Committee for Risk Assessment

RAC

Annex 2

Response to comments document (RCOM)

to the Opinion proposing harmonised classification and labelling at EU level of

imazalil

EC number: 252-615-0

CAS number: 35554-44-0

CLH-O-0000002720-08-03/A2

Adopted

4 June 2013

ANNEX 2 - COMMENTS AND RESPONSE TO COMMENTS ON CLH PROPOSAL ON IMAZALIL

COMMENTS AND RESPONSE TO COMMENTS ON CLH: PROPOSAL AND JUSTIFICATION

ECHA has compiled the comments received via the internet that refer to several hazard classes and entered them under each of the relevant categories/headings as comprehensively as possible. Please note that some of the comments might occur under several headings, when splitting the information provided is not reasonable.

Substance name: Imazalil
EC number: 252-615-0
CAS number: 35554-44-0
Dossier submitter: Germany

GENERAL COMMENTS

Date	Country	Organisation	Type of Organisation	Comment number
05/10/2012	Netherlands	Member State		1

Comment received

Several studies have been performed with salts of imazalil. Please provide a justification for the read-across towards imazalil.

Some of the conclusions in the CLH proposal differ from the conclusions in the DR(AR) and the JMPR. Examples are the NOAELs for developmental and reproductive toxicity. Although an explanation for these differences is not so important for a CLH proposal, a statement that the DR(AR) conclusion were not always taken over would be informative to the user of the CLH report.

Dossier Submitter's Response

According to the Technical Notes for Guidance and the Technical Guidance Document for the Risk Assessment of Biocides read across can be performed if the substance used in the study is closely related to the substance evaluated. Imazalil and Imazalil salts are structurally nearly similar. Hence, read across is possible in the present case.

The NOAELs are based on the evaluations from the biocide risk assessment. However, it should be noted that the present procedure focuses on classification and labelling rather than on the correct derivation of NOAELs.

RAC's response

Noted. The read-across from the salts of imazalil to imazalil is considered acceptable because of the good water solubility of both imazalil sulphate and imazalil nitrate.

Date	Country	Organisation	Type of Organisation	Comment number
04/10/2012	France	Member State		2

Comment received

FR agrees with the general conclusion dealing with the classification of the substance.

Pages 6 and 8: 1.2 Harmonized classification and labelling proposal, Tables 3 and 5:
The column "reason for classification" is filled in with "data lacking" when no classification is proposed for toxicological endpoints. We do not consider that there is a lack of data to conclude on the toxicological classification of imazalil. Therefore it is more appropriate to fill in this column with "data conclusive but not sufficient for classification".

Pages 19 to 45: 5 Human health hazard assessment:
From a general point of view, this section is not enough detailed and do not permit a complete assessment of the presented studies. In particular it would be useful to have tabulated details of results showing e.g. incidences, dose-response, statistical significance of findings for each study including mechanistic studies.

Dossier Submitter's Response

Thank you. We have noted this.

RAC's response

RAC also finds that very little detail is presented in the CLH dossier on the available studies, complicating the interpretation of the effects as to their potential classification.

ANNEX 2 - COMMENTS AND RESPONSE TO COMMENTS ON CLH PROPOSAL ON IMAZALIL

Date	Country	Organisation	Type of Organisation	Comment number
24/09/2012	Sweden	Member State		3
Comment received				
SE supports classification of Imazalil (Cas No 3554-44-0) as specified in the proposal. SE agrees with the rationale for classification into the proposed hazard classes and differentiations (N;R51-53 according to DSD, and Aquatic Chronic I with M=10 according to CLP).				
Dossier Submitter's Response				
Thank you for your support.				
RAC's response				
The support is noted.				

CARCINOGENICITY

Date	Country	Organisation	Type of Organisation	Comment number
05/10/2012	Spain	Member State		4
Comment received				

p. 32 and 34 Summary and discussion of carcinogenicity. Other effects
Imazalil is currently included in Annex VI of 1272/2008/EEC Regulation (CLP), and it is not classified for carcinogenicity.

The dossier submitter proposes to classify Imazalil under de DSD and CLP classification criteria as Car, cat 3, R 40: Limited evidence of a carcinogenic effect, and as Carc. 2 H351: Suspected of causing cancer. The Spanish CA does not support this proposal.

Neoplastic lesions were observed in thyroid and liver in rat, in long-term toxicity studies of 24 months, (Van Deun et al, 1999) and in liver in mouse in studies of 24 months instead 18 months (Vertraeten et al, 1993). Hepatic adenomas in male rats increased only at the highest dose of 120 mg/kg p.c /day. In male mouse the incidence of total liver neoplasms increased by 50% from doses 34,4 mg/kg p.c./day (mainly adenomas 46%) in male. There was also an increase of male liver carcinomas (22%) at the dose of 105 mg/kg p.c/day.

The mechanism of rat thyroid tumour formation is known. An increase in hepatic UDP-GT was observed with the corresponding increase in the thyropropin TSH and alteration of thyroid hormones T3 or T4 in blood (Verbeek et al, 2000). This type of disturb in rats thyroid hormone balance is not considered relevant to humans (ECHA-Guidance on the Application of Regulation (EC) Nº 1272/2008).

Regarding the mechanism of hepatic tumour formation, the results of the mode of action (MOA) supplementary studies showed that Imazalil and Phenobarbital (PB) may share some similarities in the pattern of metabolic enzyme induction and histopathological changes in the liver (CYP isoenzymes induction, liver weight increase, hepatocyte hypertrophy and develop of foci). However, data for concordance analysis with PB are limited. The target molecule within the cell or the nuclear receptor which imazalil acts on is not known and it is uncertain whether it can activates the CAR receptor (also activated by phenobarbital), the XPR receptor or both. There are a number of data gaps, such as the lack of available data regarding CAR involvement in the induction of CYP isoforms following Imazalil exposure and there is no data regarding the concordance of key events between rodents and humans.

Therefore, for this compound there is not robust data for a PB-like MOA and there is not a satisfactory demonstration that other molecular mechanisms are not relevant. Relationships between imazalil activation pathways and their involvement in carcinogenesis should be further established. Therefore, based on the data available, the mode of action for formation of liver tumours in rodents remains unclear.

Besides, the increases in the incidence of neoplasms, although appears in two different species does not seem to be sufficient evidence to classify imazalil regarding carcinogenicity, for the following reasons:

- The neoplasms are primarily benign (adenomas)
- No dose response effect was observed
- Liver carcinomas were only observed in mice treated for more than 18 months and this effect is more likely due to aging.

The comparison of imazalil carcinogenicity data with the corresponding classification criteria is not trivial because the data are complex and some kind of borderline and the criteria leave a margin for different interpretations.

On overall, we considered that it is not justified to classify the substance Imazilil as Car, cat 3, R 40: Limited evidence of a carcinogenic effect, and as Carc. 2 H351: Suspected of causing cancer.

Dossier Submitter's Response

It is justified to propose classification and labelling of the substance as Carc cat. 3, R40 and as Carc. 2, H351. The mechanism of hepatocarcinogenesis remains unclear. As stated by the Spanish CA 'there is not robust data for a PB-like MOA and there is not a satisfactory demonstration that other molecular mechanisms are not relevant.' Furthermore, mechanistic data recently provided by the applicant, show that humanized mice react to the substance the same way as wildtype mice, supporting the hypothesis that tumors are indeed relevant to humans. As stated by the Spanish CA 'the increase in the incidence of neoplasms…appears in two different species.' This could serve as an argument for an even higher classification. However, acknowledging other arguments (primarily benign nature of tumors), R40/H351 seems more appropriate.

RAC's response

RAC agrees that the thyroid tumours do not warrant classification. As to the liver tumours, it was concluded that imazalil shows some similarities with phenobarbital, albeit imazalil is less potent. This could point to imazalil being a CAR(/PXR)-activator. Even so, currently there is no generally agreed framework by which to assess the relevance to humans of non-genotoxic rodent liver carcinogens acting via CAR(/PXR) activation and cell proliferation, or to assess the relevance of experiments with humanized and knockout PXR/CAR rodents. Furthermore, the evidence presented on imazalil-induced cell proliferation is not sufficient to allow the conclusion that this will not be operative in humans. Because the relevance to humans of the mechanism behind imazalil-induced liver tumour formation in rodents cannot be convincingly excluded, RAC supported the proposal of the dossier submitter to classify imazalil for carcinogenicity as Carc. 2 - H351 (CLP) and Carc. Cat. 3; R40 (DSD).

Date	Country	Organisation	Type of Organisation	Comment number
05/10/2012	Netherlands	Member State		5

Comment received

Only very limited information is provided on the actual incidences (number and percentage) of the tumours in the different carcinogenicity studies. Could you provide a table containing this information including historical control data? Further, in the DAR and its updates the tumorigenic effects in the mice are described as neoplastic nodules and hepatocytic neoplasms whereas in the CLH proposal hepatocytic adenoma and carcinoma are mentioned. Could you please explain the change in terminology.

We agree that based on the available evidence the increase in thyroid tumors is not relevant to humans.

We agree that based on the provided evidence the increase in liver tumors cannot be dismissed as not relevant to humans as the mechanism remains unclear. We also agree that the mechanism is most likely non-genotoxic. We agree with the proposed classification with R40 / CLP Cat 2. An additional argument for CLP Cat 2 instead of Cat 1B is that in rats only an increase in adenomas was observed whereas the CLP criteria for sufficient evidence (CLP Cat 1B) require an increased incidence in malignant or appropriate combination of benign and malignant neoplasms in two species or two independent studies.

Dossier Submitter's Response

We agree that it is justified to propose classification and labelling of the substance as Carc. Cat. 3, R40 and as Carc. 2, H351. The mechanism of hepatocarcinogenesis remains unclear. There is no satisfactory demonstration that the molecular mechanism of tumor formation is not relevant to humans. Furthermore, mechanistic data recently provided by the applicant, show that humanized mice react to the substance the same way as wildtype mice, supporting the hypothesis that tumors

are indeed relevant to humans.

The use of historical control data has to fulfil several quality criteria under Reg EC No 1272/2008. I.e. the data should not be older than five years and from the same laboratory. Furthermore averages of HC have to be taken into account rather than maxima. In any case the control of the study is regarded of higher relevance than HC. Considering this argument, HC is only of limited value in the present case.

RAC's response

IND in their comments provided some additional data as to statistical significance and historical control ranges.
RAC agrees that the thyroid tumours do not warrant classification. As to the liver tumours, it was concluded that imazalil shows some similarities with phenobarbital, albeit imazalil is less potent. This could point to imazalil being a CAR(/PXR)-activator. Even so, currently there is no generally agreed framework by which to assess the relevance to humans of non-genotoxic rodent liver carcinogens acting via CAR(/PXR) activation and cell proliferation, or to assess the relevance of experiments with humanized and knockout PXR/CAR rodents. Furthermore, the evidence presented on imazalil-induced cell proliferation is not sufficient to allow the conclusion that this will not be operative in humans. Because the relevance to humans of the mechanism behind imazalil-induced liver tumour formation in rodents cannot be convincingly excluded, RAC supported the proposal of the dossier submitter to classify imazalil for carcinogenicity as Carc. 2 - H351 (CLP) and Carc. Cat. 3; R40 (DSD).

Date	Country	Organisation	Type of Organisation	Comment number
04/10/2012	France	Member State		6

Comment received

Page 29: Contrary to what is written in this section, the 24-month oral study in rats (Van Deun et al., 1999) was already assessed by the experts of the European Food Safety Authority in the context of the renewal of inclusion of imazalil under plant protection product directive at EU level.

Dossier Submitter's Response

It is unclear what is meant by this comment. On page 29 no specific statement is made saying that the study cited above was not assessed.

RAC's response

Noted.

Date	Country	Organisation	Type of Organisation	Comment number
26/09/2012	United Kingdom	Member State		7

Comment received

We agree with the proposed carcinogenicity classification as there remains some uncertainty that the observed rodent liver tumours could be relevant for human health.

We agree that there is sufficient mechanistic information to discount the rat thyroid tumours as not being relevant for human health. However, we suggest that the EU Specialised Experts conclusions (ECBI 49/99. Rev 2) should be used as the basis for dismissing the rat thyroid tumours.

Dossier Submitter's Response

Thank you. We have noted this.

RAC's response

RAC agrees that the thyroid tumours do not warrant classification. As to the liver tumours, it was concluded that imazalil shows some similarities with phenobarbital, albeit imazalil is less potent. This could point to imazalil being a CAR(/PXR)-activator. Even so, currently there is no generally agreed framework by which to assess the relevance to humans of non-genotoxic rodent liver carcinogens acting via CAR(/PXR) activation and cell proliferation, or to assess the relevance of experiments with humanized and knockout PXR/CAR rodents. Furthermore, the evidence presented on imazalil-induced cell proliferation is not sufficient to allow the conclusion that this will not be operative in humans. Because the relevance to humans of the mechanism behind imazalil-induced liver tumour formation in rodents cannot be convincingly excluded, RAC supported the proposal of the dossier submitter to

classify imazalil for carcinogenicity as Carc. 2 - H351 (CLP) and Carc. Cat. 3; R40 (DSD).

Date	Country	Organisation	Type of Organisation	Comment number
04/10/2012	Belgium	Janssen PMP, a division of Janssen Pharmaceutical N.V.	Company - Manufacturer	8

Comment received

p. 32, p. 41 ff the conclusion on classification as carcinogen cat 2 based on the mechanistic data and potential relevance of Imazalil to human health:

Imazalil produced in a mouse carcinogenicity study a statistically significant increase in combined hepatocellular adenoma/carcinoma in females with an incidence that was beyond the historical background range of the test laboratory. When considered separately, the adenomas and carcinomas were not significantly increased. The incidence of hepatocellular tumors in male mice remained within the boundaries of the historical controls from the same laboratory. In rats, no corresponding tumor profile was observed (a statistically significant increase in hepatocellular adenoma was limited to male rats at the highest dose level far beyond the maximum tolerated dose (MTD) and should not be considered for cancer risk assessment - there was no increase in the incidence of hepatocellular adenoma in females and hepatocellular carcinoma in males and females).
The mode of action of the mouse liver tumors was investigated experimentally in vitro and in vivo. Sufficient experimental evidence is provided to prove that Imazalil acts through receptor (PXR/CAR) activation to produce hepatomegaly in the mouse. Oxidative stress and peroxisome proliferation as causes for the increased incidences of hepatocellular tumors can be confidently excluded. In mechanistic toxicity studies in vitro and in vivo, Imazalil was shown to produce cell proliferation in mouse hepatocytes after single and multiple (4 days) exposure. In vivo, cell proliferation was only seen at exposure levels that produced hepatocellular toxicity. In contrast to the mouse, Imazalil did not produce any increase in cell proliferation in the rat in vivo and was not found to be hepatotoxic at the dose levels explored in the mechanistic studies. Sufficient evidence is provided to prove that Imazalil produces hepatomegaly through PXR/CAR activation in the rat.
Since it has been shown in the literature that rodent hepatocellular carcinogens with a mechanism of action based on PXR/CAR activation produce tumors through cell proliferation in rodent but not in human hepatocytes and that cell proliferation is a prerequisite for liver cell carcinogenicity, an in vitro study was conducted to address the relevance of this mechanism to humans. In this assay Imazalil was incubated for 96 hours with female mouse and female human hepatocytes up to toxic exposure levels. In contrast to mouse hepatocytes, the results clearly indicate that imazalil does not produce cell proliferation in female human hepatocytes. This finding indicates that the mechanism of hepatocellular carcinogenicity in female mice is not operative in human cells and that therefore Imazalil should not be classified as a carcinogen.
(for details please refer to the documents attached)

(ECHA note: The documents attached are confidential and only the reference is provided in this document)

Dossier Submitter's Response

It is justified to propose classification and labelling of the substance as Carc. Cat. 3, R40 and as Carc. 2, H351. The mechanism of hepatocarcinogenesis remains unclear. There is no satisfactory demonstration that the molecular mechanism of tumor formation is not relevant to humans. The study with primary hepatocytes of mice and men has only been performed with a set of human hepatocytes from one donor. Furthermore, mechanistic data provided by the applicant, show that humanized mice react to the substance the same way as wildtype mice, supporting the hypothesis that tumors are indeed relevant to humans.
The use of historical control data has to fulfil several quality criteria under Reg EC No 1272/2008. I.e. the data should not be older than five years and from the same laboratory. Furthermore averages of HC have to be taken into account rather than maxima. In any case the control of the study is regarded of higher relevance than HC. Taken this into account the argument on HC is only of limited relevance.

RAC's response

It was concluded that imazalil shows some similarities with phenobarbital, albeit imazalil is less potent. This could point to imazalil being a CAR(/PXR)-activator. Even so, currently there is no

generally agreed framework by which to assess the relevance to humans of non-genotoxic rodent liver carcinogens acting via CAR(/PXR) activation and cell proliferation, or to assess the relevance of experiments with humanized and knockout PXR/CAR rodents. Furthermore, the evidence presented on imazalil-induced cell proliferation is not sufficient to allow the conclusion that this will not be operative in humans. Because the relevance to humans of the mechanism behind imazalil-induced liver tumour formation in rodents cannot be convincingly excluded, RAC supported the proposal of the dossier submitter to classify imazalil for carcinogenicity as Carc. 2 - H351 (CLP) and Carc. Cat. 3; R40 (DSD).

TOXICITY TO REPRODUCTION

Date	Country	Organisation	Type of Organisation	Comment number
05/10/2012	Netherlands	Member State		9

Comment received

No classification for effects on fertility and development is proposed based on the occurrence of adverse effects such as dystocia, reduced number of live born pups (2-gen study) and increases in resorptions and decreases in live pups (developmental studies) only at dose levels associated with maternal toxicity or insignificantly below the maternal LOAEL. However, in the rabbit study, the maternal NOAEL is 10 mg/kg bw, whereas the NOAEL for developmental toxicity is 5 mg/kg bw (based on increased resorptions, quantity not specified). It is unclear why this factor of 2 is considered insignificant. In addition, the criteria state that classification should also be considered when the reproductive effects occur together with other toxic effects if the effects on reproduction are not a secondary non-specific consequence of these other toxic effects. Please provide details on the level of reprotoxic and maternal/systemic effects and a justification for repro classification or not. Furthermore, some of the described maternal effects such as reduced body weight and reduced body weight gain could also be considered secondary to the reduced implantation and the resorptions. Further, imazalil belongs to the class of imidazoles and part of the effects observed with imazalil resemble those of classified fungicides. Is there information on placenta weight?

Dossier Submitter's Response

The necessity of classifying Imazalil with R63 was discussed during the pesticide evaluation process at a PRAPeR meeting (round 15, session 71, Oct 2009). The majority of experts agreed not to consider Imazalil for classification, also because effects on fetuses in the teratogenicity study in rabbits mentioned above are not significant. Please find details on this teratogenicity study in rabbits below (Dirkxs et al. 1992b; table taken from the DAR, by the Dutch CA):

Dose (mg/kg bw/day)	0	5	10	20	dr
Maternal effects					
Mortality	0/15	0/15	1/15	8/15	dr
Clinical signs	No treatment-related findings				
Pregnant animals	15/15	11/15	14/15	13/15	
Abortions	No treatment-related findings				
Body weight (day 19)			d (-5.3%)	dc (-7.0%)[1]	
Food consumption			dc (-18.3%)	dc (-23.1%)[2]	dr
Organ weight	Not required by OECD 414, version 12 May 1981				
Pathology					
Number of corpora lutea	No treatment-releated findings				
Litter response (mean per female)					
Implantations	7.5	6.8	6.5	7.3	
Litter size	6.9	6.2	5.0	3.6	dr
Live fetuses	6.4	6.2	5.0	3.6	dr

Fetal weight	41.3	42.3	45.2	39.0	
Post implantation loss	0.67	0.64	1.46	3.71	dr
Sex ratio		dc			
Examination of the fetuses					
External observations					
Skeletal findings	No treatment-reletad findings				
Visceral findings	No treatment-related findings				

dr dose related
dc/ic statistically significantly decreased/increased compared to the controls
d/i decreased/increased, but not statistically significantly compared to the controls
a/r absolute/relative organ weight
\+ present in one/a few animals
++ present in most/all animals
[1] day 6-19
[2] day 6-19, for the 10 mg group also day 0-5 (-10.4%)

Reduced food consumption of 18.3% accompanied by a reduction in body weight gain by 5.3 % at the dose level of 10 mg/kg bw was considered adverse during the pesticides evaluation. Hence, both maternal and developmental NOAEL were considered to be at 5 mg/kg bw/d. Furthermore it should be noted that none of the litter effects reached statistical significance, even if for the parameters 'litter size', 'live fetuses' and 'post implantation loss' dose response was observed. However, the effects were most pronounced (but still not significant) in the highest dose level of 20 mg/kg bw/d, were maternal mortality was already more than 50%.
An elongated duration of pregnancy and as a result a higher percentage of dystocia as described in the rat two generation study (Dirkx et al 1992a) occurring at the highest dose level of 80 mg/kg bw/d could also be attributed to maternal toxicity observed at this dose level.

RAC's response

With the limited information provided by the dossier submitter (data on e.g. number of animals affected, magnitude of the effects are not available), it is difficult to judge whether the effects observed are indeed effects, and whether there for instance is a causal relationship. Hence, RAC was provided with too little study details to allow proper evaluation of the endpoints 'effects on sexual function and fertility' and 'developmental toxicity'.

Date	Country	Organisation	Type of Organisation	Comment number
26/09/2012	United Kingdom	Member State		10

Comment received

We think better justification is required to support the overall conclusion that imazalil is not a reproductive toxicant.

We note that in section 5.9.1 dystocia and slight decreases in implantations were observed, suggesting an adverse effect on development/fertility. Similarly, in section 5.9.2, increased resorptions were reported, in both rats and rabbits. Such effects are usually regarded as an adverse effect on development.

Given these reported changes, it might be helpful to provide quantitative information to enable the reader to come to a more robust conclusion.

Dossier Submitter's Response

See comment above.

RAC's response

With the limited information provided by the dossier submitter (data on e.g. number of animals affected, magnitude of the effects are not available), it is difficult to judge whether the effects observed are indeed effects, and whether there for instance is a causal relationship. Hence, RAC was provided with too little study details to allow proper evaluation of the endpoints 'effects on sexual function and fertility' and 'developmental toxicity'.

ACUTE TOXICITY

Date	Country	Organisation	Type of Organisation	Comment

				number
05/10/2012	Spain	Member State		11

Comment received

p. 23 Summary and discusión of acute toxicity

Imazalil is listed in Annex VI of 1272/2008/EEC Regulation (CLP) – included in 28th ATP of Directive 67/548/EEC (DSD). The current classification for acute toxicity is as follows: Acute Tox. 4* H332, Harmful if inhaled (minimum classification), Acute Tox. 4 H302*, Harmful if swallowed (minimum classification), and Eye Dam.1 H318, Causes serious eye damage, under the CLP Regulation, and as Xn; R 20/22-41 under the DSD Regulation.

The Spanish CA supports the proposed classification of imazalil for acute inhalation toxicity as Xn, R20: Harmful by inhalation (limits 1 < CL50 ≤ 5 mg/l/4h) and as Acute Tox 4 (H332: Harmful if inhaled) (limits 1 < ATE ≤ 5 mg/l/4h) according to DSD and CLP classification criteria, respectively. This classification is based on the LD50 value in male (LD50 = 1,84 mg/l/4h) obtained in the inhalation toxicity study in rats (Blagden SM, 1990). It is no longer necessary to maintain the current reference that indicates a minimum classification (*).

Considering the results obtained in the oral toxicity study (LD50 = 227 mg/kg bw/day; Goodwine WR, 1990a) the existing classification as Acute Tox 4*, H302: Harmful if swallowed minimum classification, (limits 300< LD50 ≤ 2000 mg/kg bw/day) seems inappropriate according to CLP classification criteria and imazalil should be classified for acute oral toxicity as Acute Tox 3, H301: Toxic if swallowed (limits 50 < ATE ≤ 300 mg/kg pc/día).

Dossier Submitter's Response

The LD_{50} in the oral toxicity study by Goodwine et al has indeed been found to be below 300 mg/kg bw/d. Hence, classification for acute oral toxicity 3 (H301) was proposed.

RAC's response

The support is noted.

Date	Country	Organisation	Type of Organisation	Comment number
04/10/2012	Belgium	Janssen PMP, a division of Janssen Pharmaceutical N.V.	Company - Manufacturer	12

Comment received

p. 23 the conclusion on classification as "Toxic if swallowed" based on an LD50 between 50 and 300 mg/kg bw:
Classification with toxicity category 4 for acute oral toxicity of imazalil, "Harmful if swallowed, H302", is considered appropriate based on an acute oral LD50 exceeding 300 mg/kg bw.
(for details please refer to the documents attached)

(ECHA note: The documents attached are confidential and only the reference is provided in this document)

Dossier Submitter's Response

See comment above.

RAC's response

The original studies with an acute oral LD50 > 300 mg/kg bw, as referred to in the (confidential) document, were not provided by IND. Not having the original studies available and noting that all acute oral studies had the same reliability score in IUCLID, RAC saw no reason to dismiss the study with the lower LD_{50} value.

SPECIFIC TARGET ORGAN TOXICITY – REPEATED EXPOSURE

Date	Country	Organisation	Type of Organisation	Comment number
05/10/2012	Spain	Member State		13

Comment received

p. 27 Summary and discussion of repeated dose toxicity

In the CH report there is no proposal regarding Specific target toxicity – Repeated exposure (CLP Regulation) and repeated dose toxicity (DSD).
However, the Spanish CA proposes the classification of imazalil as Xn, R 48/22; Danger of serious damage to health by prolonged oral exposure, according to DSD (limits C ≤ 50 mg/Kg p.c./day) and as STOT RE cat.2 H 373 according to 1271/2008/EEC Regulation (limits 10 < C ≤ 100 mg/kg p.c./day), based on hepatic injury observed in subacute and subcronic toxicity studies (Gur et al, 1991; Van Deun et al, 1996: report R023979 Exp 3514 and Exp 3672) at doses from 18,78 mg/kg p.c./day in rat and 32 mg/kg p.c./day in mouse.
The most severe effect observed was hepatocyte fatty vacuolisation, considered an effect that supports classification for specific target organ toxicity following repeated exposure (CLP guidance; chapter 3.9.2.7).

Dossier Submitter's Response

R48/22 would be based on severe effects observed in organs below the dose limit of 50 mg/kg bw/day. As mentioned above fatty vacuolisation was the most severe effect. In our view this is not sufficient for classification, also taking into account that more severe liver effects observed in the carcinogenicity studies (tumors) would also be covered by the proposed R40.

RAC's response

In most studies the effects on the main target organ liver do not qualify for classification because the effective dose levels are above the cut-off levels for classification. In other studies, it seems questionable whether at the (lower) effective dose levels there is clear evidence of marked liver dysfunction (e.g. in the form of *severe* fatty change), as the criteria would require. Yet, RAC was provided with too little study details to allow proper evaluation of the endpoint 'specific target organ toxicity – repeated exposure' (CLP)/'repeated dose toxicity' (DSD) via the oral route.

HAZARDOUS TO THE AQUATIC ENVIRONMENT

Date	Country	Organisation	Type of Organisation	Comment number
01/10/2012	Belgium	Member State		14

Comment received

Based on the results of the aquatic toxicity test (most sensitive species : Selenastrum capricornutum :72hErC50=1.20 mg/l (mm), Daphnia magna : 21dNOEC reprod <0.01mg/l (nom)), the fact that the substance is not rapidly degradable, it is justified to classify, following the classification criteria of the 2nd ATP, as Aquatic chronic 1, H410 with a chronic M factor of 10 (0,001 < NOEC ≤ 0,01 mg/l).

Based on the classification and labelling criteria in accordance with dir. 67/548/EEC, lowest LC50 = 1.20 mg/l(mm), BCF<100 and not readily degradable, imazalil should be classified as N, R51/53.

In conclusion : we support the proposed classification for the environment by the DE MSCA.

Dossier Submitter's Response

Thank you for the support and the explanations according 2nd ATP

RAC's response

The support is noted.

Date	Country	Organisation	Type of Organisation	Comment number
26/09/2012	United Kingdom	Member State		15

Comment received

The aquatic chronic toxicity classification is based on two invertebrate studies, both of which present NOECs <0.1 mg/l in the Aquatic Chronic 1 range. We feel that further details should be provided about these studies, particularly the key study, to support the M factor. This is important as the NOEC for the key study is a "less than value". At present it is difficult to compare the two chronic invertebrate studies and understand whether there is a significant different between the tests and their results. Additional data about the level of effects were observed at the 0.0071 mg/l exposure concentration (Weytjens, 1989) would also be helpful.

Dossier Submitter's Response

Thank you for your comment. Further details for the chronic invertebrate studies are given below:

Weytjens, 1989: Daphnia reproduction test with Imazalil (R 23979). Janssen Pharmaceutica N.V., Company file No. : R 23979/RD/K6
The study was performed according OECD Guideline 202 (1984) without deviations to the protocol and with GLP. All validity criteria were met throughout the whole test period.
First-instar daphnids *Daphnia magna* straus, younger than 24 hours, were exposed to Imazalil (batch no.D7303; purity: 97.6%) with nominal concentrations of 0.01; 0.03; 0.1; 0.3; 1.0 and 3.0 mg/L. The real measured concentrations were 0.007; 0.023; 0.08; 0.262; 0.763 and 2.481 mg/L at start of the test. Test duration was 21 days under semi-static conditions (medium renewal after 2-3 days). Four replicates each containing 10 young daphnids for each test concentration and the control (dilution water) was tested. The daphnids were fed daily with Chlorella and Tetramin suspension.
There was 100% mortality of the adults at the 0.763 mg/L measured concentration and above. No significant mortality (7.5%) was found at 0.023 mg/L measured concentration and below. The NOEC for mortality is therefore 0.023 mg/L. At 0.08 mg/L significant mortality of 15% occurred.
Significant reduction of reproduction (according Mann-Whitney-U test with 0.05 significant level) were already found at the lowest test concentration of 0.007 mg/L with 15% reduction of produced offspring and for the other tested concentrations with 20 and 25% reduction for 0.023 and 0.08 mg/L. Therefore, no discrete NOEC could be determined (NOEC < 0.007 mg/L).
This study is used as the key study for deriving the chronic M-factor of 10 (0.001 < NOEC ≤ 0.01 mg/L).
The second additional study has the following details:
Kuhl, R., Wydra, V. (2008) Influence of Imazalil technical to Daphnia magna in a Reproduction test. Janssen Pharmaceutica N.V., Report No. : AGR4026
The study was performed according OECD Guideline 211 (1998) without deviations to the protocol and with GLP. All validity criteria were met throughout the whole test period.
First-instar daphnids *Daphnia magna* straus, younger than 24 hours, were exposed to Imazalil technical (batch no.ZR023979G3L431; purity: 97.46%) with nominal concentrations of 0.008; 0.025; 0.08; 0.25 and 0.8 mg/L. The real measured concentrations were 90-114% during test duration. Therefore all results are related to nominal concentrations. Test duration was 21 days under semi-static conditions (medium renewal after 2-3 days)
Ten replicates each containing 1 young daphnid for each test concentration and the control (dilution water) was tested. The daphnids were fed daily with green algae (*Desmodesmus subspicatus*).
There was 80% mortality of the adults at the 0.8 mg/L nominal concentration (highest test concentration). No significant mortality (20%) was found at 0.25 mg/L measured concentration and below, because 10% mortality occurred in the control (Bonferoni-Holm test, a= 0.05). The NOEC for mortality is therefore 0.25 mg/L.
Significant reduction of reproduction (according Dunnetts Multiple t-test with 0.05 significant level) were found at the nominal concentration of 0.08 mg/L with 20.3% reduction of produced offspring per surviving adult and 72.2% reduction for the other tested concentration of 0.25 mg/L. The reproduction reduction of 12% at 0.025 mg/L Imazalil was not significant. Therefore the NOEC for reproduction of 0.025 mg/L was derived.
This study is given as additional information, because a lower valid NOEC for aquatic invertebrates (*Daphnia magna*) was already determined.

RAC's response

Based on the additional details provided on the two invertebrate studies, RAC supported the proposed M-factor of 10, but took as starting point the 'less than' value of 0.007 mg/L (i.e., LOEC ≤ 0.007 mg/L) rather than the NOEC < 0.01 mg/L as done by the dossier submitter.

Date	Country	Organisation	Type of Organisation	Comment number
24/09/2012	Sweden	Member State		16

Comment received

We agree with the dossier submitter in its conclusion on the environmental classification of the substance. According to the information found in the N-class database the current environmental classification (N; R50-53) of Imazalil was agreed by the TCC&L in November 1997 (see ECBI/52/97 rev.1). The classification was based on the data provided by the rapporteur country in the pesticide evaluation process. The classification was based on EbE50 72 hr for Selenastrum capricornutum of 0.87 mg/l (see ECBI/43/96 – Add. 4). No reference to this value was specified; however it seems that this result comes from the OECD 201 study by Van Ginneken (1996), where ErC50 of 1.2 mg/l

was also measured. Since the preferred endpoint for classification is growth rate we agree that the substance does not meet the criteria for Aquatic Acute I classification according to CLP and R50-53 according to DSD.
Thus, based on the data set available we support the dossier submitter that the substance meets the criteria for classification as N;R51-53 according to DSD and Aquatic Chronic I with M=10 according to CLP.

Dossier Submitter's Response

Thank you for your agreement and comment. Both results EbE50 72 hr for Selenastrum capricornutum of 0.87 mg/L and ErC50 72 hr of 1.2 mg/L were measured at the same OECD 201 study by Van Ginneken (1996) with Imazalil.

RAC's response

The support is noted.

Date	Country	Organisation	Type of Organisation	Comment number
04/10/2012	France	Member State		17

Comment received

FR agrees with the general conclusion dealing with the environmental classification of the substance. However, it would be interesting to have more detailed summaries of both long-term studies investigating the effects of imazalil on the reproduction and survival of Daphnia magna. Indeed, detailed summaries would be helpful to further explain the chronic M-factor of 10 based on the NOEC value < 0.01 mg/L.

Dossier Submitter's Response

Thank you for your comment. Further details for the chronic invertebrate studies are given below:

Weytjens, 1989: Daphnia reproduction test with Imazalil (R 23979). Janssen Pharmaceutica N.V., Company file No. : R 23979/RD/K6
The study was performed according OECD Guideline 202 (1984) without deviations to the protocol and with GLP. All validity criteria were met throughout the whole test period.
First-instar daphnids *Daphnia magna* straus, younger than 24 hours, were exposed to Imazalil (batch no.D7303; purity: 97.6%) with nominal concentrations of 0.01; 0.03; 0.1; 0.3; 1.0 and 3.0 mg/L. The real measured concentrations were 0.007; 0.023; 0.08; 0.262; 0.763 and 2.481 mg/L at start of the test. Test duration was 21 days under semi-static conditions (medium renewal after 2-3 days). Four replicates each containing 10 young daphnids for each test concentration and the control (dilution water) was tested. The daphnids were fed daily with Chlorella and Tetramin suspension.
There was 100% mortality of the adults at the 0.763 mg/L measured concentration and above. No significant mortality (7.5%) was found at 0.023 mg/L measured concentration and below. The NOEC for mortality is therefore 0.023 mg/L. At 0.08 mg/L significant mortality of 15% occurred.
Significant reduction of reproduction (according Mann-Whitney-U test with 0.05 significant level) were already found at the lowest test concentration of 0.007 mg/L with 15% reduction of produced offspring and for the other tested concentrations with 20 and 25% reduction for 0.023 and 0.08 mg/L. Therefore, no discrete NOEC could be determined (NOEC < 0.007 mg/L).
This study is used as the key study for deriving the chronic M-factor of 10 (0.001 < NOEC ≤ 0.01 mg/L).
The second additional study has the following details:
Kuhl, R., Wydra, V. (2008) Influence of Imazalil technical to Daphnia magna in a Reproduction test. Janssen Pharmaceutica N.V., Report No. : AGR4026
The study was performed according OECD Guideline 211 (1998) without deviations to the protocol and with GLP. All validity criteria were met throughout the whole test period.
First-instar daphnids *Daphnia magna* straus, younger than 24 hours, were exposed to Imazalil technical (batch no.ZR023979G3L431; purity: 97.46%) with nominal concentrations of 0.008; 0.025; 0.08; 0.25 and 0.8 mg/L. The real measured concentrations were 90-114% during test duration. Therefore all results are related to nominal concentrations. Test duration was 21 days under semi-static conditions (medium renewal after 2-3 days)
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Significant reduction of reproduction (according Dunnetts Multiple t-test with 0.05 significant level) were found at the nominal concentration of 0.08 mg/L with 20.3% reduction of produced offspring per surviving adult and 72.2% reduction for the other tested concentration of 0.25 mg/L. The reproduction reduction of 12% at 0.025 mg/L Imazalil was not significant. Therefore the NOEC for reproduction of 0.025 mg/L was derived. This study is given as additional information, because a lower valid NOEC for aquatic invertebrates (*Daphnia magna*) was already determined.
RAC's response
Based on the additional details provided on the two invertebrate studies, RAC supported the proposed M-factor of 10, but took as starting point the 'less than' value of 0.007 mg/L (i.e., LOEC ≤ 0.007 mg/L) rather than the NOEC < 0.01 mg/L as done by the dossier submitter.

ATTACHMENTS RECEIVED:

The attachments received are confidential

1. Imazalil, Classification and Labelling for Acute Oral Toxicity, Expert Statement, 13 May 2011, document submitted on behalf of Janssen PMP, a division of Janssen Pharmaceutical N.V.
2. Carcinogenicity Classification of Imazalil, 17 September 2012, document submitted on behalf of Janssen PMP, a division of Janssen Pharmaceutical N.V.
3. IUCLID files submitted on behalf of Janssen PMP, a division of Janssen Pharmaceutical N.V., concerning Imazalil acute oral toxicity studies and new and additional mechanistic studies in vitro

ECHA note: The documents sent as attachments are being sent separately to this document. The contents of the IUCLID files are being sent as separate attachments.