SUBSTANCE EVALUATION CONCLUSION

as required by REACH Article 48

and

EVALUATION REPORT

for

Imidazole

EC No 206-019-2

CAS No 288-32-4

Evaluating Member State(s): UK

Dated: December 2018

Evaluating Member State Competent Authority

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Year of evaluation in CoRAP: 2012

Before concluding the substance evaluation a Decision to request further information was issued on: 21 February 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

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

Reproductive toxicity – Although the Technical Committee for Classification and Labelling (TC C&L) had recommended that imidazole should be classified as a reproductive toxicant in 2007, a harmonised classification had not been taken forward. Whilst the registrants self-classified imidazole as Repr. 1B: May damage the unborn child (H360D) a review of the classification and labelling inventory showed that not all notifiers were applying the recommended classification. Imidazole was placed on the CoRAP to verify the agreed C&L was still appropriate, with a view to submitting a harmonised classification and labelling (CLH) proposal to ECHA.

Due to this hazard and some wide dispersive industrial and professional uses of imidazole, an evaluation of exposure and risk management measures employed was undertaken to determine if the risks were being adequately controlled.

At the start of the initial evaluation period in 2012, the eMSCA was informed that the lead registrant intended to submit a harmonised classification and labelling proposal for imidazole, including Repr. 1B. This proposal was submitted to ECHA in July 2012 and consequently the human health hazard evaluation looked at all available information to see if there were additional concerns and whether there was a need to further investigate fertility.

Additionally the environmental hazard and exposure information was assessed.

During the evaluation other concerns were also identified, these were:

- Mutagenicity – concern over the robustness of the available data package.

- Environmental hazard – further information was required to allow the assessment of the adequacy and reliability of the available ecotoxicity tests.

These concerns were addressed in a decision dated 21 February 2014.

2. OVERVIEW OF OTHER PROCESSES / EU LEGISLATION

As noted above the lead registrant submitted a CLH proposal for imidazole in 2012. Following discussion at RAC, classification for acute toxicity, corrosivity and developmental toxicity was agreed and the opinion published in September 2013.

Imidazole was included in the 7th ATP to Regulation (EC) No 1272/2008 on classification, labelling and packaging (CLP) of substances and mixtures published in 2015 and is listed by Index number 613-319-00-0 in Annex VI, Part 3, Table 3.1 (list of harmonised classification and labelling of hazardous substances) for;

Acute Tox. 4	H302
Skin Corr. 1C	H314
Repr. 1B	H360D

This classification should be applied, at the latest, from 1 January 2017.

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	✓

4. FOLLOW-UP AT EU LEVEL

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

The eMSCA has not identified a need for follow-up regulatory action.

4.1.1. Harmonised Classification and Labelling

Not applicable, as noted above a harmonised classification has recently been adopted. No additional hazards were identified.

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

As imidazole has a harmonised classification of Repr. 1B H360D, it meets the Article 57(c) hazard criteria for identification as an SVHC. However, the eMSCA does not consider that authorisation is an appropriate risk management approach for this substance. The majority of the registered tonnage is supplied for use as an intermediate and most intermediate use takes place under strictly controlled conditions. This use is exempt from authorisation.

For the remaining "authorisable" uses, the eMSCA has not identified concerns that warrant the imposition of this risk management measure. Overall, the eMSCA does not identify this as a relevant substance for the purposes of the SVHC Roadmap.

4.1.3. Restriction

An unacceptable risk has not been identified therefore there is no need to consider restrictions for any of the identified uses of this substance.

4.1.4. Other EU-wide regulatory risk management measures

Not applicable

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	✓
Actions by the registrants to ensure safety, as reflected in the registration dossiers(e.g. change in supported uses, applied risk management measures, etc.)	

Human health – hazard

To address the abovementioned concerns the following information was requested and submitted by the deadline stipulated in the final decision (dated 21 February 2014) :

- *In vitro* mouse lymphoma study
- A report summarising the weight of evidence assessment on the reproductive toxicity of imidazole, including information on some structural analogues

The eMSCA has assessed and summarised the submitted information in this report (section 7.9) and concludes that there are no further concerns for mutagenicity and no further testing for effects on fertility is necessary.

Human health - exposure

During the initial evaluation in 2012 it was found that the CSR contained only limited details of the processes and operating conditions for each scenario and it was not possible for the eMSCA to replicate the exposure estimates given in the CSR. As such further contextual information regarding processes, operating conditions and company RMM's were requested.

Additional information was provided and this was sufficient to enable the eMSCA to complete its exposure assessment and risk characterisation.

Using precautionary DNELs and taking a precautionary approach to the exposure assessment, the eMSCA has obtained RCRs > 1 for some activities covered by the scenarios for industrial and professional use of products containing up to 3% imidazole. All RCRs are below 1 when the exposure assessment is refined to take account of the low vapour pressure of the substance. The eMSCA therefore concludes that no further regulatory action is necessary.

Environment

During the initial evaluation it was noted that the registrants had provided limited details about the ecotoxicity studies in the registration dossier and more information was requested. The update included the following:

- updated CSR with additional information in Section 7 (Environmental Hazard Assessment);
- report summarising read-across justification of ecotoxicological effects of imidazole; and

- QSAR Toolbox output report for the fish acute toxicity endpoint.

The eMSCA has included the information in this report. Data limitations are also noted.

Considering the presented data, the eMSCA considers the acute ecotoxicity endpoints are not below 100 mg/l. On this basis, there is no concern for aquatic toxicity. Therefore, further environmental information is not required to address to acute ecotoxicity concern at this time. However, recommendations to improve the environmental read-across are presented.

The potential for endocrine disruption in the environment has not been evaluated. However, the eMSCA notes that the substance is readily biodegradable and has a low bioaccumulation potential.

The evaluation of imidazole is therefore concluded with no further regulatory follow up necessary.

5.2. Other actions

The following points are recommendations for the registrants.

For human health;

- To ensure that companies receiving exposure scenarios that include tasks assessed on a reduced duration basis implement sufficient measures to protect their workers, clarification should be provided with the scenario that the RMMs identified apply where the worker does not have any additional exposure to imidazole during the shift.

- If imidazole containing preparations are sprayed by professionals, it will be useful to emphasise the need for good ventilation in safe use information that is provided to those carrying out the work, particularly if others may be working in the same area. If this cannot be guaranteed, then advice should be provided to consider excluding other workers from the area during spraying.

For the environment;

- The read-across justification should consider ECHA's Read-Across Framework.

- Robust Study Summaries should be included for the analogue endpoints to determine the validity of read-across ecotoxicity endpoints.

- A QMRF / QPRF should be included for the acute toxicity to fish QSAR. Given the current prediction appears to be outside the model domain, alternative QSAR predictions should be considered.

Additionally a review of the classification and labelling inventory (checked June 2018) shows that some notifiers have still not updated their notifications to include the harmonised classification and labelling despite this applying from January 1 2017. MS NEAs could consider following this up with relevant notifiers.

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

Not applicable.

Part B. Substance evaluation

7. EVALUATION REPORT

7.1. Overview of the substance evaluation performed

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

Reproductive toxicity – Although the Technical Committee for Classification and Labelling (TC C&L) had recommended that imidazole should be classified as a reproductive toxicant in 2007, a harmonised classification had not been taken forward. Whilst the registrants self-classified imidazole as Repr. 1B (H360) a review of the classification and labelling inventory showed that not all notifiers were applying the recommended classification. Imidazole was placed on the CoRAP to verify the agreed C&L was still appropriate, with a view to submitting a harmonised classification and labelling (CLH) proposal to ECHA.

However, at the start of the evaluation period, the registrants informed the eMSCA that they intended to submit a harmonised classification and labelling proposal for imidazole. This proposal was submitted to ECHA in July 2012 and was largely consistent with that agreed at TC C&L in 2007 (Rep.1B, H360D: May damage the unborn child; Acute tox. 4; H302; Skin Corr.1B; H314, plus eye damage 1; H318). Consequently the human health hazard evaluation looked at all available information to see if there were additional concerns and whether there was a need to further investigate fertility.

Due to the reproductive toxicity, the high tonnage and wide dispersive uses of imidazole an evaluation of worker exposure and risk management measures employed was necessary.

During the evaluation other concerns were also identified, these were:

- Mutagenicity – concern over the robustness of the available data package.

- Environmental hazard – further information was required to allow the assessment of the adequacy and reliability of the available ecotoxicity tests.

The outcome/conclusion of the evaluation of the endpoints of concern are briefly summarised in the table below

Table 3

EVALUATED ENDPOINTS	
Endpoint evaluated	**Outcome/conclusion**
Reproductive toxicity	*Developmental toxicity - Repr. 1B H360D – agreed.* *Fertility – No information was available in the registration dossier for imidazole. The registrants provided the requested weight of evidence assessment using information from related substances. The eMSCA concluded further testing to investigate fertility was unnecessary.* *No further action necessary.*
Mutagenicity	*The registrants provided the requested study showing negative results.* *No further action necessary.*

Exposure of workers	*The registrants provided sufficient information for the eMSCA to assess the exposure and risk characterisation. During the evaluation the registrants revised the RMMs recommended for situations where imidazole containing preparations are sprayed which addressed the concern identified by the eMSCA.* *No further action necessary.*
Environmental hazard	*The registrants provided information to clarify the concern.* *No further action necessary.*

The endpoints and the respective outcome/conclusions mentioned in the table above are substantiated in more detail in the sections below and in Part A.

7.2. Procedure

The focus of the evaluation was reproductive toxicity and worker exposure although all available information was evaluated to identify any additional concerns.

For human health hazard the initial evaluation was based on information contained within the IUCLID file and the registrants' joint submission CSR. Where greater detail was required, the original study reports or publications were requested from the registrants and evaluated in full.

The study reports requested were as follows: 90-day repeat dose toxicity, developmental toxicity, skin corrosion, eye irritation, Ames tests, in vitro UDS and in vivo micronucleus study.

Additional information also came from the OECD SIDS assessment of imidazole; a range-finding 28-day study that had not been summarised in the IUCLID file and a paper on the toxicokinetics of 2-methylimidazole. A literature search carried out in July 2012 did not identify any additional information concerning imidazole's potential to cause reproductive toxicity.

For the human health exposure assessment all the data provided by the registrants regarding exposure scenarios and exposure assessment were screened. It was determined that further information would be required to complete the evaluation.

For the environment, it was noted that the registrants had provided limited details about the ecotoxicity studies in the registration dossier.

A telephone conference was held with the lead registrant in May 2012 to discuss the process. As a result of this discussion, additional study reports were provided by the lead registrant in May 2012. Further study reports were also provided upon request during the period June – November 2012 (including the results of a literature search from May 2012).

A draft of the evaluation report was sent to the registrants in November 2012, and a telephone conference was held in December 2012 to discuss the draft conclusions and next steps. Their comments were taken into account before final submission of the evaluation to the European Chemicals Agency in February 2013.

Unanimous agreement of the Member State Committee (MSC) was reached following discussion and modification of the draft decision at MSC-33 (December 2013). The final decision contained requests for:

- In vitro mouse lymphoma study (test method EU Method B.17/OECD 476)

- A weight of evidence assessment of the reproductive toxicity effects (fertility) of imidazole.

- Human health exposure information

- More detailed information on the available ecotoxicity studies

The information requested in the decision was provided by the deadline given.

7.3. Identity of the substance

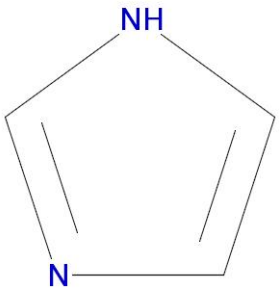
The following identity information is reported on the ECHA dissemination site

Table 4

SUBSTANCE IDENTITY	
Public name:	Imidazole
EC number:	206-019-2
CAS number:	288-32-4
Index number in Annex VI of the CLP Regulation:	613-319-00-0
Molecular formula:	$C_3H_4N_2$
Molecular weight range:	68.0773
Synonyms:	1H-Imidazole

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

Structural formula:



7.4. Physico-chemical properties

The physico-chemical properties given on the ECHA dissemination site are summarised in the table below. Where a number of records were provided for an endpoint, only the most relevant have been included in the table. The registrant considered the reliability of the studies to be 2 (reliable with restrictions) although no information was given regarding deficiencies.

In some cases the method used was not reported. It is recommended that the registrant updates their dossier to include further details.

Table 5

OVERVIEW OF PHYSICOCHEMICAL PROPERTIES	
Property	**Value**
Physical state at 20°C and 101.3 kPa	Colourless to slightly yellow , crystalline solid with a amine like odour
Vapour pressure	0.00327 hPa at 25 °C
Water solubility	633 g/l at 20 °C (no pH given)
Partition coefficient n-octanol/water (Log Kow)	Log Kow -0.02 at 25 °C
Flammability	not easily ignitable
Melting point	I89.8°C
Boiling point	268.1 °C at 1013 hPa
Granulometry	Percentile (10) = 179µm (mean) 0% < 4 µm 0% < 10 µm 5.1% < 100 µm
Density	1.03 g/cm^3 20^{o}C
Dissociation constant	6.92 at 25 °C

7.5. Manufacture and uses

7.5.1. Quantities

Currently there are 11 active registrations listed on the ECHA dissemination site; 1 joint submission covering 7 companies registering a combined tonnage of 10+ tpa, a separate joint submission covering 3 companies registering use as an intermediate only and one stand alone intermediate registration[2]. A second stand alone intermediate registration is no longer active.

7.5.2. Overview of uses

Imidazole is an aromatic heterocycle which has amphoteric properties meaning that it can function as an acid and as a base. An OECD SIAR published in 2003 indicated that imidazole was mainly used as an intermediate in the manufacture of biologically active substances such as pharmaceuticals and pesticides. It was also used as an intermediate in the manufacture of dyes and other substances (OECD 2003). Product information available on registrants' websites describes its use as an intermediate for pharmaceuticals and agricultural chemicals. It is also used as a catalyst for the modification of epoxy resins, for two component epoxy systems and as a blocking agent for polyisocyanates in powder coatings[3],[4].

[2] Dissemination site accessed June 2018.

[3] http://product-finder.basf.com/group/corporate/product-finder/en/brand/IMIDAZOLE (accesssed June 2018).

[4] https://www.ulprospector.com/en/na/Coatings/Detail/3821/109088/Imidazole (accesssed June 2018).

The main use categories are summarised in the table below.

Table 6

USES	Use(s)
Uses as intermediate	Use as intermediate, use as monomer;
Formulation	Formulation of preparations
Uses at industrial sites	Use in industrial chemical processes, use in laboratories, use as an intermediate
Uses by professional workers	Use in construction chemicals, use in coatings, use as a laboratory chemical
Consumer Uses	None identified
Article service life	Not relevant for the registered uses

7.6. Classification and Labelling

7.6.1. Harmonised Classification (Annex VI of CLP)

Table 7 gives the classification of imidazole according to the entry in table 3.1 in Annex VI of CLP Regulation (Regulation (EC) 1272/2008) as included in the 7th ATP. This classification should be applied from 1 January 2017 at the latest.

Table 7

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)**		
613-319-00-0	Imidazole	206-019-2	288-32-4	Acute Tox. 4 Skin Corr. 1C Repr. 1B	H302 H314 H360D		

7.6.2. Self-classification

- In the registration(s)*:*

The one full registration includes the correct harmonised classification as given in table 10. The 2 of the 3 intermediate registrations have not been updated to include Repr. 1B and still propose Repr. 2 H361. The 3rd intermediate registration reports no classification.

- The following hazard classes are in addition notified among the aggregated self-classifications in the C&L Inventory (checked June 2018)*:*

There are 47 aggregated notifications on the ECHA dissemination site and whilst many have notified the correct harmonised classification given in table 10 there are a number of discrepancies ranging from including additional classification for eye damage; Eye Dam. 1 H318 or STOT SE 3 H336 to reporting no classification whatsoever. Many give Repr. 2 H361 or even no classification for reproductive toxicity. One gives Repr. 1A H360.

Some have Skin Corr. 1B H314 or Skin Irrt. 2 H315 instead of Skin Corr. 1C. Some report Acute Tox. 3 H301 or H311 instead of Acute Tox. 4 H302.

7.7. Environmental fate properties

7.7.1. Degradation

7.7.1.1. **Photodegradation**

Not assessed.

7.7.1.2. **Hydrolysis**

Not assessed. Given the lack of hydroysable functional groups the substance is unlikely to hydrolyse.

7.7.1.3. **Biodegradation in water**

The results of two reliable ready tests are available in the dossier, both showing that the substance is readily biodegradable (OECD 301A, DOC die away test: 90 – 100% DOC removal after 18 days); OECD 301C, modified MITI test, 90% degradation after 4 weeks). The 301A study, conducted to GLP, was judged reliability 1 in the registration dossier, although the robust study summary contains a minimum of detail (reliability 2 might be more appropriate). The 301C study was judged reliability 2 but also lacks detail (for example although concentrations of sludge and test substance are given, no details on the source of the inoculum are available); it appears to have been taken from the NITE online database. Given the level of detail, reliability 4 might be more appropriate.

Overall it can be concluded that the substance is readily biodegradable.

7.7.2. Environmental distribution

7.7.2.1. **Adsorption/desorption**

An adsorption/desorption study following OECD test guideline 106 is available for imidazole (reliability 1). The study calculated Koc and Kd values as follows using 5 soils with 0.52 to 1.83 % organic carbon at 20°C:

Koc: > 23 — < 207 l/kg

Kd: > 0.23 — < 3.37 at 20 °C

The dossier also includes two estimated values for adsorption/desorption.

The first is calculated using SRC PCKOC v1.66, giving a Koc of 9.7 (corrected log Koc = 0.99) based on the uncharged molecule. The registrant states that the molecule will partly exist in its cationic form at lower environmental pH values as its pKa is 7.15 and, as cations generally adsorb more strongly to clay than their neutral counterparts, a Koc

of 1943 can be estimated according to the formula as given by Franco & Trapp (2008) for the fully charged molecule.

7.7.2.2. **Henry's Law Constant**

The dossier includes one estimated value for Henry's law constant (HLC), based on measured values for vapour pressure and water solubility (HLC = vapour pressure x molecular weight/water solubility). This gives a value of 0.000034 Pa.m³/mol, suggesting that the substance is unlikely to volatilize from surface waters.

7.7.2.3. **Distribution modelling**

The registrant states that the substance will preferentially reside in surface waters given its solubility, low HLC and low organic carbon – water partition coefficient (for the neutral form) when released to waste water. Specific modelling of environmental distribution has not been carried out, which seems appropriate.

Level III fugacity modelling using EPISUITE v4.10, assuming continuous release to surface water only, agrees with the registrants' summary: 0.03% will reside in air, 99.7 in water, 0.017 in soil and 0.27 in sediment.

Summary

Owing to its high solubility, low HLC and low organic carbon – water partition coefficient, the substance is likely to remain in surface waters if released to waste water (although the charged form may have a higher affinity for sediments and waste water treatment plant sludge).

7.7.3. Bioaccumulation

Aquatic bioaccumulation

No measured data are available.

It is noted that the measured log Kow for imidazole is 0.02 indicating the substance has a low potential for bioaccumulation.

Terrestrial bioaccumulation

No measured data are available.

Summary

The substance is very unlikely to bioaccumulate in aquatic or terrestrial organisms given its low lipophilicity (as measured by its Kow value).

Secondary poisoning

Not relevant for this evaluation.

7.8. Environmental hazard assessment

7.8.1. Aquatic compartment (including sediment)

During the Substance Evaluation process, the eMSCA noted the lack of study details for acute ecotoxicity endpoints. Further information was requested to update the robust study summaries and provide a weight of evidence approach to support the reliability of the available experimental endpoints. This was considered to include read-across to an appropriate analogue and QSARs.

In 2015 the Registrant provided an updated dossier including an updated CSR. They confirmed that additional study details were not available for the acute ecotoxicity studies due to their age.

The Registrant also provided read-across data on two analogues which consist of an imidazole ring with additional methyl groups: 1-methylimidazole (CAS: 616-47-7) and 2-methylimidazole (CAS: 693-98-1). Information on the analogues is presented below (Table 8) with the endpoints included in the relevant sections. The analogues are proposed based on their structural similarity and similar physico-chemical properties. On this basis the Registrant proposes that read-across can be performed for the ecotoxicological endpoints. In relation to ecotoxicity, the Registrant considers the additional methyl group in the analogues is likely to increase log Kow due to a slightly lower hydrophilic nature. In turn, this is considered to increase ecotoxicity and provide a conservative approach.

The eMSCA considers that the analogues are appropriate given their similarities to imidazole in terms of structure, molecular weight, physico-chemical data and fate profiles.

The Registrant considers that the ecotoxicological endpoints for imidazole and the analogues are within a comparable range and generally indicate a low level of ecotoxicity.

The eMSCA notes that while analogue endpoints indicate low ecotoxicity, there are deficiencies with the studies such as lack of GLP, lack of analytical support, and use of non-standard fish test species (compared to OECD test guideline 203).

Further study details other than those provided in the table below were not included for the biodegradation endpoints.

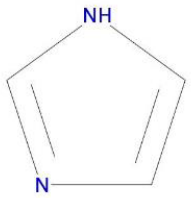
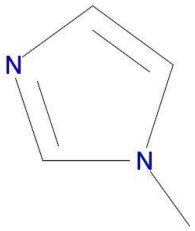
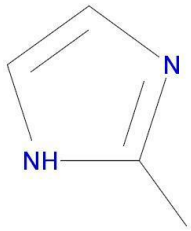
The eMSCA also notes that the read-across is not supported by a measured acute ecotoxicity endpoint for imidazole with full robust study summary details and Klimisch 1 score.

In addition, the Registrant provided a QSAR prediction for acute toxicity to fish based on the QSAR Toolbox v.3.2.1. This information is described with the endpoint data in the relevant section below. The eMSCA notes that the target chemical is not within the model domain as the target chemical logKow value is outside the applicability domain. The Registrant did not include QSAR QMRF (QSAR Model Reporting Format) or QPRF (QSAR Prediction Reporting Format).

No further QSAR predictions with alternative models were presented by the Registrant. Considering the presented data, the eMSCA considers that the acute ecotoxicity endpoints are not below 100 mg/l. On this basis, there is no concern for aquatic toxicity. Therefore, further environmental information is not required to address the initially identified acute ecotoxicity concern at this time. However, recommendations to improve the environmental read-across are presented below.

Recommendations:
- The read-across justification should consider ECHA’s Read-Across Framework.
- Robust Study Summaries should be included for the analogue biodegradation studies to support the endpoints.
- A QMRF / QPRF should be included for the acute toxicity to fish QSAR. Given the current prediction appears to be outside the model domain, alternative QSAR predictions should be considered.

Table 8 – Environmental read-across data proposed by the Registrant

	Target chemical	**Source chemical**	**Source chemical**
	Imidazole	1-methylimidazole	2-methylimidazole
			
CAS	288-32-4	616-47-7	693-98-1
SMILES	c1c[nH]cn1	Cn1ccnc1	Cc1ncc[nH]1
Formula	C3H4N2	C4H6N2	C4H6N2
Purity / Impurities (w/w)	≥99.5 ≤99.9%	≥95 ≤100%	≥98 ≤100%
Molecular weight	68.08	82.10	82.10
Physical state (20° C, 1013 hPa)	solid	liquid	solid
Melting point (°C, 1013 hPa)	89.8	-2	144
Boiling point (°C, 1013 hPa)	268.1	198.9	267
Density (g/cm3 at 20 °C)	1.23	1.035	1.096
Vapour pressure (hPa)	0.00327 (25 °C)	0.3514 (20 °C)	0.00043 (20°C)
Log Kow (at 25 °C)	-0.02	-0.19 (pH 9.25-9.85)	0.22
Water solubility (g/l at 20 °C)	663	1000	267
Biodegradation	Readily biodegradable; 90-100% DOC removal 18d; OECD 301A	Inherently biodegradable; 18% CO_2 evolution, 28d; 97% DOC removal, 35d; ISO DIN 9439	Readily biodegradable; 67% CO2 evolution, 28d;OECD 301B
Short-term toxicity to fish	LC_{50} = 283.6 mg/l 48h, nominal; *Leuciscus idus*; static, PF 94, Screening Test	LC_{50} > 100 - < 215 mg/l 96h nominal; *Leuciscus idus*; static at 21°C, 10F0163/895 106; DIN 38 412	LC_{50} = 190 mg/l 96h nominal; *Leuciscus idus*; static at 20-21°C, DIN 38 412
Short-term toxicity to aquatic invertebrates	EC_{50} = 341.5 mg/l 48h, nominal; *Daphnia magna*, static at 20.85°C, EU Method C.2	EC_{50} = 267.94 mg/l 48h, nominal; *Daphnia magna*, static at 292-294K, EU Method C.2	EC_{50} = 225.31 mg/l 48h, nominal; *Daphnia magna*, static at 292-294K, EU Method C.2
Toxicity to aquatic algae and cyanobacteria	EC_{50} = 133 mg/l 72h, nominal; *Desmodesmus subspicatus*, static at 20°C, DIN 38 412/9	EC_{50} = 180.7 mg/l 72h, nominal; *Desmodesmus subspicatus*, static at 20°C, OECD 201	EC_{50} = 256.3 mg/l 72h, nominal; *Desmodesmus subspicatus*, static at 20°C; DIN 38 412/9

7.8.1.1. **Fish**

Short-term toxicity to fish

The following information is reported in the dossier (see table 9 below).

Table 9 Acute toxicity to fish for imidazole from registration

Method	**Results**	**Remarks**
Leuciscus idus freshwater static equivalent or similar to Screening Test	LC_{50} (48 h): 283.6 mg/L test mat. (nominal)	2 (reliable with restrictions) key study experimental result **Test material (Common name): Imidazole**

From the robust study summary it appears a pre-test was carried out with nominal test concentrations of 10, 100, 500, 1000 and 1600 mg/l. Two main tests appear to have been conducted, the first with concentrations of 100, 160, 250, 400 mg/l and the second with concentrations of 250, 275, 302, 331, 364, 400 mg/l. Few details on the test are available, which is judged reliability 2 (reason: discrepancy between documented test parameters and standard methods, but scientifically acceptable). It is also noted that the test duration is half that recommended in the current OECD 203 Test Guideline.

In the May 2015 Registration update the Registrant confirmed that additional study details were not available due to the age of the study.

The Registrant provided data on two analogues which were assigned Klimisch 2 (reliable with restrictions) to support read-across for the endpoint:

- 1-methylimidazole 96-h LC50 >100 to <215 mg/l

- 2-methylimidazole 96-h LC50 190 mg/l

The two analogue studies are of standard 96-hour duration with the same non-standard fish species (compared to OECD test guideline 203) as the imidazole study. The eMSCA notes that while analogue endpoints indicate low ecotoxicity, there are deficiencies with the studies such as lack of GLP and absence of analytical support.

The eMSCA also notes that exposure concentrations maybe influenced by the ready biodegradable nature of the substances and the relatively high vapour pressure for 1-methylimidazole. This may lead to endpoints based on mean measured concentrations lower than quoted nominal concentrations.

The QSAR Toolbox prediction provided by the Registrant gives a LC50 of 140 mg/l although it is noted that the target chemical does not fall within the model applicability domain due to the log Kow being below the model applicability of 0.609. The eMSCA also notes that 2 analogue chemicals were manually removed from the model by the user [CAS: 68694-11-1 triflumizole; and CAS: 2232-08-8 1-(p-toluenesulfonyl)imidazole]. This is presumed to be due to their lack of overall structural similarity. The Registrant did not include QMRF (QSAR Model Reporting Format) or QPRF (QSAR Prediction Reporting Format) details. Overall, the eMSCA does not consider the QSAR is valid to support read-across or the acute toxicity to fish endpoint.

Other QSAR models were not considered by the Registrant.

Overall, the eMSCA considers the experimental value for imidazole and analogue data do not indicate an acute toxicity to fish LC50 below 100 mg/l.

Long-term toxicity to fish

No data are available. The eMSCA notes that the lack of acute aquatic ecotoxicity and low bioaccumulation potential indicate that experimental data are not required at this time.

7.8.1.2. **Aquatic invertebrates**

Short-term toxicity to aquatic invertebrates

The following information is reported in the dossier (see table 10 below).

Table 10 Acute toxicity to invertebrates for imidazole from registration

Method	**Results**	**Remarks**
Daphnia magna freshwater static equivalent or similar to EU Method C.2 (Acute Toxicity for Daphnia)	EC_{50} (48 h): 341.5 mg/L (nominal) based on: mobility	2 (reliable with restrictions) key study experimental result **Test material (Common name): Imidazole**

In the Registrant's IUCLID the experimental study is judged reliable with restrictions because it is "comparable to guideline study with acceptable restrictions (exposure concentrations in the test and the stability of imidazole were not confirmed by analysis)". However, from review of the robust study summary the study appears to be valid (although the information is fairly limited).

In the May 2015 Registration update the Registrant confirmed that additional study details were not available due to the age of the study.

The Registrant provided data on two analogues which were assigned Klimisch 2 (reliable with restrictions):

- 1-methylimidazole 48-h EC_{50} 267.94 mg/l
- 2-methylimidazole 48-h EC_{50} 225.31 mg/l

The two analogue studies indicate low acute toxicity to invertebrates. However, the eMSCA notes there are deficiencies with the studies such as lack of GLP and absence of analytical support.

The eMSCA also notes that exposure concentrations maybe influenced by the ready biodegradable nature of the substances and the relatively high vapour pressure for 1-methylimidazole. This may lead to endpoints based on mean measured concentrations lower than quoted nominal concentrations.

The Registrant did not include any QSAR predictions in the May 2015 update to support read-across.

Overall, the eMSCA considers that the available experimental value for imidazole and analogue data do not indicate an acute toxicity EC_{50} for invertebrates below 100 mg/l.

Long-term toxicity to aquatic invertebrates

No data are available. The eMSCA notes that the lack of acute aquatic ecotoxicity indicates that experimental data are not required at this time.

7.8.1.3. **Algae and aquatic plants**

The following information is reported in the dossier (see table 11 below).

Table 11 Algal inhibition for imidazole from registration

Method	Results	Remarks
Scenedesmus subspicatus (new name: Desmodesmus subspicatus) (algae) freshwater static DIN 38412, Part 9	EC_{50} (72 h): 133 mg/L (nominal) based on: growth rate NOEC (72 h): 25 mg/L (nominal) based on: growth rate LOEC (72 h): 50 mg/L (nominal) based on: growth rate EC_{10} (72 h): 63.7 mg/L (nominal) based on: growth rate	2 (reliable with restrictions) key study experimental result **Test material (Common name): Imidazole**

The experimental study in the registrant's IUCLID is judged reliable with restriction because it is "comparable to guideline study with acceptable restrictions (exposure concentrations in the test and the stability of imidazole were not confirmed by analysis)".

In the May 2015 Registration update data was provided on two analogues which were assigned Klimisch 2 (reliable with restrictions):
- 1-methylimidazole 72-h E_rC_{50} 180.7 mg/l
- 2-methylimidazole 72-h E_rC_{50} 256.3 mg/l

The two analogue studies indicate low acute toxicity to algae. However, there are deficiencies with the studies such as lack of GLP and absence of analytical support.

The eMSCA also notes that exposure concentrations maybe influenced by the ready biodegradable nature of the substances and the relatively high vapour pressure for 1-methylimidazole. This may lead to endpoints based on mean measured concentrations lower than quoted nominal concentrations.

The Registrant did not include any QSAR predictions in the May 2015 update to support read-across.

Overall, the eMSCA considers that the experimental value for imidazole and analogue data do not indicate an acute toxicity EC_{50} for algae below 100 mg/l.

7.8.1.4. **Sediment organisms**

No data are available. The eMSCA notes that the lack of acute aquatic ecotoxicity and low bioaccumulation potential indicate that experimental data are not required at this time.

7.8.1.5. **Other aquatic organisms**

No data.

7.8.2. Terrestrial compartment

No data are available. The eMSCA notes that the lack of acute aquatic ecotoxicity and low bioaccumulation potential indicate that experimental data are not required at this time.

7.8.3. Microbiological activity in sewage treatment systems

Not considered in this evaluation.

7.8.4. PNEC derivation and other hazard conclusions

The main PNECs are summarised in the table below.

Table 12

PNEC DERIVATION AND OTHER HAZARD CONCLUSIONS		
compartment	**PNEC**	**Remarks/Justification**
Water	0.13 mg/L	Derived using the EC_{50} data for algae and an assessment factor of 1000
Sediment	0.336 mg/kg dry weight	Derived using equilibrium partitioning and the algal EC_{50}
Soil	0.0425 mg/kg dry weight	Derived using the equilibrium partitioning approach.
Air		Not considered
Sewage treatment plant		Not considered
Secondary poisoning	13.3 mg/kg oral	A $PNEC_{oral}$ can be calculated in view of the high repeat dose toxicity in the rat and the potential for environmental exposure. Although it is noted that food chain exposure is likely to be very low due to degradability and low bioaccumulation potential. The pre-natal developmental toxicity study in the rat gave a NOAEL of 60 mg/kg bw/d. The $PNEC_{oral}$ can be calculated using a conversion factor of 20 to convert the NOAEL to a NOEC and the default assessment factor of 90.

7.8.5. Conclusions for classification and labelling

Overall, the substance is of low acute toxicity for the aquatic environment. The substance is not classified in Annex VI of the CLP Regulation for the environment and the registrant does not propose any environmental classification. Based on the ready biodegradation, low log Kow value and all acute aquatic toxicity end points above 100 mg/l, this is justified.

7.9. Human Health hazard assessment

During the initial evaluation a screen of all the available information was carried out to identify any other concerns in addition to considering whether there was a need to further investigate fertility. It was concluded that the Registrants should provide an *In vitro* mouse lymphoma study (test method EU Method B.17/OECD 476) and a weight of evidence assessment of the reproductive toxicity effects (fertility) of imidazole. This information was provided in an updated dossier in 2015 and the results are summarised in the following sections.

Additionally the updated dossier contained the following information not considered in the initial assessment;

- an in vitro skin absorption study carried out according to OECD Guideline 428 (Skin Absorption: In Vitro Method)

- An Ames test from 1979

Neither study changed conclusions reached in the initial evaluation and are only mentioned in the relevant sections.

Unpublished studies included in the registration dossier are not referenced in this report.

7.9.1. Toxicokinetics

The toxicokinetic information available for imidazole during the initial evaluation is summarised in tables 13 and 14.

Non-human information

Table 13: Overview of experimental studies on absorption, metabolism, distribution and elimination

Method	**Results**	**Remarks**	**Reference**
Pre-OECD 417 Rat (Wistar)/ 5 male Oral/gavage Non-radiolabelled Imidzaole or the salt imidazole and salicyclic acid* Imidazole: 0.24 mmol/l (16.6 mg/kg bw imidazole) Salt: 0.24 mmol/kg bw (equivalent to 16.3 mg imidazole/kg bw), 0.48 mmol/kg bw (equivalent to 32.7 mg imidazole/kg bw) or 0.97 mmol/kg bw (equivalent to 66.03 mg imidazole/kg bw) Limit of detection: 0.02 mmol/l (equivalent to 1.36 ug/ml) imidazole	Plasma levels Imidazole 0.25 h: 0.13 mmol/l (8.8 mg/l) 0.5 h: 0.13 mmol/l (8.8 mg/l) 1 h: 0.09 mmol/l (6.1 mg/l) 2 h: 0.03 mmol/l (2.0 mg/l) 4 h: not detectable Salt – low dose 0.25 h: 0.15 mmol/l (10.2 mg/l) 0.5 h: 0.14 mmol/l (9.5 mg/l) 1 h: 0.10 mmol/l (6.8 mg/l) 2 h: 0.03 mmol/l (2.0 mg/l) 4 h: not detectable Salt-medium dose 0.25 h: 0.24 mmol/l (16.3 mg/l) 0.5 h: 0.25 mmol/l (17.0 mg/l) 1 h: 0.22 mmol/l (15.0 mg/l) 2 h: 0.13 mmol/l (8.9 mg/l) 4 h: not detectable Salt- High dose 0.25 h: 0.30 mmol/l (20.4 mg/l) 0.5 h: 0.39 mmol/l (26.6 mg/l) 1 h: 0.31 mmol/l (21.1 mg/l) 2 h: 0.21 mmol/l (14.3 mg/l) 4 h: 0.18 mmol/l (12.3 mg/l) 8 h: not detectable	2 (reliable with restrictions) Key study **Test material (EC name): imidazole**	Pagella PG et al. (1983)
Equivalent to OECD 417	Urinary excretion profile over 24 h (% administered dose): Imidazole: 14 % Hydantoin: 39 %	2 (reliable with restrictions) Key study	Ohta K et al. (1996)

Rat (Wistar) / 3 males Intravenous Dose: 3 µmol/kg bw (0.204 mg/kg) [2-^{14}C]-imidazole	Hydantoic acid: 31 % Unidentified metabolites: 4 % Pre-treatment with cytochrome P450 inhibitor – 49 % imidazole Pre-treatment with Cytochrome P450 inducers: No effect Distribution After 24 h, Liver: 0. 35 nmol/g Kidney: 0.12 nmol/g Aorta: 0.1 nmol/g (bound to elastin) Plasma, blood, heart, lung, brain, muscle, skin and cartilage < 0.03 nmol/g or ml Fat: non-detected	**Test material (EC name): imidazole**	

* salicyclic acid is a synonym for 2-hydroxybenzoic acid

Oral

One study is available in the rat via the oral route using non-radiolabelled imidazole. Supporting information also comes from a study in the rat in which radiolabelled imidazole was administered via the intravenous (*i.v.*) route.

Absorption

Imidazole was detected in the plasma of Wistar rats dosed orally (gavage) with approximately 17 mg/kg bw of imidazole, indicating imidazole is absorbed. Plasma levels peaked between 15-30 min after administration suggesting absorption is rapid. A similar profile was observed following administration of imidazole salicyclic acid salt at 16 mg imidazole/kg bw. The amount of imidazole in the plasma increased with dose (33 and 66 mg imidazole/kg bw) suggesting absorption is not saturated at these dose levels (Pagella *et al*., 1983).

Distribution

No specific information is available on distribution following oral administration of imidazole. However, the presence of imidazole in the plasma suggests systemically available imidazole will be well distributed around the body, particularly to well perfused organs (Pagella *et al*., (1983)).

Distribution was investigated twenty-four hours after intravenous injection of 2-^{14}C-imidazole. Radioactivity was detected in the liver, kidney and aorta (bound to elastin) and residual amounts were detected in a number of other tissues (plasma, blood, heart, lung, brain, muscle, skin and cartilage) (Ohta, 1996). These results support the prediction imidazole will be well distributed and are consistent with the results of the 90-day repeated dose toxicity study, in which the liver and kidney were identified as target organs (see section 5.6.1.1).

Metabolism

No specific information is available on metabolism following oral administration of imidazole. However, within 24 hours of receiving an *i.v.* injection of 0.204 mg/kg bw imidazole, 14 % of the radioactivity of the administered dose was excreted in the urine as unchanged parent, 39 % as hyantoin, 31 % as hydantoic acid and 4 % structurally

unidentified metabolites. No significant effect on the relative proportions was observed following pre-treatment with P450 inducers (3-methylcholanthrene and phenobarbitone). However, pre-treatment with P450 inhibitor (SKF525) resulted in a higher proportion of unchanged imidazole, suggesting P450 enzymes are involved in the metabolism of imidazole. A similar metabolism pattern of imidazole can be predicted for the oral route of administration, at dose levels below those saturating liver metabolism.

Excretion

Imidazole was not detected in plasma 4 hours after oral administration of up to 32.7 mg imidazole/kg bw (administered on its own or as the imidazole salicyclic acid salt) and 8 hours after administration of 66.6 mg imidazole/kg bw of the salt, suggesting the rate of excretion is dose dependent and relatively rapid.

Following systemic absorption, the *i.v.* study results show elimination was primarily via renal excretion. A similar elimination pattern may also be predicted for orally administered imidazole.

Dermal

At the time of the initial evaluation no information was available for the dermal route.

Inhalation

No information is available for the inhalation route.

Human information

Information in humans is available from a study investigating differences in bioavailability between two different doses of the drug Selezen (imidazole salicylic acid salt).

Table 14 Overview of experimental studies on absorption, metabolism, distribution and elimination in humans

Method	**Results**	**Remarks**	**Reference**
Non-guideline Human 18 males volunteers (age: 18-25)/group Oral tablet or drops Doses/conc: ORAL: 750 mg of active substance (248 mg imidazole: 502 mg salicyclic acid*) DROPS: 400 mg of active substance **Single dosing:** 1 tablet or 40 drops in the morning after an overnight fast	Neither dosing regime showed any difference between tablet and drops. **Single administration:** (mean; single dose tablets and drops) Tablets Cmax: 3.59 ± 0.96 µg imidazole/ml plasma Tmax: 0.79 ± 0.54 h T ½: 2.89 ± 1.13h Drops Cmax: 3.3 ± 1.22 µg imidazole/ml plasma Tmax: 0.71 ± 0.59 h T ½: 2.48 ± 1.19h Protein binding (% dose) – 5-15 %	2 (reliable with restrictions) Key study **Test material (EC name): imidazole**	Kuemmerle H-P et al. (1987)

Blood sampling: every 15 min up to 240 min, then after 360, 480 min and 24 hour Urine sampling: every 2h up to 6-8 h, then between 8-24 h, 24-36h and 36-48 h **Multiple dosing:** Either 3 tablets/day or 3 x 40 drops/day for three days, followed by one dose (either a tablet of 40 drops) on the morning of the fourth day (10 treatments in total) Blood sampling: Blood sampling: every 15 min up to 240 min, then hourly until 12h and then every 12 h. On day 4: every 15 min up to 240 min, then 300, 360 min and 8, 24 and 36 h Urine sampling: 0-12h and 12-24 h for days 1-3. Day 4 was the same as for single dosing	**Multiple administration:** (mean; single dose tablets and drops) Tablets Cmax: 2.87 ± 0.84 µg imidazole/ml plasma (first dose), 3.11 ± 0.78 µg imidazole/ml plasma (last dose). Tmax: 1.04 ± 0.5 h (first dose), 0.68 ± 0.51 h (last dose). T ½: 2.85 ± 1.25 h (first dose), 1.86 ± 0.78 h (last dose). Drops Cmax: 2.67 ± 1.22 µg imidazole/ml plasma (first dose), 2.30 ± 0.61 µg imidazole/ml plasma (last dose). Tmax: 0.96 ± 0.67 h (first dose), 0.51 ± 0.52 h (last dose). T ½: 3.47 ± 2.64 h (first dose), 2.12 ± 0.91 h (last dose). Excretion Renal elimination of imidazole (substance itself) was 10-15 % of administered dose		
Non-guideline – pilot study Human 4 male volunteers Single application of imidazole salicyclic acid salt gel 5 % (5 g gel) to the forearm (25 cm^2) No details of duration of exposure Urine sampling: 0-4 h, 4-8h and 8-12 h after topical application	Urinalysis Small peaks, below the level of detection, were observed. These could not be attributed to salicyclic acid or salicyluric acid. Imidazole 2-hydroxybenzoate or imidazole was not detected in the urine at any time point.	3 (unreliable study) supporting study **Test material (EC name): imidazole**	Kuemmerle H-P et al. (1987)

Absorption

Plasma concentrations of imidazole peaked between 0.7 - 1 hour following single or multiple administrations of tablets or drops of the drug to male volunteers, suggesting absorption of imidazole is fast.

Distribution

No information is available on the distribution of the imidazole salicylic acid salt; however, as imidazole was detected in the plasma it is considered to be well distributed around the body. 5-15 % of imidazole was found bound to protein.

Metabolism

Little information is available on metabolism in the study. None of the administered drug (salt of imidazole and salicylic acid) was detected, but imidazole, itself, was detected in both the plasma and urine. Imidazole's major metabolites, hydantoin and hyantoic acid, were also present in both the urine and plasma, but their levels could not be quantified due to methodological limitations. The decrease in plasma half-life following multiple administrations suggests imidazole may have an enzyme-inducing effect.

Excretion

The plasma half-life of imidazole, following single administration, was similar for both forms of administration and less than 3 hours, suggesting excretion was fast. Renal elimination of imidazole was between 10-15 % of the administered dose. The proportion excreted as the metabolites, hyantoin and hyantoic acid, could not be quantified in this study.

Dermal

In a pilot study summarised in Kuemmerle et al. (1987), a 5 % gel of imidazole salicylic acid salt (82 mg imidazole in 5 g gel) was applied to the forearm (area about 25 cm^2) of four male volunteers (duration unknown). Plasma levels were not investigated, but neither the parent compound nor any metabolite was detected in the urine in the 12 h period following application suggesting limited dermal uptake.

Inhalation

There is no information available via the inhalation route.

Summary and discussion on toxicokinetics

Rat

Oral

Information is available from an oral study and is supported by a study conducted via the i.v. route.

Following oral administration, imidazole levels in the plasma peaked between 15-30 min, suggesting absorption is rapid. The amount of imidazole present in the plasma increased with dose, suggesting absorption is not saturated at doses ≤ 66 mg imidazole/kg bw. Its presence in plasma suggests it will be well distributed. Imidazole was extensively metabolised to hydantoin and hydantoic acid when given intravenously; a process involving P450s. Metabolism via the oral route is expected to be qualitatively similar. Based on the rate at which plasma levels of imidazole fell, elimination is expected to be relatively rapid and is expected to occur primarily via the urine.

The registrants have proposed an absorption value of 100 % by the oral route for rats. We agree with this proposal as the degree of absorption does not appear to be saturated at doses up to 66 mg imidazole/kg bw (a dose higher than the NOAEL selected for risk characterization, see section 5.13) and absorption of other imidazoles has been shown to be high (e.g. bioavailability in F344 rats was estimated to be 97 % or more following oral administration of 25 to 100 mg/kg bw/day of 2- methyl imidazole – Johnson et al (2002)).

As none of the toxicity studies in animals were conducted via the dermal and inhalation routes, the extent of dermal or inhalation absorption in animals does not need to be estimated.

Human

Oral

Absorption was fast (0.7 - 1 hour) following oral administration of the drug selezen (imidazole salicylic acid salt) in both tablet and drop form. Detection of imidazole in the plasma suggests it will be well distributed. The proportion of unchanged imidazole detected in the urine was 10-15 %, similar to that observed in the rat. The major metabolites, hyantoic acid and hyanduric acid were below the level of detection in plasma and urine; therefore, although the metabolic profile of imidazole may be the same as in the rat, it cannot be confirmed. The plasma half-life of both administered forms was < 3 hours, suggesting excretion is rapid.

There is no specific information on the extent of absorption; however, as the toxicokinetic profile appears similar to the rat, the eMSCA agrees with the registrants' proposal to assume 100 % absorption by the oral route for humans (this is also the default worst-case assumption).

Dermal

At the time of the initial evaluation information on dermal absorption came from a pilot study in humans, in which a 5 % gel of a salt containing 82 mg imidazole was administered to the forearm of four human volunteers. No parent compound or metabolite was detected in the urine in the following 12 h period.

The pilot study suggests absorption is limited; however, it is not possible to estimate the extent of absorption from this study. The Registrants proposed to use the default value of 100% dermal absorption in humans.

In the updated dossier the Registrants included an *in vitro* study (unpublished, 2013) carried out according to OECD Guideline 428 (Skin Absorption: In Vitro Method) ; according to OECD Guidance Document No. 28 for the conduct of skin absorption studies, March 2004. Single topical application to *ex vivo* human skin gave the following dermal absorption rates;

92.09 % (930 µg/cm²) (24 hours - 8 hour exposure)

67.66 % (99 µg/cm²) (24 hours - 8 hour exposure)

This study has not been assessed as the Registrant has taken the default assumption of 100 % dermal absorption into risk characterisation, which the eMSCA agrees with.

Inhalation

No information is available for inhalation. Therefore, the eMSCA agrees with the registrants' proposal to use the default assumption of 100 % inhalation absorption in humans.

7.9.2. Acute toxicity and Corrosion/Irritation

Imidazole has the harmonised classifications Acute Tox 4 (H302) & Skin Corr. 1C (H314) (included in the 7th ATP to the CLP Regulation).

The registrants had also proposed the classification Eye Dam. 1 (H318). Whilst RAC agreed that the available test data supported classification for Eye Dam. 1 (H318), the current guidance and practice means this classification is not required in addition to Skin Corr. 1C. However this may change in future.

7.9.3. Sensitisation

Skin sensitisation testing was waived on the basis the substance is corrosive. This is in accordance with the column 2 adaptation in Annex VII, section 8.3. No information on respiratory sensitisation was available.

7.9.4. Repeated dose toxicity

A 90-day sub-chronic study, a pre-guideline 28-day study and a 28-day range-finding study are available in the rat. A full evaluation of repeated dose information was conducted to inform on the need for further investigation on fertility.

In the updated dossier the Registrant included a robust study summary for a 90-day oral toxicity study conducted using the analogue substance 1-methyl imidazole. This was considered as part of the weight of evidence assessment.

Repeated dose toxicity: oral

The registration dossier contains robust study summaries for a 90-day oral toxicity study and, an older, 28-day study (1976). The 90-day study was identified as the key study, which accords with the OECD HPV assessment of imidazole.

The full study report of the 90-day study was requested and assessed. The assessment of the 28-day study has been based upon information included in the dossier (due to its age the full study report could not be obtained). In addition, the registrants provided the methodology and results of a 28-day range finding study, which have been evaluated and summarised below and in the IUCLID dossier for information.

Table 15: Overview of experimental studies on repeated dose toxicity after oral administration

Method	Results	Remarks	Reference
28-day range finding study Rat (Wistar) 5/sex/dose Oral gavage 0, 125, 250, 500 mg/kg bw/day Non-GLP	**500 mg/kg bw/day** Anogenital staining (urine), acanthosis in the forestomach (4 males/ 4females), focal erosion of the stomach lining (2 males/ 2 females), dark faeces Liver: 30 % ↑ in absolute/relative weight (females), 27 % ↑ in relative weight (males) Kidney: 25 % ↑ in absolute/relative weight (females), 68 % ↑ in relative weight (males) Spleen: ~ 30 % ↑ in absolute/relative weight (females), 40 % ↑ in relative weight (males)	2 (reliable with restrictions) Supporting study **Test material (EC name): imidazole**	Unpublished

	Blood parameters (males/females): 17/19% ↓ in hb, 14/16 % ↓ in HCT , 10/10% ↓ in MCV in 13/12 % ↓ in MCH, 3/3 % ↓ in MCHC Clinical Chemistry and Urinalysis: ↑ in P_i , ↓ chloride and ↓ albumin in males and females, ↑ cholesterol and total bilirubin in females **250 mg/kg bw/day** acanthosis in the forestomach (4 males, 3 females) Liver: 16 % ↑ in absolute/relative weight (females) Kidney: 11 % ↑ in absolute/relative weight (females), 22 % ↑ in relative weight (males) Spleen: 22 % ↑ in relative weight (males) Blood parameters (males/females): 8/7% ↓ in hb, 7/4 % ↓ in MCV, 9/6 % ↓ in MCH, 2/2 % ↓ in MCHC **125 mg/kg bw/day** Acanthosis in the forestomach (1 male) Blood parameters (males/females): 7/3% ↓ in hb		
90-day (oral: gavage) Rat (Wistar) 10/sex/dose 0, 20, 60, 180 mg/kg bw/d OECD Guideline 408	**180 mg/kg bw/day** Bodyweight: 4.1 % ↑ in females Kidney: 12/10 % ↑ kidney weight in males/females, 9 % ↑ relative kidney weight in males. Slight/moderate/diffuse accumulation of α2u-microglobulin in the epithelia and tubule lumina of the proximal tubules of the renal cortex of all males. Liver: 7 % ↑ liver weight in females, 7.5 % ↑ relative liver weight in males, Hypertrophy observed in the liver in 9 males and 2 females Clinical chemistry and urinalysis: ↓ Cl, ↓ globulin in males/females ↓ protein and albumin in females, ↑ transitional epithelial cells **60 mg/kg bw/day** Bodyweight: 4.8 % ↑ in males	1 (reliable without restriction) key study **Test material (EC name): imidazole**	Unpublished

	Kidney: 13 % ↑ kidney weight in males **20 mg/kg bw/day** No adverse effects observed NOAEL: 60 mg/kg bw/day based on liver and kidney effects at the next dose		
28 days (oral: gavage) Rat (Sprague-Dawley) 5/sex/dose 0, 62.5, 125, 250, 500 mg/kg bw/day Pre-guideline study	**500 mg/kg bw/day** Clinical signs and Bodyweight: marked salivation (scattered appearance of blood), unsteady gait and unkempt fur, ↑ female bodyweight Liver: 15/33 % ↑ relative liver weight in males/females, hepatomegaly in most males and 5/10 females Kidney: 10 % ↑ relative kidney weight in males, grading pattern in males Blood: ↓ Hb and Hct in both sexes, ↓ Red blood cell number in females **250 mg/kg bw/day** Clinical signs and bodyweight: salivation, ↑ female bodyweight Liver: 12/15 % ↑ relative liver weight in males/females, hepatomegaly in most males Kidney: 7 % ↑ relative kidney in males, grading pattern in males Blood: ↓ Hb, HC and RBC number in females **125 mg/kg bw/day** Bodyweight: ↑ female bodyweight Liver: 19 % ↑ relative liver weight in females, hepatomegaly in most males Kidney: faint grading pattern in kidney Blood: ↓ Hb in females **62.5 mg/kg bw/day** No toxicologically relevant effects reported NOAEL: 125 mg/kg bw/day	2 (reliable with restrictions) Supporting study **Test material (EC name): imidazole**	Unpublished

	(nominal) (male/female)		

A 28-day range finding study has been conducted. This study was not performed to GLP and a full study report was not written. The data summarised in table 15 and in the text below are based on a methodology document and tables of results. In this study, Wistar rats were dosed with 125, 250 or 500 mg/kg bw/day for 28-days. Irritation of the forestomach was observed at all dose levels. Significant increases in relative liver and kidney weight were observed in both sexes at 250 mg/kg bw/day and above. Absolute liver and kidney weight were also increased in females at these dose levels. Effects on red blood cell parameters were observed from 125 mg/kg bw/day. Although considered marginal at this dose, the severity of the effects increased with dose and was considered marked at 500 mg/kg bw/day. No NOAEL was derived as this was a range-finding study.

The 90-day study was conducted according to OECD TG 408. Imidazole was given daily by oral gavage to Wistar rats (10/sex/dose) at doses of 20, 60 and 180 mg/kg bw/day. No irritation of the gastrointestinal tract was reported in this study. The liver and kidney (in males) were identified as target organs.

At the top dose (180 mg/kg bw/day), relative liver weight in males (+7.5%) and females (+2.5%) was increased, which correlated with minimal to slight centrilobular liver cell hypertrophy in males (9/10) and females (2/10). The increase in absolute liver and kidney weights (+7/10 %, respectively) in females is considered to be secondary to the increase in bodyweight observed in these animals (+4.4 %) as no marked effects on relative weight were observed.

In top dose males, a significant increase in absolute and relative kidney weight was observed (+12/9 %, respectively). This was accompanied by an accumulation of alpha 2-microglobulin in the epithelia and lumina of the proximal tubules of the male renal cortex. The alpha 2-microglobulin was detected by Mallory Heindenhain staining technique and the specificity for alpha 2-microglobulin was demonstrated by immunohistochemical staining.The accumulation of alpha 2-microglobulin is considered a rat-specific phenomenon and has no toxicological relevance for humans.

Other effects observed at this dose level were minor changes in blood chemistry parameters (decreased serum globulin and chloride in males and total protein, globulin and chloride in females) and urinalysis (increase in the number of transitional epithelial cells detected in the urinary sediments).

No effects were observed in male and female reproductive organs (no effect on weight of the ovaries, uterus, testes and epididymides or histopathology of the uterus, ovaries, oviducts, vagina, female mammary gland, left testes, left epididymis, prostate gland, seminal vesicles), nor were any changes observed in sperm parameters (sperm number in cauda epididymis and testis, motility and morphology) in males or in the estrus cycle in females.

In addition, no substance-related effects were observed in the functional observational battery or motor activity measurements at any dose level.

No toxicologically significant effects were noted at 20 and 60 mg/kg bw/day. At 180 mg/kg bw/day (the highest dose tested), the magnitude of the liver effects is not sufficient to be considered adverse and, therefore, a NOAEL of 180 mg/kg bw/day can be derived from this study. The effects in the kidney have been dismissed in establishing the NOAEL as these are not considered relevant to humans.

Supporting information is also available from an old 28-day study. In this study, Sprague-Dawley rats (5/sex/dose) were administered 62.5, 125, 250 or 500 mg/kg bw/day of imidazole for 28-days. The liver (both sexes) and kidney (in males only) were

identified as target organs with effects observed in both organs from 125 mg/kg bw/day (see table above). Female bodyweight was also increased at doses > 125 mg/kg bw/day (no quantitative information available). No treatment related histopathological changes were noted in the kidney, heart, kidney, testes or ovaries at any dose. Red blood cells were also identified as a target organ. Haemocrit and red blood cell count were reduced in females at 250 mg/kg bw/day and above. In males, similar effects were also observed, but at the high dose only. At 125 mg/kg bw/day, haemoglobin was significantly reduced in females. No quantitative data is available as to the extent of this effect; however, as only minor reductions were observed in the range finding study (see above) and no effects were observed in the 90-day study (conducted to 180 mg/kg bw/day), it is unlikely these effects were toxicologically significant at this dose level. Similarly, the effects on the liver at 125 mg/kg bw/day were not confirmed in the two more modern studies and therefore the NOAEL is 125 mg/kg bw/day based on the increase in relative liver weight at 250 mg/kg bw/day.

Repeated dose toxicity: inhalation

No information available

Repeated dose toxicity: dermal

No information available

Repeated dose toxicity: other routes

No information available

Human information

No information available.

Summary and discussion of repeated dose toxicity

Information on repeated dose toxicity of imidazole is available from a 90-day study, a pre-guideline 28-day study and a 28-day range finding study. All studies were conducted via the oral route.

In the 28-day range finding study, conducted with Wistar rats, the liver and kidney were identified as target organs and gastric irritation was observed at all dose levels (≥ 125 mg/kg bw/day). No specific investigation was carried out on the male kidneys. In this study, effects on red cell parameters were observed from 125 mg/kg bw/day, although the severity of the effects at this dose level was marginal. No NOAEL was established as this was a range-finding study.

In the 90 day study, the liver and kidney were identified as target organs. In males, specific investigations demonstrated the effects in the kidney were due to the accumulation of alpha-2 microglobulin, a rat specific effect not considered of relevance in humans. A NOAEL of 180 mg/kg bw/day was identified.

In the 28-day study, the liver (in both sexes) and the kidney (in males only) were identified as target organs. No specific investigations were carried out on the male kidneys. In this study, effects on red cell parameters were also observed. A NOAEL of 125 mg/kg bw/day was identified from this study.

The NOAEL of 180 mg/kg bw/day derived from the 90-day study will be taken forward for risk characterisation. It is recognised that a lower NOAEL (125 mg/kg bw/day) was identified from the 28-day sub-acute study; however, this lower NOAEL is likely to be due to differences in the dose spacing between the two studies. In addition, the NOAEL of 180 mg/kg bw/day was derived from a better quality study.

In the updated dossier the registrant has included information on a 90-day study on 1-methyl imidazole as supporting information. This is the key analogue used in their weight of evidence assessment for reproductive toxicity effects. The results of this study are summarised in table 19 .

7.9.5. Mutagenicity

This endpoint was evaluated to check the adequacy of the data base and is not relevant to the original focus of the evaluation. In the initial evaluation two Ames tests, a mammalian cell gene mutation assay (HPRT) and an unscheduled DNA synthesis (UDS) assay were available. As limited information was available in the registration dossier for all studies apart from the in vitro mammalian gene mutation study, the study reports were requested and assessed.

The eMSCA requested an *in vitro* mouse lymphoma assay following the initial evaluation. Results of this and other studies evaluated are summarised in tables 16 and 17 below.

In vitro data

Table 16: Overview of experimental in vitro studies

Method	Results	Remarks	Reference
Ames OECD TG 471 (1983) *Salmonella typhimurium* TA1535, TA100, TA1537, TA98 2 experiments (standard and plate-incorporation) +/- metabolic activation Five doses between 20- 5000 µg/plate	Negative No cytotoxicity observed Positive controls included	1 (reliable without restriction) key study Test material (EC name): imidazole	Unpublished
Ames Non-guideline Test substances : Imidazole, hydantoin, hydantoic acid, and N-acetyl-imidazole *Salmonella typhimurium* TA97, TA98, TA100, TA102 2 experiments +/- metabolic activation Five doses between 0.62- 10 mg/plate	Negative (imidazole and its metabolites) - data presented as the mean of 2 experiments, using three plates per test point No cytotoxicity observed Positive controls included	2 (reliable with restrictions) Supporting study Test material (EC name): imidazole	Forster R et al. (1992)
Mammalian cell gene mutation Chinese hamster lung fibroblasts (V79) OECD 476 2 experiments: Six doses between 22 - 700 µg/ml (equivalent to 10 mM)	Equivocal* Positive controls included No cytotoxicity observed	1 (reliable without restriction) Key study Test material (EC name): imidazole	Unpublished

Unscheduled DNA synthesis OECD 482 Rat hepatocytes Six doses between 0.24 – 4 mg/ml	Negative Positive controls included 50 % survival was at approximately 1 mg/ml	2 (reliable with restrictions) Supporting study Test material (EC name): imidazole	Forster R et al. (1992)
mammalian cell gene mutation assay mouse lymphoma (OECD TG 476) Test concentrations: 1st Experiment: With and without S9 mix (4-hour exposure period): 0, 87.5, 175.0, 350.0, 700.0 µg/mL 2nd Experiment Without S9 mix (24-hour exposure period): 0, 87.5, 175.0, 350.0, 700.0 µg/mL With S9 mix (4-hour exposure period): 0, 100.0, 200.0, 400.0, 700.0 µg/mL Positive control substance(s): methylmethanesulfonate; cyclophosphamide	negative; with and without S9; cytotoxicity: no ; vehicle controls positive controls included	1 (reliable without restriction) Key study Test material (EC name): imidazole	Unpublished

* The results of this study was considered negative by the registrants

The results of both[5] Ames studies were negative. The key Ames test was conducted according to OECD 471 (1983) and, although negative, does not, therefore, cover all the strains recommended in the current 1997 guideline (an additional strain of either *E. coli* WP2 uvrA, or *E. coli* WP2 uvrA (pKM101), or *S. typhimurium* TA102 is now required). The strain *S. typhimurium* TA102 was included in the second Ames test, the results of which have been published in a peer-reviewed paper (Forster et al, 1992). However, it should be noted that although the results for this strain were negative (as were the results of all the other strains tested), the results from both initial and repeat experiments (each consisting of three plates) have been averaged together and no information on the variability of the data is available to determine whether this approach is justified. In the absence of such data, overall confidence in the results of this study is reduced.

The results of the HPRT assay are considered equivocal by the eMSCA on the basis of isolated increases in mutation frequency above the laboratory's three-fold threshold; in particular an increase observed at the top concentration in one test with S9. The result of an *in vitro* UDS study was negative.

The potential of imidazole to cause gene mutations *in vitro* has been investigated in a modern mouse lymphoma assay which was requested following the initial evaluation. No toxicologically significant increases in mutation frequency were observed. The positive controls gave the expected results. Overall, imidazole is not mutagenic *in vitro* on the basis of this study.

[5] The dossier update included an additional Ames test from 1979. This was also negative and did not contain information on additional strains. It has not been summarised in this report.

No information on *in vitro* cytogenetics is available. However, this is not considered a concern as the results from a reliable *in vivo* micronucleus study are available (see table 17 below).

In vivo data

Table 17: Overview of experimental in vivo genotoxicity studies

Method	Results	Remarks	Reference
Micronucleus study (bone marrow) Imidazole hydrochloride OECD 474 Single oral dose Mouse, NMRI, 5/sex/dose 500, 1000, 2000 mg/kg bw	Negative Clinical signs observed ≥ 500 mg/kg bw including irregular respiration and piloerection in all doses groups and squatting posture and death of one animal in the high dose group Positive controls included	1 (reliable without Restriction key study Test material (EC name): imidazole	Unpublished

One study investigating the potential of imidazole to cause cytogenetic damage to the bone marrow of mice is available. No increase in micronucleus formation was observed following oral administration. No change in the P/N ratio was observed; however, detection of imidazole in the blood (see section 5.1) suggests the bone marrow will have been exposed. In addition, death of one animal in the high dose group suggests the maximum tolerated dose was exceeded.

No information is available on Imidazole's potential to cause gene mutations *in vivo*.

Summary and discussion of mutagenicity

The mutagenic profile of imidazole has been well investigated in vitro and in vivo (mouse bone marrow micronucleus test). Although the majority of the studies were negative, including the micronucleus test, not all recommended strains were investigated in the guideline Ames test and the results of the HPRT assay were considered equivocal. However, the mouse lymphoma test, requested as a result of this evaluation, gave negative results providing additional reassurance that imidazole is not mutagenic in vitro.

Overall, there are no remaining concerns for mutagenicity, and no further information is needed.

7.9.6. Carcinogenicity

No information available.

Imidazole is considered to be non-genotoxic based on the available information and no effects of concern (e.g. hyperplasia) were observed in the 90-day repeated dose study. Therefore, no further information is required.

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

As noted earlier in this document during the initial evaluation the registrants submitted a CLH dossier including the classification Repr. 1B. Consequently the human health hazard evaluation looked at whether there was a need to consider further fertility testing. To assess this, study reports for all relevant endpoints (developmental toxicity and repeated dose toxicity) were requested and evaluated in full.

Developmental toxicity

The registration dossier contains robust study summaries for a standard developmental study in rats and an *in vitro* study for imidazole.

The full study report for the developmental study was requested as was the paper for the *in vitro* study. Both were assessed and are summarised below.

Table 18: Overview of experimental studies on developmental toxicity

Method	Results	Remarks	Reference
OECD 414 Oral (gavage) Rat (Wistar) 25/dose 0, 20, 60, 180 mg/kg bw/day day 6-19 gestation	**180 mg/kg bw/day** *Maternal toxicity* Salivation (6 dams), vaginal hemorrhage (1 dam)*, 13 % ↓ food consumption days 6-8, 45% ↓ bw gain days 6-8 and 34% ↓ bw gain days 17-20** *Developmental toxicity* Total resorption in 3 dams (mainly late) → 43 % post implantation loss *v.* 8 % in controls → 6.3 % live fetuses/litter *v.* 9 % in controls 14 % ↓ mean fetal bw, 13/142 runts **** *Teratogenicity* *Malformations* Total: 16 out of 132 fetuses, 11 % fetuses/litters External malformations: cleft palate (3 pups) and anasarca (11 pups), (total 13/132 pups, 9 % fetuses/litter) Soft tissue malformations: misshapen kidney (1 pup) (1/59 pups, 1.1 % fetuses/litter) Skeletal malformations: shortened scapula (4 fetuses), bent radius/ulna (2 fetuses), misshapen sacral vertebra (1 fetus), malpositioned and bipartite sternebra (2 fetuses), (total 7/73fetuses, 8 % fetuses/litter)	1 (reliable without restriction) key study Test material (EC name): imidazole	Unpublished

<table>
<tr>
<td></td>
<td>Variations

No external variations, soft tissue variations (dilated renal pelvis and/or ureters) and skeletal variations (affecting skull, ribs, vertebral column and sternum) observed in 86/132 fetuses, 70 % fetuses/litter

60 mg/kg bw/day

Malformations

Total, 1/202 fetuses (misshapen sacral vertebra), 0.5 % fetuses/litter

Variations

120/202 fetuses, 61 % fetuses/litters

20 mg/kg bw/day

Malformations

Total, 2/194 fetuses (misshapen sacral vertebra, absent cervical vertebra), 1 % fetuses/litter

Variations

98/194 fetuses, 51 % fetuses/litters

Control

Malformations

Total, 1/195 fetuses (malpositioned and bipartite sternbra), 0.6 % fetuses/litter

Variations

101/195 fetuses, 52 % fetuses/litters

NOAEL: 60 mg/kg bw/day based on malformations at the next dose level</td>
<td></td>
<td></td>
</tr>
<tr>
<td>In vitro study

Embryos from Sprague Dawley rats and CD-1 mice were explanted on gestation day 10.5 (rats) or day 8.5 (mice) and cultured for 48 hours on a roller culture

Embryos (between 6-10) incubated with 30 or 60 µg/ml imidazole for 48 hour and assessed for viability, growth, development and presence of abnormalities.</td>
<td>Rat

<table>
<tr><th>Dose (ug/ml)</th><th>Dead (%)</th><th>Abnormal (%)</th></tr>
<tr><td>0</td><td>0</td><td>0</td></tr>
<tr><td>30</td><td>20</td><td>62.5</td></tr>
<tr><td>60</td><td>37.5</td><td>80</td></tr>
</table>

<table>
<tr><th>Dose (ug/ml)</th><th>Yolk sack diameter (mm)</th><th>No of Somites</th><th>Crown rump length (mm)</th></tr>
<tr><td>0</td><td>4.3 ± 0.2</td><td>36 ± 1</td><td>4.3 ± 0.1</td></tr>
<tr><td>30</td><td>4.3 ±</td><td>33 ± 2</td><td>3.6 ±</td></tr>
</table>
</td>
<td>2 (reliable with restrictions)

Supporting study

Test material (EC name): imidazole</td>
<td>Daston G.P et al, 1989</td>
</tr>
</table>

	0.3		0.3*
60	4.3 ± 0.3	34 ± 2	3.6 ± 0.4

Mouse

Dose (ug/ml)	Dead (%)	Abnormal (%)
0	0	0
30	33.3	50
60	83.3	100

Dose (ug/ml)	Yolk sack diameter (mm)	No of Somites	Crown rump length (mm)
0	4.1 ± 0.1	37 ± 1	3.9 ± 0.2
30	3.8 ± 0.2	35 ± 2	3.5 ± 0.1
60	4.1 ± 0.1	34 ± 1	3.4 ± 0.2

In both species there was significant embryolethality. The abnormalities produced at both doses were decreased brain size and clear blisters.

The developmental toxicity of imidazole was investigated in a guideline pre-natal developmental study. In this study, pregnant Wistar rats (25/dose) were administered, via oral gavage, 0, 20, 60 or 180 mg/kg bw/day imidazole between days 6-19 of gestation.

At 180 mg/kg bw/day, maternal toxicity manifested as significantly reduced food intake (-13 %) on days 6-8 and was reflected in reduced bodyweight gain (-45 %) over the same period. Bodyweight gain was also reduced on days 17-20 (-34 %); however, as terminal body weight was comparable across all groups, this decrease is likely attributable to a significant decrease in gravid uterus weight (-26 %), high rate of resorptions (see below) and lower mean fetal body weight (see below), rather than maternal toxicity. No signs of maternal toxicity were observed at 60 or 20 mg/kg bw/day.

At 180 mg/kg bw/day, there was an increase in the number of late resorptions (3.1 % v. 0.1 % in the controls). Three dams in this dose group resorbed all implants primarily during the last days of treatment and thus had no live foetuses at termination. Consequently, post-implantation loss was higher than in control (43 % vs. 8 % in control) and was outside the historical control range (mean value: 7.0 %; range: 3.8 – 11 %). Mean foetal weight was also reduced (-14 %) at this dose level. No effect on sex distribution was observed.

In addition, in the top dose, the incidence of external malformations (anasarca and/or cleft palate) was significantly increased. About 9 % of the high dose foetuses/litter were

affected (13/132 fetuses; in 7/22 litters) while no such changes were observed in the control. No incidences of these malformations were recorded in the available historical control data, increasing concern for these effects.

The total proportion of skeletal malformations (shortened scapula, bent radius, bent ulna, malpositioned and bipartite sternebrae) was also statistically significantly increased with about 8 % foetuses/litter (7/73 fetuses in 5/21 litters) affected compared to 1 % in the control. This incidence is outside the historical control range (foetuses/litter range: 0.0-5.3%; mean 1.6 %). Soft tissue malformations were limited to a single finding of misshapen kidney in one male fetus from the high dose group.

Soft tissue variations (dilated renal pelvis and ureter) were significantly increased in foetuses from high dose dams compared to controls (27 % vs. 6.4 %) and were above the historical control range (4.4 % - 22.2 %; mean 11.6 %). Incidences of skeletal variations, mainly delays of the ossification process, were also statistically significantly increased from 91 % in the control group to 98.4 % in the high dose group, slightly above the upper limit of the historical control range (92.6 %; range 87-98.1 %). These increases in variations are indicative of reversible delays in the kidney and skeleton development.

The effects observed at 60 or 20 mg/kg bw/day were not significantly different from the control. The NOAEL for maternal toxicity, developmental toxicity and teratogenicity was 60 mg/kg bw/d.

Developmental toxicity was also observed in an *in vitro* whole embryo culture test employing rat and mouse embryos (Daston et al., 1989). In this study, exposure to 30 and 60 µg/ml imidazole resulted in embryo lethality (up to 83% in mice at 60 ug/ml) and abnormalities (decreased brain size and clear blisters) in up to 100 % of embryos (see table for more details).

Classification

On the basis of these effects imidazole meets the CLP criteria for classification as a category 1B developmental toxicant (H360D). This was confirmed by RAC and the harmonised classification Repr. 1B, H360D (May damage the unborn child) was included in the 7th ATP to the CLP regulation.

Effects on Fertility

No standard fertility study is available for imidazole.

In the 90-day sub-chronic study (section 7.9.4), no effects in male and female reproductive organs (no effect on weight of the ovaries, uterus, testes and epididymides or histopathology of the uterus, ovaries, oviducts, vagina, female mammary gland, left testes, left epididymis, prostate gland, seminal vesicles) were observed, nor were any changes observed in sperm parameters (sperm number in cauda epididymis and testis, motility and morphology) or in the females estrus cycle up to the highest dose tested (180 mg/kg bw/day)[6].

The results of the 90-day sub-chronic toxicity study showed no signs of reproductive toxicity at the highest dose tested (180 mg/kg bw/day). The results suggest that no major effects on fertility are likely to be observed at doses below 60 mg/kg bw/day.

It is recognised, however, that none of the studies available during the initial evaluation inform on whether pup development, sexual maturation or mating behaviour may be

[6] Dose levels in repeated dose studies are limited by the corrosive nature of the test substance (acanthosis of the forestomach was observed in the 28-day range-finding study).

adversely affected at a dose below the critical NOAEL of 60 mg/kg bw/day. Although it is possible a lower NOAEL could be identified from the multigeneration study for effects not investigated in the available studies, the overall toxicological profile of the substance indicates that the occurrence of an unexpectedly, very potent effect on reproduction (occurring at dose levels much lower than those at which liver and developmental effects occur) is unlikely.

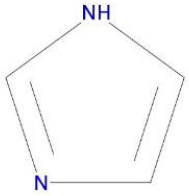
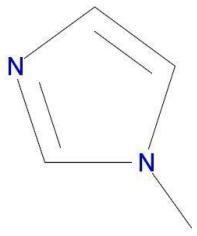
In the course of the initial evaluation the registrant noted that a number of reproductive toxicity screening studies would soon be available on some closely-related structural analogues which could be used to address the fertility concern in a weight of evidence assessment. The final decision contained a request for documentation and justification concerning these substances that the registrants believe would be applicable in an appropriate weight of evidence assessment of the reproductive toxicity effects of imidazole.

In the updated dossier the registrants included a read-across/weight of evidence assessment using information from imidazole, 1-methyl imidazole (CAS no 616-47-7) , and limited supporting evidence from other related imidazoles (2-methylimidazole (CAS no. 693-98-1), 2-ethyl-4-methylimidazole (CAS no. 931-36-2), 1,2-dimethylimidazole (CAS no. 1739-84-0), and 1-(3-Aminopropyl) imidazole (CAS no. 5036-48-6). The updated dossier also contains summaries of reproductive toxicity screening studies (OECD TG 421 or 422) conducted on these substances.

The registrants consider imidazole and its mono-alkylated analogue 1-methylimidazole to be read-across analogues based on structural, physico-chemical and predicted metabolic similarities as well as comparable toxicological properties. Modelling (Tissue Metabolism Simulator OASIS TIMES v. 2.27.17.6 (Laboratory of Mathematical Chemistry at the Bourgas University)) suggests that the target substance is a predicted metabolite of the source substance.

The available mammalian toxicity data for imidazole and 1-methylimidazole are summarised in the table below.

Table 19: Comparison of toxicity data on imidazole and 1-methylimidazole

	Target chemical	**Source chemical**
	Imidazole	1-methylimidazole
		
CAS	288-32-4	616-47-7
SMILES	c1c[nH]cn1	Cn1ccnc1
Formula	C3H4N2	C4H6N2
Purity / Impurities (w/w)	≥99.5 ≤99.9%	≥95 ≤100%
Acute toxicity (oral)	**LD50 970 mg/kg bw** (rat, similar to OECD TG 401). Clinical symptoms were described as convulsions and disequilibria with lateral posture. Necropsy not reported.	**LD50 1144 mg/kg bw** (rat, similar to OECD TG 401). Clinical symptoms reported as convulsions. No abnormalities at necropsy

Acute toxicity (inhalation)	Waiver: corrosive	Waiver: corrosive
Acute toxicity (dermal)	Waiver: corrosive	LD50 400-640 mg/kg bw (rabbit, similar to OECD TG 402)
Skin/eye irritation	Skin and Eye corrosive (rabbit, similar to OECD TG 404 and 405)	Skin and Eye corrosive (rabbit, similar to OECD TG 404 and 405)
Skin sensitization	Waiver: corrosive	Waiver: corrosive
Repeated dose toxicity	**Sub-acute:28-day, rat** Oral, gavage: doses 0, 62.5, 125, 250, 500 mg/kg bw/d NOAEL: 62.5 mg/kg bw/d LOAEL: 125 mg/kg bw/d Target organs: Liver (increased weight), red blood cell changes (Female ≥125 mg/kg bw/d Male ≥500 mg/kg bw/d) **Sub-chronic:** **OECD TG 408**, rat Oral, gavage: doses 20, 60, 180 mg/kg bw/d **NOAEL: 60 mg/kg bw/d** Target organs: - Liver (increased weight) and changed blood chemistry - Kidneys (alpha 2-Macroglobulin accumulation)	**Sub-acute: OECD TG 422,** rat Oral, gavage: doses 10, 30, 90 mg/kg bw/d NOAEL: 30 mg/kg bw/d LOAEL: 90 mg/kg bw/d Target organs: Increased urea levels in both sexes and effects on other urinalysis parameters in males **Sub-chronic:** **OECD TG 408,** rat Oral, gavage: doses 10, 30, 90 mg/kg bw/d NOAEL: 90 mg/kg bw/d Target organs: - Liver (increased organ weight and slightly changed blood chemistry) - Kidneys (slight effects on functionality)
Mutagenicity	Not mutagenic In vitro (OECD 476, OECD 471, similar to OECD 482) In vivo (OECD 474)	Not mutagenic In vitro (OECD 471, OECD 476, OECD 487)
Carcinogenicity	No data	No data
Reproductive toxicity	No data	**OECD TG 422,** rat Oral, gavage: doses 10, 30, 90 mg/kg bw/d **NOAEL parental 30 mg/kg bw/d** **NOAEL reproduction and developmental: 90 mg/kg bw/d** Target organs: Parental tox: increased urea levels in both sexes and effects on other urinalysis parameters in males
Developmental toxicity	**OECD TG 414**, rat Oral, gavage: doses: 0, 20, 60, 180 mg/kg bw/d **NOAEL (maternal toxicity, fetotoxicity, and teratogenicity): 60 mg/kg bw/d** Target organs: (at 180 mg/kg bw/d) Maternal toxicity: decreased food consumption bw gain and uterus weight	**OECD TG 414***, rat Oral, gavage: doses: 0, 10, 30, 90 mg/kg bw/day **NOAEL (maternal toxicity, fetotoxicity, and teratogenicity): 90 mg/kg bw/d** (preliminary) **OECD TG 422**, rat No indication of developmental toxicity from OECD TG 422 (see

	Fetotoxicity: reduced mean foetal weight and increased number of resorptions Teratogenicity: increased rate of variations and malformations	above)

* preliminary and not fully complete results

The eMSCA considers information from the close analogue, 1-methyl imidazole and the weight of evidence assessment well justified.

Summary and discussion of reproductive toxicity

Developmental toxicity

In a standard developmental toxicity study, oral administration of 180 mg/kg bw/day imidazole during days 6-19 of gestation resulted in developmental toxicity characterised by external (ansarca and cleft palate) and skeletal malformations (shortened scapula, bent radius, bent ulna, malpositioned and bipartite sternebrae) and late resorptions. Maternal toxicity at this dose level consisted of a reduction in food consumption and bodyweight gain during days 6-8 of gestation. No adverse effects were noted at 60 or 20 mg/kg bw/day.

As agreed by RAC in 2013 imidazole is classified as a category 1B developmental toxicant (H360D). This classification was included in the 7th ATP to the CLP Regulation.

The eMSCA considers that the existing pre-natal developmental toxicity study is a modern guideline study, in which a clear NOAEL was identified. The study is adequate to support a robust risk assessment and in the absence of any substance-specific information suggesting that imidazole would be a significantly more potent developmental toxicant in another species, the eMSCA does not consider that further investigations of developmental toxicity are necessary.

Fertility

No standard fertility study is available for imidazole.

The registrants have developed a read-across/weight of evidence assessment using information from imidazole, 1-methyl imidazole, and limited supporting evidence from other related imidazoles. The eMSCA considers information from the close analogue, 1-methyl imidazole and the weight of evidence assessment well justified.

The repeated dose toxicity of imidazole, and the structural analogue1-methylimidazole, has been thoroughly investigated in modern 90-day studies. There was no evidence of toxicity to the reproductive organs and tissues in either of these 90-day studies. 1-methylimidazole has also been tested in a standard reproductive toxicity/repeated dose screening study (OECD TG 422) and no evidence of developmental or reproductive toxicity was reported at doses of up to 90 mg/kg/day, the highest dose tested.

The eMSCA concludes that there are no concerns for adverse effects on fertility from investigation of the reproductive tissues and organs from the 90-day study conducted with the registered substance. In addition, the eMSCA concludes that no additional concerns for adverse effects on fertility are raised from the weight of evidence assessment of other close structural analogues, in particular 1-methyl imidazole.

Overall, the eMSCA does not consider there is concern for fertility warranting further investigation.

7.9.8. Hazard assessment of physico-chemical properties

Imidazole is a highly pure (>99 %w/w) slightly yellow crystalline solid with a moderately high melting point (89.8 °C) and a low volatility (3.27 x 10^{-10} Pa at 25 °C). It is very soluble in water (663 g/l at 20 °C), with an octanol/water partition coefficient (Log Pow = -0.02 at 25 °C) which indicates its is very unlikely to bio-accumulate and does not require classification with regards to flammability, oxidising and explosive properties.

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

Table 20: Available dose-descriptors for imidazole as a result of the hazard assessment

Endpoint		**Dose descriptor**	**Qualitative assessment**	**Remarks on study**
Acute toxicity	oral	LD50: 970 mg/kg bw		The LD50 (oral, rat) value derived from the key-study was ca. 970 mg/kg bw.
Irritation / Corrosivity	skin	corrosive		Imidazole is corrosive to skin under occlusive conditions and irritating to corrosive to the rabbit eye. The test methods were comparable with the corresponding OECD test guidelines 404 and 405.
Irritation / Corrosivity	eye	highly irritating		
Irritation / Corrosivity	respiratory tract			
Sensitisation	skin			Imidazole is corrosive to the skin and for animal welfare reasons an *in vivo* skin sensitisation test is not required according to REACH (1907/2006/EC) Annex VII, 8.3, column 2.
Repeated dose toxicity: sub-acute / sub-chronic / chronic	oral	NOAEL: Target organs: liver; : kidneys 180 mg/kg bw/day		In a 90-day oral gavage study Wistar rats were treated with imidazole at dose levels of 0, 20, 60 and 180 mg/kg bw/d. Liver and kidney were identified as the target organs. A NOAEL of 60 mg/kg bw/d was derived.
Mutagenicity	*in vitro / in vivo*		Genetic toxicity: negative	No mutagenicity was observed in a guideline Ames tests, an *in vitro* UDS test and in an *in vivo* mouse micronucleus test. The result of the HPRT Test with V79 cells was considered negative/equivocal and another *in vitro* study is

				proposed to confirm imidazole is non-mutagenic.
Carcinogenicity	oral			No data available
Reproductive toxicity: fertility impairment	oral			No changes of the male and female reproductive organs including sperm quality and estrus cycle were noted in any of the dose groups up to and including 180 mg/kg bw/d in a rat 90-d oral gavage study.
Reproductive toxicity: developmental impairment	oral	NOAEL: 60 mg/kg bw/day		Imidazole, when tested in the rat according to OECD Guideline 414 at dose levels of 20, 60, and 180 mg/kg bw/d, was developmentally toxic and teratogenic at 180 mg/kg bw/d. The NOAEL for maternal toxicity, developmental toxicity and teratogenicity was 60 mg/kg bw/d.

Long term DNELs have to be calculated for workers. There is no exposure to consumers and therefore DNELs have not been calculated.

Table 21: Selection of the critical DNEL(s)/DMEL(s) and/or qualitative/semi-quantitative descriptor for critical health effects

Exposure pattern	Route	Descriptor	eMSCA DNEL / DMEL	(Corrected) Dose descriptor *	Most sensitive endpoint	Justification
Long-term - systemic effects	dermal	DNEL (Derived No Effect Level)	1.2 mg/kg bw/day	NOAEL: 60.0 mg/kg bw/day (based on AF of 100)	repeated dose toxicity	see "discussion"
Long-term - systemic effects	inhalation	DNEL (Derived No Effect Level)	8 mg/m³	NOAEC: 106.0 mg/m³ (based on AF of 25)	repeated dose toxicity	see "discussion"
Long-term - local effects	dermal					Local effects were qualitatively assessed (see" discussion").
Long-term - local effects	inhalation					Local effects were qualitatively assessed (see" discussion").

* The (corrected) dose descriptor starting points have been automatically calculated by multiplying the values of the fields "D(N)MEL" and "Assessment factor" provided in the Endpoint summary of

IUCLID section 7. Toxicological information. It reflects the value after any corrections, e.g. route-to-route extrapolation. See column "Justification" for the rationale behind such modifications and the use of assessment factors.

Discussion

The eMSCA has derived DNELs for both the inhalation and dermal route as follows:

There are no chronic studies available. Information on repeated dose systemic effects is available from an oral 90-day repeated dose study in rats (NOAEL of 180 mg/kg bw/day) and an oral pre-natal developmental toxicity study in rats (NOAEL of 60 mg/kg bw/day). As it is not clear at this stage which study will derive the most conservative NOAEL, the NOAELs from both studies have been used to calculate the DNELs for the dermal and inhalation routes, as specified in the guidance (Chapter R8: characterisation of dose [concentration]-response for human health).

- Dermal exposure:

Route to route extrapolation
In the absence of a dermal toxicity study in animals, route to route extrapolation will be used to calculate a dermal NOAEL from an oral NOAEL.

To convert the oral NOAELs to a dermal NOAEL, the eMSCA assumed 100 % absorption for the oral route in the rat and 100 % absorption through human skin.

The value chosen for absorption via the oral route (100 %) is consistent with the information from the toxicokinetic studies.

Starting value taken from:	Dermal NOAEL
Oral sub-chronic study in rats	180 x 100/100 = 180 mg/kg bw/day
Oral pre-natal developmental toxicity study	60 x 100/100 = 60 mg/kg bw/day

Default Assessment factors
To convert the rat dermal NOAELs to the human equivalent, the eMSCA has applied the same default assessment factors as applied by the registrant:

- A default assessment factor of 4 to take account of differences in sensitivity between experimental animals and humans (interspecies differences);
- A default assessment factor of 5 to take account of differences in sensitivity within the human population (intraspecies differences in workers); and
- An additional factor of 2 to convert the dermal NOAEL derived from the sub-chronic study to a chronic NOAEL (not to be applied to the NOAEL from the pre-natal developmental toxicity study).

In addition, the eMSCA is of the opinion that an additional interspecies factor is required. This factor is to cover mainly toxicodynamic differences rather than toxicokinetic differences. As there is no information available suggesting the adverse effects seen in the studies would not occur in humans or that humans are less sensitive to these effects than rats this factor cannot be omitted.

- A default of assessment factor of 2.5 to take account of any remaining differences between rats and humans.

Starting value taken from:	Dermal DNEL
Oral sub-chronic study in rats	180 / (4 x 5 x 2.5 x 2) = 1.8 mg/kg bw/day
Oral pre-natal developmental toxicity study	60 / (4 x 5 x 2.5) = 1.2 mg/kg bw/day

The lowest DNEL was derived using the NOAEL from the oral pre-natal developmental toxicity study.

Inhalation exposure:

No studies are available via the inhalation route in animals. Therefore, the eMSCA has used route to route extrapolation to convert the oral NOAELs in rat to an equivalent inhalation NOAEC (8 hr).

The rat oral NOAELs were converted by taking into account the respiratory volume in rats (correction factor of 0.38 m3/kg (8 hours)) and then multiplying by the ratio of oral absorption in rats (100 %) vs. inhalation absorption in humans (in the absence of information this was assumed to be 100 %). The resulting value was then corrected for the difference in calorie demand of animals at rest and calorie demand under light activity (correction factor of 0.67).

Starting value taken from:	Inhalation NOAECs
Oral sub-chronic study in rats	180 x (1/0.38) x (100/100) x 0.67 = 317 mg/m³
Oral pre-natal developmental toxicity study	60 x (1/0.38) x (100/100) x 0.67 = 105.79 mg/m³

Assessment factors
To convert the rat inhalation NOAECs to the human equivalents, the eMSCA has applied the following default assessment factors:

- A default assessment factor of 5 for intraspecies differences (workers) was applied.
- An additional factor of 2 to convert the inhalation NOAEC derived from the sub-chronic study to a chronic NOAEL (not to be applied to the NOAEL from the pre-natal developmental toxicity study).

Based on the same arguments given for derivation of the dermal DNEL (see above), the eMSCA believes an assessment factor for remaining interspecies differences (factor of 2.5) should also be applied.

Starting value taken from:	Inhalation DNEL
Oral sub-chronic study in rats	317/ (5 x 2.5 x 2) = 12 mg/m³
Oral pre-natal developmental toxicity study	105.8/ (5 x 2.5 x 2) = 8 mg/m³

The lowest DNEL was derived using the NOAEL from the oral pre-natal developmental toxicity study

Conclusion

The DNEL values of **1.2 mg/kg bw/day (dermal)** and **8 mg/m³ (inhalation)** are taken forward to risk characterisation.

Long term exposure - local effects
Not evaluated at this stage.

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

Imidazole was included in the 7th ATP to Regulation (EC) No 1272/2008 on classification, labelling and packaging (CLP) of substances and mixtures published in 2015 and is listed

by Index number 613-319-00-0 in Annex VI, Part 3, Table 3.1 (list of harmonised classification and labelling of hazardous substances) for;

Acute Tox. 4 H302

Skin Corr. 1C H314

Repr. 1B H360D

On the basis of this evaluation the eMSCA considers that no further information is needed and no other classification is warranted.

7.10. Assessment of endocrine disrupting (ED) properties

7.10.1. Endocrine disruption – Environment

The potential for endocrine disruption in the environment was not evaluated. However, the eMSCA notes that the substance is readily biodegradable and has a low bioaccumulation potential.

7.10.2. Endocrine disruption - Human health

During the evaluation, the potential for imidazole to have endocrine disrupting activity was identified and the need to generate information to further investigate this was evaluated.

Table 22: Overview of experimental studies on endocrine disruption

Method	Results	Remarks	Reference
Non-guideline study Rats (10/group, sex unspecified) 3 doses between 10-300 mg/kg bw Controls – saline Subcutaneous injection Samples of serum and testicular interstitial fluid (TIF) collected 2h later.	*Serum testosterone levels* Serum testosterone decreased in a dose-related manner and was approximately 60 %* lower than control at the top dose level. *TIF testosterone levels* TIF testosterone levels decreased in a dose-related manner and were approximately 80 %* lower than controls at the top dose level. *TIF volumes* TIF volume decreased in a dose-related manner and was approximately 40 %* lower than controls at the top dose level. Serum LH Serum LH was decreased at the top dose by between approximately 40 – 50 %* compared to the control.	Reliability 3 (not reliable) Test material (EC name): imidazole	Adams *et al* (1998)

*Values are only approximate as estimated from graphs within the paper.

In a paper by Adams *et al* (1998), increasing single doses of imidazole (0-300 mg/kg bw) were administered to rats (10 male rats/group) via subcutaneous injection and samples of serum and TIF (testicular interstitial fluid) investigated at a single time point only (after 2 hours). Treatment with imidazole appeared to lead to a dose dependent decrease

in serum testosterone levels, TIF testosterone levels and TIF volume; however, detailed results were not provided and the values in the table are estimated from the graphs within the paper. Serum LH levels also appeared decreased at the top dose (300 mg/kg bw), suggesting imidazole can suppress LH secretion from the pituitary gland. A similar effect on testosterone levels was observed with other imidazoles.

This study suggests imidazole may have endocrine disrupting properties on sex hormones when given subcutaneously to rats. However, it is noted that although the apical multigeneration study is not available for imidazole, there were no adverse effects potentially related to endocrine disruption in the available standard oral studies (e.g. no effects on sperm parameters in the 90-day study; no effects on sex ratio and repro organs in the pre-natal developmental toxicity study). It is possible that administration via the subcutaneous route results in larger plasma peaks and slower metabolic clearance than would be seen orally, and casts doubt on whether any effect would be observed via more relevant routes of exposure. Alternatively, the effects reported by Adams *et al* (1998) may be chance findings not related to treatment. To clarify these findings, the potential endocrine disrupting properties of imidazole on sex hormones could be further explored by performing a battery of validated *in vitro* assays (e.g. aromatase assay, steroidogenesis assay and AR binding assay) as outlined in the OECD's draft guidance on Standardised Test Guidelines for Evaluating Chemicals for Endocrine Disruption (April 2012). However, as these findings are of questionable significance and since such testing would not have any significant impact on the current regulatory position of imidazole as it is already classified as Repr 1B; H360D (May damage the unborn child), further testing is not considered necessary.

7.10.3. Conclusion on endocrine disrupting properties

No further testing necessary.

7.11. PBT and VPVB assessment

Not relevant for the substance evaluation. The substance is not persistent or bioaccumulative within the meaning of the Annex XIII criteria.

7.12. Exposure assessment

7.12.1. Human health

The exposure assessments submitted by the registrants cover workers engaged in the manufacture and use of imidazole at industrial sites, also professionals using products containing imidazole. Imidazole is not supplied to consumers on its own or in mixtures and, based on the information provided by registrants, exposure to this substance via articles is not expected. The following table identifies the scenarios listed on the ECHA dissemination site[7] .

Table 23: Exposure scenarios listed in the ECHA dissemination site

Short description of exposure scenario	Life Stage to be covered				Process category (PROC)
	Man	Form	End Use	Servi	

[7] Site accessed June 2018.

			Industrial	Professional	Consumer		
Manufacture of substance	X						1, 2, 8B
Formulation of Preparations		X					1,2,3, 5, 8A, 8B, 9, 28
Use as Intermediate, Use as monomer			X				1, 2, 3, 4, 8A, 8B, 9, 28
Use in laboratories			X	X			15
Use in industrial chemical processes			X				1,2,3,4,5,7, 8A, 8B, 9, 10, 13, 14, 21, 28
Use in construction chemicals, use in coatings				X			2, 3, 4, 5, 8A, 8B, 9, 10, 11, 13, 14,19, 21, 28

7.12.1.1. **Worker**

The worker exposure assessment is based on modelled data using the EASY TRA model and the Advanced REACH Tool (ART) version 1.5. Calculations have been performed to estimate full-shift inhalation and dermal exposure. Estimates have not been made of potential short-term peak exposure. Since the available data do not permit DNELs to be calculated for short-term effects arising as a result of the corrosivity of this substance (this includes effects to the skin and eyes and also potentially to the respiratory tract if the substance is inhaled) a qualitative assessment has been made to assess the likelihood that adverse short-term effects will be avoided.

The eMSCA has been able to replicate the modelled exposure assessments using the information provided in the updated chemical safety report (CSR). Since the eMSCA does not have access to the Easy TRA tool which is an adaptation of the ECETOC TRA tool, the eMSCA used the ECETOC TRA tool version 3 to replicate calculations performed with the Easy TRA tool. The eMSCA confirmed that the scenarios for which the ECETOC TRA tool version 3 and ART version 1.5 have been used are within the stated range of applicability for these tools.

Several processes are carried out at temperatures up to 115°C. The registrants have confirmed that the vapour pressure of imidazole at this temperature remains within the vapour pressure band of the low vapour pressure category adopted within the ECETOC TRA tool (0.01 – 500 Pa) and hence the exposure values calculated assuming ambient temperature also cover activities at operating temperatures of up to 115°C.

Where imidazole is used in mixtures, the concentration in mixtures has been taken into account using a linear approach which is permitted within the EASY TRA tool rather than the concentration band approach that is used within the ECETOC TRA tool. This will result in a less precautionary exposure prediction. However, given that the vapour pressure of imidazole (0.327 Pa) is at the lower end of the low vapour pressure band used within the TRA tool, there is the potential for calculations made using the default concentration modifiers in the TRA tool to overestimate exposure in this case. To explore how the use of the exact concentration rather than the concentration band approach affects exposure estimates and RCRs, the eMSCA calculated exposures using both approaches.

Dermal exposure predictions have not been adjusted to take account of LEV which will increase conservatism in the dermal exposure assessment.

Manufacture

Imidazole is manufactured in a closed process, breached only for sampling and maintenance. The starting materials are reacted together at elevated temperature and pressure. The CSR provides details of the measures that are applied to ensure containment of the substance during manufacture and storage. The PROCs selected for this scenario include PROCs 1, 2, and 8b. LEV is in use at drum filling stations and eye protection and gloves with an assumed effectiveness of 80%, or 95% for transfers, must be worn for any task where there is a potential for exposure. Prior to maintenance activities, the plant equipment is depressurized and purged. Maintenance workers wear full face protection, gloves, chemical protective suits, protective helmets and rubber boots.

The exposure estimates have been generated assuming the substance is undiluted and all tasks are performed for a full shift. This is likely to result in conservative exposure estimates for both the inhalation and the dermal routes and the eMSCA will use the registrants' exposure estimates for its own risk characterisation.

Formulation of preparations

Imidazole may be supplied to formulators as solid flakes, as a high temperature molten solid or in solution. The registrants do not have detailed information about the operating conditions and risk management measures that are applied by downstream users formulating imidazole into preparations and have therefore chosen a range of potentially applicable PROCs to ensure that all foreseeable use conditions have been assessed. The PROCs selected include PROCs 1, 2, 3, 5, 8a, 8b and 9. However, the registrants consider it is unlikely that transfers will be performed under the conditions assumed for PROC 8a since it is important that exact quantities of imidazole are included in blends and this would typically be achieved with specialist transferring equipment.

Calculations have been performed assuming the substance is handled either as solid flakes or as a molten solid at temperatures up to 115°C or a solution. Where the substance is handed as solid flakes, no requirements for additional ventilation were identified. Eye protection and gloves with an assumed effectiveness of 80% (or greater for blending activities covered by PROC 5 and transfers) are required. Where the substance is handled as a molten solid or in solution, LEV is required for blending activities covered by PROCs 3 and 5 and for transfers, supported by enhanced general ventilation for PROCs 5 and 8a. Eye protection and gloves with an assumed effectiveness of 80% (or 95% for blending activities covered by PROC 5 and transfers) are also required.

The exposure estimates have been generated assuming the substance is undiluted and all tasks are performed for a full shift. The eMSCA considers that this is a worst case approach since some of the activities will be performed with mixtures rather than undiluted imidazole. Depending on the concentration of imidazole in mixtures, both inhalation and dermal exposures could be substantially lower than the levels that have been predicted. The eMSCA will use the registrants' exposure estimates for its own risk characterisation.

Use as an intermediate, use as a monomer

Imidazole is used as an intermediate in the manufacture of pharmaceuticals, pesticides and dyes. The eMSCA expects that the majority of these processes are carried out under strictly controlled conditions since across all registrations the greatest proportion of the registered tonnage is registered for use as a transported isolated intermediate according to Article 18. However, to cover the possibility that not all use takes place under strictly

controlled conditions (SCC), the following PROC codes have been assessed; PROC 1, 2, 3, 4, 8a, 8b, and 9. Calculations have been performed assuming the substance is handled either as solid flakes or as a molten solid at temperatures up to 115°C or a solution. Where the substance is handed as a solid, no requirements for additional ventilation were identified. Eye protection and gloves with an assumed effectiveness of 80% (or greater for blending activities covered by PROC 4 and transfers) are required. Where the substance is handled as a molten solid or in solution, LEV is required for blending activities covered by PROC 4 and for transfers if this activity is performed for more than 4 hours per day. Eye protection and gloves with an assumed effectiveness of 80% (or 95% for blending activities covered by PROC 4 and transfers) are also required.

Exposure estimates have been generated for each PROC code assuming the substance is handled undiluted and the activities are performed for more than 4 hours per day. Additional estimates have been derived for transferring tasks covered by PROCs 8a and 8b assuming the work is performed for durations shorter than 4 hours and in some cases less than 15 minutes. In these cases, although the requirements for eye protection and gloves remain, no need was identified for LEV. The eMSCA will use the registrants' exposure estimates for its own risk characterisation.

It is important that companies receiving these exposure scenarios understand that exposure values calculated with durations < 4 hours represent an 8-hour time weighted average assuming the worker does not come into further contact with imidazole during the working day. If this is not the case then, depending on the types of tasks and potential for exposure, the employer may need to consider if additional controls are required.

Note to registrants: To ensure that companies receiving exposure scenarios including tasks assessed on a reduced duration basis implement sufficient measures to protect their workers, clarification should be provided with the scenario that the RMMs identified apply where the worker does not have any additional exposure to imidazole during the shift.

Use in laboratories (industrial and professional)

Scenarios have been provided for laboratory use covering analyses for quality control purposes in industrial settings and professional use as a laboratory reagent. Calculations have been performed assuming the substance is handled either as solid flakes or as a molten solid at temperatures up to 115°C or a solution. For all situations, eye protection and gloves with an assumed effectiveness of 80% are required. No ventilation requirements have been identified where the substance is handled as solid flakes. Where the substance is handled as a molten solid or in solution, it should be handled in a fume cupboard. The exposure estimates have been generated assuming the substance is handled undiluted and the activities are performed for more than 4 hours per day. This is likely to provide worst case estimates. The eMSCA will use the registrants' exposure estimates for its own risk characterisation.

Use in industrial chemical process

This scenario covers the use of solvent mixtures containing imidazole at concentrations of up to 3% in metal surface treatment products and polymer preparations and compounds e.g. as a curing agent for epoxy resins and polyurethane foams. The registrants expect that most uses for imidazole containing products will take place using dedicated, possibly automated production lines. However, to ensure that all foreseeable downstream use conditions have been assessed, the registrants have included included PROCs 1, 2, 3, 4, 5, 7, 8a, 8b, 9, 10, 13, 14 and 21 in their assessment. Eye protection and gloves with an assumed effectiveness of 80% are required in all cases except PROC 7 for which gloves with an assumed effectiveness of 90% are required in addition to eye protection. The exposure assessment for PROC 7 (spraying) also takes account of the use of local exhaust ventilation (LEV).

The TRA tool has been used to generate exposure estimates for all PROCs except PROC 7. For PROCs assessed with the TRA tool, with the exception of PROC 21 (which covers processing of solid articles containing up to 3% imidazole), all calculations assume the substance is handled as a low volatility liquid mixture at ambient temperature. It is assumed that each activity is performed for a full shift. Since imidazole is only present at concentrations of up to 3% in products covered by this scenario, the registrants have used this concentration to adjust their exposure estimates rather than using the concentration band approach adopted within the TRA tool. The registrants exposure estimates are therefore over 6 times lower than those obtained by the eMSCA using the concentration band approach. The significance of this difference will be considered in the risk characterisation (see section 7.13).

The ART was used to assess full-shift exposure from activities covered by PROC 7. Based on information provided by the registrants about the process, the assessment parameters chosen which include the use of LEV (fixed capturing hood) appear to be reasonable. The eMSCA will therefore use the registrants exposure value for its own risk characterisation. The use of LEV to limit release at source should also be sufficient to avoid possible site of contact effects due to the corrosivity of imidazole which might arise if spraying was performed without this control measure.

Professional use in construction chemicals, use in coatings

This scenario covers the use of mixtures containing imidazole at concentrations of up to 3%. Typical products where imidazole may be used include polyurethane and epoxy resin-based coatings and adhesives which may be applied by brush, spatula or roller. Since the registrants do not have detailed information about the conditions at downstream user sites using these products they have chosen to cover PROCs, 2, 3, 4, 5, 8a, 8b, 9, 10, 11, 13, 14, 19 and 21 in their assessment to ensure that all foreseeable use conditions have been assessed. Although PROC 11 has been included because it is a possible method of application for polyurethane and epoxy systems (for example spraying is mentioned in this safety brochure produced by PlasticsEurope[8]), the registrants are not aware that this method of application is used for imidazole-containing products. Eye protection and gloves with an assumed effectiveness of 80% are required for all activities except those covered by PROCs 11 and 19 for which gloves with an assumed effectiveness of 90% are required in addition to eye protection. For PROC 11 (spraying), RPE with an assigned protection factor of 10 is also required (or LEV if RPE is not in use) and it is necessary for there to be good general ventilation in the work area, particularly if other work is being performed nearby. These measures are consistent with the controls recommended in the safety brochure for work with epoxy systems.

The TRA tool has been used to generate exposure estimates for all PROCs except PROC 11 for which the ART tool (version 1.5) was used. For PROCs assessed with the TRA tool, with the exception of PROC 21 (which covers processing of solid articles), all calculations assume the substance is handled as a low volatility liquid mixture at ambient temperature and it is assumed that each activity takes place for a full shift. As before, the registrants have adjusted their exposure estimates using the exact concentration rather than the concentration band approach. The eMSCA has therefore performed additional calculations to generate exposure estimates using the concentration band approach.

The ART was used to assess full-shift exposure from activities covered by PROC 11. The Registrants used many of the same parameters for this assessment as they used for PROC 7 in the scenario covering industrial use in chemical processes. Assessments were performed for the worker carrying out the spraying task (near-field) and a colleague

[8] https://www.epoxy-europe.eu/wp-content/uploads/2016/09/EPOXY_SafetyBrochure_2017.pdf (site accessed June 2018)

carrying out different work in the same area (far-field). The 75th percentile value was taken forward to the risk characterisation. The eMSCA is satisfied with the approaches that have been taken to generate full-shift exposure estimates.

No quantitative assessment has been made of potential short-term peak exposure during spraying activities. In this case the recommended RPE (or LEV) will mitigate against inhalation of aerosols that may be generated by the worker carrying out spraying activities. There is a small possibility that site of contact effects may arise in workers covered by the far-field assessment if RPE is used to protect the worker carrying out spraying. It is therefore important to ensure that spraying operations are only carried out in well ventilated conditions (the registrants specify a minimum of 3 air changes per hour).

Cleaning and maintenance

Occasional controlled exposure during cleaning and maintenance of process equipment has been assessed using PROCs 3 or 4. PROC 28 has been assigned to cover manual cleaning and repair e.g. where there is a need to enter normally closed systems or change filters. A quantitiative exposure assessment has not been performed for this PROC code. Based on a qualitative assessment, it is recommended that workers wear gloves, eye protection, suitable coveralls and respiratory protection e.g. EN 143 or 149, type P3 or FFP3 when performing manual cleaning and maintenance activities.

7.12.1.2. **Consumer**

Not applicable

7.12.2. Environment

The registrants have not performed an environmental exposure assessment as the substance is not classified for the environment. This is in accordance with the REACH guidance.

7.12.3. Combined exposure assessment

Not applicable

7.13. Risk characterisation

7.13.1 Human Health

Workers

Assessment for systemic effects

Imidazole is classified for reproductive toxicity (Repr. 1B) owing to its potential to harm the unborn child and it is this property that drives the values for the long-term systemic DNELs of 8 mg/m3 (inhalation) and 1.2 mg/kg/day (dermal) calculated by the eMSCA. In the registrants' risk characterisation, all RCRs are below 1. Using the eMSCA's DNELs and the registrants exposure values, all RCRs remain below 1. It is only in the case where exposures are calculated using the default concentration band approach implemented within the ECETOC TRA tool that RCRs above 1 are calculated. Table 24 identifies scenarios where the combined inhalation and dermal RCRs calculated using the eMSCA's DNELs and exposure values are greater than 1.

Table 24: Scenarios where combined (inhalation + dermal) RCR values are >1 using the eMSCA's DNELs and the concentration band approach.

SCENARIO	PROC CODE		Combined RCR
Industrial use of solvent mixtures containing up to 3% imidazole in industrial chemical processes	8A	transfers using non-dedicated facilities	1.17
	10	roller application or brushing	1.62
	13	treatment of articles by dipping and pouring	1.17
Professional use of solvent mixtures containing up to 3% imidazole in construction chemicals and coatings	5	mixing or blending in batch processes (multistage and/or significant contact)	1.17
	8A	**transfers at non-dedicated facilities**	**2.23**
	8B	transfers at dedicated facilities	1.17
	10	**roller application or brushing**	**2.69**
	11	**professional spraying (near field)**	**2.14**
	11	**professional spraying (far field)**	**1.87**
	13	treatment of articles by dipping and pouring	1.17
	19	**hand-mixing with intimate contact (only PPE available)**	**4.13**

Bold text indicates cases where either the inhalation and/or dermal RCR was also >1.

In deciding how to react to these RCRs, the eMSCA takes into account the fact that the exposure estimates driving these RCRs have been calculated using the default concentration ranges adopted within the TRA tool. This is likely to be a precautionary approach because the vapour pressure of imidazole places it at the low end of the relevant vapour pressure range. It is also worth noting that each assessment assumes that activities are performed for a full shift whereas workers may carry out a variety of different tasks during a work-shift some of which will not require the use of imidazole containing products (e.g. preparing surfaces before applying products). The assumption that all activities are peformed for a full shift will therefore introduce another element of precaution, but one that is impossible to quantify because it is highly specific to the combinations of tasks performed by each worker. For these reasons, the eMSCA does not identify a risk to workers in relation to the systemic hazards of imidazole.

Assessment for site of contact effects

Imidazole is also classified as corrosive (Skin Corr. 1B) and is harmful if swallowed (Acute Tox 4). The available data do not permit DNELs to be calculated for corrosivity or short term toxicity. A qualitative assessment indicates that measures to protect the skin and eyes are necessary where there is the potential for skin/eye contact and these are recommended by the registrants.

There are no data to demonstrate whether or not imidazole will cause adverse effects in the respiratory tract if inhaled and if so, to assess the likely concentration-response relationsip for this hazard. However, this hazard could be anticipated on the basis of its corrosivity. Given the low vapour pressure of imidazole (0.00327 hPa), under conditions of use that do not require elevated temperatures and do not generate aerosols, airborne exposures are likely to be below a level that might be of concern for adverse local effects in the respiratory tract.

Where aerosols may be generated during use e.g. spraying, the eMSCA cannot exclude the possibility that site of contact irritation or inflammation might occur in the respiratory tract if short-term peak exposures are not appropriately managed. In the case of industrial spraying, the registrants recommend the use of LEV to limit peak exposures. With this risk management measure using the eMSCA's DNELs and exposure calculations, RCRs are below 1.

It is not clear if imidazole is used for spraying applications by professionals; this assessment was included by the registrants for completeness. If imidazole containing preparations are sprayed by professionals, the registrants recommend the use of RPE (or LEV if RPE is not in use) to limit exposure for the worker carrying out the spraying task. If others are working in the same area at the time spraying is performed, there is a small possibility that these workers might experience site of contact effects if RPE rather than LEV is used to protect the worker carrying out spraying. It is therefore important to ensure that such spraying operations are only carried out in well-ventilated conditions (the registrants specify a minimum of 3 air changes per hour) and if necessary other work is halted during spraying.

Conclusion

Using its own DNELs and taking a precautionary approach to the exposure assessment, the eMSCA has obtained RCRs > 1 for some activities covered by the scenarios for industrial and professional use of products containing up to 3% imidazole. The eMSCA does not consider that these RCRs provide evidence for an unacceptable risk and concludes that no further regulatory action is necessary.

Consumers

Not applicable

Indirect exposure of humans via the environment

Not applicable

Environment

Not relevant for this evaluation.

Overall risk characterization

Human health (combined for all exposure routes)

The registrant states that simultaneous direct exposure to imidazole from more than one workplace emission source can be excluded, and there is no consumer use, therefore combined emissions from different exposure scenarios was considered not applicable.

Environment (combined for all exposure routes)

Not relevant for this evaluation

7.14. References

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7.15. Abbreviations

%	Percentage
AC	Article Category
BCF	Bioconcentration factor
BMF	Biomagnification factor
ChV	Chronic value
Cmax	Maximal plasma concentration
C&L	Classification and labelling
CLH	Harmonised classification and labelling
CSR	Chemical Safety Report
DMEL	Derived Minimum Effect Level
DNEL	Derived No Effect Level
DOC	Dissolved oxygen concentration
ECETOC TRA	European Centre for Ecotoxicology and Toxicology of Chemicals Targeted Risk Assessment
ECx	Effect Concentration X (concentration causing an effect in x% of the population)
ERC	Environmental release category
ES	Exposure Scenario
EU	European Union
EUSES	European Union System for the Evaluation of Substances
EU TGD	European Union Techincal Guidance Document
GLP	Good Laboratory practice
Hb	Haemoglobin
HCT	Hemocrit
HPRT	Hypoxanthine-guanine phosphoribosyltransferase
i.v.	Intravenous
LC50	Lethal concentration expected to kill 50% of the animals exposed
LD50	Lethal dose expected to kill 50% of animals dosed
LEV	Local exhaust ventilation
LH	Luteinizing hormone
LOEC	Lowest observable effect concentration
LOAEL	Low Observed Adverse Effect Level
Log Pow	Octanol-water partition coefficient
m	metre(s)
MCH	Mean cell haemoglobin
MCHC	Mean cell haemoglobin concentration
MCV	mean corpuscular volume
mg	milligram
mg/kg bw	milligram per kilogram of bodyweight
mg imidazole/kg bw	milligrams of imidazole per kilogram of bodyweight
mg m-3	milligrams per cubic metre
mmol/l	millimoles per litre
mmol/kg bw	millimoles per kilogram of bodyweight
min	minute
MS	Member State of the EU
NOEC	No observable effect concentration
NOAEL	No observed adverse effect level
N/A	not applicable
Nmol/g	nanomoles per gram
PBT	Persistent, Bioaccumulative and Toxic
PC	Product category
PEC	Predicted environmental concentration
Pi	Inorganic phosphate
PNEC	Predicted no effect concentration

PPE	Personal Protective Equipment
PROC	Process Category
QSAR	Quantitative Structure-Activity Relationship
RAC	Risk Assessment Committee
RBC	Red blood cells
RCR	Risk Characterisation Ratio
REACH	Registration, Evaluation, Authorisation and Restriction of Chemicals (EU Regulation No. 1907/2006)
RMM	Risk management measure
RPE	Respiratory protective equipment
SIAR	SIDS (Screening information dataset) initial assessment report
spERC	Specific Environmental release category
STP	Sewage treatment plant
SU	Sector of Use
t	Tonne
TK	Thymidine kinase
T1/2	Elimination half-life
TC C&L	Technical Committee on Classification and Labeling
TIF	Testicular interstitial fluid
Tmax	Time taken after administration for maximal plasma concentration to be reached
UDS	Unscheduled DNA synthesis
UK	United Kingdom
µmol/kg bw	microgram per kilogram bodyweight
vPvB	Very persistent and very bioaccumulative