation.

The evaluating MSCA considers that the validity criterion concerning procedural control is not fulfilled. It is noted that degradation percentages exceeding 100% are reported for procedural controls (119.4-128.11%) (

Table 13) and also the values calculated by the evaluating MSCA from the raw data (119.8-126.1%; data not shown) are similar. In the procedural control, the only external carbon source is the reference substance and pass level is calculated as 60% of the ThOD of reference substance. In ready biodegradation tests, some of the carbon from the test chemical is incorporated into new cells and therefore percentage of carbon dioxide produced (and oxygen consumed) is lower than the percentage of carbon being used. Therefore, even a 100% degradation based on oxygen consumption would be exceptional. The present study reports even higher degradation, suggesting technical problems or error in documentation.

An increase in oxygen content was detected in procedural control after day 14 (on day 14, O_2 content 57.9% of the initial level; on day 21-28 O_2 content 74.4-78.8% of the initial level). No explanation to the >100% degradation or increase in O_2 content is given in the test report. The evaluating MSCA considers the procedural control not valid as it is possible that the O_2 measurements on days 5 and 14 are not reliable and as the reported (38.6-58.6%) and calculated (38.3-59.5%) degradation percentages for days 21-28 are below the pass level.

In toxicity control more than 25% degradation was detected after 14 days, and therefore according to the Registrant(s) the test substance is identified as non-toxic in a ready biodegradability test. However, it is noted that a decrease in degradation percentage (

Table 13) and increase in O_2 content was detected in toxicity control after day 14 (on day 14, O_2 content was 54.3% of the initial level; on days 21-28, O_2 content was 59.6-63.0% of the initial level) and no explanation is given in the test report. The evaluating MSCA therefore considers the toxicity control not reliable as it is possible that the O_2 measurements on days 5 and 14 are not reliable and as the reported (19.1-25.1%) and calculated (18.8-24.8%) degradation percentages for days 21-28 are partly or fully below 25%.

General validity criteria of OECD 301

Test is considered valid if the difference of extremes of replicate values of the removal of the test chemical at the plateau, at the end of the test or at the end of the 10-d window, as appropriate, is less than 20% and -if the percentage degradation of the reference compound has reached the pass levels by day 14.

If in a toxicity test, containing both the test substance and a reference compound, less than 35% degradation (based on total DOC) or less than 25% (based on total ThOD or ThCO2) occurred within 14 days, the test substance can be assumed to be inhibitory.

Specific validity criteria of OECD 301 D

Oxygen depletion in the inoculum blank should not exceed 1.5 mg dissolved oxygen/l after 28 days. Values higher than this require investigation of the experimental techniques. The residual concentration of oxygen in the test bottles should not fall below 0.5 mg/l at any time. Such low oxygen levels are acceptable only if the method of determining dissolved oxygen used is capable of measuring such levels accurately.

Table 13. Cumulative degradation (%) based on O_2 consumption in OECD 301D ready biodegradation test (reported values, Fraunhofer 2015)

Day	Test item	Procedural control	Toxicity control
0	0,0	0.0	0.0
5	5.8	128.1	not determined
9	4.7	122.5	31.0
14	4.1	119.4	30.7
21	3.0	58.6	25.1
28	-0.9	38.6	19.6

A reliability score of 3 is applied ("not reliable") for the following reasons: -the procedural control is not valid due to biodegradation values exceeding 100% and due to substantial increase in oxygen content after day 14 (as explained above and in

Table 13), indicating possible technical problems, or error in documentation .

Table 14: Deviations/problems identified in the OECD 301D test (Fraunhofer 2015)

Deviation/problem identified	Remarks by evaluating MSCA
Biodegradation percentages exceeding 100% in procedural control.	This is not commented in test report. However, values exceeding 100% suggest a technical problem or an error in the documentation.
Increase in oxygen content in procedural control after day 14.	This is not commented in test report. In principle, the exceeding of pass level at day 14 in procedural control would be sufficient to fufil the validity criterion concerning procedural control. However, the increased oxygen content, together with the fact that >100% degradation values were presented, without any explanation, the procedural control is considered not reliable.
Increase in oxygen content in toxicity control and day 14.	This is not commented in test report. In principle, the biodegradation exceeding 25% after 14 days would be sufficient to rule out inhibition by test substance. However, increased oxygen content in toxicity control, together with the fact that >100% degradation values were presented without any explanation in the procedural control, the toxicity control is considered not reliable.
Reproducibility of the calculated cumulative O2 consumption and percentage degradation values from the raw data presented.	The evaluating MSCA is unable to reproduce the exact values presented in Table 7 and Table 8 of the Annex 1 of the report from the raw data presented. However, the deviation between calculated and reported values is not critical for the conclusion in this case and does not make the study inacceptable for the purpose. It is not indicated what ThOD value was used for calculation (a THOD based on mean MCH concentration or based on concentrations of each replicate) The concentration measurements were conducted for six test item vessels and six toxicity control vessels; however, in Table 8 only three replicates for test item and toxicity control are presented and it is not reported which of the six vessels are used for the calculation.

A reliability score of 3 is applied ("not reliable") as the procedural control is not valid due to biodegradation values exceeding 100% and due to substantial increase in oxygen content after day 14, indicating possible technical problems, or error in documentation

Comparison with requirements set in the substance evaluation decision

In Table 15, the OECD 301D ready biodegradation study (Fraunhofer 2015) is compared with the requirements and recommendations given in the substance evaluation decision.

Table 15: Comparison of the OECD 301D ready biodegradation test with the requirements and recommendations in the decision on substance evaluation of MCH

Requirement/recommendation in the Decision	Remarks by evaluating MSCA	Fulfilment of the requirement/recommendation (Yes/Partially/No)
II. Information requited Pursuant to Article 46(1) of the REACH Regulation the Registrant(s) shall submit the following information using the indicated test methods and instructions (in accordance with Article 13 (3) and (4) of the REACH Regulation) and the registered substance subject to the present decision: 1. Ready biodegradability study - closed bottle test (Test method EU C.4-E/OECD 301D) with chemical analysis to verify the test substance concentration.	The submitted study was conducted according to the OECD 301D guideline; however, the study is not reliable as the procedural control is not considered valid. The study included chemical analysis to verify the test substance concentration.	Partially.
An OECD 301 D (Closed Bottle Test), in which completely full test bottles without headspace are used, is considered most suitable for volatile substances like MCH. It can be applied to substances with high biological oxygen demand provided that the test substance concentration is adjusted to ensure that enough oxygen is available in the water/test system.	It is mentioned in the report that completely full, closed bottles are used. Test substance concentration was 2.45 mg/l. Oxygen concentration in the test vessels did not fall below 0.5 mg/l, thereby fulfilling the validity criterion.	Yes.
Care must be taken when administering the substance into the test vessel in order to ensure that the substance enters the test vessel.	Test item was applied using stock solutions prepared by stirring in a sealed vessel; after this equilibration phase the dissolved test item concentration was measured by chemical analysis. To verify test item concentration in the test vessels, six vessels of the test item assays and six vessels of the toxicity control assays were sampled at test start and test end; the samples were taken with a syringe through the septum of the sealed vessels. Test item concentration was determined by GC/MS.	Yes
In order to ensure bioavailability, the test can be performed under continuous mixing.	The vessels were statically incubated.	No (Not a mandatory requirement)
A toxicity control must be included and if inhibition by test substance is suspected the test shall be repeated using a lower test substance concentration as instructed in the test guideline.	Toxicity control was included in the study. No inhibition by test substance was reported; however, evaluating MSCA considers that the toxicity control is not reliable	No.

	due to reported increase in oxygen content.	
Concerning the test substance concentration, the instructions given in Annex II of OECD 301 test guideline shall be taken into account. In Annex II of OECD 301 it is stated that if inhibition due to toxicity is to be avoided, it is suggested that the test substance concentrations used in ready biodegradability testing should be less than 1/10 of the EC5O values (or less than EC2O values) obtained in toxicity testing. For MCH, this would imply a test substance concentration of 2.9 mg/I (based on microbial toxicity EC50 of 29 mg/I).	The mean test substance concentration was 2.45 mg/l in the test item vessels and 3.02 mg/l in the toxicity control assays. Therefore, test substance concentration in the toxicity control (3.02 mg/l) slightly exceeded the recommended level (2.9 mg/l).	Yes
The maintenance of the test substance concentrations during the test shall be verified with analytical determinations of MCH e.g. in sterile controls containing no inoculum, but prepared and treated otherwise similarly to the actual test bottles.	The maintenance of the test substance concentrations during the test was verified with analytical determinations of MCH in six test item vessels and six toxicity control vessels. Sterile controls were not performed; however, inclusion of sterile control was not a mandatory requirement.	Yes
The chemical analysis shall be conducted on a sufficient number of days (at least on days 0, 14 and 28) and with a sufficient amount of replicates (at least three for each day).	The analytical determinations of MCH were performed at test start (day 0) and test end (day 28), but not at day 14, for six test item vessels and six toxicity control vessels. The lack of measurement at day 14 is not considered a critical deviation from the requirement as sufficient information was obtained from the measurements at day 0 and 28 (i.e., the presence of test substance in test vessels throughout the test was verified). The amount of replicates analysed is sufficient.	Yes (Fulfilled with acceptable deviation)
Specific chemical analysis can also be used to assess primary degradation of the test substance and to determine the concentration of intermediate substances formed. For this purpose additional bottles with the test substance and inoculum can be prepared.	The analytical determinations of MCH were performed for test item vessels and toxicity control vessels and no additional bottles were performed for primary degradation. Intermediate substances were not determined. The change in MCH concentration can be biotic and/or abiotic and as sterile control was not included, the relative contribution of biotic and abiotic phenomena cannot be differentiated from these results.	Partially (Not a mandatory requirement)

Regarding biological oxygen demand, it is noted, that, for instance, at a concentration of 2 mg/I of the test substance, oxygen depletion should not be a problem as 6.84 mg/l O_2 is enough to fully decompose the substance (Water at a temperature of 20 °C contains approximately 9 mg/I of O_2).	The test substance concentration was 2.4 mg/l. Oxygen concentration in the test vessels did not fall below 0.5 mg/l, thereby fulfilling the validity criterion.	Yes (Not a mandatory requirement)

Conclusion on the OECD 301D test (Fraunhofer 2015) in terms of the information requirement on ready biodegradation given in the substance evaluation decision

The submitted OECD 301D test is considered not reliable because of problems with the procedural control. In addition, the requirement of a toxicity control in the substance evaluation decision is not fulfilled as the toxicity control is not valid and therefore it cannot be evaluated whether or not inhibition by test substance occurred.

However, it is acknowledged that oxygen content decreased in the procedural control and in toxicity control, indicating that biodegradation of reference substance occurred. In addition, the presence of test substance in the test item vessels throughout the test was verified by chemical analysis, which is a significant improvement compared to the other available ready biodegradation tests. No ultimate biodegradation of the test substance was detected after 28 days. The highest individual ultimate degradation percentage was 5.6%. The observed decrease in concentration of MCH (30% during 28 days) is in line with the observation that pass level was not reached based on oxygen consumption and indicates that primary degradation and/or abiotic removal of MCH occurred.

In summary, the evaluating MSCA notes that the Registrant(s) have submitted the required test and have taken note of the requirements given in the substance evaluation decision. The poor degradability is in line with other available ready biodegradation tests on MCH In the present study the presence of test substance in test vessels was analytically demonstrated. However, the test has deficiencies and does not, on its own, allow final conclusions to be done on ready biodegradability of MCH.

Non-standard published studies

Studies concerning biodegradation of MCH available in the scientific literature were reviewed and are summarized (Table 16). In addition to data concerning MCH, also data concerning cyclohexane was reviewed because cyclohexane was used as a read across source substance (read-across is discussed in the next chapter).

Several microorganisms are able to utilize MCH as a sole carbon source (Anderson et al. 1980, Rouviere and Chen 2003, Stirling et al. 1977, Lloyd-Jones and Trudgill 1989, Tonge and Higgins 1975, Trower et al. 1985). However, in these studies the microorganisms have been pre-exposed to MCH, CH, or other hydrocarbons, or, the pre-exposure is not known. Therefore the growth and the degradation rates reported in those studies are not relevant for biodegradation in environmental sites with no pre-exposure.

In many cases microbial growth on MCH did not occur despite pre-exposure to MCH or other hydrocarbons (Lloyd-Jones and Trudgill 1989, Koma et al. 2005, Beam and Perry 1974). Lloyd-Jones (1989) observed that although a three-organism consortium grew on MCH, the individual strains did not. They also observed that the ability to grow on MCH was linked to the presence of plasmids. Koma et al. (2005) observed that MCH was not utilized as a sole carbon and energy source but degradation occurred when an n-alkane (hexadecane) was added.

It seems that the ability to ultimately degrade MCH may develop as a response to exposure of microorganisms to MCH or, possibly, to other hydrocarbons. However, there is no information on the pre-exposure time needed. Commensalism between micoorganisms, occurrence of plasmids, or presence of other hydrocarbons may be needed for MCH biodegradation.

Primary biodegradation of MCH in water/microbial culture has been observed in water (Prince et al. 2007), soil (Bushnaf et al. 2011) , and in microbial cultures (Koma et al. 2005, Van Hamme et al. 2001). For one of these studies, half-life values (median 7.4 d, mean 13.8 d) are reported (Prince et al. 2007). However, these results are not relevant for the environmental risk assessment of MCH because the reported degradation rates may be influenced by cometabolism. Under anaerobic conditions biodegradation was not detected (Vieth and Wilkes, 2006).

Information on biodegradation intermediates and biodegradation pathways obtained from non-guideline studies is reviewed in the next Chapter.

In conclusion, the non-standard published studies do not indicate that MCH would undergo "rapid and ultimate degradation in most environments" as is expected for readily biodegradable substances (ECHA 2012) and are therefore consistent with the low biodegradation in the ready biodegradability tests on MCH.

Table 16: Non-standard published biodegradation studies

Study decscription	Studied microorganisms (or their source)	Biodegradation and growth results for MCH (and cyclohexane, if available)	Remarks by evaluating MSCA	Reference
The study describes primary aerobic biodegradation of an unleaded, unoxygenated, regular gasoline by unacclimated inocula from uncontaminated fresh and sea water, and from a domestic sewage treatment plant. Cometabolic substrates were present in gasoline. Primary degradation was determined by purge-and-trap gas chromatography coupled with mass spectrometry. Mineralization or growth was not determined.	Water samples used as inocula were collected from a New Jersey rainwater retention pond (4000 m^2 surface area, up to 3 m deep) approximately every month throughout the year, from the New Jersey shore in June and November, and from an activated sludge wastewater treatment facility treating only domestic wastewater in August. The samples were not pre-exposed to hydrocarbons. None of the samples showed any detectable hydrocarbons by the methods used (detection limit 2 ppb in 10 mL water).	MCH primary biodegradation half-life, median 7.4 d, mean 13.8 d. Cyclohexane primary biodegradation half-life, median 8.2 d. mean 28.5 d.	The relevance of the study for biodegradation estimations under REACH is compromised by the facts that MCH was not the only test substance and that other hydrocarbons, which may serve as cometabolic substrates, were present.	Prince et al. (2007)
The impact of biochar (2% on dry weight basis) on the fate of volatile petroleum hydrocarbons in a mixture of 12 petroleum hydrocarbons was studied in an aerobic sandy soil with batch and column studies. Biodegradation rates were determined from hydrocarbon concentrations determined by gas chromatography. Mineralization or	Soil obtained from a construction site in Newcastle (UK). It was not documented whether the microorganisms were pre-exposed to hydrocarbons.	MCH degradation was higher in the biochar amended soil in both batch and column studies. First-order biodegradation rate kw (1/s) soil with no biochar: $(4.1\pm4.0)\times10^{-4}$; soil with biochar $(1.6\pm0.3)\times10^{-3}$ Cyclohexane degradation was higher in the biochar amended soil. First-order biodegradation rate kw (1/s) soil with no biochar: $(6.4\pm0.6)\times10^{-4}$; soil with biochar $(1.1\pm0.2)\times10^{-3}$	The relevance of the study for biodegradation estimations under REACH is compromised by the facts that MCH was not the only test substance and that other hydrocarbons, which may serve as cometabolic substrates, were present.	Bushnaf et al. (2011)

Study decscription	Studied microorganisms (or their source)	Biodegradation and growth results for MCH (and cyclohexane, if available)	Remarks by evaluating MSCA	Reference
growth was not determined.				
Growth, respiration and enzyme studies were conducted on a pseudomonad strain isolated from soil (Nottingham, UK)	A *Pseudomonas* sp. capable of growth on cyclohexane first isolated from a soil sample of an ash wood by classical enrichment techniques using cyclohexane vapour as a sole carbon source	The bacterium was able to grow on MCH. The rate of oxygen uptake when growing on MCH (endogenous rate subtracted) was 4.0 µmol O_2 h^{-1} mg $drywt^{-1}$ The bacterium was able to grow on CH. The rate of oxygen uptake when growing on CH (endogenous rate subtracted) was 2.4 µmol O_2 h^{-1} mg $drywt^{-1}$	The microorganisms were exposed to cyclohexane during the enrichment procedure and, therefore, the results are not representative of degradation by microorganisms not exposed to hydrocarbons.	Anderson et al. (1980)
The article describes studies on the oxidation and assimilation of n-alkyl-substituted cycloalkane substrates by several hydrocarbon-utilizing microorganisms	The bacterial cultures used in this study were: *Mycobacterium vaccae* strain JOB5; *M. rhodochrous* strains OFS and 7E1C; *Nocardia asteroides* strain A-116; and *M. convolutum* strain R-22. Stock cultures were maintained on mineral salts medium with propane (50:50) [vol/vol] with air) as substrate. The microorganisms had been isolated on *n*-alkanes. These microorganisms utilized hepta-decylcyclohexane and dode-cylcyclohexane as the sole source of carbon and energy.	These microorganisms were not able to use neither MCH nor ethylcyclohexane as growth substrate. Biodegradation of cyclohexane was not studied.	No degradation of MCH was observed despite of the pre-exposure to hydrocarbons.	Beam and Perry (1974)
The degradation pathways for cyclic alkanes (*c*-alkanes) in *Rhodococcus* sp. NDKK48 were investigated.	*Rhodococcus* sp. NDKK48 was isolated from soil as a bacterium that degrades the *c*-alkane fraction of car engine oil. The isolation procedure is described in another paper (Koma et al. 2003). The bacterium was isolated using cyclic	The bacterium could not utilize MCH for growth. MCH was co-oxidised in the presence of hexadecane. Co-oxidation was required for primary and secondary oxidations. MCH was degraded via a ring oxidation pathway, and the degradation pathway	MCH (nor cyclohexane) did not serve as growth substrate and cometabolism was necessary for the degradation, despite the pre-exposure to cyclic alkanes. Moreover, information concerning the degradation	Koma et al. (2005)

Study decscription	Studied microorganisms (or their source)	Biodegradation and growth results for MCH (and cyclohexane, if available)	Remarks by evaluating MSCA	Reference
	alkane fraction of car engine base oil as a sole carbon and energy source.	contained part of the Bayer-Villiger oxidation for ring cleavage. The bacterium could not utilize cyclohexane for growth. Co-oxidation was required for primary and secondary oxidations. Cyclohexane was degraded by the same pathway as MCH.	mechanisms and intermediates is obtained from this study.	
This article describes properties of a bacterial consortium isolated from oil refinery waste by elective culture with MCH.	A three-organism bacterial consortium consisting of *Rhodococcus, Flavobacterium* and *Pseudomonas* spp isolated from oil refinery waste.	The bacterial consortium consisting of three strains was able to grow on MCH but the individual strains were not. The consortium was capable of growth with a wide range of aclicyclic hydrocarbons and related compounds but was unstable, rapidly losing the ability to grow with MCH when placed on non-selective media. It was reported that unstable plasmids were involved in growth on methylcyclohexane. Loss of the plasmids was concomitant with loss of ability to grow on MCH	The studied bacteria had likely been exposed to hydrocarbons in the environment and an elective culture with MCH was used. Therefore, the results are not representative of degradation by microorganisms not exposed to hydrocarbons. The individual strains were not able to grow on MCH and cyclohexane (despite pre-exposure). Also information on the degradation intermediates of MCH and cyclohexane is obtained from this study.	Lloyd-Jones ja Trudgill (1989)
Isolation of a new L-proteobacterium capable of growing on cyclohexane from an oil refinery wastewater sludge. This strain grows on a range of light hydrocarbons (C5-C10) as well as on some aromatic compounds such as toluene and m-cresol.	*Brachymonas petroleovorans* CHX was isolated from the wastewater plant of a petroleum refinery.	The bacterium was able to grow on MCH and on cyclohexane.	The bacterium was isolated from environment where exposure to hydrocarbons is likely and, therefore, therefore, the results are not representative of degradation by microorganisms not exposed to hydrocarbons.	Rouviere and Chen (2003)
Several soil and mud samples were examined for methylcyclohexane-utilizing microorganisms but	The organism chosen for detailed study, tentatively identified as a *Nocardia*, was isolated from	The bacterium was able to grow on MCH as sole carbon and energy source. The rate of oxygen uptake when growing on MCH	The microorganisms were exposed to MCH during the enrichment procedure and, therefore, the results	Stirling et al. (1977)

Study decscription	Studied microorganisms (or their source)	Biodegradation and growth results for MCH (and cyclohexane, if available)	Remarks by evaluating MSCA	Reference
only two (both from estuarine mud flats) proved positive. The study described the isolation and properties of a bacterium which grows on MCH or cyclohexane as sole carbon and energy source.	estuarine mud flats near Sittinbourne, Kent, UK, by classical enrichment techniques using MCH vapour as sole carbon source. CH also served as a growth substrate and the detailed properties of the bacterium were determined after growth on cyclohexane. The organism was identified as auxotrophic for biotin.	(endogenous rate subtracted): 36 µl O_2 min^{-1} (mg dry wt organisms) $^{-1}$. In addition, the bacterium was able to grow on cyclohexane as sole carbon and energy source. The rate of oxygen uptake when growing on MCH (endogenous rate subtracted): 44 µl O_2 min^{-1} (mg dry wt organisms) $^{-1}$.	are not representative of degradation by microorganisms not exposed to hydrocarbons.	
This article describes the growth of *Nocardia petroleophila* on MCH as sole carbon and energy source and the identification of catabolites.	The studied bacterial strain *Nocardia petroleophila* (NCIS9438) was maintained on nutrient agar and was grown routinely in liquid culture. MCH (0.5% or 1% v/v) was added to a mineral salts medium. The origin of the bacterial strain, and the information on exposure to MCH or other hydrocarbons prior the growth experiments, were not reported.	The bacterium was able to grow on MCH. Biodegradation of cyclohexane was not studied.	Due to unknown origin and pre-treatment of the studied bacterial strain, it is not known whether the results are representative to microorganisms not exposed to MCH or other hydrocarbons. However, information on the degradation intermediates is taken into account.	Tonge and Higgins (1974)
This article describes the isolation and properties of a *Xanthobacter* sp. capable of growth upon cyclohexane as the sole carbon source.	Bacterial culture (*Xanthobacter sp.*) isolated from forest soil. The study was conducted with micro-organisms pre-exposed to CH during enrichment and maintenance.	The bacterium was able to grow on MCH and on cyclohexane.	The microorganisms were exposed to MCH during the enrichment procedure and, therefore, the results are not representative of degradation by microorganisms not exposed to hydrocarbons.	Trower et al (1985)
Volatile hydrocarbon biodegradation by a mixed-bacterial culture during growth on crude oil	The study was performed using a mixed-bacterial culture isolated from petroleum-	Primary biodegradation of MCH was observed. Inoculum age rather than concentration had the most profound	The relevance of the study for biodegradation estimations under REACH is	Van Hamme and Ward (2001)

Study decscription	Studied microorganisms (or their source)	Biodegradation and growth results for MCH (and cyclohexane, if available)	Remarks by evaluating MSCA	Reference
was investigated using solid phase microextraction (SPME). Mineralization or growth was not determined.	contaminated soil and maintained in cyclone fermenters on various hydrocarbon substrates (diesel fuel, crude oil, motor oil, refinery sludge). After storage (-80°C), the culture was pregrown on crude oil (Bow River, Canada) with a surfactant and yeast nitrogen base. Biodegradation flasks were prepared using inoculum sampled at different time points from the pregrown flask.	impact on biodegradation. Biodegradation of cyclohexane was not studied.	compromised by the facts that MCH was not the only test substance and that other hydrocarbons, which may serve as cometabolic substrates, were present. In addition, the microorganisms had been exposed to hydrocarbons during the enrichment procedure and, therefore, the results are not representative of degradation by microorganisms not exposed to hydrocarbons.	
In-reservoir anaerobic biodegradation rates of hydrocarbons were determined by stable carbon isotope analyses (oil field, Norwegian North Sea).	Field study focusing on the biodegradation of oil hydrocarbons by natural microbial population of the oil reservoir.	No change (<1‰) in the isotope ratio ($d^{13}C$) was observed for MCH nor for cyclohexane. It was evaluated by the authors that biodegradation of the cycloalkanes such as cyclohexane and methylcyclohexane is "at best marginal".	The study concerns anaerobic degradation and was and conducted in a field site in the presence of other oil hydrocarbons.	Vieth and Wilkes (2006)

Biodegradation intermediates and pathways

Several intermediates of MCH biodegradation have been reported (Tonge and Higgins, 1974, Trudgill 1984, Lloyd-Jones and Trudgill 1989, Koma et al. 2005). These intermediates include cyclohexylmethanol, cyclohexylformaldehyde, cyclohexanecarboxylic acid, cyclohexanol, 2-methylcyclohexanol, 3-methylcyclohexanol, 4-methylcyclohexanol, 2-methylcyclohexanone, 3-methylcyclohexanone, 4-methylcyclo-hexanone, 4-methyl-2-oxepanone, and 3-methyladipic acid (Table 17). Ready biodegradability data in ECHA database is available for only three of these intermediates: 2-methylcyclohexanol, cyclohexanol, and cyclohexanecarboxylic acid. Cyclohexanol and 2-methylcyclohexanol are reported to be readily biodegradable (based on experimental data) whereas cyclohexanecarboxylic acid is reported to be "Possible Ready Biodegradable" based on QSAR estimation (VegaNIC v.1.0.).

Different biodegradation pathways for MCH have been proposed (Figure 1, Figure 2). One of the pathways (Pathway 1) is the ring oxidation pathway in which biodegradation of MCH starts with the oxidation of carbon in the alicyclic ring, to yield methylcyclohexanol, methylcyclohexanone, 4-methyl-2-oxepanone, and methyladipic acid, which is possibly further metabolized by beta-oxidation and citric acid cycle (Table 18, Figure 1). The position of the methyl group in the alicyclic ring or in the carbon chain in relation to the other functional group of the degradation intermediate, may vary. In some cases the first

step in MCH biodegradation may be oxidation of the methyl group to yield cyclohexylmethanol, with possible formation of cyclohexylformaldehyde and cyclohexanecarboxylic acid (Pathway 2), which is then metabolized further. Alternatively, when cyclohexylmethanol is oxidized, the extracyclic carbon can be eliminated at the cyclohexylformaldehyde stage (Pathway 3) with the formation of cyclohexanol and cyclohexanone. Methylcyclohexanone and cyclohexanone (from Pathways 1 and 3) and cyclohexanecarboxylic acid (from Pathway 2) may be channelled to beta-oxidation through further reaction steps (Figure 2) and used as carbon and energy source. Trudgill (1984) reported that a five-organism consortium was able to simultaneously utilize each of the three above-mentioned metabolic pathways for transformation of MCH.

It is further noted that, for cyclohexane, a degradation route including aromatization of the ring and subsequent formation of phenol has been proposed (Yi et al. 2011). Apparently, it has not been studied whether aromatization could occur with MCH. However, this might be possible as in many studies the same organisms have been able to degrade both MCH and cyclohexane, with similar reaction steps occurring with both substances. Moreover, it is noted that some of the intermediates (cyclohexanol and cyclohexanone) that may occur in MCH metabolism (Trudgill 1984, Lloyd-Jones and Trudgill 1989) are also involved in the aromatization pathway proposed for cyclohexane (Yi et al. 2011).

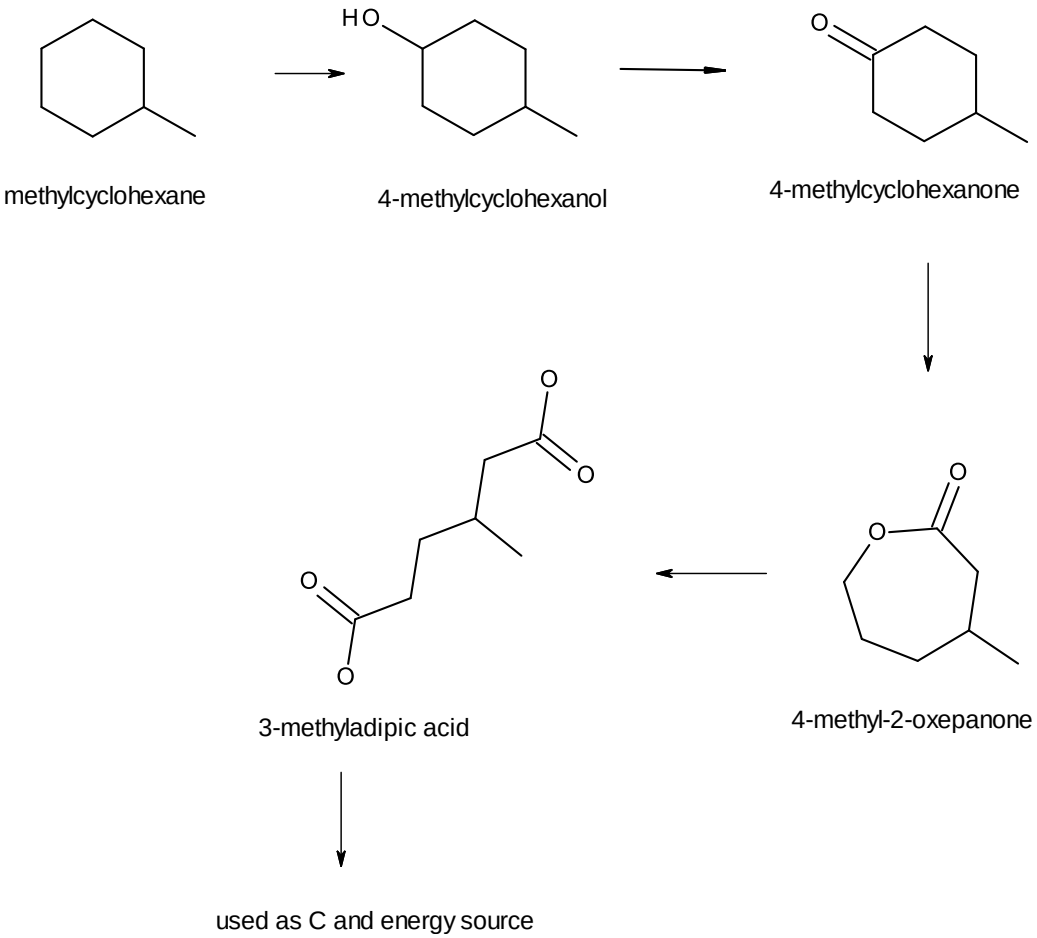


Figure 1: Methyl cyclohexane degradation pathway in Rhodococcus sp. based on Koma et al. (2005).

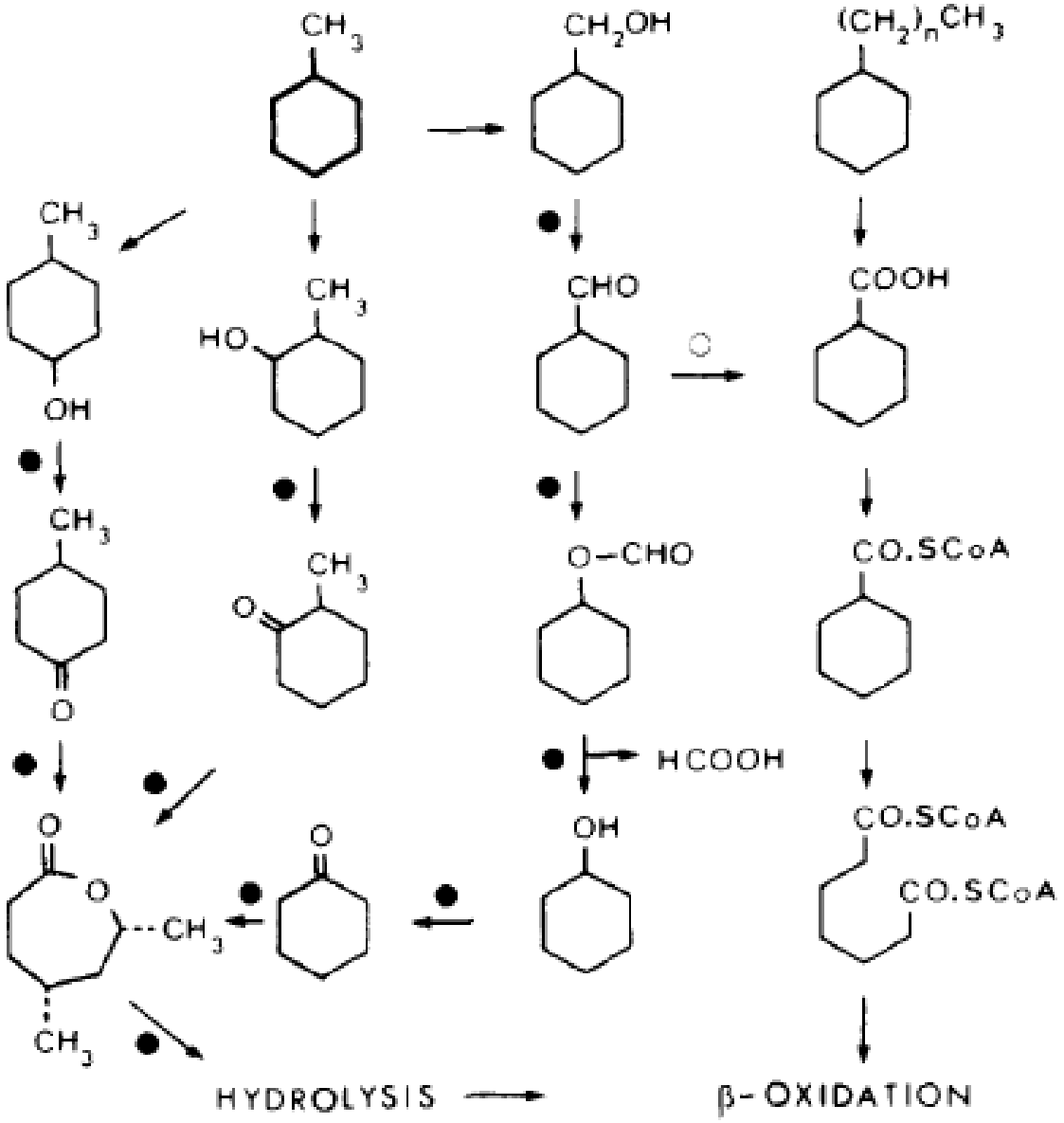


Figure 2: Proposed pathways of methylcyclohexane oxidation by the three-organism bacterial consortium consisting of *Rhodococcus, Flavobacterium and Pseudomonas spp*. (Reprinted from Lloyd-Jones, G. and Trudgill, P.W. 1989. The degradation of alicyclic hydrocarbons by a microbial consortium. International Biodeterioration 25, 197-206 with permission from Elsevier. http://www.sciencedirect.com/science/journal/02653036)

Table 17: Intermediates in the biodegradation of methyl cyclohexane and cyclohexane via the ring oxidation pathway*.

chemical category (in stepwise order according to the metabolic route)	*methylcyclohexane*	*cyclohexane*
cyclic alcohol	2-methylcyclohexanol (Lloyd-Jones and Trudgill 1989) 3-methylcyclohexanol (Tonge & Higgins, 1974) 4-methylcyclohexanol (Lloyd-Jones and Trudgill 1989, Koma et al.2005)	cyclohexanol (Trower et al., 1985, Koma et al. 2005
cyclic ketone	2-methylcyclohexanone (Lloyd-Jones and Trudgill 1989) 3-methylcyclohexanone (Tonge & Higgins, 1974) 4-methylcyclohexanone (Lloyd-Jones and Trudgill 1989, Koma et al. 2005)**	cyclohexanone* (Trower et al. 1985, Koma et al. 2005)
lactone	4-methyl-2-oxepanone (synonyms: 4-methyl-ε-caprolactone; 4-methyl-1-oxa-2-oxocycloheptane) (Koma et al. 2005) 5-methyl-2-oxepanone (Lloyd-Jones and Trudgill 1989) 7-methyl-2-oxepanone (Lloyd-Jones and Trudgill 1989)	2-oxepanone (synonyms: ε-caprolactone; 1-oxa-2-oxocyclohepta-ne) (Trower et al. 1985; Koma et al. 2005)
hydroxy acid	not reported	6-hydroxyhexanoic acid (Trower et al. 1985)
keto acid	not reported	6-oxohexanoic acid (Trower et al. 1985
dicarboxylic acid	3-methyl adipic acid (Koma et al. 2005)	adipic acid (Trower et al., Koma et al. 2005)

*The data is from studies with the bacterials strains *Nocardia petroleophila* (NCIB9438) (Tonge and Higgins 1974), *Xanthobacter* sp. (Trower et al. 1985), and *Rhodococcus* sp. NDKK48 (Koma et al. 2005) and with bacterial consortium consisting of *Rhodococcus, Flavobacterium* and *Pseudomonas* spp. (Lloyd-Jones and Trudgill 1989)

** It has been reported that MCH can be co-oxidized to 4-methylcyclohexanone by a soil isolate growing on 2-methylbutane (Ooyama and Foster (1965) as cited in Stirling and Watkinson (1977)).

Evaluation of the analogue approach for estimation of biodegradation of MCH

The Registrant has used a read across adaptation using analogue approach as a part of a weight of evidence approach to fulfill the data requirement on ready biodegradability. The source substance for this read-across is cyclohexane (CH). In a dossier update in June 2014 a further analogue substance was included: 1-isopropyl-4-methylcyclohexane. The validity of the read across to cyclohexane was first assessed. For this purpose, the studies available on biodegradability of cyclohexane and methyl cyclohexane were reviewed.

The available ready biodegradability tests for cyclohexane are summarized in Table 18. According to the EU-RAR cyclohexane is readily biodegradable, indicating that it was used

as carbon and energy source by microorganisms in the ready biodegradability test. No robust study summary is given in the registration dossier for this study (Exxon 1995) and only the information available in the EU risk assessment (EU, 2004) is given. The test was an OECD 301 F (Manometric respirometry) test and 77 % of MCH was degraded in 28 days. More detailed information on the same study was obtained from the ECHA database (cyclohexane) (Table 18).

It is noted that, besides the study by Exxon (1995), there were also other ready biodegradability studies in the EU risk assessment report for cyclohexane (EU, 2004), which are not included in the registration dossier of MCH. These studies are:

> Manometric-Respirometry-Test (OECD GL 301 F): 6 % degradation after 28 days (BASF,1990).
> 99MITI-I-Test (OECD GL 301 C): 0.6 % after 14 days (CITI, 1992).

The part of the discussion by EU (2004) is cited here:
"As the test duration in the MITI test was only 14 days, the result is not conclusive, especially as a long lag-phase was observed in the respirometry test by Exxon (1995). Regarding the interpretation of the biodegradability potential of cyclohexane, two opposing results remain. The test by BASF (1990) was performed in 1989, at a time when the official OECD method had not yet been adopted. It is not clear whether significant deviations from the finally adopted method remained in the draft protocol.

According to Verschueren (1983), the first step of cyclohexane biodegradation is oxidation to cyclohexanol. Cyclohexanol can clearly be considered as readily biodegradable (CITI 1992).

Furthermore, the possible biodegradation of cyclohexane has been proven in a non-standardised test. The test was performed with sterile saltwater inoculated with hydrocarbon oxidizing bacteria. The inoculum concentration is not reported. The biodegradation rate of cyclohexane was 70 % after 35 days, approximately the same biodegradation rate as for n-octane and n-hexadecane under the same conditions (Zobell, 1966)."

The conclusion of the EU risk assessment (EU, 2004) was that cyclohexane is readily biodegradable in the aquatic environment.

Of the ready biodegradability tests cited in EU (2004), the Exxon (1995) BASFand BASF (1990) tests are considered suitable for the assessment. However, in the case of the BASF study, the deficiencies in documentation need to be taken into account. For example, the initial test substance concentration is not known and therefore it is not known whether the low degradability was affected by toxicity.

In addition, in ECHA database, a ready biodegradability test according to ISO 14593 is available. In that test cyclohexane was not readily biodegradable but, however, biodegradation was detected and a biodegradation level 60 - 70 % was reached after 49 days. It is noted that full study report has not been used for the present assessment.

In case there are conflicting results for ready tests it is recommended to consider differences in stringency of tests and to check the origin of the inoculum in order to check whether or not differences in the adaptation of the inoculum may be the reason (ECHA 2012b, OECD 2006). For example, it is mentioned that very high concentrations (100 mg/L) used for some 301 tests increases the probability of inhibition or mass transfer issues for low solubility materials.

According to the CSR, cyclohexane can inhibit microbial activity at concentrations relevant to ready biodegradability tests. In the CSR, the most sensitive group of microorganisms was aerobic heterotrophs: the result for this group was used to derive an EC50/LC50 for aquatic micro-organisms of 29 mg/L. Therefore, the results of the ready biodegradability tests may have been influenced by toxicity.

Table 18. Ready biodegradability tests on cyclohexane

Method	Result	eMSCA Remarks and **conclusion**	Reference
OECD 301 F	77 % degradation after 28 days	EU(2004) has the following information: Lag-time ca. 12 days (12-13 days in 2 of the replicates and 20 in the third replicate). The 10-day window criterion was fulfilled. The log-phase was very short in 2 of the 3 replicates, between 3 and 4 days, and approx. 7 days in the third replicate. The same study is included in the ECHA database with more detailed information: Initial test substance concentration was 34 mg/l. Fresh, non-adapted activated sludge was used as the inoculum, obtained from domestic sewage outlet with no known contaminants present. Inoculum was aerated for 2 hours, blended and allowed to settle before use. **This test is used for the assessment.**	Exxon 1995 (as cited in EU (2004)) and ECHA database (with reference to study report dated 1995-08-02)[a]
ISO 14593	<10 % biodegradation (% TIC/ThIC) of cyclohexane was measured after 28 days. > 60 < 70 % biodegradation after 49 days	Initial test substance concentration was 23 mg/l. Activated sludge from a laboratory wastewater treatment plant treating municipal sewage was used as inoculum. It was concluded in the database that test substance is biodegradable under the test conditions, but not readily biodegradable according to the OECD criteria. It is also mentioned that "there are some limitations in design and/or reporting, however it is considered reliable and suitable for use for this endpoint.". This test was not included in the EU risk ssessment (EU, 2004) and eMSCA has not evaluated the study. According to the registration data in ECHA database validity criteria were fulfilled. **This test is used for the assessment.**	ECHA database (with reference to study report dated 2002-03-12)
OECD 301 F	6 % degradation after 28 days	The same study is included in EU (2004) and in the ECHA database. Initial test concentration is not defined in either of the references. It is noted that initial test substance concentration defined in the guideline (100 mg/l) is above the EC50/LC50 for aquatic micro-organisms[c]. In EU (2004) it is mentioned that "The test by BASF (1990) was performed in 1989, at a time when the official OECD method had not yet been adopted. It is not clear whether significant deviations from the finally adopted method remained in the draft protocol." **This test is used for the assessment, however the validity of test has not been verified. The deficiencies in documentation need to be taken into account.**	BASF 1990 (as cited in EU (2004)) and ECHA database with reference to BASF AG 1990 Labor Oekologie unveroeffentlichte Untersuchung :Respirometrischer Test 1990-04-10)[b]
OECD 301 C	0.6 % degradation after 14 days	Initial CH concentration not reported. It is noted that initial test substance concentration defined in the guideline (100 mg/l) is above the EC50/LC50 for aquatic micro-organisms[c]. Moreover, according to EU (2004), as the test duration in the MITI test was only 14 days, the result is not conclusive, especially as a long lag-phase was observed in the respirometry test by Exxon (1995). It is also noted that the treatment of the inoculum according to the MITI test seriously impacts the diversity of the microbes (ECHA 2012b, p. 179) which may affect the result. **This test is not used for the assessment due to the limitations mentioned above.**	CITI (1992) as cited in EU (2005)

[a]Based on the similarity of data (results and other information) it is assumed that this is the same study as Exxon (1995) cited in EU (2004). However it is noted that no reference details are given in the ECHA database.

[b]Based on the similarity of data (results and other information) it is assumed that this is the same study as BASF (1990) cited in EU (2004). However it is noted that no reference details are given in the ECHA database. Method information in ECHA database is: EEC Directive 79/831 Annex V Part C: Methods for the Determination of Ecotoxicity 5.2 Degradation - Biotic Degradation:Manometric respirometry.
[c]In the CSR, the most sensitive group of microorganisms was aerobic heterotrophs, which was used to derive an EC50/LC50 for aquatic micro-organisms of 29 mg/L.

In conclusion, the tests suitable for the assessment are:

OECD 301F (Exxon 1995): Readily biodegradable. This study was included in the EU risk assessment (EU, 2004).

OECD 301F (BASF 1990): Not readily biodegradable. Inhibition by test substance is not ruled out. This study was included in the EU risk assessment (EU, 2004).

ISO 14953: Not readily biodegradable. Inhibition by test substance is not ruled out. This study was not included in the EU risk assessment (EU, 2004) and it has not been evaluated by the eMSCA for validity. According to the registration data in ECHA database validity criteria were fulfilled.

Considering the apparent conflict between the negative test results ISO 14953 and OECD 301F (BASF 1990) and the positive result from OECD 301 F (Exxon 1995) it needs to be taken into account that consistent positive test results from test(s) should generally supersede negative test results (ECHA 2012b). **Therefore, it is concluded that cyclohexane is readily biodegradable.**

Of the identified intermediates of CH biodegradation (Trower et al. 1985, Koma et al. 2005) ready biodegradability data is available at least for cyclohexanol, cyclohexanone, 2-oxepanone and adipic acid, which are all reported to be readily biodegradable (ECHA database). The reported ready biodegradability of these intermediates is consistent with the ready biodegradability of CH.

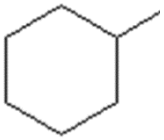
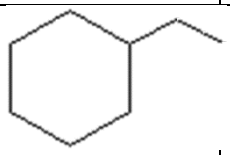
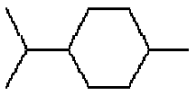
Primary biodegradation data for CH are available. Primary biodegradation half-lives for CH were 8.2 d (mean) and 28.5 d (median) in a hydrocarbon mixture (Prince et al. 2007; (

Table 16). In addition, the predicted primary biodegradation half-life for CH was 55 days) (BIOHCWIN model) (see 7.7.1.2.1.1). The primary biodegradation half-lives appear to be in some contradiction with ready biodegradability of CH. However, it is noted that the primary biodegradation half-lives may have been affected by the presence of other hydrocarbons. Although in mixtures cometabolic processes are expected to increase the degradation of CH, it is noted that when many other hydrocarbons are present, the situation can be more complicated due to interactions between different microorganisms and available substrates and thus even negative effects on CH degradation could be possible. Mixture studies are not representative of conditions in ready biodegradability tests and should not be used in evaluation of ready biodegradability.

In a dossier update in June 2014 the category approach was extended to include 1-isopropyl-4-methylcyclohexane, in addition to cyclohexane. In

Table 19 the properties of 1-isopropyl-4-methylcyclohexane as well as another relevant analogue substance ethylcyclohexane are presented. The comparison shows that it is not possible to make a sound conclusion on the ready biodegradability of MCH within this category.

Table 19: Ready biodegradability test results of methylcyclohexane and some other cyclocalkanes

substance/ property	cyclohexane (CAS 110-82-7)	methylcyclo-hexane (CAS 108-87-2)	ethylcyclohexane (CAS 1678-91-7)	1-isopropyl-4-methyl-cyclohexane (CAS 99-82-1)
Molecular weight (g/mol)	84.16	98.19	112.21	140.27
				
ready biodegra-dability conclusion	readily biodegradable		not readily biodegradable	readily biodegradable
Ready biodegra-dability test description	77 % (OECD 301 F)	0 % (see Annex 3)	0 % (OECD 301 C)	87 % (ISO 10708)
Vapour pressure at 25.0 °C	12 930 Pa	6180 Pa	1710 Pa	352 Pa
Solubility (20 - 25 °C)	55-58 mg/l	14 mg/l	6.3 mg/l	0.62 mg/L
Henry's law constant	14 900	43 600	30 400	178 000

Pa m3/mol (20 - 25 °C)				
Reference	EU RAR (2004); ECHA database	Registration dossier	NITE 2014a, OECD 2014, Episuite	ECHA database, Episuite

eMSCA's evaluation of justification for analogue approach

Regarding the use of an analogue approach (read-across to cyclohexane), the available data on MCH and CH biodegradation does not allow valid conclusions to be made on the behavior of MCH in ready biodegradability testing. The reason is that there is no sufficient evidence to support the proposed similarity of CH and MCH in terms of their susceptibility to ultimate biodegradation.

Cyclohexane contains only secondary carbon atoms while MCH contains one tertiary carbon, five secondary carbons, and one primary carbon. In the case of n-alkanes, branching in general reduces the rate of biodegradation because tertiary and quaternary carbon atoms interfere with degradation mechanisms or block degradation altogether (Atlas and Bartha 1996). Alicyclic hydrocarbons may be degraded by similar mechanisms as n-alkanes (Atlas and Bartha 1996). Therefore it has to be taken into account that branching, and the presence of tertiary carbon atom, may have an effect on the biodegradation of alicyclic hydrocarbons, including MCH. Moreover, the biodegradation products differ between MCH and CH. Table 17 lists the degradation intermediates as proposed for one possible degradation route (the ring oxidation pathway). This degradation pathway has been identified to occur with both of the substances. Because of the methyl group, degradation products of MCH may include more isomers, for example methylcyclohexanols and methylcyclohexanones, with different positions of the methyl group in relation to the other functional group of the alicyclic ring (Table 17). These isomers may differ in their susceptibility to biodegradation and their ability to serve as microbial growth substrates (Tonge and Higgins 1974, Lloyd-Jones and Trudgill 1989, Koma et al. 2005) (Table 17). In addition, it is noted that the available information on biodegradation potential and rates in non-guideline studies on MCH and CH (Beam and Perry 1974, Koma et al. 2005, Lloyd-Jones and Trudgill 1989, Tonge and Higgins 1974, Trower et al. 1985) cannot be used to evaluate the read-across for ready biodegradability. The reasons are that these studies concern microorganisms pre-exposed to MCH, CH, or other hydrocarbons, or that the pre-exposure is not known.

Therefore, the eMSCA concluded that in the case of ready biodegradability the justification of the analogue approach (read across), with CH as the source substance, is **not scientifically valid.**

eMSCA conclusion on the use of analogue approach for ready biodegradability

The source substance (cyclohexane) used in the proposed analogue approach is deemed readily biodegradable. However, justification for using analogue approach is not scientifically valid. Therefore the use of the proposed analogue approach for ready biodegradability is **not acceptable.**
The extension of the category approach to 1-isopropyl-4-methylcyclohexane does not change the conclusion.

7.7.1.2.2.1. Simulation tests (water and sediments)

No data available.

7.7.1.2.2.2. Summary and discussion of biodegradation in water and sediment

The evaluation is based on water biodegradation studies whereas no tests on biodegradation in sediment were available. The available data includes QSAR predictions, ready biodegradability tests, and non-guideline studies. No simulation tests are available.

Six ready biodegradability tests are available and in none of these tests biodegradation is observed. The Registrant(s) have concluded that the standard testing guidelines for ready biodegradation cannot be applied for the substance due to its low water solubility (14 mg/l), high volatility (Henry's law constant 33 400 - 43 600 (Pa m^3 mol^{-1} at 25°C) and high biological oxygen demand (3.42 mg O_2 per mg test item). They also have indicated that the bioavailability of test substance was limited during several of the tests (tests 1-5; Table

6). It is noted that these have been conducted taking specific caution to avoid volatilisation, the test substance concentrations (3.2-11.5 mg/L) are below water solubility, and no oxygen depletion has been observed in the tests. It is acknowledged that the substance is very volatile and that it is possible that in the ready biodegradability tests the substance has not been sufficiently bioavailable for micro-organisms. No measured concentration data from liquid and gas phases have been provided to verify the assumed poor bioavailability in tests 1-5. Therefore, it cannot be overruled that the reason for not detecting biodegradation in the tests is that the substance is not susceptible to biodegradation in ready biodegradability tests. As no biodegradation at all has been observed and the poor bioavailability has been proposed but not experimentally indicated, eMSCA considers that the reliability score for each of these tests is 4 ("not assignable"). Another ready biodegradation test was submitted in response to substance evaluation decision (test 6, Table 6). That test was evaluated as not reliable; however, it is noted that the maintenance of test substance was verified by chemical analysis and no ultimate biodegradation of test substance was detected.

In addition, non-guideline studies (Table 16) do not indicate that MCH would undergo "rapid and ultimate degradation in most environments" as is expected for readily biodegradable substances (ECHA 2012) and therefore do not support the proposed ready biodegradability of MCH. Although several microorganisms are able to utilize MCH as a sole carbon source in these studies the microorganisms have been pre-exposed to MCH or other hydrocarbons, or, the pre-exposure is not known. Therefore the growth and the degradation rates reported in those studies are not relevant for biodegradation in environmental sites with no pre-exposure.

Primary biodegradation of MCH in water/microbial cultures has been observed in studies conducted with hydrocarbon mixtures. However, these results are not relevant for the environmental risk assessment of MCH because the reported degradation rates may be influenced by cometabolism. Under anaerobic conditions biodegradation was not detected.

A weight of evidence adaptation has been used by the Registrant(s) for the data requirement for ready biodegradability. Based on BIOWIN QSAR models and read across to cyclohexane (CH), which is considered readily biodegradable in the EU risk assessment (EU RAR 2004), the Registrant(s) have concluded that the substance is readily biodegradable. However, the BIOWIN models cannot be applied to assess the ready biodegradability of MCH. Neither can the read across to CH be applied as it is not scientifically justified for ready biodegradability. Addition of ethylcyclohexane and 1-isopropyl-4-methylcyclohexane to the category does not change the conclusion.

Because there is no data indicating that MCH would be degradable in the water or sediment compartments (with the exception of degradation observed in the presence of other hydrocarbons), the substance should be treated as not biodegradable in water and sediment unless further information can sufficiently demonstrate biodegradation in relevant environmental conditions

7.7.1.2.3. Biodegradation in soil

No soil biodegradation tests according to standard methods are available for MCH. One non-standard published soil biodegradation study is available (Bushnaf et al. 2011). The study showed that MCH was primarily degraded by soil microorganisms in a hydrocarbon mixture and that biochar addition increased the rate of MCH degradation. In addition, several studies conducted with microbial cultures isolated from soil are available (Anderson et al. 1980, Koma et al. 2005, Stirling et al. 1977, Trower et al. 1985, Van Hamme and Ward 2001). All these mentioned non-standard studies are presented in Chapter 7.7.1.2.2). The results suggest that the ability of soil microorganisms to degrade MCH either ultimately or primarily, possibly involving cometabolic reactions, may develop as a response to exposure of microorganisms to MCH or, possibly, to other hydrocarbons.

Because there is no data indicating that MCH would be degradable in the soil compartment when present without other hydrocarbons, the substance should be treated

as not biodegradable in soil unless further information can sufficiently demonstrate biodegradation in relevant environmental conditions.

7.7.1.3. Summary and discussion on degradation

Abiotic degradation

Hydrolysis is not considered a relevant degradation mechanism for MCH as it has no functional groups liable to hydrolysis.

The estimated half time in air due to photodegradation is 37.9 hours. Therefore, indirect photodegradation in the atmosphere may be an important environmental fate process for this substance. The predicted half-life in air is below the criterion for persistent organic pollutants (POP) (2 d) as defined in the Annex D of the Stockholm convention (Stockholm Convention, 2001) and therefore the substance is not expected to have long-range transport potential.

General considerations on biodegradation
The present assessment of biodegradation is based on studies on biodegradation in water, including ready biodegradability tests, QSAR predictions, and non-standard published studies on microbial growth and biodegradation. Alicyclic hydrocarbons are generally regarded as recalcitrant to biodegradation unless they have a sufficiently long side chain (Atlas and Bartha 1996). Some microorganisms are able to utilize MCH as a sole carbon and energy source and others degrade MCH in a cometabolic process which requires the presence of other hydrocarbon(s). The ability to degrade MCH as a sole carbon and energy source has been observed with some microorganisms which have been exposed previously to MCH or other hydrocarbons. Biodegradation pathways and biodegradation intermediates have been identified for MCH. In the standard ready biodegradability tests no biodegradation of MCH is observed; however, bioavailability of test substance may have been limited.

Conclusions on biodegradation
Microorganisms capable of utilizing MCH as a sole carbon source have been found. However, in these studies the microorganisms have been pre-exposed to MCH or other hydrocarbons, or, the pre-exposure is not known. There are no test data showing any biodegradation in conditions relevant to environmental risk assessment under REACH. Moreover, there is no information on the pre-exposure time needed to induce the capability of microorganisms to biodegrade MCH. The proposed ready biodegradability of MCH was claimed by the Registrant(s) based on read-across to cyclohexane studies as well as the overall BIOWIN model prediction; however, these were deemed not scientifically justified. Addition of other potential read-across substances, 1-isopropyl-4-methylcyclohexane and ethylcyclohexane, does not change the conclusion.

There are six ready biodegradability tests available. Although in all of these tests there are deficiencies, in none of these test biodegradation of MCH is observed.

Based on the available data, the eMSCA concludes that MCH should be regarded **as not biodegradable in water, sediment, and soil** in the context of environmental assessment under REACH. For classification according to the CLP regulation, MCH should be regarded as "**not rapidly degradable**".

7.7.2. Environmental distribution

7.7.2.1. Adsorption/desorption

There are no experimental data available on adsorption/desorption. Based on the estimated Koc values (Table 20) it can be concluded that methylcyclohexane is moderately/significantly adsorptive.

Table 20. Estimated Koc (log Koc) values for methylcyclohexane

Koc (L/kg)	logKoc	KOCWIN v.2.00 method
233.9	2.37	MCI method
1304	3.12	Kow method using log Kow 3.59
2328	3.37	Kow method using log Kow 3.88

7.7.2.2. Volatilisation

Based on the Henry's law constants (Table 21) it can be concluded that methylcyclohexane is very easily volatilized from water.

Table 21 Studies on volatilisation

Henry's law constant [Pa m^3 mol^{-1}] (at 25°C)	Method and reference
43 600 (at 25°C)	Experimental. Hine J. and Mookerjee, JK: (1975) as cited in HENRYWIN v.3.20
34 300 (at 25°C)	Calculated. HENRYWIN v.3.20; Bond estimation method
33 400 (at 25°C)	Calculated. HENRYWIN v.3.20; Group estimation method
43 342 (at 25°C)	Calculated using vapour pressure (at 25°C) 6180Pa and water solubility (at 25°C) at 14 mg/l.

7.7.2.3. Distribution modelling

Multimedia environmental models (Mackay Level I and Level III)

The distribution of methylcyclohexane in the environment was estimated using fugacity models. The level I Mackay model assumes a closed system where no degradation occurs. The evaluator performed a level I Mackay modelling using software "Level I Version 3.00" with standard settings (Table 22). Essentially the same result was obtained as reported in the registration dossier. According to a Mackay level I calculation MCH will be distributed almost exclusively to the atmosphere (99.9 %) (Table 24).

The level III Mackay model assumes a standard environment which is in steady-state but not in equilibrium (input in and output from the model environment are occurring, as well as fluxes between the different environmental compartments). Level III model takes into account degradation processes. The modelling was done by assuming emissions to water compartment only. Degradation half-life of 37.9 h for air was used (4.1.1.2.1). For water, soil and sediment compartments, a half-life of 7.31 days was used, originating from BIOHCWIN model prediction (primary degradation half-life), which compares well with the experimental primary degradation half-life. In addition a theoretical half-life of 100000 days (274 years) was used to estimate a situation where degradation in soil, water, and sediment is practically negligible (Table 23).

According to a Mackay level III modelling using the minimum half-life, under steady-state MCH will be mostly distributed to the water (91 %) while only 8% of the substance will end up in the atmosphere, 0.6 % in sediment and less than 0.01 % in soil (Table 24). It is noted that the minimum biodegradation half-life may be based on mixture studies so this half-life may represent conditions where other hydrocarbons are present. Moreover, this minimum half-life represents primary degradation only. Assuming a longer

degradation half-life for water, sediment and soil in the model changed the relative distribution of MCH between compartments so that, with half-life of 100 000 days, percentage of the substance in water is decreased to (68 %) while the percentage in sediment (26 %) is notably increased compared to the scenario with the minimum half-lives, whereas percentages of MCH in air (6%) and soil (<0.1%) compartments are less changed (Table 24).

Degradation in air is probably the main reason why the proportion of MCH in air is small in level III results compared to level I model (degradation processes are not included in level I model).

Table 22: Parametres used for distribution modelling for the Level I Mackay model

Media	air, soil, water, sediment
Calculation programme:	Level I Fugacity-Based Multimedia Environmental Equilibrium Partitioning Model. Version 3.00. 2004. www.trentu.ca/cemc
Environment	EQC Standard Environment
Input data:	
Amount of chemical	100000 kg
Molar mass	98.19 g/mol
Data temperature	25 °C
Water solubility	14 g/m^3
Vapour pressure	6180 Pa
Melting point	-126.6 °C
Log Kow	3.88

Table 23: Parametres used for distribution modelling for Level III Mackay model

Media	air, soil, water, sediment
Calculation programme:	eMSCA: Level III Fugacity-Based Multimedia Environmental Model. Version 2.80.1. Trent University. 2004. www.trentu.ca/cemc CSR: Mackay, Level 1, v3.00
Environment	EQC Standard Environment
Emission rates	Emission to: water 1000 kg/h soil 0 kg/h air 0 kg/h sediment 0 kg/h
Molar mass	98.19 g/mol

Data temperature	25 °C
Water solubility	14 g/m^3
Vapour pressure	6180 Pa
Log Kow	3.88
Melting point	-126.6 °C
Reaction half-life estimates, minimum values	air: 37.9 h water: 7.31 d (175.44 h) soil: 7.31 d sediment: 7.31 d suspended sediment: negligible aerosols: negligible aquatic biota: negligible
Reaction half-life estimates, theoretical maximum	air: 37.9 h water: 100000 d (2400000 h) soil: 100000 d (2400000 h) sediment: 100000 d (2400000 h) suspended sediment: negligible aerosols: negligible aquatic biota: negligible

Table 24: Results of distribution modelling

Model used	Half-life in air (d)	Half-life in water, sediment and soil (d)	Distribution in environmental compartments (%)			
			Air	Water	Soil	Sediment
Mackay Level I (CSR)	not applicable	not applicable	99.9	0.01	0.09	0.002
Mackay Level I (eMSCA)	not applicable	not applicable	99.9	0.0114	0.0768	0.00171
Mackay Level III (eMSCA)	37.9	7.31	8.03	91.3	0.0055	0.640
Mackay Level III (eMSCA)	37.9	100000*	5.96	67.9	0.0048	26.2

*theoretical value assuming that no degradation occurs

Fate of MCH in waste water treatment plant (STPWIN model)

To estimate the behavior of MCH in waste water treatment plant, STPWIN model was used (model included in EpiSuite v 4.0). The modelling parametres are presented in Table 25.

Table 25: Parametres used for STPWIN modelling

Calculation programme:	STPWIN model (included in EpiSuite v 4.0)
Henry's law constant	0.430299 (43 600 Pa m3/mol)/(101325 Pa / atm)
Water solubility	14 g/m^3

Vapour pressure	6180 Pa
Log Kow	3.88
Melting point	-126.6 °C
Boiling point	100.9 °C
Reaction half-life estimates, minimum values	Bio P (primary clarifier): 7.31 d (175 h) Bio A (aeration vessel): 7.31 d (175 h) Bio S (settling tank): 7.31 d (175 h)
Reaction half-life estimates, theoretical maximum	Bio P (primary clarifier): 100000 d (240000 h) Bio A (aeration vessel):: 100000 d (2400000 h) Bio S (settling tank): 100000 d (2400000 h)

The model results (Table 26) indicate that 83-85% of MCH is emitted to air, 14% is adsorbed to sludge and 0.5% is released to water. Biodegradation (0.01-3%) is relatively low. It is noted that the minimum biodegradation half-life may be based on mixture studies so this half-life may represent conditions where other hydrocarbons are present. Moreover, this minimum half-life represents primary degradation only.

Table 26: Results of STPWIN modelling

Half-lives (hours)			Total removal	Total bio-degra-dation	Total sludge adsorption	Total to air
BIO P (primary clarifier)	BIO A (aeration vessel)	BIO S (settling tank)				
175.44	175	175	99.48	3.07	13.67	82.74
99999*	99999*	99999*	99.46	0.01	14.09	85.37

*theoretical value assuming that no degradation occurs

7.7.2.4. Summary and discussion of environmental distribution

Based on the estimated Koc values it can be concluded that MCH is moderately/significantly adsorptive. Therefore, sorption of MCH to soil and sediment organic matter can be expected. Based on the Henry's law constants it can be concluded that MCH is very easily volatilized from water. According to Level I Mackay model, MCH will be distributed mostly to air compartment. However, Mackay level III model which takes into account degradation processes, indicates that 68-91% will be distributed in the water compartment, 6-8% to air, 0.6-26% to sediment and less than 0.01% to soil, when emissions are assumed to water compartment only. The ranges represent values obtained assuming minimum or maximum biodegradation half-lives (7.31 d and 100000 d) in water, soil, and sediment. The results indicate that in a closed system MCH tends to occur in the gas phase but in a dynamic system where inflow of MCH to the water compartment occurs, MCH is present also in the water and may end up in sediment. In waste water treatment plant, a majority of MCH will be removed from water (83-85% emitted to air, 14% distributed to sludge and 0.1-3% biodegraded) whereas 0.5% will remain in the effluent, as estimated using the STPWIN model.

7.7.3. Bioaccumulation

7.7.3.1. Aquatic bioaccumulation

METI 1986 study on aquatic bioaccumulation

An aqueous (fresh water) flow-through bioaccumulation study with *Cyprinus carpio* was conducted with two (nominal) concentrations of 10 and 100 µg/L (METI 1986). Only uptake of the substance was measured. Depuration was not measured. The original study report is in Japanese. Therefore, the evaluation of the test is mainly based on the robust study summary provided in the registration dossier. In addition, further details translated from the original report were provided by the Registrant(s) during the evaluation.

The continuous flow-through test was conducted in 100 l glass tanks designed for volatile organic compounds with a test solution renewal rate of 1 155 l/day. The number of organisms per vessel was 13. For each concentration one tank was used (1 replicate). The concentration of oxygen varied between: 4.8 - 6.6 mg/l (100 µg/l) and 4.9 - 6.5 mg/l (10 µg/l). Assuming oxygen saturation point of 8 mg/l at 25 °C, this would correspond to > 60 % saturation.

The lipid content of the fish was 4.1 %. The fish were fed twice a week (amount of feed 2 % of body weight)

Sampling (of fish) was performed at study initiation and after 2, 4, 6 and 8 weeks exposure time. Two fish were sampled at each time point. After measurement of weight and length, fish samples were incubated with deionized water, sodium hydroxide and methanol for 20 - 25 hours on a hot bath shaker (50°C). The length and weight of the fish at study initiation were 23.6 g and 9.6 cm, respectively. At the end of the study the fish weight was 33.1 g (conc. 100 µg/L) and 31.65 g (conc. 10 µg/L). The fish weight increase was approximately 35 - 40 % during the tests. However, between sampling points week 2 and week 8 the growth was only approximately 5 - 10 %. Fish lengths were not reported.

MCH was not detected in the control fish except for one sample were 37.6 ng/g was measured (Table 29). Compared to the concentrations measured in control fish (> 1000 ng/g) this is considered acceptable. The control fish were somewhat bigger (around 30 g) than the test fish and grew 2.7 % during the test.

The test medium was sampled twice a week. Test medium samples were mixed with methanol and incubated in a closed bottle on a hot bath shaker for 10 min (40 °C). Measured concentration of the test substance maintained within ± 20 % of the mean of the measured values during the uptake phase (

Table 27).

The test substance was identified and quantified by means of GC-FID. The detection limit for the test media was 3.0 µg/l (nominal 100 µg/l) or 0.3 µg/l (nominal 10 µg/L). For the fish samples the detection limit was 200 µg/l (nominal 100 µg/L) or 21 µg/l (nominal 10 µg/L). Reproducibility is given as 97.3 % for test media (10 µg/L), 102 % for fish sample, in which 30 µg of test item was added.

Based on the reported BCF-values, it seems that steady state is reached during the test (Table 30).

The test is considered reliable with restrictions (2) as most of the validity criteria according to OECD 305 test guideline are met:

- the water temperature variation was within ± 2 °C and thus acceptable
- the concentration of oxygen did not fall below 60 % saturation
- the concentration of the test substance maintained within ± 20 % of the mean of the measured values during the uptake phase

- the concentration of the test substance was below its limit of solubility in water

However, there is no information on mortality or other adverse effects in control/treated fish. Presumably no such effect occurred, as BCF values are reported for all sampled fish and based on the reported fish weights the fish have grown in both control and treated groups.

Table 27. Measured concentrations of the test substance during the bioaccumulation test

Nominal concentration µg/L	Week 2 µg/L	Week 4 µg/L	Week 6 µg/L	Week 8 µg/L	Mean µg/L
100	77.0	77.9	78.6	79.4	78.2
10	7.52	7.51	7.61	7.70	7.59

Table 28. Concentration of MCH in fish (ng/g)

(Translation as provided by the Registrant by email 6.12.2013. "Flask" probably refers to "fish" as there was only one tank (replicate) used per concentration and two fish were sampled each sampling week.)

			Absolute content of MCH in fish (ng)	Fish weight (g)	Concentration of MCH in fish (ng/g)*
1st concentration	2-wk	Flask No. 1	269000	28.9	9300
		Flask No. 2	272000	31.3	8680
	4-wk	Flask No. 1	198000	26.7	7400
		Flask No. 2	769000	35.8	21500
	6-wk	Flask No. 1	558000	36.3	15400
		Flask No. 2	730000	34.7	21000
	8-wk	Flask No. 1	538000	31.7	17000
		Flask No. 2	879000	34.5	25500
2nd concentration	2-wk	Flask No. 1	30100	28.9	1007
		Flask No. 2	49300	31.3	1195
	4-wk	Flask No. 1	41100	26.7	1158
		Flask No. 2	55100	35.8	1505
	6-wk	Flask No. 1	53800	37.5	1435
		Flask No. 2	42900	35.1	1222
	8-wk	Flask No. 1	48100	34.6	1390
		Flask No. 2	52300	28.7	1822

Table 29. Concentration of MCH in control fish

flask		Content of MCH (ng)	Fish weight (g)	MCH concentration on fish ng/g
0 hr	1	-	30.6	-
	2	-	32.5	-
8 week	1	1140	30.3	37.6
	2	-	34.5	-

Table 30. Measured BCF values (values normalized to 5 % lipids in brackets.)

Nominal concentration µg/L	Week 2	Week 4	Week 6	Week 8
100	121 (148) 113 (138)	95 (116) 276 (337)	196 (239) 268 (327)	214 (261) 321 (392)
10	134 (163) 225 (275)	154 (188) 200 (244)	188 (229) 161 (196)	181 (221) 237 (289)

Other studies

In a study by Gossett et al. (1983) sediments and animals collected from near the discharge zone of the Los Angeles County wastewater treatment plant were analyzed for 27 selected organic compounds (including methylcyclohexane) that had been identified in the effluent. In the study it was found that the sediment and tissue concentrations of methylcyclohexane were positively correlated with each other and with the n-octanol/water partition coefficients, whereas the sediment/tissue concentrations were negatively correlated with the effluent concentrations. Concentration of methylcyclohexane in the effluent was 20 µg/l. Methylcyclohexane was not detected in the tissue samples (detection limit 0.3 µg/kg w.w.), neither in the sediment samples (detection limit 0.5 µg/kg dry weight).

In a study by Benville et al. 1985 acute toxicity of seven alicyclic hexanes, including methylcyclohexane, to Striped Bass and Bay Shrimp was investigated. At the end of the 96-hour experiment, concentrations of these alicyclic hexanes were measured in the tissues of the Bass and the Shrimps. Based on the concentrations in water and in tissue, the following tentative BCF values are derived by the evaluator. In shrimp the maximum BCF is 48 l/kg and minimum BCF = 2.2 l/kg. In bass the maximum BCF is 240 l/kg and minimum BCF is 17.8 l/kg. It has to be emphasized that the purpose of this study was not to derive BCF values and therefore these values can be used only tentatively.

Estimated data

Table 31. Estimated BCF values

BCF (l/kg wet-wt)	BAF (l/kg wet-wt)	Method
109	-	BCFBAF v.3.00 Regression-based estimate using logKow=3.59 (logBCF = 0.6598 * logKow - 0.333)
169	-	BCFBAF v.3.00 Regression-based estimate using logKow=3.88 (logBCF = 0.6598 * logKow - 0.333)
212	216	BCFBAF v.3.00 Arnot-Gobas (lower trophic), using logKow=3.59, assuming biotransformation rate 0.1861/days and half-life 0.571 days)
379	395	BCFBAF v.3.00 Arnot-Gobas (lower trophic), using logKow=3.88, assuming biotransformation rate 0.1861/days and half-life 0.571 days
411	620	BCFBAF v.3.00 Arnot-Gobas (upper trophic), using logKow=3.59, assuming zero biotransformation
791	1563	BCFBAF v.3.00 Arnot-Gobas (upper trophic), using logKow=3.88, assuming zero biotransformation
207		CAESAR version 2.1.13 ("the compound can be safely classified as not bioaccumulative (BCF < 2000)")
112		Meylan version 1.0.2

7.7.3.2. Terrestrial bioaccumulation

There is little information available to assess the potential for terrestrial bioaccumulation. Nevertheless, based on a Kaw value of 2.365 (KOAWIN v.1.10 estimate), which is below 6 and a log Kow values below 4.00, there is no indication of potential to bioaccumulate in the terrestrial environment.

7.7.3.3. Summary and discussion of bioaccumulation

Based on a reliable (with restrictions) aqueous bioaccumulation study (METI 1986) showing BCF values below 400 it can be concluded that methylcyclohexane is not bioaccumulative. The conclusion is further supported by QSAR estimates for BCF and bioaccumulation factors (BAF). The estimated BCF values are generally below 500 and always below 800. The highest BAF value (assuming zero biotransformation) is 1563 and thus below the B criterion 2000.

The conclusion for low bioaccumulation, is further supported by the study by Benville et al. (1985) from which BCF values of 2.2 - 48 l/kg for Bay Shrimp and 17.8 - 240 l/kg for Striped Bass can be derived. It must be emphasized, however, that the purpose of this study was not to determine BCF values, therefore the values can be used only tentatively.

In addition the study by Gossett et al. (1983), in which methylcyclohexane was detected in wastewater treatment plant effluent, but not in sediment and organisms of the receiving environment, seems to support the general conclusion that MCH does not have significant bioaccumulation potential.

7.8. Environmental hazard assessment

7.8.1. Aquatic compartment (including sediment)

7.8.1.1. Fish

7.8.1.1.1. Short-term toxicity to fish

There are three acute fish tests available in the registration dossier (ECHA). All results are summarised in the following table.

Table 32 Summary of short-term toxicity tests on fish

Method	Results and remarks by Registrant/s	Results and/or remarks by eMSCA	Reference
Oryzias latipes freshwater semi-static Japanese GLP Standard : Circular on Test Methods of New Chemical Substances (Japan), Fish, acute toxicity test	LC50 (96 h): 2.07 mg/L test mat. (meas. (TWA)) based on: mortality (1.64 - 2.57mg/L) 1 (reliable without restriction) key study experimental result Test material (EC name): methylcyclohexane CAS#: 108-87-2	Results are based on time weighted mean concentrations (calculated with the method as described in OECD 211, Annex 6) **key study** **2 (reliable with restrictions)**	MOE (2008a)
Morone saxatilis saltwater static Standard Methods for the examination of Water and Wastewater	LC50 (96 h): 5.8 mg/L test mat. (meas. (arithm. mean)) based on: mortality 2 (reliable with restrictions) supporting study experimental result equivalent or similar to OECD Guideline 203 (Fish, Acute Toxicity Test) Test material (EC name): methylcyclohexane CAS#: 108-87-2	The correct LC50 (96 h) is 4.46 mg/L (= 5.8 mikrol/l). Benville et al. determined the 96 h LC50 value of 5.8 mikrol/l which was incorrectly reported in mg/l in the registration dossier. Test procedures were from Standard Methods for the examination of Water and Wastewater as described in Connors, Jenkins and Greenberg, 1981. No data on GLP	Benville et al. (1985a)

Method	Results and remarks by Registrant/s	Results and/or remarks by eMSCA	Reference
		Poor documentation (test method was not sufficiently described) 4 (not assignable)	
Oryzias latipes freshwater semi-static Japanese GLP Standard : Circular on Test Methods of New Chemical Substances (Japan), bioconcentration test no GLP	LC50 (48 h): 5.02 mg/L test mat. based on: mortality 2 (reliable with restrictions) supporting study experimental result Test material (EC name): methylcyclohexane CAS#: 108-87-2	Deviations from the guideline: test concentrations not stated and no analytical measurement was performed. Results are based on nominal concentrations and taking into account the high volatility potential of methylcyclohexane measured concentrations should have been used. Due to poor documentation and t lack of measured concentrations the test reliability is considered to be not assignable. 4 (not assignable)	METI (1986b)

Acute tox study in *Oryzias latipes* of methylcyclohexane (MOE, 2008a), key study

Acute toxicity of methylcyclohexane to *Oryzias latipes* was studied in a 96 h test under semi-static conditions. The original study report is in Japanese. Therefore, the evaluation of the test is mainly based on the robust study summary provided in the registration dossier.

The test was conducted according to Japanese GLP standard and guideline: Circular on Test Methods of New Chemical Substances (Japan), fish, acute toxicity test. Test was performed with 5 l glass tanks which were closed with teflon sheet. Only one test tank per concentration was used in the test (1 replicate). Ten ≤ 6 months old Medeka fish (*Oryzias latipes*) were placed in each tank after 11 days acclimation in flow-through conditions. During acclimation period < 5% mortality was observed for 7 days before exposure. Mean length and weight of the fish were 2.08 cm (range 1.80 - 2.47 cm) and 0.078 g (range 0.045 - 0.141 g), respectively. There was no aeration and no food was given during the test. Photoperiod was 16 hours in the light, 8 hours in the dark and light intensity was < 1000 lux.

Test item methylcyclohexane (purity 99.8%, Lot/batch No.: GI01) was mixed with acetone and this solution was diluted in the test water, stirred for 5 min and sonicated for 10 - 15 min, until oil drop disappeared. Both blank control and vehicle control (acetone) were used in the test. Range finding test was performed twice and based on the results nominal test concentration were chosen to be: 2.0; 3.6; 6.3; 11; 20 mg/L. The measured time weighted mean concentrations were 0.638; 1.18; 2.04; 3.26; 7.26 mg/L. Same concentrations values can be achieved also by arithmetic mean. Mean measured concentrations were 30-36 % of the nominal concentrations. Test solutions were renewed once in 24 hours and measurements were taken from the freshly prepared test water and immediately prior to renewal or at the end of the exposure. During every 24 hour exposure period test concentrations remained 82-101% of the new freshly prepared test solutions. Test water temperature varied 24.2 - 24.5 °C (24 ± 1 °C) and

pH ranged 7.3 - 7.8 during the test (no pH adjustment). The hardness was 52 mg CaCO3/L and dissolved oxygen varied 6.5 - 8.3 mg/L. Assuming oxygen saturation of 8.40 mg/l at 24 °C, this would correspond to > 60 % saturation throughout the test.

General conditions were recorded every day and mortality after 96 h test duration. Behavioural abnormalities were found in the following treatments: 1.18, 2.04 and 3.26 mg/L (nominal 3.6, 6.3 and 11 mg/l, respectively). Mortality of control was 0% in blank control and 10% in vehicle control (one of the ten fish died). No other adverse effects were observed. 96 h-LC50 value of 2.07 mg/L on the basis of time weighted mean concentrations was determined for *Oryzias latipes* under semi-static test conditions.

The study is used as a key study in this assessment for evaluating the acute toxicity of methylcyclohexane to fish. The test was evaluated to be reliable with restrictions (Klimisch score 2). When testing volatile substances test concentrations should, where possible, be prepared individually by addition of test substance directly to the test vessels rather than by dilution of a stock solution (OECD, 2000). This substance preparation approach might have caused the low initial measured test concentrations.

Table 33 Observed symptoms in the acute fish test with *Oryzias latipes*

Nominal concentration [mg/L]	**Mean measured concentration (*1) [mg/L]**	**Symptoms (number of fish showing corresponding symptoms)**			
		24 h	**48 h**	**72 h**	**96 h**
Control	-	Normal (10)	Normal (10)	Normal (10)	Normal (10)
Solvent Control	-	Normal (10)	Normal (10)	Normal (10)	Normal (9) Death (1)
2.0	0.638	Normal (10)	Normal (10)	Normal (10)	Normal (10)
3.6	1.18	Normal (9) AS (1)	Normal (8) AS (2)	Normal (9) IS (1)	Normal (6) AS (3) Death (1)
6.3	2.04	Normal(4) AS (6)	Normal(3) AS (7)	Normal(2) AS (6) Death (2)	AS (4) IS (3) Death (3)
11	3.26	AS (7) IS (1) Death (2)	AS (1) IS (1) Death (8)	Death (10)	Death (10)
20	7.26	Death (10)	Death (10)	Death (10)	Death (10)

(*1): The time weighted mean values equal arithmetic mean values
AS: Abnormal swimming
IS: Impossible to swim

Acute toxicity of seven alicyclic hexanes to striped bass, Morone saxatilis, and bay shrimp, Crangon franciscorum, in seawater (Benville et al. 1985)

The acute toxicity of methylcyclohexane (analytical purity 99%) to striped bass, *Morone saxatilis*, was studied for 96 h under static conditions. According to Benville et all., the test procedures followed were from Standard Methods for the examination of Water and Wastewater as described in Connors, Jenkins and Greenberg, 1981. There were no data on whether the study was conducted in compliance with GLP.

Juvenile striped bass (mean weight = 8.5 g, mean total length = 9.2 cm) were obtained from freshwater at the US Bureau of Reclamation fish screening facility at Tracy, California. They were acclimated in 200 l tanks under flow-through conditions for 2 weeks

in saltwater before toxicity testing. Mortality from transportation and salinity change during the first 24 h was 20% and for acclimation period less than 1%.

The actual toxicity test was conducted with five oval fiberglass aquaria (dimensions of 110 cm length x 50 cm width x 40 cm height) and each of them were filled with 180 l of filtered seawater. Five nominal concentrations ranging in geometric progressions from 1 to 16 ml were achieved by mixing the test material (alicyclic aliquot was used) with sea water and adding this solution slowly to the test tanks. There was no aeration in the test aquaria during the test. Ten fish per aquaria were used and no information on replicates was provided. Two 10 ml seawater samples were pipetted at mid-depth from each aquarium before and after introducing the animals. Each succeeding day a 100 ml water sample was taken, extracted and analyzed. Test water temperature varied 15 - 20 °C during the study. Dissolved oxygen and pH was not stated. According to the test report measured test concentrations were 0.54 - 7.3 mg/l but no details were given.

Mortalities were recorded daily and according to Benville et al. the 96 h LC50 value was determined to be 5.8 mikrol/l based on the measured concentration (arithm.mean). However, this value seems to be incorrectly reported as 5.8 mg/l in the registration dossier.The correct LC50 value for methylcyclohexane is 4.46 mg/l (= 5.8 mikrol/l, according to the evaluating MSCA).

It is unclear whether the high volatility potential of methylcyclohexane was taken into account in the test system or not. The authors acknowledge themselves in the article that using a continuous flow method of dosing instead of static test would have probably showed higher toxicities (lower LC50 value). In addition, the test water temperature varied more than ± 2 °C and there were no data on dissolved oxygen and pH. As the used test method was not sufficiently described in the test report in order to assess the reliability of the study, it is considered to be not assignable with Klimisch score 4. The study is used as a weight of evidence for this assessment.

Acute tox test of methylcyclohexane as a pretest for a bioaccumulation test, METI 1986

An acute toxicity test with Medeka fish (*Oryzias latipes*) was conducted in freshwater under semi-static condition for 48 hours as a pre-test for a bioaccumulation test. The original study report is in Japanese and therefore, the evaluation of the test is based on the robust study summary provided in the registration dossier.

The test was performed according to Japanese guideline: Circular on Test Methods of New Chemical Substances (Japan), bioconcentration test, and according to robust study summary it was not in compliance with GLP. Test solution was prepared by mixing methylcyclohexane (analytical purity: > 99%, Lot/batch No.: FBT01) with HCO-20 and deionized water. Vehicle control (HCO-20) and blank control were also used in the test. Test organism Medaka fish (mean length = 3.3 cm, mean weight = 0.28 g at study initiation) were obtained from Nakajima Fish Farm, Tamana, Japan. Acclimation period was 53 days before the exposure and the conditions were same as in the test. Only healthy stock was used for exposure.

Test vessel material was glass and the fill volume was 3.85 l. Type of the vessel was not mentioned. Ten fish were used per concentrations but the test concentrations (measured/nominal) were not stated nor were there any information on the replicates. Renewal rate of the test solution (frequency/flow rate) was once in 8 - 16 hours.

Test water temperature was 25 ± 2 °C. Dissolved oxygen was at the test initiation: 7.9 mg/L and at the end: 6.1 - 6.5 mg/L. Assuming oxygen saturation of 8.24 mg/l at 25 °C, this would correspond to > 60 % saturation throughout the test. pH varied at the initiation: between 7.9 - 8.0 and at the end: 7.6 - 7.9.

Mortality was recorded after 48h test duration. A 48 h LC50 value of 5.02 mg/L (nominal) was determined for *Oryzias latipes* under semi-static test conditions.

Deviations from the guideline were noted: test concentrations were not stated and no analytical measurement was performed. Results are based on nominal concentrations and taking into account the high volatility potential of methylcyclohexane measured concentrations should have been used. The test method should have been better described. Due to poor documentation and the lack of analytical measurements of the test concentrations the test is considered to be not assignable with Klimisch score 4.

7.8.1.1.2. Long-term toxicity to fish

There is one long-term fish study available in the registration dossier. The results are summarised in the following table.

Table 34 Summary of long-term toxicity tests on fish

Method	Results and remarks by Registrant/s	Results and/or remarks by eMSCA	Reference
(I) *Jordanella floridae;* (II) *Salmo gairdneri (new name: Oncorhynchus mykiss)* freshwater Continous-flow (I) Flagfish bioassay: embryo and sacfry stages (sublethal effects); (II) Rainbow trout assay: fry-stages	NOEC (7 d): >= 0.83 mg/L dissolved (test substance) (meas. (arithm. mean)) based on: number hatched (flagfish) NOEC (87 d): >= 0.83 mg/L dissolved (test substance) (meas. (arithm. mean)) based on: fry development (flagfish) 3 (not reliable) disregarded study experimental result Test material (EC name): methylcyclohexane CAS#: 108-87-2	(I) Failure in dosing and dilution apparatus caused significant volatilization of the test substance. (II) Failure in the refrigeration equipment let the exposure tanks' temperature to rise to 20 °C which caused significant mortality. Test had to be terminated after 23 days. Due to relevant methodological deficiencies in both tests the study is considered as not reliable. 3 (not reliable)	SERL (1976)

JP-4 and JP-9 fuel toxicity studies using freshwater fish and aufwuchs (SERL1976)

The evaluation of the study is based only on the robust study summary provided in the registration dossier. There were two separate tests included in the study: (I) Continuous-flow embryo and sac-fry stages biossay with flagfish, and (II) Continuous-flow juvenile stage bioassay with rainbow trout. According to the robust study summary no guideline or GLP standards were followed when conducting the tests. In both tests the test material was methylcyclohexane.

(I) Continuous-flow embryo and sac-fry stages biossay with flagfish

In the continuous-flow embryo and sac-fry stages bioassay test animals were flagfish (*Jordanella floridae*) which were obtain from a commercial aquarium. Five females were placed in a spawning aquarium. Eggs were fertilized by one male. The exposure tanks were 80 l stainless steel tanks (121.92 cm x 30.48 cm x 30.48 cm) filled with removable

size 40-010 mesh stainless steel screens. Eggs (92 per vessel) were exposed in 4 glass jars with their bottoms replaced with a size 40 -100 mesh stainless steel screen within the exposure tanks. Only one replicate was used per concentration and control. Sac-fry stages were exposed in fry chambers (30.5 x 15.2 x 30.5 cm) with size 40-010 mesh stainless steel screens at each end to allow free circulation of water, which were located about 30.48 cm from the inlet end of the exposure tank. Exposure period were for the eggs 7 days and 87 d for the fry stages. Measured concentrations were: 0.34 (± 0.38); 0.67 (± 0.65) and 0.83 (± 0.73) mg/L as dissolved test substance. Hardness was 24 - 30 mg/L as CaCO3, test water temperature was 25 ± 0.5 °C, pH varied 7.4 - 7.5 and dissolved oxygen was > 7 mg/L during the study.

Studied endpoints in the flagfish bioassay were number of hatched embryos after 7 d exposure time and fry development after 87 d exposure time. NOEC value of >= 0.83 mg/l was determined for both embryo stage and sac-fry stage bioassays. The results are based on the measured concentration (arithm. mean).

(II) Continuous-flow juvenil stages bioassay with rainbow trout

Test animal in the continuous flow juvenile stages bioassay was *Salmo gairdneri* (new name: *Oncorhynchus mykiss*), Rainbow trout. Test animals were obtained from the American River Fish Hatchery of the California State Department of Fish and Game. Before use in a bioassay, the fish were acclimated for one month to dechlorinated Richmond Field Station tap water. Test tanks were the same as in the embryo and sac-fry stages biossay with flagfish.

Fertilized eggs were transferred from spawning aquaria to egg cups in the continuous-flow exposure tank. After hatching, young fry were transferred to fry chambers within the exposure tanks. After about 1 month, the fry had grown sufficiently to permit their release into the effluent-end chamber of the exposure tanks. Total exposure duration was 23 d for juvenile rainbow trout. Thirty fish per test vessel were used and only one replicate per control and concentrations. Measured concentrations were: 0.31; 0.80; 0.84 and 1.19 mg/L as dissolved test substance. Hardness was 24 - 30 mg/L as CaCO3, test water temperature was 15 °C, pH varied 7.4 - 7.5 and dissolved oxygen was > 7 mg/L during the test.

Studied endpoint in the rainbow trout bioassay was mortality after 23 d duration. LC50 value of 1.3 mg/l (23 d) was determined. The experiment had to be terminated after 23 days, allowing no sufficient assessment of "no effect concentration".

According to the robust study summary relevant methodological deficiencies were found:

I) Flagfish flow through test: There was significant temporal variation in the exposure tank test substance concentration, questioning the reliability of the general test performance and the calculated NOEC values as those are related to the mean measured concentrations (Table 35). It was stated in the robust study summary that the variation was attributable largely to inconsistencies in the dosing and dilution apparatus. The test substance analytical technique has a coefficient of variation of only 8% and does not therefore explain the variation in measured concentrations. In addition, the variation was speculated to be mainly affected by the high volatility of the test substance. Significant volatilization of test substance took place between the final head tank of the contacting device and the effluent from the diluter.

II) Rainbow trout flow through test: Due to a failure in the refrigeration equipment which allowed the temperature of the exposure tanks to rise to 20 °C which caused significant mortality, the experiment had to be terminated after 23 days, allowing no sufficient assessment of "no effect concentration".

Table 35 Test substance concentration in dilution apparatus and exposure tanks during flagfish bioassay

Sample location	No. of analyses	Mean concentration of test substance [mg/L]	Standard deviation [mg/L]	Coefficient of variation [%]
Head tank	6	3.41	2.22	65
Diluter effluent	7	1.85	0.82	44
100% exposure tank	8	0.83	0.73	88
50% exposure tank	6	0.67	0.65	97
12% exposure tank	6	0.34	0.38	112
control	6	0.01	0.01	100

Due to all deficiencies in the test systems the study is evaluated with Klimisch score 3, not reliable and it is not used for this assessment.

7.8.1.2. Aquatic invertebrates

7.8.1.2.1. Short-term toxicity to aquatic invertebrates

There are two short-term toxicity studies to aquatic invertebrates available. The results are summarised in the following table.

Table 36 Summary of short-term toxicity tests on aquatic invertebrates

Method	**Results and remarks by Registrant/s**	**Results and/or remarks by eMSCA**	**Reference**
Daphnia magna freshwater semi-static Japanese GLP standard and guideline: Circular on Test Methods of New Chemical Substances (Japan), Daphnia, acute immobilisation test	EC50 (48 h): 0.326 mg/L test mat. (meas. (TWA)) based on: mobility (0.276 - 0.389 mg/L) 1 (reliable without restriction) key study experimental result Test material (EC name): methylcyclohexane CAS#: 108-87-2	Results are based on time weighted mean concentrations (calculated with the method as described in OECD 211, Annex 6) **key study** **2 (reliable with restrictions)**	MOE (2008b)
Crangon franciscorum saltwater static	LC50 (96 h): 3.3 test mat. (meas. (arithm. mean)) based on: mortality (2.9 - 3.9 mg/L) 2 (reliable with restrictions)	The correct LC50 (96 h) value is 2.54 mg/l (=3.3 mikrol/l). Benville et al. determined the 96 h LC50 value of 3.3 mikrol/l which was incorrectly reported as mg/l in the registration dossier.	Benville et al. (1985b)

Method	Results and remarks by Registrant/s	Results and/or remarks by eMSCA	Reference
equivalent or similar to EPA OPP 72-3 (Estuarine/Marine Fish, Mollusk, or Shrimp Acute Toxicity Test)	supporting study experimental result Test material (EC name): methylcyclohexane CAS#: 108-87-2	Test procedures were from Standard Methods for the examination of Water and Wastewater as described in Connors, Jenkins and Greenberg, 1981. No data on GLP Poor documentation (test method was not sufficiently described) 4 (not assignable)	

Daphnia magna immobilisation test of methylcyclohexane (2008, MOE)

Acute toxicity of methylcyclohexane to *Daphnia magna* was studied in a 48 h test under semi-static conditions. The original study report is in Japanese. Therefore, the evaluation of the test is mainly based on the robust study summary provided in the registration dossier. The test was conducted according to Japanese GLP standard and guideline: Circular on Test Methods of New Chemical Substances (Japan), Daphnia, acute immobilization test.

The test was performed with 100 ml glass beakers which were closed with a teflon sheet. Test animals (water flea, *Daphnia magna*) were obtained from Incorporated Administrative Agency, National Institute for Environmental Studies, Tsukuba, Japan. Five animals aged ≤ 24 hours at study initiation were used per test vessel and four replicates per concentrations (including blank and vehicle control). Acclimation period for parental animals was 2 - 4 weeks (10 Jun - 01 Jul 2008) and the acclimation conditions were same as in the test. Type and amount of food during acclimation period was: *Chrolella vulgaris*, 6 mgC/2L/day. During the test there was no feeding and no aeration. Observed mortality during the acclimation period was < 20% during 2 weeks before exposure.

Test solution was prepared by mixing the test substance methylcyclohexane (batch No. GI01, purity 99.8%) with acetone and sonicated for 1 min. This stock solution (I) was diluted in acetone and stirred gently. Stock solution (II) was diluted then in test water. There were four replicates per concentration, control and vehicle control (acetone). Nominal concentration were: 0.05; 0.1; 0.2; 0.4 and 0.8 mg/L. Test solutions were renewed once in 24 hours and measurements were taken directly in each vessel from the freshly prepared test water and immediately prior to renewal or at the end of the exposure. Mean measured concentrations (time-weighted mean) were: 0.037; 0.08; 0.153; 0.298 and 0.603 mg/l (74-80 % of the nominal concentrations). During every 24 hour exposure period the test concentrations remained 78-84% of the new freshly prepared test solutions.

Test water temperature varied 19.9 - 20.2 °C (20 ± 1 °C), pH was 8.1 - 8.4 (no pH adjustment) and dissolved oxygen ranged 8.6 - 8.8 mg/L. Assuming oxygen saturation of 9.07 mg/l at 20 ˚C, this would correspond to > 60 % saturation throughout the test. Hardness was not stated. Photoperiod was 16 hours in the light, 8 hours in the dark and used light intensity was < 800 lux. Mortality of blank control and vehicle control was 0% and no other adverse effects were observed. Immobility was observed on each day during the test.

A 48 h EC50 value of 0.326 mg/L on the basis of time weighted mean concentrations was determined for *Daphnia magna* under semi-static test conditions.

The study is used as a key study in this assessment for evaluating the acute toxicity of methylcyclohexane to aquatic invertebrates. The test was evaluated to be valid with restrictions (Klimisch score 2). When testing volatile substances test concentrations should, where possible, be prepared individually by addition of test substance directly to the test vessels rather than by dilution of a stock solution (OECD, 2000).

Table 37 The number of immobilized Daphnia magna (percent immobility)

Nominal Concentration [mg/L]	Measured *1 Concentration [mg/L]	Cumulative Number of Immobilized Daphnia (Percent Immobility)	
		24 h	48 h
Control	Control	0 (0)	0 (0)
Solvent Control	Solvent Control	0 (0)	0 (0)
0.05	0.037	0 (0)	0 (0)
0.1	0.08	0 (0)	1 (5)
0.2	0.153	0 (0)	2 (10)
0.4	0.298	0 (0)	5 (25)
0.8	0.603	4 (20)	20 (100)

*1: Time weighted mean values equal arithmetic mean values

Acute toxicity of seven alicyclic hexanes to striped bass, Morone saxatilis, and bay shrimp, Crangon franciscorum, in seawater. (Benville et al. 1985)

The acute toxicity of methylcyclohexane (analytical purity 99%) on bay shrimp, *Crangon franciscorum* was studied for 96 h under static conditions. According to Benville et al., the test procedures followed were from Standard Methods for the examination of Water and Wastewater as described in Connors, Jenkins and Greenberg, 1981. There were no data on whether the study was conducted in compliance with GLP.

Bay shrimp (mean weight = 1.7 g, mean total length = 6.4 cm) were obtained from a local bait dealer in San Rafael, California. Test animals were acclimated in 200 l tanks under flow-through conditions for 2 weeks in saltwater before toxicity testing. Mortality from transportation and salinity change during the first 24 h and for acclimation period was less than 1%.

The actual toxicity test was conducted with five oval fiberglass aquaria (dimensions of 110 cm length x 50 cm width x 40 cm height) and each of them were filled with 180 l of filtered seawater. Five nominal concentrations ranging in geometric progressions from 1 to 16 ml were achieved by mixing the test material (alicyclic aliquot was used) with sea water and adding this solution slowly to the test tanks. No information on replicates was provided. Ten animals per aquaria were used. No aeration was used during the testing period. Two 10 ml seawater samples were pipetted at mid-depth from each aquarium before and after introducing the animals. Each succeeding day a 100 ml water sample was taken, extracted and analyzed. Test water temperature varied 15 - 20 °C during the study. Dissolved oxygen and pH was not stated. According to the test report measured test concentrations were 0.54 - 7.3 mg/l but no details were given.

Mortalities were recorded daily and according to Benville et al. the 96 h LC50 value was determined to be 3.3 mikrol/l based on the measured concentration (arithm.mean). However, this value seems to be incorrectly reported as 3.3 mg/l in the registration dossier, and the correct LC50 value for methylcyclohexane is 2.54 mg/l (= 3.3 mikrol/l, according to the evaluating MSCA).

It is unclear whether the high volatility potential of methylcyclohexane was taken into account in the test system or not. The authors acknowledge themselves in the article that using a continuous flow method of dosing instead of static test would have probably showed higher toxicities (lower LC50 value). In addition, the test water temperature varied more than ± 2 °C and there were no data on dissolved oxygen and pH. As the used test method was not sufficiently described in the test report in order to be able to

assess the reliability of the study, this study is considered to be not assignable with Klimisch score 4. It is used as a weight of evidence for this assessment.

7.8.1.2.2. Long-term toxicity to aquatic invertebrates

No long-term studies available.

7.8.1.3. Algae and aquatic plants

There is one toxicity study to algae available and the results are summarised in the following table.

Table 38 Summary of toxicity test on algae

Method	Results and remarks by Registrant/s	Results and/or remarks by eMSCA	Reference
Pseudokirchneriella subcapitata (algae) freshwater static Circular on Test Methods of New Chemical Substances (Japan), Alga, growth inhibition test	EC50 (72 h): 0.134 mg/l (meas. geometric mean, based on growth rate) NOEC (72 h): 0.0221 mg/l (meas. geometric mean, based on growth rate) 1 (reliable without restriction) key study experimental result Test material (EC name): methylcyclohexane CAS#: 108-87-2	Results are based on geometric mean concentrations. Test substance concentrations declined to ca. 1 % of the initial concentrations during first 24 hours. 2 (reliable with restrictions)	MOE (2008c)

Growth inhibition test in Pseudokirchneriella subcapitata of methylcyclohexane (MOE, 2008)

The effect of methylcyclohexane on the growth of green alga Pseudokirchneriella subcapitata was studied in a 72 h test under static conditions. The original study report is in Japanese. Therefore, the evaluation of the test is mainly based on the robust study summary provided in the registration dossier. The test was conducted according to Japanese GLP standard and guideline: Circular on Test Methods of New Chemical Substances (Japan), Alga, growth inhibition test.

Test was performed with 500 ml, glass Erlenmayer flasks (closed); the measured total available volume was 490 ml. Test solution volume was 100 mL and the headspace was 390 ml. Test organism green alga Pseudokirchneriella subcapitata (Strain: ATCC22662) was obtained from American Type Culture Collection. Age of inoculum at the test initiation was 3 days. Acclimation period before the exposure was 3 days and the condition were same as in the test. No abnormality (any deformed or abnormal cell) was observed during the acclimation period. In the actual test the initial cell density was 5000 cells/ml. In the control treatments end cells density was 739000 ± 56400 cells/ml in blank control and 832000 ± 57000 cells/ml in solvent control. Three vessels were used per concentrations except for control and vehicle control where 6 replicates were used

per each. There was no pH adjustment in the test. Photoperiod was continuous and the light intensity and quality were 62 - 65 µE/m2/s.

Test item methylcyclohexane (purity: 99.8%, batch No.: GI01) was mixed with acetone by inverting and swirling at first. This stock solution (I) was diluted in acetone by inverting again. Stock solution (II) was diluted in test water, sonicated and stirred. Based on range finding study which was performed twice nominal concentration were chosen to be: 0.32; 0.6; 1.2; 2.2; 4.2 and 8.0 mg/L. Mean measured concentration were: 0.0054; 0.0087; 0.0221; 0.0465; 0.0861; 0.1380 mg/L (geometric mean). At the test initial measured concentrations were 55-76 % of nominal concentrations and after 24 hours they declined to 0.4-0.8 % of nominal (appr. 1 % of 0 hour measured concentrations) (Table 39)

Table 39 Measured and nominal concentrations in the growth inhibition study on alga.

Test Group	**Nominal Concentrations (mg/l)**	**Measured Concentrations (mg/l) (Percent of Nominal) [Percent of Initial 0 Hours]**				**Mean Measured Concentrations (mg/l) (geom.mean)**
		0 Hours	**24 Hours**	**48 Hours**	**72 Hours**	
Conc. 1	0.32	0.184 (58 %)	0.00183 (0.5%) [1 %]	0.00173 (0.5%)[0.9 %]	0.00146 (0.5%)[0.8 %]	**0.0054*** (1.69%**)[2.9%***]
Conc. 2	0.60	0.434 (72 %)	0.00256 (0.4%)[0.6 %]	0.00256 (0.4%)[0.6 %]	0.00205 (0.3%)[0.5 %]	**0.0087***(1.45%**)[2 %***]
Conc. 3	1.2	0.912 (76 %)	0.00715 (0.6%)[0.8 %]	0.00677 (0.6%)[0.7 %]	0.00543 (0.5)[0.6%]	**0.0221*** (1.84%**)[2.4%***]
Conc. 4	2.2	1.6 (74 %)	0.0169 (0.8%) [1%]	0.0170 (0.8%)[1.1 %]	0.0120 (0.5%)[0.8 %]	**0.0465***(2.1%**)[2.9 %***]
Conc. 5	4.2	2.61 (62 %)	0.0295 (0.7%)[1.1 %]	0.0315 (0.8%)[1.2 %]	0.0227 (0.5%)[0.9 %]	**0.0861*** (2.05%**)[3.3%***]
Conc.6	8.0	4.39 (55 %)	0.0483 (0.6%)[1.1 %]	0.0472 (0.6%)[1.1 %]	0.0362 (0.5%)[0.8 %]	**0.1380*** (1.73%**)[3.1%***]

* Mean measured concentrations based on geometric mean ** Percent of nominal concentrations

***Percent of Initial measures concentrations at 0 Hour

Samples were taken directly in each vessel. Temperature was measured after 0, 24, 48 and 72 hours test duration. The pH-value was measured at the study initiation and at the end of the study. Hardness was not stated. Test temperature was 20.8 - 23.0 °C and pH

varied between 7.8 - 10.6. The pH-value varied in a higher range as recommended (within 1.5). It was concluded in the robust study summary that there was no impact in this test because it can be assumed that growth rate of alga was high under active carbonic acid assimilation.

Algal growth was recorded after 72 h test duration. The NOECr value was determined by STUDENT-t test for Homogeneous Variances with Bonferroni-Holm Adjustment, subsequent to Bartlett test for homogeneity of variances. An EC50 (72h) of 0.134 mg/l and a NOEC (72h) of 0.0221 mg/L on the basis of measured concentrations (geometric mean) is determined for Pseudokirchnerella subcapitata under static conditions.

The measured concentrations declined to appr. 1 % of the initial concentrations (0 hour) during the first 24 hours and stayed on that level until the end of the test. Considering the high volatility potential of methylcyclohexane (Henry's law constant 33 400 - 43 600 Pa m3 mol-1 at 25oC) the headspace was rather large in relation to the used test solution volume in the test vessels (headspace 390 ml vs. test solution volume 100 ml). It is impossible to confirm how the declining process took place in the test solution; whether methylcyclohexane has disappeared very quickly at the test initial or has the concentrations declined slowly towards the 24 h measuring point. Used concentrations which are only 2-3 % of the measured concentrations at 0 hour underestimate the exposure at the beginning of the test. However, as a dose/response curve is identifiable from the test results and as the results are based on the geometric mean measured concentrations, which is considered a reasonable way to account for the decline in the concentrations at the beginning of the test (OECD, 2000), the test is considered reliable with restrictions. The validity criteria of OECD test guideline 201 regarding the variation of growth rate in control cultures during the test and between replicates have not been discussed in the robust study summary of the updated dossier. However, based on the raw data submitted, the validity criteria are considered to be fulfilled.In conclusion, this study is considered reliable with restrictions Klimisch score 2.

Table 40 Growth inhibition [%] in the inhibition study on alga Pseudokirchneriella subcapitata

Nominal Concentration (Measured (a) conc.) [mg/L]	Growth rate (0-72 h) Inhibition (*1) [%]
Control	-
Solvent control	-
0.32 (0.0054)	- 0.4
0.60 (0.0087)	- 0.4
1.2 (0.0221)	- 1.0
2.2 (0.0465)	8.3**
4.2 (0.0861)	8.2**
8.0 (0.1380)	53.8**

(a): Geometric mean

*1: Values are the growth inhibition (%) relative to the solvent control.

**: Indicates a significant difference (α = 0.01) from the solvent control.

7.8.1.4. Sediment organisms

There are no sediment toxicity studies available.

7.8.1.5. Other aquatic organisms

There are no studies available evaluating the toxicity of methylcyclohexane (CAS No. 108 -87 -2) to aquatic microorganisms. Therefore, an analogue approach to the structurally

similar substance cyclohexane (CAS: 110-82-7) has been used in the registration dossier and results are presented in the following table.

Table 41 Summary of effects on micro-organisms

Method	Results and remarks by Registrant/s	Results and/or remarks by eMSCA	Reference
(I) Activated sludge, domestic; (II) methanogens enrichment culture; (II) Nitrosomonas freshwater static (I) Aerobic toxicity assay with aerobic heterotrophs (activated sludge); (II) Anaerobic toxicity assay for methanogens according to Owen et al. (1979); (III) Toxicity to Nitrosomonas (IV) Microtox® screening assay (Bioluminescence of Photobacterium phosphoreum)	IC50 (15 h): 29 mg/L test mat. (estimated) based on: growth inhibition (Aerobic heterotrophs; mean of 2 - 4 experiments) IC50 (96 h): 150 mg/L test mat. (estimated) based on: growth inhibition (Methanogens; mean of 2 - 4 experiments) IC50 (24 h): 97 mg/L test mat. (estimated) based on: growth inhibition (Nitrosomonas; mean of 2 - 4 experiments) IC50 (5 min): 200 mg/L test mat. (estimated) based on: bioluminescence (Photobacterium phosphoreum in Microtox screening assay; mean of 2 - 4 experiments) 2 (reliable with restrictions) key study read-across from supporting substance (structural analogue or surrogate) Test material (EC name): cyclohexane (See endpoint summary for justification of read-across)		Blum, D.J.W. and Speece, R.E. (1991) EUROPEAN COMMISSION - European Chemicals Bureau (2004)

For cyclohexane, a publication is available investigating the toxicity to different trophic and functional groups of sewage treatment plants, such as aerobic heterotrophs, methanogens and Nitrosomonas (Blum and Speece, 1991). The referred data has been previously used in the EU Risk assessment of cyclohexane (ECB, 2004). Considering that methylcyclohexane is expected to pose toxicity via non-specific narcotic type mechanism, this read across approach presented in the registration dossier seems to be plausible for evaluating the toxicity of methylcyclohexane on aquatic micro-organisms and it is also used in this assessment. In addition, toxicity control test of available ready biodegradability studies on methylcyclohexane are used for the assessment of microbial toxicity of methylcyclohexane.

Results on cyclohexane (Blum and Speece, 1991) indicate that aerobic heterotrophs, which predominate in activated sludge, are the most sensitive group showing a 15 h-IC50 of 29 mg/L. Anaerobic bacteria (96h-IC50 = 150 mg/L) and specific functional organism groups, such as Nitrosomonas (24 h-IC50 = 97 mg/L) and Photobacterium phosphoreum, used in a Microtox®assay, demonstrated to be less sensitive. After correction for differences in

molar mass (cyclohexane: 84.16 g/mol; methylcyclohexane: 98.19 g/mol), a 15 h-IC50 of 33.8 mg/L is calculated for methylcyclohexane. Thus, given these results, methylcyclohexane can be considered as harmful to aquatic microorganisms.

In a toxicity control tests of an available ready biodegradability study (Harlan 2012) methylcyclohexane was demonstrated to be inhibitory at the concentrations of 100 mg/l (reference substance aniline showed < 25 % degradation). No such effect could be found when the test was repeated at the concentrations of 10 mg/l (reference substance aniline attained 67 % degradation after 14 days). Similar results were obtained by Harlan 2013, where sodium benzoate was used as the reference substance. PNEC for STP has been derived with interpolation of results from cyclohexane micro-organism study by using assessment factor 100. It is also noted that the risk characterization rates are rather low (1.6x 10 -2 - 2.9 x 10 -9) for all scenarios presented in the chemical safety report.

In conclusion, applied weight of evidence approach is considered to be sufficient to fulfill the information need for the purpose of this substance evaluation and the eMSCA considers that there is no need to conduct toxicity studies on micro-organisms with methylcyclohexane.

7.8.2. Terrestrial compartment

No information available.

7.8.3. Microbiological activity in sewage treatment systems

Information on aquatic micro-organisms is discussed in Chapter 7.8.1.5.

7.8.4. PNEC derivation and other hazard conclusions

In the Registrant's CSA a PNEC aquatic was derived using the *Daphnia magna* EC50 value of 0.326 mg/l and an assessment factor of 100. A PNEC value of 3.26 µg/l is thus derived. The choice of the assessment factor has been justified as follows:

"Three short-term results covering three trophic levels (fish, Daphnia and algae) are available. According to chapter R.10 of the 'Guidance on information requirements and chemical safety assessment' (ECHA, 2008), an assessment factor of 1000 applies to short-term results. However, as it is assumed that methylcyclohexane acts by a non-specific mechanism and as the acute values for all three trophic levels are very similar, a lower assessment factor AF = 100 can be applied on the most sensitive experimental result. In addition, an invalid study on the chronic toxicity to fish and the valid NOEC for algae, support the lowering of the assessment factor to 100. Therefore, the PNEC aqua (freshwater) will be 326/100 = 3.26 µg/L, based on the acute EC50 for aquatic nvertebrates (Daphnia magna)."

It is noted that from the *Pseudokirchnerella subcapitata* growth inhibition test a lower EC50 is derived (0.134 mg/l). The PNEC should be based on this value. However, the difference is not considered crucial in terms of risk assessment.

7.8.5. Conclusions for classification and labelling

The current harmonized environmental classification for methylcyclohexane according to CLP is Aquatic chronic 2 - H411 and according to DSD N, R51-53.

Reliable acute ecotoxicity tests are available for fish, aquatic invertebrates and algae. The aquatic Acute Category 1, **H400**, classification is applicable for methylcyclohexane

based on the lowest ErC50 value of 0.134 mg/l obtained for algae.In the case of the H400 classification according to CLP, an **M-factor of 1** is applicable based on 0.1< L(E)C50 ≤1 mg/l.

There are no reliable long term tests available with the exception of a 72 hour algae test. Since there is no adequate chronic data available for all three trophic levels the environmental hazard classification for methylcyclohexane is assessed using two approaches according to CLP (2nd ATP).

1. In the case of non-rapidly degradable substances, for which there are adequate chronic toxicity data available, aquatic Chronic Category 1, **H410,** classification is applicable based on the NOEC value of 0.0221 mg/l (≤ 0.1 mg/l) for algae. In the case of the H410 classification according to CLP, an **M-factor of 1** is applicable based on 0.01< NOEC ≤0.1 mg/l.

2. When adequate chronic toxicity data are not available classification is based on the combination of acute aquatic toxicity data and environmental fate data. Methylcyclohexane is regarded as "not rapidly degradable" for CLP classification based on the available data (see Chapter 4.1.3). The lowest EC50 value (for those trophic levels where adequate chronic toxicity data is not available) obtained for *Daphnia magna* is 0.326 mg/l resulting aquatic Chronic Category 2, **H411**, classification for methylcyclohexane.

The most stringent outcome shall be chosen. Therefore, the eMSCA concludes that according to criteria in CLP Regulation and the available information the environmental hazard classification for methylcyclohexane should be:

Classification categories	Aquatic **Acute Category 1, M factor 1**	
	Aquatic **Chronic Category 1, M factor 1**	
Hazard Statement	**H400**	'Very toxic to aquatic life',
	H410	'Very toxic to aquatic life with long lasting effects'

7.9. Human Health hazard assessment

Not assessed (apart from toxicokinetics, which has been evaluated as supporting information for the assessment of bioaccumulation as a part of the environmental fate properties.)

7.9.1. Toxicokinetics

The following studies on toxicokinetics are available for methylcyclohexane:

Elliot et al. 1965: The Metabolism of Methylcyclohexane

In this study, the kinetics of ^{14}C-labelled methylcyclohexane was investigated in rabbits given single oral doses of approx. 206-237 mg/kg bw. About 58-68 h after administration, 65.4% of the dose was excreted in the urine, 12.8% in expired air (5.6% as CO_2, 7.2% as methylcyclohexane), 0.5% in the faeces; 4.2% remained in tissues. The low output of $^{14}CO_2$ shows that reactions leading to complete oxidation of

methylcyclohexane are of minor importance. In a separate experiment, 42 and 2% of the dose were found to be excreted as glucuronide and sulphate conjugates, respectively, in rabbits given ca. 432 mg/kg bw. The major metabolites found in the urine were the glucuronide conjugates of trans-4-methylcyclohexanol (14.7% of the dose), cis-3-methylcyclohexanol (11.5%), and trans-3-methyl-cyclohexanol (10.5%). Minor metabolites included glucuronides of cis-4-methylcyclohexanol (2.4%) and of cis- and trans-2-methyl-cyclohexanol (0.5% and 1.2%, respectively). No 1-methylcyclohexanol was found. Small amounts of cyclohexylmethanol (≤ 0.3%) and free and conjugated benzoic acid (1.9%; about 0.5 and 1.5% being free benzoic and hippuric acid, respectively) suggested some minor aromatisation of the cyclohexane ring via hydroxylation and carboxylation of the methyl group.

Frommer et al. 1970: Hydroxylation of Aliphatic Compounds by Liver Microsomes, I. The Distribution Pattern of Isomeric Alcohols (summary cited as in the registration dossier, the original article was not available)

"In this study, hydroxylation of methylcyclohexane was investigated in an in vitro system. In the presence of NADPH, oxygen and liver microsome of phenobarbital-treated (80 mg/kg bw/day, for 3 days) rats, mice, rabbits or guinea pigs, methylcyclohexane is hydroxylated to all its isomeric alcohols. The hydroxylation pattern was not significantly affected in rats pretreated with phenobarbital when compared with untreated controls. In all cases, incubations with microsomes of different species resulted in the formation of ***cistrans-3-methylcyclohexanol*** *as the major hydroxylation product."*

Parnell et al. 1988: The metabolism of methylcyclohexane in Fischer 344 rats

This study investigated among other things the metabolites from the urine of male Fischer 344 rats. Urinary metabolites identified in samples collected during 48 h following a single oral administration of methylcyclohexane at 800 mg/kg bw and hydrolyzed with glucuronidase/sulphatase included **cyclohexylmethanol, trans-3-methylcyclohexanol, trans-4-methylcyclohexanol, cis-2-hydroxy-cis-4-methylcyclohexanol, cis-2-hydroxy-trans-4-methylcyclohexanol, and trans-2-hydroxy-cis-4-methylcyclohexanol** in relative abundancies of 10.1:2.0:1.0:2.1:15.7:23.4 as determined by gas-liquid partition chromatography. No cyclohexanecarboxylic acid was found, which indicates that if cyclohexylmethanol is formed, it is readily conjugated and eliminated. The results of the study suggest that metabolism of the ring structure (dihydroxylation) is strongly favoured in comparison with metabolism of the methyl group.

Zahlsen et al. 1992. Inhalation kinetics of C6 to C10 aliphatic, aromatic and naphthenic hydrocarbons in rat after repeated exposures

This study investigated the inhalation kinetics of C6 to C10 aliphatic, aromatic and naphthenic hydrocarbons in rats after a 3-day inhalation exposure. Methylcyclohexane served as the model substance for C7 naphthenic hydrocarbons. Groups of animals were exposed to 100 ppm (400 mg/m³) methylcyclohexane, 12 h/day, for 1, 2 and 3 days. Methylcyclohexane concentrations were determined in blood, brain, liver, kidney, and perirenal fat immediately after each exposure as well as 12 hours following the final exposure. Methylcyclohexane concentrations in blood and highly vascularized organs showed no particular pattern of increase or decrease during the 3-day exposure period, suggesting the achievement of a steady state level. The naphtenes (including methylcyclohexane) seemed to achieve during the exposure period steady state levels also in perirenal fat. The naphtenes showed very high concentrations in fat, but no significant accumulation during the exposure. During the 3-day exposure period, the highest methylcyclohexane concentrations were found in the perirenal fat tissue (356-550 µmol/kg), followed by the kidney (94.7-127.7 µmol/kg), brain (44.4-47.2 µmol/kg), liver (30.1-32.7 µmol/kg), and blood (5.8-6.4 µmol/kg). The distribution pattern of the various hydrocarbon species in the brain was different from that in blood. After the 12-hour recovery period following the last exposure, concentrations decreased to 0.1

µmol/kg in blood (-98.4%), 0.5 µmol/kg in brain (-98.9%), 0.5 µmol/kg in liver (-98.4%), 2.9 µmol/kg in kidney (-97.4%) and 231 µmol/kg in perirenal fat (-49.3%).

7.9.1.1. Summary and discussion on toxicokinetics

Major metabolites of methylcyclohexane have been identified as (glucuronide conjugated) trans/cis methylcyclohexanols (Elliot et al. 1965, Parnell 1988, Frommer et al. 1970). It seems that the metabolism of the ring structure (dihydroxylation) is favoured in comparison with metabolism of the methyl group. The hydroxylation and carboxylation of the methyl group can lead to aromatization of the cyclohexane ring, but only minor amounts of free and conjugated benzoic acid have been identified (Elliot 1965). In a study where single oral doses of ^{14}C-labelled methylcyclohexane were administered to rabbits the majority of the dose (65.4 %) was excreted in the urine after about 80 hours. 12.8 % was expired in air, 0.5 % in the faeces and 4.2 % remained in tissues. (Elliot et al. 1965). In an inhalation study by Zahlsen et al. (1992) methylcyclohexane seemed to achieve steady state in blood, liver, kidney, and perirenal fat. Concentrations were high in fat, but no significant accumulation during the 3 day exposure was observed.

7.10. Assessment of endocrine disrupting (ED) properties

Not assessed.

7.11. PBT and VPVB assessment

Persistence

Abiotic degradation

Hydrolysis is not considered a relevant degradation mechanism for MCH as it has no functional groups liable to hydrolysis.

The estimated half life in air due to photodegradation is 37.9 hours. Therefore, indirect photodegradation in the atmosphere may be an important environmental fate process for this substance. The predicted half-life in air is below the criterion for persistent organic pollutants (POP) (2 d) as defined in the Annex D of the Stockholm convention (Stockholm Convention, 2001) and therefore the substance is not expected to have long-range transport potential.

Biodegradation

There are only screening level data available, which do not allow a direct comparison to P criteria. Non guideline studies show that microorganisms capable of utilizing MCH as a sole carbon source have been found. However, in these studies the microorganisms have been pre-exposed to MCH or other hydrocarbons, or, the pre-exposure is not known. There are six ready biodegradability tests available. In none of these tests biodegradation of MCH is observed. However, due to uncertainties related to the maintenance of the test substance bioavailability and deficiencies in documentation the reliability of five of these studies could not be assigned. In the sixth study, requested by the SEv-decision, maintenance of the test substance concentrations during the test was demonstrated with measured concentrations. Neither in this test was the substance readily biodegradable.

The eMSCAS considers that the use of Biowin QSAR predictions is not possible as the substance is not within the applicability domain of Biowin 3 model. Neither is a category approach using read across to cyclohexane, 1-isopropyl-4-methylcyclohexane and ethylcyclohexane considered scientifically justified.

Based on the available information, the eMSCA cannot conclude on P although the substance fulfils the P screening criterion as it is not readily biodegradable. Generation of new data for the P assessment is, however, not considered necessary for the purpose of this substance evaluation, because it is concluded that the substance is not B.

Bioaccumulation

Based on a reliable (with restrictions) aqueous bioaccumulation study (METI 1986) showing bioconcentration values (BCFs) values below 400, the eMSCA concludes that methylcyclohexane does not meet the criteria for bioaccumulation (BCF < 2000). The conclusion is further supported by QSAR estimates for BCFs and bioaccumulation factors (BAF). The estimated BCF values are generally below 500 and always below 800. The highest BAF value (assuming zero biotransformation) is 1563 and thus below the B criterion 2000.

The conclusion for low bioaccumulation is further supported by two monitoring studies (Benville at al. 1985, Gosset et al. 1983). From the study by Benville et al. (1985) BCF values of 2.2 - 48 l/kg for Bay Shrimp and 17.8 - 240 l/kg for Striped Bass can be derived. It has to be emphasized, however, that the purpose of this study was not to determine BCF values, therefore the values can be used only tentatively. In the study by Gossett et al. (1983) methylcyclohexane was detected in wastewater treatment plant effluent, but not in sediment and organisms of the receiving environment, which seems to support the general conclusion that methylcyclohexane does not have significant bioaccumulation potential.

Ecotoxicity

There are only screening level data available, which do not allow a direct comparison to T criteria. Acute ecotoxicity tests which the eMSCA considers reliable (with restrictions) are available for algae, fish and aquatic invertebrates. For *Daphnia magna* an EC50 value of 0.326 mg/L is obtained. For fish an EC50 value of 2.07 mg/l is obtained. An EC50 value of 0.134 mg/l (and a NOEC of 0.0221 mg/l (based on growth rate)) was obtained for green alga. There are no reliable long term tests available for methylcyclohexane with the exception of the 72 h algae test.

Based on the available information, the eMSCA cannot conclude on T. Generation of new data for the T assessment is, however, not considered necessary for the purpose of this substance evaluation, because it is concluded that the substance is not B.

Toxicity

Not assessed. Assessment not considered necessary, because the eMSCA concluded that the substance is not B.

7.12. Exposure assessment

During the evaluation, the Registrant's environmental exposure assessment and environmental exposure scenarios were evaluated and additional information was requested during informal communications to clarify discrepancies and ambiguity in the reported tonnages and operational conditions.

In the dossier updated in February 2014, the Registrant recalculated the PEC values using EasyTRA, which is a model based on EUSES algorithms. The exposure scenarios were updated based on these updated calculations. The inconsistencies in tonnages and operational conditions identified in the previous dossier (based on Petrorisk modelling) were clarified.

Regarding the additional concerns on personal protective equipment, further information was requested in a Sev-decision (December 2014). The Registrant updated his dossier (7.10.2015;) by including in all exposure scenarios where gloves were recommended a

footnote giving reference to a breakthrough test on one brand of gloves (Showa 720R Nitrile gloves).

The eMSCA considered at the time that the information submitted did not provide sufficiently detailed information as required by the Sev-decision for several reasons, most importantly because,

- there were no specific recommendations for gloves in the chemical safety report (CSR), neither were specific recommendations mentioned in IUCLID section 11 (guidance on safe use).

- the information submitted consisted of a reference to a breakthrough test report on a specific brand of gloves (Showa 720R), whereas the recommendation for suitable gloves should be general and specify, as a minimum, the glove material.

After communications with the Registrant, the Registrant made a further update of the dossier (2.6.2016) and modified the footnote by replacing the reference to a specific brand of gloves (Showa 720R) with general properties of gloves (1.1 mm Nitrile gauntlets or 0.9 mm Nitrile disposable gloves). In Section 11 of the IUCLID dossier a recommendation "to wear suitable gauntlets (1.1 mm thickness, nitrile rubber) and/or suitable gloves (0.9 mm thickness, nitrile rubber) if a risk assessment indicates this is necessary" was included. For further details see Annex 1.

The eMSCA notes, however, that no clear exposure scenario specific recommendations for gloves are given in the CSR. The eMSCA further notes that the tested/recommended gloves are quite thick (0.9 mm/1.1 mm) and it is not clear whether such thick gloves are suitable for all exposure scenarios (e.g. laboratory work). The eMSCA recommends to include exposure scenario specific recommendations for protective gloves in the CSR.

7.13. Risk characterisation

Not assessed in detail.

7.14. References

Anderson, M., Hall A.R., Griffin, M. 1980. Microbial metabolism of alicyclic hydrocarbons: cyclohexane catabolism by a pure strain of *Pseudomonas* sp. *Journal of General Microbiology* (1980), 120,89-94.

Atlas and Bartha 1998. Microbial Ecology - Fundamentals and Applications in Biotechnology. Benjamin/Cummings Science Publishing

BASF AG (1990). Manometric Respirometry Test. Labor Oekologie, Unpublished Report from 11/4/90.

Beam, H.W., Perry. J.J. 1974. Microbial Degradation and Assimilation of *n* -Alkyl-Substituted Cycloparaffins. Journal of Bacteriology 118(2):394.

Benville, P. E. JR, Jeannette, A.W. and Eldridge, M.B. 1985: Acute toxicity of sevn alicyclic hexanes to striped bass, *Morone saxatilis*, and bay shrinp, *Crangon franciscorum*, in seawater. California fish and game 71(3): 132-140.

Blum DJW and Speece RE (1991). A database of chemical toxicity to environmental bacteria and its use in interspecies comparisons and correlations. Journal Water Pollution Control Federation **63**(3), 198-207.Bushnaf, K.M., Puricelli, S.,Saponaro, S, Werner, D., 2011. Effect of biochar on the fate of volatile petroleum hydrocarbons in an aerobic sandy soil. Journal of Contaminant Hydrology 126 (2011) 208–215.

CITI (1992). Biodegradation and Bioaccumulation Data of Existing Chemicals based on the CSCL Japan, Edited by The Chemicals Inspection & Testing Institute, Japan.

Connor, J.J., Jenkins, D. and Greenberg, A.E. 1981. Bioassay procedures for fish p. 723-743 in Standard Methods for Examination of Water and Wastewater. 15th Edition, American Public Health Association, Washington D.C.

ECHA, European Chemicals Agency: http://echa.europa.eu. (search for chemicals)

ECHA 2008. Guidance on information requirements and chemical safety assessment. Chapter R.10: Characterisation of dose [concentration]-response for environment.

ECHA 2008a. "Ready biodegradability prediction: yes or no". Guidance for information requirements and chemical safety assessment. Chapter R.7b: Endpoint specific guidance. ECHA. May 2008 (version 1.1). (pp. 170-171)

ECHA 2008b. "Table R11-2 Screening criteria for P and vP". Guidance for information requirements and chemical safety assessment. Chapter R.11: PBT Assessment. ECHA. May 2008 (page 14).

ECHA 2012a. Guidance on Information Requirements and chemical safety assessment. Chapter R.16: Environmental Exposure Estimation. Version 2.1. October 2012.

ECHA 2012b. Guidance for information requirements and chemical safety assessment. Chapter R.7b: Endpoint specific guidance. November 2012 (Version 1.2).

Elliot, T.H., Tao, R.C.C., Williams, R.T. 1965. The Metabolism of Methylcyclohexane. Biochem J 95:70-76

European Commission - European Chemicals Bureau. 2004. European Union Risk Assessment Report Cyclohexane. CAS 110-82-7; EINECS No: 203-806-2. Volume 41. http://esis.jrc.ec.europa.eu/doc/risk_assessment/REPORT/cyclohexanereport031.pdf

Exxon (1995). Ready Biodegradability: OECD 301F Manometric Respirometry Test. Project No. 103394A. Final Report. Exxon Biomedical Sciences Inc.

EU RAR 2004. European Union Risk Assessment Report:

Cycloheane; CAS 110-82-7; EINECS No: 203-806-2. European Chemicals Bureau - Institute for Health and Consumer Protection.

Fraunhofer 2013. Ready Biodegradability of Methylcyclohexane - Biodegradability in a Closed Bottle Test and a CO_2 - Headspace (Sealed Vessel) Test. Report 2013-07 HUN-1. July 30th 2013.

Frommer 1970. Hydroxylation of Aliphatic Compounds by Liver Microsomes, I. The Distribution Pattern of Isomeric Alcohols. Hoppe-Seyler's Z. Physiol. Chem. 351 (8) 903-918.

Harlan 2012. Methylcylcohexane (MCH). Assessment of Ready Biodegradability; ManometricRespirometry test. Harlan Laboratories Ltd. Study no. 41206062

Harlan 2013. Methylcylcohexane (MCH). Assessment of Ready Biodegradability; CO2 in SealedVessels. Harlan Laboratories Ltd., Study no. 4127023

Howard, P. Meylan, W., Aronson, D., Stiteler, W., Tunkel. J. Comber, M., Parkerton, T.F. 2005. A new biodegradation prediction model specific to petroleum hydrocarbons. Environ. Toxicol. Chem. 24, 1847-1860.

Hydroqual 2011. PETRORISK version 6.02. Available at: www.concawe.org.

Gossett, R.W., Brown, D.A., Young, D.R., 1983. Predicting the bioaccumulation of organic compounds in marine organisms using octanol/water partition coefficients. Marine Pollution Bulletin, 14 (10) 387-92.

Kirkwood, K. M., Chernik, P., Foght, J. M., and Gray, M. R. (2008). Aerobic biotransformation of decalin (dacahydronaphthalene) by Rhodococcus spp. Biodegradation 19:785-794.

Ko, S. H. and Lebeault, J. M. (1999). Effect of a mixed culture on co-oxidation during the degradation of saturated hydrocarbon mixture. Journal of Applied Microbiology 87, 72–79.

Koma, D., Sakashita, Y., Kubota, K., Fuji, Y., Hasumi, F., Chung, S.-Y., Kubo, M. 2003.

Degradation of car engine base oil by Rhodococcus sp. NDKK48 and Gordonia sp. NDKY76A. Biosci. Biotechnol. Biochem., 67, 1590-1593.

Koma, D., Sakashita, Y., Kubota,K., Fujii, Y., Hasumi, F., Chung, S.Y., Kubo; M. 2005. Degradation pathways of cyclic alkanes in Rhodococcus sp. NDKK48. Appl Microbiol Biotechnol (2005) 66: 92–99.

Knoell 2013. Methylcyclohexane. Assesment of persistence/degradability for the registration under Regulation (EC) No 1907/2006 - a weight of evidence approach.. Sponsored by Huntsman (Europe). report date 22th July 2013.

Lloyd-Jones, G., Trudgill, P.W. 1989. The degradation of alicyclichydrocarbons by a microbial consortium. International Biodeterioration 25, 197-206.

METI (1986). Bioconcentration test of methylcyclohexane. http: //www. safe. nite. go. jp/jcheck/. Testing laboratory: Chemicals Evaluation and Research Institute, Kurume Laboratory, Kurume, Japan. Report no.: 50718. Owner company: Ministry of Economy, Trade and Industry, Japan. Report date: 1986-07-12.

MOE (2008a). Acute tox study in Oryzias latipes of methylcyclohexane. http://www.safe.nite.go.jp/jcheck/direct.do?table_name=seidoku&cas_no=108-87-2 . Testing laboratory: Mitsubishi Chemical Safety Institute Ltd., Yokohama Laboratory, Yokohama, Japan. Report no.; A060517. Owner company: Ministry of the Environment, Government of Japan. Rpeort date: 2008-08-18.

MOE (2008b). Daphnia magna immobilisation test of methylcyclohexane. http://www.safe.nite.go.jp/jcheck/direct.do?table_name=seidoku&cas_no=108-87-2. Testing laboratory: Mitsubishi Chemical Safety Institute Ltd., Yokohama Laboratory, Yokohama, Japan. Report no.: A060516. Owner company: Ministry of the Environment, Government of Japan Report date: 2008-08-18.

MOE (2008c). Growth inhibition test in Pseudokirchneriella subcapitata of methylcyclohexane. http://www.safe.nite.go.jp/jcheck/direct.do?table_name=seidoku&cas_no=108-87-2. Testing laboratory: Mitsubishi Chemical Safety Institute Ltd., Yokohama Laboratory, Yokohama, Japan. Report no.: A060515. Owner company: Ministry of the Environment, Government of Japan. Report date: 2008-12-22.

OECD (2000). Guidance document in aquatic toxicity testing of difficult substances and mixtures. OECD Series on testing and assessment, Number 23.

OECD, 2006. OECD guidelines for the testing of chemicals. Revised introduction to the OECD guidelines for testing of chemicals, section 4. Part 1: Principles and strategies related to the testing of degradation of organic chemicals. Adopted: 23 March 2006.

Ooyama, J., Foster, W. 1965. Bacterial oxidation of cycloparafic hydrocarbons. *Antonie van Leeuwenhoek* 31,45-65.

Parnell, M.J., Hennigsen, G.M., Hixson, C.J., Yu, K.O., McDonald, G.A., Serve, M.P. 1988 Chemosphere 17 (7) 1321-1327.

Prince, R.C., Haitmanek, C., Lee, C.C. 2007. The primary aerobic biodegradation of gasoline hydrocarbons. Environ Sci Technol. 2007 May 1;41(9):3316-21. (Supporting information including half-life estimates available at http://pubcs.acs.org).

Rouviere, P.E., Chen, M.W. 2003. Isolation of Brachymonas petroleovorans CHX, a novel cyclohexane-degrading L-proteobacterium. FEMS Microbiology Letters 227 (2003) 101-106.

SERL (1976). JP-4 and JP-9 fuel toxicity studies using freshwater fish and aufwuchs. Testing laboratory: Sanitary Engineering Research Laboratory, University of California at Berkley, USA. Report no.: AMRL-TR-76-50. Owner company: Aerospace Medical Research Laboratory, Aerospace Medical Devision, Wright-Patterson AFB, Ohio, USA

Stirling L.A., Watkinson, R.J., Higgins, I.J. 1977. Microbial Metabolism of Alicyclic Hydrocarbons: Isolation and Properties of a Cyclohexane-degrading Bacterium. Journal of General Microbiology (1977), 99, 119-125.

Stockholm Convention (2001). Annex D. Available at http://chm.pops.int/Convention/tabid/54/language/en-US/Default.aspx (accessed November 2012)

Tonge, G.M., Higgins, I. 1974. Microbial Metabolism of Alicyclic Hydrocarbons. Growth of *Nocardia petroleophila* (NCIB9438) on Methylcyclohexane*Journal of General Microbiology* (r974), 81, *52* 1-524.

Trower, M.K., Buckland, R.M., Higgins, R., Griffin, M. 1985. Isolation and Characterization of a Cyclohexane-Metabolizing *Xanthobacter* sp. *Appl. Environ. Microbiol.* 1985, 49(5):1282.

Trudgill, P. W 1984, Microbial degradation of the alicyclic ring. In D.T. Gibson (ed.) Microbial degradation of organic compounds. Marcel Dekker, New York, pp. 131-180.

Van Hamme, J.D., Ward, O.P. 2001. Volatile hydrocarbon biodegradation by a mixed-bacterial culture during growth on crude oil. Journal of Industrial Microbiology and Biotechnology June 2001, Volume 26, Issue 6, pp 356-362.

Verschueren K (1983). Handbook of Environmental Data on Organic Chemicals. 2nd Edition. Van Nostrand Rienhold Company, New York, 417-419.

Vieth, A., Wilkes, H. 2006. Deciphering biodegradation effects on light hydrocarbons in crude oils using their stable carbon isotopic composition: A case study from the Gullfaks oil field, offshore Norway. Geochimica et Cosmochimica Acta 70 (2006) 651–665.

Zahlsen K., Eide, I., Nilsen, A.M., Nilesen O.G. 1992. Pharmacology & Toxicology 71: 144-149. Inhalation kinetics of C6 to C10 aliphatic, aromatic and naphthenic hydrocarbons in rat after repeated exposures

Zobell CF, Prokop JF (1966). Microbial oxidation of mineral oils in Barataria Bay bottom deposits. Zeitschrift für allgemeine Mikrobiologie, **6**(3), 143-162.

Yi, T., Lee, E.H., Ahn, Y.G., Hwang, G.S., Cho, K.S. 2011. Novel biodegradation pathways of cyclohexane by Rhodococcus sp. EC1. Journal of Hazardous Materials. 191, 393-396.

7.15. Abbreviations

B	bioaccumulative
CH	cyclohexane
BAF	Bioaccumulation factor
BCF	Bioconcentration factor
BMF	Biomagnification factor
ChV	Chronic Value
CSA	Chemical Safety Assessment
CSR	Chemical Safety Report (Registrants)
EC_{50}	Effect Concentration 50 %
ECHA	European Chemical Agency
eMSCA	evaluating Member State Competent Authority
GC-FID	Gas Chromatography - Flame Ionization Detection
MCH	methylcyclohexane
K_{ow}	Octanol-water distribution co-efficient
LC_{50}	Lethal Concentration 50 %
MCH	methylcyclohexane
MSCA	Member State Competent Authority
NOEC	No Observed Effect Concentration
P	persistent
PNEC	Predicted no Effect Concentration
QSAR	Quantitative Structure-Activity Relationship
SEV	Substance Evaluation
T	toxic
vB	very bioaccumulative
vP	very persistent

Annex 1. Further information regarding the request for documentation for the recommended personal protective equipment (PPE)

In the dossier (Feb. 2014), there were in total 19 exposure scenarios which included up to 15 contributing scenarios. Where gloves were recommended, they were recommended as follows, "Protective gloves: Gloves APF 10 90 %".

Based on a proposal for amendment from a MSCA, a request for documentation for the recommended personal protective equipment (PPE) was included in the Sev-decision (19 December 2014).

In the decision the information request is reasoned as follows:

> *"To ensure the safe use of a substance, Annex I Section 5.1.1. requires a description of the risk management measures to reduce or avoid direct and indirect exposure of humans. Gloves are reported in the CSR and IUCLID Section 11 as required personal protective equipment to prevent dermal exposure to the substance. Generally, gloves that are capable of preventing exposure to the skin for a pre-determined duration shall be specified. Typically this information, as a minimum, has to specify the glove material and, depending on the exposure scenarios, may also need to include the breakthrough time and thickness of the glove material.*
>
> *Therefore, pursuant to Article 46(1) the Registrant(s) are required to provide in the CSR a description of the gloves to be used when handling the pure substance.* ***The information provided by the Registrant(s) shall be sufficiently detailed to allow suppliers to fulfil their obligations specified under Annex II for the compilation of the safety data sheets."***

For the complete statement of reasons, see the decision. For a summary record on key actions/dates regarding the information request see table 1.

In response to the decision, the Registrant performed a test on breakthrough of MCH (in accordance with ASTM F739-07, EN 374-3:2003, ISO 6529:2013, EN 374-1:2003) on one type of gloves (Showa 720R Nitrile gloves) (Andersen 2015) and updated his dossier. In the updated dossier (07.10.2015), there were in total 19 exposure scenarios which included up to 14 contributing scenarios (see table 2). Where gloves were recommended, they were recommended as follows:

> *"Protective gloves: Gloves APF 10 90 %[1].*
>
> *[1]Standard default effectiveness value was taken based on Showa 720R Nitrile gloves (breakthrough time for methylcyclohexane of > 480 min; assessed according to ASTM F739, EN 374-3 and ISO 6529; Andersen, N.R., 2015)."*

That is, a footnote was added in which a reference to the performed breakthrough test was given. See also table 2.

The eMSCA was of the opinion that the information submitted did not provide sufficiently detailed information as required by the decision for the following reasons:

- there were no specific recommendations for gloves (as a minimum the glove material) in the chemical safety report (CSR) exposure scenarios, neither were specific recommendations mentioned in IUCLID section 11 (guidance on safe use).

- a reference to a breakthrough test report on a specific brand of gloves (Showa 720R) was given in the CSR. As the time to breakthrough for any glove material may be different for different glove manufacturers and the availability of gloves from a specific manufacturer changes over time, it is not possible to recommend gloves of a specific brand/manufacturer. The recommendation for suitable gloves has to be general and specify, as a minimum, the glove material, and, may also include the breakthrough time and thickness.

- it is not clear what is meant by the specification " APF 10 90 %[1]". Presumably this refers to Assigned protection factors (APF) used by ECETOC TRA model. However, this is not sufficient. The recommendation for suitable gloves has to specify, as a minimum, the glove material, and, may also include the breakthrough time and thickness.

- The same "recommendation" APF 10 90 %[1]" with reference to the Showa 720R breakthrough test is given for all scenarios which include a recommendation for protective gloves. These scenarios vary from e.g. "Use of laboratory reagents in small scale laboratories" to "Transfer of chemicals from/to vessels/ large containers at non dedicated facilities". It is unlikely that one kind of glove would be fit for all these very varying scenarios. Recommendations for protective gloves should be scenario specific.

After informal communications with the Registrant, the Registrant made a further update of the dossier (2.6.2015) and modified the footnote by replacing the reference to a specific brand of gloves (Showa 720R) with general properties of gloves as follows (additions in bold),

"Protective gloves: Gloves APF 10 90 %[1]

*[1]Standard default effectiveness value was taken based on **1.1 mm Nitrile gauntlets** (breakthrough time for methylcyclohexane of > 480 min; assessed according to ASTM F739, EN 374-3 and ISO 6529; Andersen, N.R., 2015) **or 0.9 mm Nitrile disposable gloves (breakthrough time for cyclohexane of > 480 min; assessed according to ASTM F739-99A)**."*

Section 11 of IUCLID (Instructions for Safe Use) was modified as follows (additions in bold),

Hand protection:

Chemical-resistant, impervious gloves complying with an approved standard should be worn at all times when handling chemical products if a risk assessment indicates this is necessary. Considering the parameters specified by the glove manufacturer, check during use that the gloves are still retaining their protective properties. It should be noted that the time to breakthrough for any glove material may be different for different glove manufacturers. In the case of mixtures, consisting of several substances, the protection time of the gloves cannot be accurately estimated.

Chemical-resistant, impervious gloves complying with an approved standard should be worn at all times when handling chemical products if a risk assessment indicates this is necessary.

- Wear suitable gauntlets. 1.1 mm thickness, nitrile rubber, > 480 minutes (breakthrough time), Test type: ASTM F739 / EN 374-3 / ISO 6529.

- Wear suitable gloves. 0.9 mm thickness, nitrile rubber, > 480 minutes (breakthrough time), Test type: ASTM F739-99A.

It should be noted that the time to breakthrough for any glove material may be different for different glove manufacturers. In the case of mixtures, consisting of several substances, the protection time of the gloves cannot be accurately estimated. Considering the parameters specified by the glove manufacturer, check during use that the gloves are still retaining their protective properties.

The eMSCA notes that no clear exposure scenario specific recommendations for gloves are given in the CSR. The eMSCA further notes that the tested/recommended gloves are quite thick (0.9 mm/1.1 mm) and it is not clear whether such thick gloves are suitable

for all exposure scenarios (e.g. laboratory work). **The eMSCA recommends to include exposure scenario specific recommendations for protective gloves in the CSR.**

Table 1. Record of key actions/dates

Date	Action
17.12.2014	Sev-decision with a request for documentation for the recommended personal protective equipment (PPE). Dead-line for submitting information 26.6.2015.
25.6.2015 - 6.7.2015	Email form Registrant's contact point to eMSCA informing that testing on protective gloves on-going. Informal email communication between eMSCA and Registrant's contact point to agree that missing information would be up-dated by end of September 2015.
26.6.2015	Dead-line of Sev-decision for submitting information
7.10.2015	Dossier up-date including reference to a breakthrough test on Showa 720R nitrile gloves.
12.10.2015	Email from eMSCA to Registrant's contact point asking for full study reports and clarification for missing documentation for recommended protective gloves.
15.10.2015	Full study reports (Andersen 2015) submitted by email to eMSCA.
26.1.206	Email from eMSCA to Registrant asking for clarification on the documentation for recommended PPE.
17.3.2016	Teleconference between eMSCA and Registrant.
2.6.2016	Dossier update by Registrant.

Table 2. Methylcyclohexane -protective gloves in exposure scenarios (October 2015).

Exposure scenario	**Protective gloves**
9.1 Manufacture of substances: Industrial	
Contributing scenario 9.1.2	NO
Contributing scenarios 9.1.3 - 9.1.8	Gloves APF 10 90 %[1]
9.2 Use as Intermediate: Industrial	
Contributing scenario 9.2.2	NO
Contributing scenarios 9.2.3 - 9.2.8	Gloves APF 10 90 %[1]
9.3 Distribution: Industrial	
Contributing scenario 9.3.2	NO
Contributing scenarios 9.3.3 - 9.3.8	Gloves APF 10 90 %[1]

9.4 Formulation & packing of preparations and mixtures: Industrial	
Contributing scenario 9.4.2	NO
Contributing scenarios 9.4.3 - 9.4.11	Gloves APF 10 90 %[1]
9.6 Uses in Coatings: Consumer	-
9.7 Use in Cleaning Agents: Industrial	
Contributing scenario 9.7.2	NO
Contributing scenarios 9.7.3 - 9.7.10	Gloves APF 10 90 %[1]
9.8 Use in Cleaning Agents: Professional	
Contributing scenario 9.8.2	NO
Contributing scenarios 9.8.3 - 9.8.0	Gloves APF 10 90 %[1]
9.9 Use in Cleaning Agents: Consumer	-
9.10 Lubricants: Industrial	
Contributing scenario 9.10.2	NO
Contributing scenarios 9.10.3 - 9.10.13	Gloves APF 10 90 %[1]
9.11 Lubricants: Professional - high environmental release	
Contributing scenario 9.11.2	NO
Contributing scenarios 9.11.3 - 9.11.14	Gloves APF 10 90 %[1]
9.12 Agrochemical uses: Professional	
Contributing scenario 9.12.2	NO
Contributing scenarios 9.12.3 - 9.12.8	Gloves APF 10 90 %[1]
9.13 Agrochemical uses: Consumer	-
9.14 Polymer processing: Professional	

Contributing scenario 9.14.2	NO
Contributing scenarios 9.14.3 - 9.14.8	Gloves APF 10 90 %[1]
9.15 Solvent in other application: Industrial	
Contributing scenario 9.15.2	NO
Contributing scenarios 9.15.3 - 9.15.10	Gloves APF 10 90 %[1]
9.16 Solvent in other application: Professional	
Contributing scenario 9.16.2	NO
Contributing scenarios 9.16.3 - 9.16.10	Gloves APF 10 90 %[1]
9.17 Solvent in other application: Consumer	-

[1] Standard default effectiveness value was taken based on Showa 720R Nitrile gloves (breakthrough time for methylcyclohexane of > 480 min; assessed according to ASTM F739, EN 374-3 and ISO 6529; Andersen, N.R., 2015).”