EUROPEAN CHEMICALS AGENCY

Helsinki, 19 December 2014

Decision/annotation number: Please refer to the REACH-IT message which delivered this communication (in format SEV-D-XXXXXXXXX-XX-XX/F)

DECISION ON SUBSTANCE EVALUATION PURSUANT TO ARTICLE 46(1) OF REGULATION (EC) NO 1907/2006

For methylcyclohexane, CAS No 108-87-2 (EC No 203-624-3)

Addressees: Registrant(s)[1] of methylcyclohexane (Registrant(s))

This decision is addressed to all Registrants of the above substance with active registrations on the date on which the draft for the decision was first sent for comments, with the exception of the cases listed in the following paragraph. A list of all the relevant registration numbers subject to this decision is provided as an annex to this decision.

Registrants holding active registrations on the day the draft decision was sent are *not* addressees of this decision if they are: i) Registrant(s) who had on that day registered the above substance exclusively as an on-site isolated intermediate under strictly controlled conditions and ii) Registrant(s) who have ceased manufacture/import of the above substance in accordance with Article 50(3) of Regulation (EC) No 1907/2006 concerning the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH Regulation) before the decision is adopted by ECHA.

Based on an evaluation by the Finnish Safety and Chemicals Agency as the Competent Authority of Finland (evaluating MSCA), the European Chemicals Agency (ECHA) has taken the following decision in accordance with the procedure set out in Articles 50 and 52 of the REACH Regulation.

This decision is based on the registration dossier on 27 June 2014 , i.e. the day until which the evaluating MSCA granted an extension for submitting dossier updates which it would take into consideration.

This decision does not imply that the information provided by the Registrant(s) in the registration(s) is in compliance with the REACH requirements. The decision neither prevents ECHA from initiating compliance checks on the dossier(s) of the Registrant(s) at a later stage, nor does it prevent a new substance evaluation process once the present substance evaluation has been completed.

I. Procedure

Pursuant to Article 45(4) of the REACH Regulation the Competent Authority of Finland has initiated substance evaluation for methylcyclohexane, CAS No 108-87-2 (EC No 203-624-3) based on registration(s) submitted by the Registrant(s) and other relevant and available information and prepared the present decision in accordance with Article 46(1) of the REACH Regulation.

On the basis of an opinion of the ECHA Member State Committee and due to initial grounds for concern relating to Environment/Suspected PBT; Lack of experimental data;

[1] The term Registrant(s) is used throughout the decision, irrespective of the number of registrants addressed by the decision.

Exposure/Wide dispersive use; Consumer use; Aggregated tonnage, methylcyclohexane was included in the Community rolling action plan (CoRAP) for substance evaluation to be evaluated in 2013. The updated CoRAP was published on the ECHA website on 20 March 2013. The Competent Authority of Finland was appointed to carry out the evaluation.

In the course of the evaluation, the evaluating MSCA noted additional concerns regarding the environmental exposure and effects assessment.

The evaluating MSCA considered that further information was required to clarify the following concerns: environmental risk assessment. Therefore, it prepared a draft decision pursuant to Article 46(1) of the REACH Regulation to request further information. It submitted the draft decision to ECHA on 18 March 2014.

On 29 April 2014 ECHA sent the draft decision to the Registrant(s) and invited them pursuant to Article 50(1) of the REACH Regulation to provide comments within 30 days of the receipt of the draft decision.

By 5 June 2014 ECHA received comments from the Registrant(s) of which it informed the evaluating MSCA without delay.

The evaluating MSCA considered the comments received from the Registrant(s).

On the basis of this information, Section II was amended and Section III was modified. Information requirements regarding a growth inhibition study on algae and information on operational conditions and tonnages were removed from the draft decision because the concerns related to environmental exposure and effects were clarified in the updated registration dossier.

In accordance with Article 52(1) of the REACH Regulation, on 4 September 2014 the evaluating MSCA notified the Competent Authorities of the other Member States and ECHA of its draft decision and invited them pursuant to Articles 52(2) and 51(2) of the REACH Regulation to submit proposals to amend the draft decision within 30 days of the receipt of the notification.

Subsequently, a Competent Authority of a Member State submitted a proposal for amendment to the draft decision.

On 10 October 2014 ECHA notified the Registrant(s) of the proposal for amendment to the draft decision and invited them pursuant to Articles 52(2) and 51(5) of the REACH Regulation to provide comments on the proposal for amendment within 30 days of the receipt of the notification.

The evaluating MSCA reviewed the proposal for amendment received and amended the draft decision.

On 20 October 2014 ECHA referred the draft decision to the Member State Committee.

By 10 November 2014, in accordance to Article 51(5), the Registrant provided comments, agreeing to the proposal for amendment.

A unanimous agreement of the Member State Committee on the draft decision was reached on 24 November 2014 in a written procedure launched on 13 November 2014.

ECHA took the decision pursuant to Article 51(6) of the REACH Regulation.

II. Information required

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.

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).

Pursuant to Article 46(2) of the REACH Regulation, the Registrant(s) shall submit to ECHA by **26 June 2015** an update of the registration(s) containing the information required by this decision[2], including robust study summaries and full study reports, and an update of the Chemical Safety Report.

III. Statement of reasons

1. Ready biodegradability study

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. In their comments to the draft decision and in their dossier update, the Registrant(s) have aimed at further strengthening their read across by including 1-isopropyl-4-methylcyclohexane as an analogue substance. In addition, the Registrant(s) further justify in their comments the use of BIOWIN QSAR predictions. Due to specific reasons described in Annex 1 to this decision, it is, however, not possible to conclude based on the BIOWIN predictions and the read across to CH and 1-isopropyl-4-methylcyclohexane on the ready biodegradability of methylcyclohexane (MCH). In addition, non-guideline studies (Annex 2) do not indicate that MCH would undergo "rapid and ultimate degradation in most environments" as is expected for readily biodegradable substances (ECHA 2012a) and do not therefore support the proposed ready biodegradability of MCH.

In the registration dossier there are five ready biodegradation studies available (tests 1-5 in Annex 3). In none of these tests biodegradation is observed. Despite the fact that tests 2 - 5 have been conducted taking specific caution to avoid volatilisation, and the test substance concentrations are below water solubility, the Registrant(s) claim that "the maintenance of substance bioavailability to the microorganism cannot be ensured during the tests." Regarding the OECD 310 and OECD 301F tests, the Registrant(s) claim that the test substance would very likely accumulate in the headspace of the test bottles. However, no measured concentration data from liquid and gas phases have been provided to verify the assumed poor bioavailability. In the OECD 301 D closed bottle test, in which completely full bottles are used, the Registrant(s) have reported that "during test substance application no visible droblet was formed" (Knoell 2013). Therefore, the Registrant(s) are unsure whether the test substance was introduced into the test system at all (Knoell 2013). Further, the Registrant(s) have concluded that the standard testing guidelines for ready biodegradation

[2] The deadline set by the decision already takes into account the time that registrants may require to agree on who is to perform any required tests and the time that ECHA would require to designate a registrant to carry out the test(s) in the absence of the aforementioned agreement by the registrants (Article 53(1) of the REACH Regulation).

cannot be applied to 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).

In their comments the Registrant(s) disagree with the requirement to conduct a closed bottle test (OECD 301D) with chemical analysis to verify the test substance concentration for MCH. Although the Registrant(s) acknowledge that a chemical analysis of test substance in medium may provide more supportive information on the biodegradation properties of MCH, they argue that it will not provide further conclusive answers on the disappearance of the chemical in the air compartment (which the Registrant(s) consider to be the "compartment of concern"). Moreover, they expect that the same technical difficulties will be encountered as noted in the previous tests. The Registrant(s) refer to the toxicity of the substance, the high biological oxygen demand and the other physico-chemical properties that pose technical challenges to testing. In the updated dossier, the Registrant(s) consider MCH readily biodegradable, but failing 10 day window and use the term rapidly degradable due to the combination of both the rapid degradation in air (which, according to the Registrant(s), is the "target compartment") and the weight of evidence (based on read across and BIOWIN predictions) which they have used due to the technical difficulties observed in various biodegradation tests.

In response to the comments by the Registrant(s) on the requirement to conduct the OECD 301D ready biodegradability test, it is noted that volatilization to air is not relevant when considering the need for data on ready biodegradation in accordance with REACH information requirements. As doubts of MCH bioavailability in the available ready biodegradation tests have been presented by the Registrant(s), the reliability of the tests could not be assigned during the substance evaluation (reliability score 4 ("not assignable")). Due to the large uncertainties related to the use of the proposed weight-of-evidence read across and BIOWIN predictions, it is neither possible to adapt the standard information requirement for ready biodegradation with this approach. Therefore, the requirement for an OECD 301D with chemical analysis to verify test substance concentration is maintained. It is acknowledged that the substance is a difficult substance to test. However, a ready biodegradation test is considered technically possible as long as specific care is taken in the test design to ensure the maintenance of the test substance in the test system.

In their comments the Registrant(s) disagree with the conclusion that the use of BIOWIN models for predicating ready biodegradability for MCH is not scientifically justified and the QSAR for all BIOWIN models for MCH should be assigned as "disregarded" with a reliability of 3.

In response to the comments by the Registrant(s) on the use of BIOWIN predictions, it is acknowledged that BIOWIN 5 and 6 models are acceptable for MCH and these models suggest ready biodegradability of MCH. However, the prediction by BIOWIN 5 is not strong as the probability value (0.5315) is close to the cut-off value given in the model (0.5). In addition, not ready biodegradability predictions by BIOWIN models seem to be more certain than ready biodegradability predictions (ECHA 2012a p. 174). The current practice is to use the outcome of these biodegradation models to predict that a substance is not readily degradable, rather than *vice versa* (Guidance on Application of the CLP Criteria, November 2013, section 4.1.3.2.3.2.). Besides, it is recommendable to use the predictions in combination with other information. Therefore, in the present case QSAR results alone are not sufficient to conlude on the ready biodegradability.

In their comments the Registrant(s) disagree with the conclusion that read-across to cyclohexane (CH) is not scientifically justified. The Registrant(s) propose to integrate a

second read-across substance (1-isopropyl-4-methylcyclohexane) in the existing weight of evidence approach with the conclusion that MCH is readily biodegradable, but failing the 10 day window, as a conservative approach. In addition, the Registrant(s) disagree on the statement that similar to n-alkanes, branching and the presence of tertiary carbons may block degradation altogether and may have an effect on the biodegradation of alicyclic hydrocarbons. The Registrant(s) mention that biodegradation behaviour of MCH in comparison with CH may be far more affected by the microbial metabolism pathway than the structure of the stereoisomers. Furthermore the Registrant(s) mention that numerous non-standard tests (Tonge and Higgins, 1974, Elshahed et al., 2001 and Lloyd-Jones and Trudgill, 1989) reveal that multiple microorganisms are either capable to utilise CH and MCH as sole carbon and energy source for growth or co-oxidise them completely in different manner.

In response to the comments by the Registrant(s) on the read-across to CH it is noted that amending the read-across by adding a further relevant analogue substance, ethylcyclohexane (Annex 1) indicated that it is not possible to make a sound conclusion on the ready biodegradability of MCH within this category because the ready biodegradability information is not consistent between the analogue substances. The comments concerning stereoisomers are not considered relevant because in the original draft decision reference was made to position isomers (not stereoisomers) and because the information concerning stereoisomers mentioned in the comments are not considered to affect the conclusion on the proposed use of read-across. Concerning the mentioned non-standard tests it is acknowledged that in the studies by Tonge and Higgins (1974) and Lloyd-Jones and Trudgill (1989) microrganisms capable of growth on MCH and/or CH were reported. However, due to the pre-exposure of the studied microorganisms to MCH or due to the lack of information on the pre-exposure (Annex II) the growth reported in these studies as well as in several other studies listed in Annex II is not comparable to the conditions of ready biodegradability tests which employ non-adapted inocula and aim at determining biodegradation in a relatively short time (28 days). In addition, co-oxidation is not relevant for the assessment of ready biodegradability because in ready biodegradability tests the test substance serves as the sole carbon source. Furthermore it is noted that the study by Elshahed et al. (2001) did not include tests on MCH nor CH and is therefore not relevant for the assessment of the proposed read-across.

It is further noted, that in their updated dossier, the Registrant(s) have demonstrated safe use of the substance when PEC values were re-calculated using easy-TRA and assuming that the substance is "not biodegradable". However, the risk assessment and exposure scenarios are based on assuming that the substance is readily biodegradable failing the 10 day window. However, as no reliable information is available on the ready biodegradability of MCH, such information needs to be generated.

Due to the uncertainties related to test substance introduction and maintenance in the available ready biodegradation tests, the available tests are not considered reliable by the Registrant(s). Therefore, as it is not possible to adapt the information requirement for ready biodegradability with the weight of evidence approach as proposed by the Registrant(s), a new ready biodegradation test is considered necessary.

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. Care must be taken when administering the substance into the test vessel in order to ensure that the substance enters the test vessel. In order to ensure bioavailability, the test can be performed under continuous mixing. 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. 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 EC50 values (or less than EC20 values) obtained in toxicity testing. For MCH, this would imply a test substance concentration of 2.9 mg/l (based on microbial toxicity EC_{50} of 29 mg/l).

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 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). 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. Regarding biological oxygen demand, it is noted, that, for instance, at a concentration of 2 mg/l 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/l of O_2).

The ready biodegradability study is a standard information requirement under REACH (Annex VII, 9.2.1.1.).

Therefore, pursuant to Article 46(1) of the REACH Regulation, the Registrant(s) are required to carry out the following study using the registered substance subject to this decision:

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

Article 14(6) as well as Annex I, 0.1., 5.1.1., 5.2.4. and 6.2. of the REACH Regulation require registrants to identify and apply appropriate measures to adequately control the risks identified in a Chemical safety Report (CSR). The exposure shall be estimated and risks shall be characterised in the CSR under the assumption that relevant risk management measures (RMM) have been implemented.

According to Annex I, 0.3., 0.5. and 5.1.1., the applied Risk Management Measures (RMM) have to be described in the CSR. The CSR needs to contain sufficient information to allow ECHA to gain assurance that the risks are adequately controlled and that appropriate risk management measures can be prescribed by actors in the supply chain. Accordingly, the supplier is required to describe the relevant RMM in detail in the Safety Data Sheet (SDS) in order to minimise the exposure for workers handling the registered substance (e.g. the type of gloves to be worn shall be clearly specified based on the hazard of the substance or mixture and potential for contact and with regard to the amount and duration of dermal exposure in accordance with Annex II, section 8.2.2.2. (b)(i)). The information provided in the SDS shall be consistent with information in the Chemical Safety Report (Annex II, section 0.1.2. of the REACH Regulation).

ECHA notes that the substance is classified for skin irritation (Cat. 2). Gloves are reported in the CSR and in IUCLID section 11 as reguired personal protective equipment to prevent dermal exposure to the substance. ECHA notes, however, that specific detailed information on the recommended personal protective equipment is missing both from the CSR and from

the information on safe use within the IUCLID dossier (section 11). In the exposure scenarios assigned protection factors (APFs) are referred to (e.g.: APF 10 90 %). In the summary of risk management measures, "gloves approved to relevant standards e.g. EN 374" are refered to. No further specification are available in the CSR nor in IUCLID section 11.

Personal Protective Equipments are produced in different types of materials, thickness, design etc. However, not all materials are well suited to protect against exposure to all substances. A concern is raised if workers are not properly informed to use the right type of personal protective equipment (e.g. gloves) to protect themselves against exposure to chemicals. The use of unsuited material may even result in higher level of exposure than not using any protection at all as the inside of contaminated gloves may be covered with migrated substance – and the skin inside a glove is often humid – corresponding to exposure under occlusion.

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.

IV. Adequate identification of the composition of the tested material

In relation to the required experimental studies, the sample of the substance to be used shall have a composition that is within the specifications of the substance composition that are given by all Registrant(s). It is the responsibility of all the Registrant(s) to agree on the tested material to be subjected to the test(s) subject to this decision and to document the necessary information on composition of the test material. The substance identity information of the registered substance and of the sample tested must enable the evaluating MSCA and ECHA to confirm the relevance of the testing for the substance subject to substance evaluation. Finally, the test(s) must be shared by the Registrant(s).

V. Avoidance of unnecessary testing by data- and cost-sharing

In relation to the experimental studies the legal text foresees the sharing of information and costs between Registrant(s) (Article 53 of the REACH Regulation). Registrant(s) are therefore required to make every effort to reach an agreement regarding each experimental study for every endpoint as to who is to carry out the study on behalf of the other Registrant(s) and to inform ECHA accordingly within 90 days from the date of this decision under Article 53(1) of the REACH Regulation. This information should be submitted to ECHA using the following form stating the decision number above at:
https://comments.echa.europa.eu/comments_cms/SEDraftDecisionComments.aspx

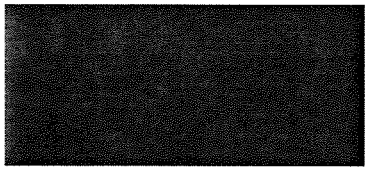
Further advice can be found at http://echa.europa.eu/datasharing_en.asp.

If ECHA is not informed of such agreement within 90 days, it will designate one of the

Registrant(s) to perform the stud(y/ies) on behalf of all of them.

VI. Information on right to appeal

An appeal may be brought against this decision to the Board of Appeal of ECHA under Articles 52(2) and 51(8) of the REACH Regulation. Such an appeal shall be lodged within three months of receiving notification of this decision. Further information on the appeal procedure can be found on the ECHA's internet page at http://echa.europa.eu/regulations/appeals. The notice of appeal will be deemed to be filed only when the appeal fee has been paid.



Jukka Malm
Deputy Executive Director

Annex 5: List of registration numbers – This annex is confidential and not included in the public version of this decision

Annex 1. Use of QSARs and analogue approach in estimating ready biodegradability

The Registrant(s) used BIOWIN QSAR models in their Chemical Safety Assessment (CSA) for the estimation of biodegradability of MCH. The Registrant(s) included results of seven different EPISUITE 4.10 BIOWIN models in their registration dossier. 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 for estimation of biodegradability of MCH. However, BIOWIN 7 (anaerobic degradation) is not considered relevant for ready biodegradability which relates to ultimate biodegradation in aerobic conditions. 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 2012b and ECHA 2012c) 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.

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.

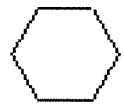
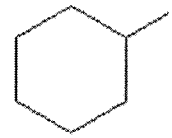
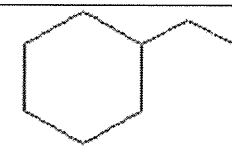
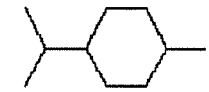
Cyclohexane contains only secondary carbon atoms while MCH contains one tertiary carbon, five secondary carbons, and one primary carbon. In the case of n-alkanes, branching in general reduces the rate of biodegradation because tertiary and quaternary carbon atoms interfere with degradation mechanisms 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. 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 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).

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.

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

Therefore, it is concluded that in the case of ready biodegradability the use of the analogue approach (read across), with CH and 1-isopropyl-4-methylcyclohexane as source substances, is not scientifically robust enough to allow conclusions.

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 Pa m3/mol (20 - 25 °C)	14 900	43 600	30 400	178 000
Reference	EU RAR (2004); ECHA database	Registration dossier	NITE 2014a, OECD 2014, Episuite	ECHA database, Episuite

Annex 2. Summary of non-standard published studies

Although 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), 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.

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 has been observed in studies conducted with hydrocarbon mixtures (Koma et al. 2005, Bushnaf et al. 2011, Prince et al. 2007, Van Hamme et al. 2001), and 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).

Studied microorganisms (or their source)	**Pre-exposure, growth results, and other information**	**Reference**
Water samples collected from a New Jersey rainwater retention pond, seawater, and from an activated sludge wastewater treatment facility treating only domestic wastewater	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). Degradation was studied in a hydrocarbon mixture. Cometabolic substrates were present. Mineralization or growth was not determined.	Prince et al. (2007)
Soil obtained from a construction site in Newcastle (UK)	It was not documented whether the microorganisms were pre-exposed to hydrocarbons. Degradation was studied in a hydrocarbon mixture. Cometabolic substrates were present. Mineralization or growth was not determined.	Bushnaf et al. 2011
Bacterial culture	The study was conducted with micro-organisms	Anderson

(Pseudomonas spp) isolated from soil	pre-exposed to CH during the enrichment procedure. The bacterium was able to grow on MCH and CH.	et al. (1980)
Bacterial cultures (*Mycobacterium vaccae* strain JOB5 (26); *M. rhodochrous* strains OFS (8) and 7E1C (9); *Nocardia asteroides* strain A-116; and *M. convolutum* strain R-22 (3))	The study was conducted with micro-organisms pre-exposed to hydrocarbons (n-alkanes, including propane), during isolation and maintenance. The bacteria were not able to grow on MCH. Biodegradation of cyclohexane was not studied.	Beam and Perry (1974)
Bacterial culture (*Rhodococcus* sp. NDKK48) isolated from soil	The study was conducted with micro-organisms pre-exposed to hydrocarbons (cyclic alkane fraction of car engine base oil) during isolation. The isolation procedure is described in another paper (Koma et al. 2003). The bacterium could neither utilize MCH nor CH for growth. Both MCH and CH were co-oxidised when either of the substances was present in the test system together with hexadecane.	Koma et al. (2005)
A three-organism bacterial consortium consisting of *Rhodococcus, Flavobacterium* and *Pseudomonas* spp isolated from oil refinery waste	The study was conducted with micro-organisms pre-exposed to MCH in laboratory. The consortium was able to grow on MCH and CH but the individual strains were not.	Lloyd-Jones ja Trudgill (1989)
Bacterial culture (*Brachymonas Petroleovorans CHX*) isolated from waste water plant of an oil refinery	The study was conducted with micro-organisms pre-exposed to CH during enrichment. The bacterium was able to grow on MCH and CH.	Rouviere and Chen (2003)
Bacterial culture (tentatively identified as a *Nocardia)* isolated from estuarine mud flats	The study was conducted with micro-organisms pre-exposed to MCH during enrichment. The bacterium was able to grow on MCH and CH.	Stirling et al. (1977)
Bacterial culture (*Nocardia petroleophila* (NCIS948)	It was not documented whether the microorganisms were pre-exposed to hydrocarbons. The bacterium was able to grow on MCH. Biodegradation of cyclohexane was not studied.	Tonge and Higgins (1974)
Bacterial culture (*Xanthobacter sp.*) isolated from forest soil	The study was conducted with micro-organisms pre-exposed to CH during enrichment and maintenance.	Trower et al (1985)

	The bacterium was able to grow on MCH and CH.	
Mixed-bacterial culture isolated from petroleum-contaminated soil	The study was conducted with micro-organisms pre-exposed to hydrocarbons (diesel fuel, crude oil, motor oil, refinery sludge) in laboratory. Degradation was studied in a hydrocarbon mixture. Cometabolic substrates were present. Mineralization or growth was not determined. Biodegradation of cyclohexane was not studied.	Van Hamme and Ward (2001)
Oil reservoir (field study)	The study was conducted in anaerobic environment. Other hydrocarbons were present. Biodegradation was determined by stable isotope analyses. Biodegradation of CH and MCH was "at best marginal".	Vieth and Wilkes (2006)

Annex 3. Ready Biodegradation test results on methylcyclohexane

TS = test substance, conc. = concentration.

No	Test method	Results	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(s): "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 biodegradation 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.	Fraunhofer 2013

			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 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

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