Committee for Risk Assessment

RAC

Opinion

proposing harmonised classification and labelling
at EU level of

Imazalil (ISO)

EC number: 252-615-0

CAS number: 35554-44-0

CLH-O-0000002720-08-03/F

Adopted

4 June 2013

4 June 2013

CLH-O-0000002720-08-03/F

OPINION OF THE COMMITTEE FOR RISK ASSESSMENT ON A DOSSIER PROPOSING HARMONISED CLASSIFICATION AND LABELLING AT EU LEVEL

In accordance with Article 37 (4) of (EC) No 1272/2008, the Classification, Labelling and Packaging (CLP) Regulation, the Committee for Risk Assessment (RAC) has adopted an opinion on the proposal for harmonised classification and labelling (CLH) of:

Chemical name: Imazalil (ISO);

EC number: 252-615-0
CAS number: 35554-44-0

The proposal was submitted by **Germany** and received by the RAC on **21 August 2012.**

In this opinion, all classifications are given firstly in the form of CLP hazard classes and/or categories, the majority of which are consistent with the Globally Harmonised System (GHS) and secondly, according to the notation of 67/548/EEC, the Dangerous Substances Directive (DSD).

PROCESS FOR ADOPTION OF THE OPINION

Germany has submitted a CLH dossier containing a proposal together with the justification and background information documented in a CLH report. The CLH report was made publicly available in accordance with the requirements of the CLP Regulation at ***http://echa.europa.eu/harmonised-classification-and-labelling-consultation*** on **23/10/2012**. Concerned parties and Member State Competent Authorities (MSCA) were invited to submit comments and contributions by **5 October 2012**.

ADOPTION OF THE OPINION OF THE RAC

Rapporteur, appointed by RAC: **Marja Pronk**

The opinion takes into account the comments provided by MSCAs and concerned parties in accordance with Article 37(4) of the CLP Regulation.

The RAC opinion on the proposed harmonised classification and labelling was reached on **4 June 2013** and the comments received are compiled in Annex 2.

The RAC Opinion was adopted by **consensus**

OPINION OF THE RAC

The RAC adopted the opinion that Imazalil (ISO); 1-[2-(allyloxy)-2-(2,4-dichlorophenyl)ethyl]-1H-imidazole should be classified and labelled as follows:

Classification and labelling in accordance with the CLP Regulation:

	Index No	International Chemical Identification	EC No	CAS No	Classification		Labelling			Specific Conc. Limits, M-factors
					Hazard Class and Category Code(s)	Hazard statement Code(s)	Pictogram, Signal Word Code(s)	Hazard state-ment Code(s)	Suppl. Hazard state-ment Code(s)	
Current Annex VI entry	613-042-00-5	imazalil (ISO); 1-[2-(allyloxy)-2-(2,4-dichlorophenyl)ethyl]-1*H*-imidazole	252-615-0	35554-44-0	Acute Tox. 4* Acute Tox. 4* Eye Dam. 1 Aquatic Acute 1 Aquatic Chronic 1	H302 H332 H318 H400 H410	GHS05 GHS07 GHS09 Dgr	H302 H332 H318 H410		
Dossier submitters proposal	613-042-00-5	imazalil (ISO); 1-[2-(allyloxy)-2-(2,4-dichlorophenyl)ethyl]-1*H*-imidazole	252-615-0	35554-44-0	Add: Carc. 2 Modify: Acute Tox. 3 Delete: Aquatic Acute 1	H351 H301 ~~H400~~	Add: GHS08 Modify: GHS06	H351 H301		Add: M=10
RAC opinion	613-042-00-5	imazalil (ISO); 1-[2-(allyloxy)-2-(2,4-dichlorophenyl)ethyl]-1*H*-imidazole	252-615-0	35554-44-0	Add: Carc. 2 Modify: Acute Tox. 3 Delete * from Acute Tox. 4 Delete: Aquatic Acute 1	H351 H301 H332 ~~H400~~	Add: GHS08 Modify: GHS06	H351 H301		Add: M=10
Resulting Annex VI entry if agreed by COM	613-042-00-5	imazalil (ISO); 1-[2-(allyloxy)-2-(2,4-dichlorophenyl)ethyl]-1*H*-imidazole	252-615-0	35554-44-0	Carc. 2 Acute Tox. 3 Acute Tox. 4 Eye Dam. 1 Aquatic Chronic 1	H351 H301 H332 H318 H410	GHS08 GHS06 GHS05 GHS09 Dgr	H351 H301 H332 H318 H410		M=10

Classification and labelling in accordance with DSD

	Index No	International Chemical Identification	EC No	CAS No	Classification	Labelling	Concentration Limits
Current Annex VI entry	613-042-00-5	imazalil (ISO); 1-[2-(allyloxy)-2-(2,4-dichlorophenyl)ethyl]-1*H*-imidazole	252-615-0	35554-44-0	Xn; R20/22 Xi; R41 N; R50-53	Xn; N R: 20/22-41-50/53 S: (2-)26-39-60-61	
Dossier submitters proposal	613-042-00-5	imazalil (ISO); 1-[2-(allyloxy)-2-(2,4-dichlorophenyl)ethyl]-1*H*-imidazole	252-615-0	35554-44-0	Add: Carc. Cat. 3; R40 Modify: N; R51-53	Xn; N R: 40-51/53 S: 36/37	Add: N; R51-53: C ≥ 25%; R52-53: 2.5% ≤ C < 25%
RAC opinion	613-042-00-5	imazalil (ISO); 1-[2-(allyloxy)-2-(2,4-dichlorophenyl)ethyl]-1*H*-imidazole	252-615-0	35554-44-0	Add: Carc. Cat. 3; R40 Modify: N; R51-53	Xn; N R: 40-51/53 S: (2-)26-36/37/39-46-61	
Resulting Annex VI entry if agreed by COM	613-042-00-5	imazalil (ISO); 1-[2-(allyloxy)-2-(2,4-dichlorophenyl)ethyl]-1*H*-imidazole	252-615-0	35554-44-0	Carc. Cat. 3; R40 Xn; R20/22 Xi; R41 N; R51-53	Xn; N R: 20/22-40-41-51/53 S: (2-)26-36/37/39-46-61	

SCIENTIFIC GROUNDS FOR THE OPINION

RAC general comment

A comment was received during public consultation on the need for a justification for the use of read across from salts of Imazalil. The dossier submitter responded to this comment, referring to the Technical notes for the Guidance and the Technical guidance document for the Risk assessment of Biocides, stating that read across can be performed if the substance used in the study is closely related to the evaluated substance, and since Imazalil and Imazalil salts are structurally nearly similar, read across is justifiable

RAC evaluation of physical hazards

Summary of the Dossier submitter's proposal
Not evaluated in the CLH dossier.

Comments received during public consultation
This endpoint was not specifically commented on.

Assessment and comparison with the classification criteria
The RAC concluded that the physico-chemical properties of Imazalil do not warrant classification.

RAC evaluation of acute toxicity

Summary of the Dossier submitter's proposal
Imazalil already has a harmonised classification for Acute toxicity in Annex VI to the CLP Regulation, as category 4* for both oral and inhalation toxicity according to CLP and as DSD: Xn; R20/22. The proposal in the CLH dossier is to upgrade the acute oral toxicity to category 3 and to confirm the classification for acute inhalation toxicity.

The acute toxicity of Imazalil has been assessed in rats after oral, intra-peritoneal and dermal exposure (Goodwine, 1990a; Niemegeers, 1977; Teuns *et al.*, 1990a). In addition, an inhalation study (with Imazalil smoke; Appelman and Woutersen, 1983) is available, but was not considered reliable due to deficiencies in methodology and reporting. Additional information on the inhalation toxicity of Imazalil was provided in a pesticide assessment report (Pesticide Safety Directorate/ECCO-Team, 1996).

The acute oral study by Goodwine (1990a; pre-GLP, similar to OECD TG 401, Wistar rats, 10 male (M) and 10 female (F)) was considered the key study. Imazalil (in aqueous solution) was administered by gavage and the LD_{50} was determined to be 343 and 227 mg/kg bw for male and female rats (average LD_{50} 285 mg/kg bw), respectively. Taking into account the classification criteria this would lead to a classification as Acute Tox. 3 – H301 (Toxic if swallowed) based on an LD_{50} for female rats between 50 and 300 mg/kg bw, according to CLP, and Xn; R22 (Harmful if swallowed; LD_{50} between 200 and 2000 mg/kg bw) according to DSD.

No change to the existing acute inhalation toxicity classification (Acute Tox. 4 – H332; Harmful if inhaled) is proposed. The 4h-LC_{50} values for Imazalil dust were determined to be 2.88 and 1.84 mg/l for male and female rats, respectively (Pesticide Safety Directorate/ECCO-Team, 1996), which fall within the range of 1-5 mg/l/4h for category 4 (CLP) and R20 (DSD) for dusts and mists.

Although a study on acute dermal toxicity has been presented in the CLH dossier (Teuns *et al.*, 1990a; LD_{50} value above 2000 mg/kg bw in rabbits), the classification of this endpoint has not been specifically addressed by the dossier submitter.

Comments received during public consultation
Three MSCA's supported the proposed classification and one further noted that the * indicating minimum classification could be removed for acute inhalation toxicity. One industry (IND) representative commented that category 4 for acute oral toxicity is appropriate based on an LD_{50} value above 300 mg/kg bw in two more recent (and GLP-compliant) studies than the study

referred to in the CLP report, which IND considered less reliable. IND however only reported the results of these studies (in a confidential expert statement), but did not provide the original studies. Having not had access to the original studies and noting that all three studies had the same reliability score in IUCLID, the RAC saw no reason to dismiss the study with the lower LD_{50} value.

Assessment and comparison with the classification criteria

Following a comparison of the available LD_{50} and LC_{50} values in rats with the CLP criteria, the RAC supported the conclusion of the dossier submitter that Imazalil should be classified under CLP for acute oral toxicity with Acute Tox. 3 – H301 (DSD: Xn; R22) and for acute inhalation toxicity with Acute Tox. 4 – H332 (DSD: Xn; R20). The RAC also concluded that based on the available dermal LD_{50} value, classification for acute dermal toxicity was not warranted.

RAC evaluation of specific target organ toxicity – single exposure (STOT SE)

Summary of the Dossier submitter's proposal

Not evaluated in the CLH dossier.

Comments received during public consultation

This endpoint was not specifically commented on.

Assessment and comparison with the classification criteria

In the acute toxicity studies, clinical signs were observed that could possibly warrant classification for STOT SE. In the dermal acute toxicity test with rabbits, 6 out of 10 animals showed sedation upon exposure to 2000 mg/kg bw. The sedation was transient (only observed on day 1) and slight to moderate in nature. In the oral acute toxicity study with rats, (a.o.) ataxia, tremors and excitation were observed at doses ≥160 mg/kg bw, accompanied by (a.o.) loss of righting reflex at doses ≥320 mg/kg bw. No information was available on the severity, incidence and duration of this effect. In the acute inhalation study with rats, animals showed (a.o.) lethargy, ataxia, coma and loss of righting reflex, but all surviving animals appeared normal from day 6. No information was available on the severity and incidence of these effects or at what doses they occurred.

The RAC noted that some of the effects occur at lethal dose levels, and for lethality the substance is already proposed to be classified. Some effects, however, also appear to occur below lethal dose levels. On the other hand, the RAC was provided with too little detail from the studies to allow proper evaluation of the endpoint 'specific target organ toxicity – single exposure'. Effectively, this endpoint should therefore be considered as not evaluated by the RAC.

RAC evaluation of skin corrosion/irritation

Summary of the Dossier submitter's proposal

A skin irritation study in rabbits (Goodwine, 1990b; according to OECD TG 404) was presented in the CLH dossier, but classification for this endpoint had not been specifically addressed by the dossier submitter. In the study, no formation on erythema or oedema was observed at any observation time following single application of 0.5 g dry Imazalil powder for 4 hours in 3 rabbits, and the substance was concluded to be not irritating. This result, plus the physico-chemical data, does not give any indication of corrosivity.

Comments received during public consultation

This endpoint was not specifically commented on.

Assessment and comparison with the classification criteria

Since all three test-animals scored zero for both erythema and edema over 24-48-72h in the study presented on skin irritation, the RAC concluded that Imazalil should not be classified for skin irritation.

RAC evaluation of eye corrosion/irritation

Summary of the Dossier submitter's proposal
Imazalil already has a harmonised classification in Annex VI to CLP as Eye Dam. 1, H318 according to CLP (DSD: Xi; R41). No change to this classification is proposed by the dossier submitter, but an eye irritation study in rabbits (Teuns *et al.*, 1990b) was summarised in the CLH dossier.

Comments received during public consultation
This endpoint was not specifically commented on.

Assessment and comparison with the classification criteria
In the one study presented for eye irritation, administration of 0.1 g Imazalil resulted in the following mean irritation scores over 24 to 72h for the three animals tested: corneal opacity 2/1.7/1.7, iritis 0.3/1/0.7, conjuctival erythema 1/0.7/0.3 and chemosis 1.3/0.7/0.7. The corneal opacity was not reversible in two out of three animals by observation day 21. The RAC concluded that the current CLP classification of Imazalil for eye irritation, i.e. Eye Dam. 1 – H318 (DSD: Xi; R41) is justified, given the non-reversibility of the corneal opacity.

RAC evaluation of respiratory tract irritation

Summary of the Dossier submitter's proposal
The CLH dossier mentions that no data on respiratory tract irritation are available, so this endpoint is not further addressed.

Comments received during public consultation
This endpoint was not specifically commented on.

Assessment and comparison with the classification criteria
In the absence of data, no conclusion can be drawn on the classification for respiratory tract irritation.

RAC evaluation of skin sensitisation

Summary of the Dossier submitter's proposal
Imazalil was evaluated for skin sensitisation in an adjuvant Guinea Pig Maximisation Test (GPMT) of Magnusson and Kligman (Teuns *et al.*, 1990c; according to OECD TG 406, 20 animals, DNCB used as positive control). One of 20 animals showed a mild reaction compared to a response rate of 100% with the positive control.

In a non-adjuvant Buehler test (Wnorowski, 1997; similar to OECD TG 406) one of 10 animals developed a very faint, non-confluent erythema 24 hours after challenge with Imazalil compared to 3/10 moderate, 5/10 faint and 2/10 very faint reactions in the positive control group (DNCB).

Based on these data (no further details on e.g. tested concentrations etc. were provided), it was concluded that Imazalil does not fulfil the criteria for classification for skin sensitisation under CLP/DSD.

Comments received during public consultation
This endpoint was not specifically commented on.

Additional key elements
According to the EFSA Draft Risk Aassessment Report (DAR, 2009) and Competent Authorities Report (CAR, 2009; biocides) there is a case report of a patient developing acute eczematous contact dermatitis after topical administration of an Imazalil containing preparation (Imaverol) for treatment of a fungal infection.

Assessment and comparison with the classification criteria

Very little detail is presented in the CLH report on the two skin sensitisation studies, complicating the assessment of the quality of the studies. Given that the level of response observed (5% in the GPMT, 10% in the Buehler test) does not meet the criteria for classification under CLP/DSD (≥30% in an adjuvant type guinea pig test and ≥15% in a non-adjuvant type Buehler test), it seems that the previous conclusion based on these studies (Imazalil is not a skin sensitiser) and the resulting current 'no classification' for this endpoint are justified. Further, a single case of contact dermatitis is not sufficient to warrant classification.

RAC evaluation of respiratory sensitisation

Summary of the Dossier submitter's proposal

The CLH dossier mentions that no data on respiratory sensitisation are available, so this endpoint is not further addressed.

Comments received during public consultation

This endpoint was not specifically commented on.

Assessment and comparison with the classification criteria

In the absence of data, no conclusion can be drawn on the classification for respiratory sensitisation.

RAC evaluation of repeated dose toxicity (DSD) and specific target organ toxicity (CLP) – repeated exposure (STOT RE)

Summary of the Dossier submitter's proposal

Six studies on sub-acute/sub-chronic oral toxicity of Imazalil were included in the CLH report; three in rats, two in mice and one in dog.

Rat studies:

- *3-months dietary study* (Van Deun *et al.*, 1996a; according to OECD TG 408 with some deviations), considered to be a **key study**:
  - Wistar rats (10 M, 10 F), doses of 0, 200, 400 and 800 ppm (corresponding to 0, 16/19, 32/38 and 64/76 mg/kg bw/d in M/F)

- *3-months dietary dose-range finding study* (Van Deun *et al.*, 1996b).
  - Wistar rats (10 M, 10 F), doses of 0, 800, 1600, 2400 and 3200 ppm (corresponding to 0, 64/79, 129/150, 181/236, 252/333 mg/kg bw/d in M/F)

- *6-months dietary study* (Lina *et al.*, 1983; similar to OECD TG 452, part of a 2-year study):
  - Wistar rats (10 M, 10 F), doses of 0, 25, 100 and 400 ppm (corresponding to 0, 1.25, 5 and 20 mg/kg bw/d)

Mouse studies:

- *3-month dietary study* with one month interim (Van Deun *et al.*, 1994; similar to OECD TG 408), considered to be a **key study**:
  - Swiss Albino mice (25 M, 25 F; interim 10 M/10 F), doses of 0, 50, 200 and 600 ppm (corresponding to 0, 12/14, 47/55, 138/166 mg/kg bw/d in M/F)
- *3-months dietary study* (Verstraeten *et al.*, 1993b; similar to OECD TG 408)
  - Swiss Albino mice (10 M, 10 F), doses of 0, 200, 400 and 800 ppm (corresponding doses in mg/kg bw/d not calculated)

Dog studies:

- 1 year oral (capsule) study (Verstraeten *et al.*, 1989, similar to OECD TG 452), considered to be a **key study**:
  - Beagle dogs (4 M, 4 F), doses of 0, 1.25, 2.5 and 20 mg/kg bw/d

Similar effects were seen in all three species tested.

In the rat, 20 mg/kg bw/d for 6 months and ≥ 32/38 mg/kg bw/d for 3 months resulted in e.g. increased liver weight and histological liver changes (hepatocyte hypertrophy and fatty vacuolisation), accompanied by changes in corresponding serum parameters (increased LDH and decreased AST, ALT and urea). Also a decrease in body weight, increased adrenal weight and adrenocortical cell swelling as well as some changes in haematological parameters were observed.

In mice, almost identical liver toxicity to that seen in rats was seen at ≥ 200 ppm (equivalent to approximately 30 mg/kg bw/d) or ≥ 47/55 mg/kg bw/d for 3 months, while in dogs early signs of liver toxicity (e.g. increased liver weight and increased level of alkaline phosphatase activity) were seen at the highest dose of 20 mg/kg bw/d, together with some signs of general toxicity and some changes in haematological parameters (statistically significant but either within the historical control range or borderline).

Two rabbit (New Zealand White, 5 M, 5F) studies on dermal toxicity are included in the CLH dossier (Teuns *et al.*, 1991), one preliminary study over 4 days (63, 250 and 1000 mg/kg bw/d) and one 3-week study (0, 10, 40, 160 mg/kg bw/d, 6 h/day, 5 days/week). In the preliminary study, a dose of 250 mg/kg bw/d induced slight liver toxicity and erythema (grade 1) which developed into severe skin lesions (slight to moderate fissures and scaling, grade 1 and 2). The higher dose of 1000 mg/kg bw/d increased the intensity of the liver toxicity from slight to moderate. No adverse effects were seen at the lowest dose. In the main study of 3 weeks, no relevant adverse local or systemic effects were reported.

The dossier submitter concluded that the effects on liver and haematological parameters seen at doses below the cut-off values for classification were primarily adaptive and not sufficiently severe to require classification under either CLP or DSD. Likewise, the dossier submitter proposed no classification for skin irritation, given that the dermal effects in rabbits were observed at a dose above the extrapolated cut-off values for classification under CLP/DSD.

Comments received during public consultation

One MSCA proposed a CLP classification of Imazalil as STOT RE 2 – H373 (DSD: Xn; R48/22, based on hepatic injury observed in sub-acute and sub-chronic studies at doses below the guidance values (hepatic fatty vacuolisation was mentioned as the most severe effect justifying classification). Another MSCA made a general comment that the human health part of the CLH report is not sufficiently detailed to permit a complete assessment of the presented studies. No further comments were received.

Assessment and comparison with the classification criteria

Oral

Oral repeated dose studies were available in the dossier for rat, mouse and dog. Rat studies included two 90-day studies and one 6-month study (all dosing occurring via the diet). The available mouse studies included two 90-day dietary studies. Further, a one-year dog study with Imazalil administered via capsules was available, plus three long-term studies (an 18-month rat study and two 2-year carcinogenicity studies (in rats and mice)).

Very little detail is presented in the CLH dossier on the available repeated dose studies, complicating the interpretation of the effects as to their potential classification. However, it is clear that following short- and long-term oral exposure, the liver was the main target organ in all species tested. Effects on the liver included changes in biochemical parameters, increased liver weight, hepatocyte swelling and (fatty) vacuolisation, hypertrophy and, in the long-term studies (see "RAC evaluation of carcinogenicity"), pigmented hepatocytes and focal cellular changes (e.g. eosinophilic foci, focal cystic degeneration).

In rats, the effective dose levels in the short-term studies (≥ 32/38 and 20 mg/kg bw/d for the 90-d and 6 month studies, respectively) are below the (extrapolated) guidance values for classification as STOT RE 2 (100 mg/kg bw/d for a 90-day study, 50 mg/kg bw/d for a 6-month study) or R48 (50 mg/kg bw/d for a 90-day study, 25 mg/kg bw/d for a 6-month study), whereas those for the long-term studies (15.9/20.3 and ≥ 60/14 mg/kg bw/d in the 18-month and 2-year

studies, respectively) are at or above the (extrapolated) guidance values (for STOT RE 2: 16.7 mg/kg bw/d for a 18-month study, 12.5 mg/kg bw/d for a 2-year study; for R48: 8.3 mg/kg bw/d for a 18-month study, 6.25 mg/kg bw/d for a 2-year study). The effects in the long-term studies therefore do not qualify for classification. As to the effects in the 6 month study: these were relatively minor (increased LDH in females, increased weights of liver and kidney in males and females without accompanying macroscopic or histopathological changes), and therefore also do not qualify for classification. In the 90-day studies on the other hand, the increased liver weight was accompanied by hepatocyte vacuolisation and hypertrophy and, at higher doses, by (a.o.) decreases in AST and ALT. However, the level of detail provided in the CLH dossier as to incidences and severity of these effects is not sufficient to establish whether they would qualify as significant or severe toxicity (CLP) or serious damage (DSD).

In mice, the effective dose level in the 2-yr study (≥ 33/42 mg/kg bw/d) is above the extrapolated guidance value for classification as STOT RE 2 (12.5 mg/kg bw/d for a 2-year study) or R48 (6.25 mg/kg bw/d for a 2-year study), hence the effects do not qualify for classification. In the 90-day studies, the effective dose levels (≥ 30 mg/kg bw/d) and ≥ 47/55 mg/kg bw/d) are below or at the guidance values for a 90-day study (for STOT RE 2: 100 mg/kg bw/d, for R48: 50 mg/kg bw/d). At these dose levels hepatocyte vacuolisation and degeneration was observed together with increased liver weight (in one study) or decreased AST (in another study). Again, however, too little detail is provided in the CLH dossier on incidences and severity of these effects to establish whether they would qualify as significant or severe toxicity (CLP) or serious damage (DSD).

In dogs, the early signs of liver toxicity at 20 mg/kg bw/d were not accompanied by histopathological changes and are concluded to be of insufficient severity to fulfil the criteria for STOT RE (CLP) or R48 (DSD).

In conclusion, in most studies the effects on the main target organ, liver do not qualify 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). Yet, the 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.

Dermal

Two dermal studies in rabbits were presented in the CLH dossier. In a preliminary 4-day study, hepatotoxicity was observed at 250 (slight) and 1000 (moderate) mg/kg bw/d. At 250 mg/kg bw/d, slight erythema developing into fissures and scaling was also seen. It is not clear from the description how many animals were affected and whether the skin effects were also observed at 1000 mg/kg bw/d. In the main 3-week study, no adverse (local and systemic) effects were observed up to and including the highest dose level of 160 mg/kg bw/d.

For the dermal route, the RAC concluded that Imazalil does not need to be classified for repeated dose toxicity under either CLP or DSD, given the absence of local and systemic effects in the 3-week study and the fact that the liver toxicity in the preliminary study is not sufficiently severe to warrant classification. The RAC noted that the skin effects in the preliminary study could possibly indicate the need for an R38 classification under DSD (where significant local effects on the skin after repeated dermal application are considered more appropriately classified with R38 than with R48), but concluded that there is insufficient information to decide on the significance of the effect.

Inhalation

In the absence of data for the inhalation route, no conclusion can be drawn on the classification for effects induced upon repeated inhalation exposure.

RAC evaluation of germ cell mutagenicity

Summary of the Dossier submitter's proposal

The mutagenicity of Imazalil has been evaluated in 4 *in vitro* studies (Ames test with *S. typhimurium* and a mammalian chromosome aberration assay with peripheral human lymphocytes, both similar to OECD TG 471; a mammalian cell gene mutation test with Chinese hamster lung fibroblasts, similar to OECD TG 476; and unscheduled DNA synthesis with primary rat hepatocytes, according to OECD TG 482) as well as one *in vivo* study (micronucleus test in Swiss Albino mice, similar to OECD TG 474). There were no signs of genotoxicity in any of the tests and hence it was concluded that there is no concern for the potential genotoxicity of Imazalil.

Comments received during public consultation

This endpoint was not specifically commented on.

Assessment and comparison with the classification criteria

Given that Imazalil tested negative in the studies available (4 *in vitro*, 1 *in vivo*), the RAC concluded that based on these studies Imazalil is not genotoxic and hence no classification is justified.

RAC evaluation of carcinogenicity

Summary of the Dossier submitter's proposal

Two oral carcinogenicity studies were considered reliable, one in rats and one in mice, both studies essentially following OECD TG 452. One further carcinogenicity study in rats was available (Lina *et al.*, 1984), but was not considered reliable due to deficiencies in dose selection. Further, one long-term study in dogs (Verstraeten *et al.*, 1989; 1 year, daily capsule administration) was considered useful for evaluation of the carcinogenicity of Imazalil.

No data on carcinogenicity following dermal or inhalation exposure were available.

Rat study (Van Deun *et al.*, 1999):
Wistar rats (50 M, 50 F), dietary administration for 24 months, doses of 0, 50, 200, 1200 and 2400 ppm (corresponding to 0, 2.5/3.5, 10/14, 60/80 and 120/160 mg/kg bw/d in M/F, respectively).

Mouse study (Verstraeten *et al.*, 1993a):
Swiss Albino mice (SPF) (50M, 50 F), dietary administration for 23 months, doses of 0, 50, 200 and 600 ppm (corresponding to 0, 8.1/9.9, 33/42 and 105/131 mg/kg bw/d in M/F, respectively).
Note: female survival in this study was below 50% (36-48%) for all groups, including controls.

Dog study (Verstraeten *et al.*, 1989):
For details, see "RAC evaluation of repeated dose toxicity (DSD) and specific target organ toxicity (CLP) – repeated exposure (STOT RE)".

The liver was identified as the target organ in all three species. In rats and mice, haematological/plasma parameters were affected, with a higher sensitivity in females than males. In female rats (at 200 ppm), haemoglobin values and red blood cell counts (RBC) were increased, while e.g. mean corpuscular volume, plasma potassium and urea nitrogen were decreased compared to controls. Similar effects were seen in female mice at the same dose level of 200 ppm. In male rats, gross and microscopic liver changes from 1200 ppm included eosinophilic foci, hypertrophy, vacuolisation, focal cystic degeneration. Microscopically, pigment laden hepatocytes were observed in female rats at 200 ppm, accompanied by hypertrophy at higher doses where livers were dark and showed more pronounced lobulation. In male mice, macro- and microscopic liver changes (vacuolisation, sinusoidal cell pigmentation and swelling) were seen at 200 ppm. At higher doses, the adverse effects on the haematological system and liver were enhanced in both rats and mice, and affected both males and females of both species.

In mice, an increase in the frequency of hepatocytic neoplasms and of neoplastic nodules (adenomas) was seen at 200 and 600 ppm in males and at 600 ppm in females. In male mice, incidences of hepatic carcinoma were also increased, at 600 ppm.

In male rats a statistically significantly higher incidence of thyroid follicular cell neoplasias (adenomas and carcinomas combined) was seen at 1200 and 2400 ppm, together with swelling, increased thyroid weight and cystic follicular hyperplasia. Statistically significantly increased incidences of liver adenomas were seen in male rats at 2400 ppm only.

Several mechanistic studies (for details, see background document, section 5.10) were performed in order to conclude on the mode of action for induction of the tumours and for evaluating the relevance for humans. Based on these studies it was concluded that the thyroid tumours in rats are the result of the deregulation of thyroid hormone homeostasis, and that these tumours are not relevant for humans due to quantitative species differences in sensitivity for hormonal imbalances in the thyroid-pituitary feedback mechanism.

It was further concluded that the relevance for humans cannot be excluded for the hepatic neoplasms seen in rats and mice. No mode of action for these tumours could be established with certainty. It was concluded that the mechanism involved is most likely non-genotoxic and tumour-promoting with a practical threshold and an induction of a mixed type of microsomal enzymes. The results of the studies indicate that Imazalil and phenobarbital may share some common mechanisms but a definite conclusion on the similarity of the mode of action between the two substances cannot be established. In relation to phenobarbital, the dossier submitter further refers to an International Agency for Research on Cancer (IARC, 2001) report which states that there is inadequate evidence from humans for the carcinogenicity of phenobarbital, but that there is sufficient evidence from experimental animals, resulting in a Group 2B classification (possibly carcinogenic to humans) for phenobarbital. Given this, the dossier submitter concluded that the hepatic neoplasms seen in two animal species after Imazalil exposure may be of relevance to humans and that Imazalil should hence be classified as Carc. 2 - H351 (Suspected of causing cancer) according to CLP, and Carc. Cat. 3; R40 according to DSD.

Comments received during public consultation

Two MSCA's expressed general agreement for the classification proposal, although one commented that the human health part of the CLH report is not sufficiently detailed to permit a complete assessment of the presented studies.

One MSCA considered that the increased incidence of neoplasms, although appearing in two different species, was not sufficient evidence for classification, given that they were primarily benign, there was no dose-response relationship, and liver carcinomas were only seen in mice treated for more than 18 months and are hence more likely to be due to aging.
Another MSCA stated that very limited information is provided in the CLH proposal and would have liked to see more information on e.g. actual incidences and historical control data. They also commented that different terminology was used in the CLH report compared to the DAR. The MSCA did however agree with the dossier submitter that, whereas the thyroid tumours are considered not relevant to humans, the increase in liver tumours cannot be dismissed as non-relevant to humans as the mechanism is unclear, and hence agreed that Imazalil should be classified as Carc. 2 according to CLP.
A fifth MSCA agreed that Imazalil should be classified as Carc. 2 since there is some uncertainty regarding the relevance of the liver tumours to humans. This MSCA also agreed that there is sufficient mechanistic information to discount the rat thyroid tumours as not being relevant for human health, but suggested that the EU specialised experts conclusion should be used for dismissing these tumours.

One IND representative commented on the statistical significance of the findings in the mouse carcinogenicity study and concluded that a statistically significant increase in combined hepatocellular adenoma/carcinoma in females occurred 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 the (statistically significantly increased) hepatocellular tumours in male mice were concluded to remain within the boundaries

of the historical controls from the same laboratory. IND further commented that in rats, no corresponding tumour profile was observed, and that the statistically significant increase in hepatocellular adenoma was limited to male rats at the highest dose level that was far beyond the maximum tolerated dose (MTD) and therefore should not be considered for cancer risk assessment.
IND did not agree that Imazalil should be classified for carcinogenicity, as there are data showing that the mechanism causing the liver tumours in rodents is not relevant to humans (referring a.o. to the mechanistic studies in the dossier and to new *in vitro* studies submitted during public consultation with mouse and human hepatocytes, where Imazalil caused cell proliferation in mouse hepatocytes, but not in human hepatocytes; and that cell proliferation is a prerequisite for liver cell carcinogenicity).

The dossier submitter did not provide more details (on e.g. historical control incidences), but in response to the IND comment remarked that the *in vitro* study with human hepatocytes has been performed with a set of hepatocytes from one donor only. Furthermore, the dossier submitter states that a new *in vivo* mechanistic study provided by IND during public consultation shows that humanised PXR/CAR mice react to the substance in the same way as wild type mice, supporting the hypothesis that the tumours are indeed relevant to humans.

Assessment and comparison with the classification criteria

Carcinogenicity studies (2-year) in rat and mouse (considered key studies) were available for Imazalil, with administration via the diet. In addition, an 18-month oral study in rats and a one-year oral dog study were included in the dossier. The study in dogs, in which no tumours were observed, is considered less relevant for carcinogenicity due to the limited exposure and observation duration (1-year exposure, no post-exposure observation period) and the limited number of animals (4M+4F/exposure dose). In the 18-month rat study (considered not reliable by the dossier submitter), also no increase in tumours was observed.

The CLH dossier further refers to several mechanistic studies, performed in order to conclude on the mode of action for induction of the tumours observed and for evaluating the relevance for humans.

The data presented in the CLH dossier on the above studies are fairly brief summaries only, the lack of detail complicating the interpretation of the effects in relation to conclusions on any potential classification. The RAC further noted several discrepancies between the description of the key studies in section 5.8.1 of the CLH report, the tabular presentation in table 25 of the CLH report and the summaries of these studies provided as annexes 5 and 6 to the CLH dossier. The RAC used these latter annexes as the basis for the evaluation, as they provided the most details (for incidence data on (non-)neoplastic lesions see section *Supplemental information* below).

The liver was identified as the main target organ in rats and mice. In rats, the thyroid appeared to be a second target organ.

Thyroid

In male rats, a statistically significantly higher incidence of thyroid follicular cell neoplasias (adenomas and carcinomas combined) was seen at 1200 and 2400 ppm together with swelling, increased thyroid weight and cystic follicular hyperplasia. The increase was mostly due to an increase in adenomas.

Mechanistic studies with Imazalil are available which indicate that that the observed thyroid tumours are not a primary effect of Imazalil, but are likely to be secondary to increased hepatic microsomal enzyme induction. Increases in UDPGT were observed, with concomitant changes in T3 and T4 and increases in TSH. This would reduce the relevance to humans, as it is known that humans are considerably less susceptible to the formation of thyroid tumours mediated by UDPGT induction than rodents (especially rats), in which consequent T4 reduction, TSH increase and finally increased thyroid stimulation are seen (CLP guidance 3.6.2.3.2(k), by reference to the Specialised Experts conclusions in document ECBI/49/99_Add.1_Rev.2). Given also that the thyroid tumours were mainly benign in nature and only occurred in males, that the thyroid gland related carcinogenicity is of low potency (with a T25 > 100 mg/kg bw/d), and that the mechanism

behind these thyroid tumours was not genotoxic (Imazalil tested negative in a battery of mutagenicity studies), the RAC concluded that the thyroid tumours in rats do not warrant classification.

Liver
In mice, Imazalil treatment resulted in increased liver weight in both sexes at 600 ppm. Macro- and microscopic liver changes (non-neoplastic) were seen in male mice at 200 and 600 pm and consisted of foci, vacuolisation, sinusoidal cell pigmentation and swelling. A trend towards similar lesions was reported to be seen in female mice at 600 ppm, but no data were shown. Neoplastic changes (no data on statistical significance reported) consisted of increased incidences of hepatocytic neoplasms (i.e. combined hepatocellular adenoma/carcinoma) and neoplastic nodules (i.e. hepatocellular adenoma) at 200 and 600 ppm in males and at 600 ppm in females. In male mice, the incidence of hepatocellular carcinoma at 600 ppm was also increased. Other effects included a reduced body weight (by 5-10%) and body weight gain (by 15-20%) in males at 600 ppm. Haematological parameters were only affected in females (increased haemoglobin, haematocrit and RBC at 200 and 600 ppm), but only after 1-yr of dosing, not at the end.

The CLH dossier contained several mechanistic studies in mice, studying the effect of Imazalil treatment on the liver. For cell proliferation, varying results were observed: treatment of male mice with 1200 ppm Imazalil for 4 days (Elmore, 2004) resulted in induction of cell proliferation (43-fold) whereas treatment of male mice with 1200 ppm for 2 or 13 weeks (O'Neill, 2002; as also summarised in Picirillo, 2002) resulted in inhibition of cell proliferation. In liver samples from the key 3-month study (Van Deun *et al.*, 1994; see "RAC evaluation of repeated dose toxicity (DSD) and specific target organ toxicity (CLP) – repeated exposure (STOT RE)") no effect of Imazalil treatment at 50, 200 or 600 ppm was seen on cell proliferation in males and females (Lawrence, 2001). In liver samples from the same key study, microsomal protein and cytochrome P450 content were increased and Imazalil appeared to both inhibit and induce various hepatic enzymes (Vermeir, 1994). Induction was seen for CYP2B and CYP3A activity (as measured by e.g. PROD, EROD, N-ethyl morphine demethylase; indicative for a mechanism via PXR/CAR activation), but not for CYP4B activity (as measured by e.g. lauric acid hydroxylase; indicative of peroxisome proliferation). For some enzymes this varied between time points (e.g. for PROD, induction was seen after 1 month of treatment but inhibition after three months). Other liver effects seen in the Elmore (2004) and O'Neill (2002) study included increased liver weight, hepatocytic vacuolation, hypertrophy and (minimal) necrosis, and increases in ALT and sorbitol dehydrogenase (SDH).

IND in their comments presented additional data on statistical significance for the liver tumours, as well as historical control data for hepatocellular tumours from 9 mouse carcinogenicity studies performed in the same test laboratory, starting within the same period of time and using the same strain of mice (see section *Supplemental information* below). From these data it appears that the only tumour findings that reached statistical significance were the increases in adenoma and combined adenoma/carcinoma in males at 200 and 600 ppm and the increase in combined adenoma/carcinoma in females at 600 ppm. The incidences for combined adenoma/carcinoma were outside the historical control ranges for both males and females, the incidences for adenomas in males were at and above the upper level of the historical control range. The RAC noted that IND concluded that in male mice the tumour incidences remained within the boundaries of the historical controls, but IND by mistake used the absolute incidences, not the incidence rates.

IND in their comments further referred to the results of some recent mechanistic studies with Imazalil (see section *Additional key elements* above). According to IND, Imazalil (7-day exposure) and phenobarbital induced the transcription of cyp2b10 and cyp3a11 (typical of PXR/CAR activation) in wild type mice and to a lesser extent in humanised PXR/CAR mice, albeit Imazalil was less potent than phenobarbital. In other studies (*in vitro*), Imazalil at up to toxic levels was found not to induce cell proliferation in human female hepatocytes, in contrast to female mouse hepatocytes. According to IND, this inability of Imazalil to produce replicative DNA synthesis in human hepatocytes demonstrates the non-relevance to humans of the hepatocellular tumours in mice, as cell proliferation through PXR/CAR activation is an essential step in the development of hepatocellular tumours.

The RAC noted that cell proliferation was not only investigated in the *in vitro* studies with female human and mouse hepatocytes, but also in an *in vivo* study with wild type mice and humanized PXR/CAR mice. Surprisingly, Imazalil in this latter study induced cell proliferation in hPXR/hCAR mice, as it also did in wild type mice. It is recognised, however, that except for the two genes CAR and PXR, all other genes in hPXR/hCAR mice are still murine in nature, in contrast to the "all-human" human hepatocytes.

In rats, Imazalil treatment resulted in increased liver weight in both sexes at 1200 and 2400 ppm. In male rats, gross and microscopic liver changes (non-neoplastic) at these dose levels included (eosinophilic) foci, centriacinar hypertrophy, vacuolisation, focal cystic degeneration and pigment laden hepatocytes. An increase in this latter finding was already observed at 200 ppm in female rats, and this was accompanied by centriacinar and periacinar hypertrophy at higher dose levels where livers were dark and showed more pronounced lobulation. The only neoplastic finding in the liver was a statistically significantly increased incidence in adenomas in male rats at 2400 ppm.

Other effects included reductions in body weight and body weight gain in both sexes at 1200 and 2400 ppm. Food consumption was reduced in females at 1200 ppm and in both sexes at 2400 ppm. From 200 ppm, in female rats, haemoglobin values and red blood cell counts (RBC) were increased, while e.g. mean corpuscular volume, plasma potassium, urea nitrogen, ALT and AST were decreased compared to controls. At higher doses, the adverse effects on the blood and serum parameters were enhanced and included also males.

IND in their comments presented historical control data for hepatocellular adenoma and carcinoma in male rats from 8 rat carcinogenicity studies (with 10 control groups in total) performed in the same test laboratory, starting within the same period of time and using the same strain of rats (see section *Supplemental information* below). From this it appears that the incidence of hepatocellular adenomas in male rats at 2400 ppm was greater than the historical control range. IND however commented that the increase in this type of tumour only occurred at a dose level that was far beyond the MTD as a result of bad nutritional status due to dietary aversion (resulting in a decrease in body weight gain of 19%), and that therefore they should not be taken into account. Indeed, food wastage was observed in male rats dosed at 2400 ppm (and to an even greater extent in female rats dosed at 1200 and 2400 ppm), apparently due to lack of palatability of the treated food. Whether this dose can be considered 'far beyond the MTD' in males is questionable, as the poor nutritional status was not associated with overt clinical signs of toxicity, an increase in mortality, or severely altered serum biochemistry parameters. Besides, the reduction in body weight gain in female rats was even greater, and they showed no increased tumour incidence.

The CLH dossier contained similar mechanistic studies for rats to those that were also available for mice. Treatment of male rats with Imazalil at 200, 1200 and 2400 ppm for 1, 2, 7, 14 or 28 days did not result in hepatic cell proliferation, whereas phenobarbital (1200 ppm) did (Mertens, 2011; as also summarised in Picirillo, 2011). Hepatic cell proliferation following Imazalil treatment was also not observed in a study by Elmore (2004). This study is however of low quality, as the positive control phenobarbital was also negative for cell proliferation. In male rat liver samples from the two 3-month studies (Van Deun *et al.*, 1996a/b; see "RAC evaluation of repeated dose toxicity (DSD) and specific target organ toxicity (CLP) – repeated exposure (STOT RE)") microsomal protein and cytochrome P450 content were increased and Imazalil appeared to be a mixed type of inducer, inducing various hepatic enzymes representative of CYP2B and CYP3A activity. CYP4B activity also tended to be slightly higher (Vermeir, 1995, 1996). CYPB1/2 induction (as measured by PROD) was also observed in the study by Mertens (2011), and the induction was dose- and time-dependent. Other effects seen in this latter study included increased liver weight, increased cytoplasmic homogeneity, and increased mRNA levels of phenobarbital response signature genes cyp2b1, cyp3a1, cyp3a2 and gadd45b. Phenobarbital induced similar findings, but with greater magnitude, and also increased ALT, SDH and single-cell necrosis. Neither Imazalil nor phenobarbital affected caspase-positive or 4-hydroxynonenal positive cells (indicative of apoptosis and oxidative stress, respectively), and also levels of CAR (NR1I3) did not show induction at the mRNA level.

Conclusion
The liver tumours observed form a borderline case for classification for carcinogenicity. In male rats the increase only involved adenomas and was limited to the highest dose, with no dose-response at lower doses. This is considered 'limited evidence' for carcinogenicity. The increase in liver tumours in male mice was observed against relatively high background incidences (26% for combined adenoma/carcinoma, 16% for adenoma, 10% for carcinoma) and was statistically significant for adenomas and combined adenoma/carcinoma only, with no dose-response at 200 and 600 ppm. The increase in liver tumours in female mice was limited to the highest dose and reached statistical significance only by combining adenomas (that were increased, but not statistically significantly, and without dose-response at lower doses) and carcinoma. The evidence for carcinogenic effects in mice is therefore also considered 'limited'. Given the limited evidence in both rats and mice, there are insufficient grounds for a category 1B classification for carcinogenicity. The choice is between a category 2 classification and no classification, depending on the mode of action that could account for the liver effects in rats and mice and their relevance to humans.

The mechanistic data seem to indicate that oxidative stress and peroxisome proliferation are unlikely to be involved in the development of the liver tumours following Imazalil treatment, and that there is also little evidence for cytotoxicity and (in rats) apoptosis. The mechanism is however non-genotoxic (Imazalil tested negative in a battery of mutagenicity studies), and most likely involves enzyme induction (with a practical threshold) as in several mechanistic studies Imazalil appeared to be a mixed type of microsomal enzyme inducer (indicative of CYP2B and CYP3A activity) in both rats and mice. The fact that in most studies Imazalil, similar to phenobarbital, further caused increases in liver weight and in hepatocellular hypertrophy and vacuolisation, and the up-regulation of several phenobarbital response signature genes, could point to a phenobarbital-like mode of action through PXR/CAR activation. Cell proliferation is however an additional essential step in the development of hepatocellular tumours by phenobarbital. IND argued that for phenobarbital it has been shown *in vitro* that there is a difference in ability between rodent and human hepatocytes in producing cell proliferation through CAR activation, by referring to Hirose *et al.* (2009). In this latter study, phenobarbital was able to induce CYP2b forms in both rat and human hepatocytes, but cell proliferation only in rat hepatocytes. Apparently a similar result has been observed for mouse versus human hepatocytes, given the results reported for phenobarbital in the Elcombe (2012b) study (see section *Additional key elements* above).

With reference to Ross *et al.* (2010), IND further argued that *in vivo* studies with humanised PXR/CAR mice exposed to phenobarbital confirmed the absence of cell proliferation, reason why phenobarbital-induced liver tumours in rodents are not considered relevant to human health (supported by the absence of an increased liver tumour risk in humans receiving phenobarbital for many years). Indeed, in the Ross *et al.* (2010) study, cell proliferation was only observed in wild type mice and not in hPXR/hCAR or knockout PXR/CAR mice following intraperitoneal injection of 80 mg/kg bw/d phenobarbital for 4 days. The RAC noted however that in the Elcombe (2012a) study, phenobarbital at a dietary dose equivalent to 127.8-155.3 mg/kg bw/d *did* induce cell proliferation in wild type and hPXR/hCAR mice (see section *Additional key elements* above). Apparently there is a threshold for phenobarbital-induced cell proliferation somewhere between 80 and 120 mg/kg bw/d.

For Imazalil the mechanistic data on cell proliferation are equivocal: in (male) rats, no cell proliferation was observed, whereas in (male) mice cell proliferation was shown after relatively short exposure (4-7 days) but not after longer exposure. The recent experiments with Imazalil by Elcombe showed an absence of replicative DNA synthesis in human hepatocytes, but an increase in cell proliferation (albeit not dose-related) in humanised PXR/CAR mice.

All in all, it can be 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, there is no generally agreed framework with 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 humanised 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. As the relevance to humans of the mechanism behind

Imazalil-induced liver tumour formation in rodents cannot be convincingly excluded, the RAC supported the proposal of the dossier submitter to classify Imazalil for carcinogenicity as **Carc. 2 - H351** (CLP) and **Carc. Cat. 3; R40** (DSD).

RAC evaluation of reproductive toxicity

Summary of the Dossier submitter's proposal

Reproductive toxicity was evaluated in a two-generation study in rats (Dirkx *et al.*, 1992; similar to OECD TG 416) and three developmental toxicity studies (similar to OECD TG 414), two in rabbits and one in rats.

In the two-generation study (24 M, 24 F Wistar rats) with nominal dietary doses of 0, 5, 20 and 80 mg/kg bw/d Imazalil, parental toxicity was seen at the highest dose (reduced bw and bw gain, increased incidence of pilo-erection and, in P1 males, vacuolisation of hepatocytes). At this dose, a reduced gestation rate and increased duration of gestation were also seen in females, the latter considered responsible for the concurrent increased rate of dystocia. At the highest dose, reproductive toxicity was seen as a slightly reduced number of implantations, reduced number of live pups and offspring survival and increased number of stillborn pups. No teratogenic effects were reported.

One rabbit developmental toxicity study with Imazalil nitrate given by oral gavage on gestation day (GD) 6-18 (Dirkx and Marsboom, 1985; New Zealand White rabbit, 15 F, doses equivalent to 0, 1, 2.1 and 4.1 mg/kg bw/d of Imazalil) showed no maternal or developmental effects.

In another rabbit developmental toxicity study with Imazalil sulphate given by oral gavage on GD 6-18 (Dirkx 1992; Albino rabbit, 15 F, doses equivalent to 0, 5, 10 and 20 mg/kg bw/d of Imazalil), an increased number of resorptions and reduced number of live foetuses were seen at 10 mg/kg bw/d and above. At 20 mg/kg bw/d, maternal effects were seen (reduced bw/bw gain and food consumption during dosing, and increased mortality (8/15 dams)).

In the rat developmental toxicity study (Gillardin *et al.*, 1998; Sprague-Dawley rats, 24 F), Imazalil sulphate (equal to 0, 40, 80 and 120 mg/kg bw/d of Imazalil) was given by oral gavage on GD 6-16. At and above the lowest dose, effects in dams included reduced food consumption and bw or bw gain during the dosing period. In high dose dams, reduced bw and bw gain were also observed at delivery. At and above 80 mg/kg bw/d, reduced live weight was seen in offspring, and at the highest dose level of 120 mg/kg bw/d, a reduced number of live foetuses as well as an increase in resorptions were seen.

The dossier submitter concluded that there are no indications of teratogenic effects of Imazalil, and that the other adverse effects on fertility or development were associated with maternal toxicity, or occurred at doses not significantly below the maternal LOAEL. Based on this conclusion, no classification for reproductive toxicity was proposed.

Comments received during public consultation

One MSCA made a general comment that the human health part of the CLH report is not sufficiently detailed to permit a complete assessment of the presented studies. Two other MSCA's commented that a better justification for no classification is required and that more detailed/quantitative information would be useful to properly evaluate reproductive toxicity. One of these two MSCA's further wished to see a justification why a factor of 2 between the NOAELs for maternal effects (10 mg/kg bw/d) and offspring toxicity (5 mg/kg bw/d) in one rabbit study is considered too small to warrant classification. This MSCA also indicated that classification should be considered if the effects seen are not a secondary non-specific consequence of the maternal toxicity, that Imazalil belongs to the class of imidazoles, and that the developmental effects seen resemble those seen with other classified fungicides.

In response to the comments, the dossier submitter provided additional information on one of the rabbit developmental toxicity studies, and some more justification for the 'no classification' proposed (see section *Additional key elements* below).

Assessment and comparison with the classification criteria

Very little detail is presented in the CLH dossier on the available reproductive toxicity studies, complicating the interpretation of the effects in relation to conclusions on any potential classification.

Regarding fertility effects, no effects on reproductive organs have been described for the repeated dose toxicity studies presented in the CLH dossier. In the rat two-generation study, fertility was not affected, but a reduction in gestation rate and increases in duration of gestation and rate of dystocia were observed in female animals exposed to the highest dose (80 mg/kg bw/day). At this dose, maternal toxicity was also observed, as indicated by reduced body weight and body weight gain. Furthermore, a slightly reduced number of implantations were observed at this dose. No information is presented in the CLH report to indicate whether these effects were seen in all generations. From the CAR (2009) it appears that the increased gestation duration and dystocia occurred in both generations, whereas the reduced gestation rate occurred in the first generation and the reduced implantations in the second generation.

Given the limited information available (on e.g. number of animals affected, magnitude of the effects), it is difficult to judge whether there indeed is an effect and whether there is a causal relationship, as required according to CLP section 3.7.2.3.4. Hence, the RAC was provided with too little study details to allow proper evaluation of the endpoint 'effects on sexual function and fertility'.

Developmental effects have been observed in the rat two-generation study (Dirkx *et al.*, 1992) and in a developmental toxicity study in rats (Dirkx, 1992) and rabbits (Gillardin *et al.*, 1988). The latter two studies were conducted with Imazalil sulphate, whereas in another developmental toxicity study in rabbits that showed no effects (Dirkx and Marsboom, 1985) Imazalil nitrate was administered. The read-across from these salts to Imazalil is considered acceptable because of the good water solubility of both substances.
In the rat two-generation study, an increased number of stillborn pups, a decreased number of live pups and a reduced pup survival were observed at a dose at which also parental toxicity was seen (80 mg/kg bw/d). From the CAR (2009) it appears that these effects occurred in both generations, but this information was not presented in the CLH report.
In the developmental toxicity study with rats, pup weight was reduced at the mid and high dose (80 and 120 mg/kg bw/d), and the high dose also resulted in a reduced number of live foetuses and increased resorptions. However, maternal toxicity (as evidenced by reduced bw and food consumption during dosing) was already observed at the lowest dose tested of 40 mg/kg bw/d. Similar effects were observed in the developmental toxicity study with rabbits, but here the high dose (20 mg/kg bw/d) also caused increased mortality (8 out of 15 dams). Effects on the offspring at the mid and high dose (10 and 20 mg/kg bw/d) included reduced litter-size, reduced number of live foetuses, and an increased number of post-implantation losses. The effects were dose-related but not statistically significant, and occurred in the presence of maternal toxicity (reduced bw and food consumption).

Imazalil treatment did not result in malformations in either rats or rabbits, but in both species Imazalil induced an increase in resorptions and a reduction in live foetuses at dose levels also inducing maternal toxicity. The available data for rats are too limited (no data on magnitude of the effects) to allow a proper assessment. From the developmental toxicity study in rabbits somewhat more (but still limited) information is available. The foetal effects observed at 20 mg/kg bw/d in the rabbit study are not considered relevant for classification, given the excessive mortality rate (53%) in dams. The maternal toxicity at 10 mg/kg bw/d is not considered to be excessive. It can however not be assessed with the limited data available (no information on e.g. net weight gain) whether the reduced bw and food consumption were a primary effect or secondary to the post-implantation loss. Overall, the RAC was provided with too little study details to allow proper evaluation of the endpoint 'developmental toxicity'.

The limited data available also do not allow an assessment of whether the observed reduction in offspring survival in the rat two-generation study was an effect on or via lactation.

The RAC noted that EFSA in their peer review of Imazalil (2010) concluded that Imazalil is not a reproductive toxicant or a teratogen. RAC, however, did not find the information provided detailed enough to evaluate this hazard class and hence no conclusion on reproductive toxicity was agreed.

RAC evaluation of environmental hazards

Summary of the Dossier submitter's proposal

The dossier submitter proposed to remove the current CLP classification of Aquatic Acute 1 and to add an M-factor of 10 to the existing Aquatic Chronic 1 classification. The dossier submitter also proposed to amend the current DSD classification (N; R50-53) to N; R51-53 with specific concentration limits N; R51-53: C≥ 25% and R52-53: 2.5% ≤ C < 25%.

Degradation

Hydrolysis of Imazalil was studied at pH 5.7 and 9 up to 61 days and no degradation was observed. The dossier submitter concluded that Imazalil is hydrolytically stable within the pH range 5-9.

Photodegradation of Imazalil was tested under continuous irradiation in aqueous buffer solution at pH 7. Based on the test results, degradation for 50 degrees of latitude was calculated and resulted in a DT_{50} of 11.6 days and a DT_{90} of 38.6 days. Identification of photodegradation products of Imazalil was not finalised by the date of dossier submission and the dossier submitter did not consider the reported information on photodegradation relevant for environmental classification. However, the dossier submitter did not clarify how any further information on photodegradation products would impact environmental hazard assessment of Imazalil.

Ready biodegradation was tested in a 28-day biological oxygen demand (BOD) study (modified MITI test, OECD TG 301C) in which also dissolved organic carbon (DOC) and Imazalil concentrations were measured at the end of the test. Low biodegradability (≤ 2%), no loss of DOC and no change in the Imazalil concentration were measured at the end of the study indicating that the substance is not readily biodegradable. The degradation of the reference substance (54% after 7 days and 58% after 14 days) did not fully meet the required pass level (40% after 7 days and 65% after 14 days) for an acceptable test but the dossier submitter concluded that the study allows the conclusion that Imazalil is not readily biodegradable (CLP).

Biodegradation was studied in two different types of water/sediment systems. The applied radioactivity (AR) in water decreased to <10% on day 14 and to <5% at the end of study (152 d). The main part of the AR was in the sediment (85-91%) at the end of the study. The DT_{50} values of the AR for the whole systems were 97.4 and 79.6 days. The share of CO_2 was 2.9-3.9% of AR and no other volatile carbon based degradants were observed.

The dossier submitter concluded that Imazalil appeared to be susceptible to primary degradation but not ultimate mineralisation and was considered to be not rapidly degradable (CLP) and not readily degradable (DSD).

Bioaccumulation

The log K_{ow} was measured to be 2.63 at pH 5, 3.66 at pH 7 and 3.82 at pH 9. Bioaccumulation of Imazalil was also studied experimentally (OECD TG 305 E) at two Imazalil concentrations (0.025 and 0.25 mg/L) in rainbow trout (*Oncorhynchus mykiss*) for 11 days. The reported BCF values were 48.7 and 63.8 l/kg (wet weight) and were based on steady state concentrations of Imazalil in whole fish during the exposure. The dossier submitter concluded that Imazalil does not meet the criteria for bioaccumulation potential of Imazalil according to CLP and DSD.

Acute toxicity

Two acute toxicity studies in fish, one in invertebrates and one in algae was reported. Both fish studies were performed according to the OECD TG 203. The reported LC_{50} (96 h) values were 1.48 mg/l for rainbow trout (*O. mykiss*) and 2.75 for zebra fish (*Danio rerio*) based on measured concentrations. The acute toxicity study (OECD 202) in water flea (*Daphnia magna*) resulted in an EC_{50} (48 h) of 3.5 mg/l (nominal concentrations). The reported EC_{50} values for algae (*Selenastrum capricornutum*) were E_bC_{50} = 0.87 mg/l and E_rC_{50} = 1.20 mg/l (measured concentrations).

The dossier submitter concluded that classification for Aquatic Acute toxicity is not warranted.

Chronic toxicity
One chronic study in fish, two in invertebrates and one in algae (the same as for acute toxicity) were reported. The NOEC value determined in fish was 0.225 mg/l (measured concentrations). The value was based on mortality and behaviour of young rainbow trout (*O. mykiss*) exposed to Imazalil for 28 days (OECD TG 204). The 28-d fish study was considered only as a prolonged toxicity test as no sensitive sub-lethal endpoints were examined.

Two 21-day chronic tests in water flea (*D. magna*) were reported. The first one, based on the old OECD TG 202 (part 2) was performed at six Imazalil concentrations ranging from 0.0071 mg/l to 2.5 mg/L (measured concentrations). However, effects were observed in all applied Imazalil concentrations leading to the conclusion that the NOEC is < 0.0071 mg/l. The second *Daphnia* study followed OECD TG 211 and the derived NOEC value was 0.025 mg/l. This value was based on nominal concentrations since the measured concentrations varied from 90% to 114%. Also an additional 17-day study in *Chironomus* larvae was reported and the derived NOEC for water was 0.178 mg/l.

The dossier submitter used the lowest reliable NOEC value (*D. magna* NOEC < 0.01 mg/l) in the classification for long-term environmental hazards. Since Imazalil was not rapidly degradable (CLP) the dossier submitter concluded that Aquatic Chronic 1 with an M-factor of 10 is warranted. The removal of acute toxicity from the current entry was based on the lowest available acute toxicity value (*S. capricornutum*, E_rC_{50} = 1.2 mg/l). The same acute study and the conclusion that Imazalil is not readily degradable were the reasons for the dossier submitter's proposal to replace the current DSD entry (N; R50-53) with N; R51-53 (specific concentration limits N; R51-53: C≥ 25% and R52-53: 2.5% ≤ C < 25%).

Comments received during public consultation
During public consultation, two MSCA's supported the proposed classification for the environmental hazards. A third MSCA agreed on the general conclusion but, together with a fourth MSCA, requested more detailed summaries of both long-term invertebrate studies, particularly for the key study that was used to set the M-factor because its NOEC was reported as a 'less than' value.
In response to the comments, the dossier submitter provided additional information on the two chronic invertebrate studies (see section *Additional key elements* below).

Assessment and comparison with the classification criteria

Degradation
The information provided shows that Imazalil is hydrolytically stable at environmentally relevant pHs (pH 5-9). In a ready biodegradability screening study, Imazalil does not degrade to a level of more than 70% in 28 days. Based on findings in a water/simulation test Imazalil is susceptible to primary degradation with DT_{50} > 16 days, and ultimate mineralization was not achieved. Considering the results the RAC agrees with the dossier submitter that Imazalil is not readily biodegradable and not rapidly or readily degradable (criterion under both CLP and DSD: degradation > 70% within 28 days) for purposes of classification and labelling.

Bioaccumulation
Measured log K_{ow} and BCF values are available for Imazalil. The latter are considered more important, given that Imazalil is a surface active substance (with a surface tension of 46.6 mN/m, which is < 60 mN/m), making the shake flask method to measure log K_{ow} less appropriate. A BCF value of 63.8 L/kg ww in whole fish (without lipid normalisation) was obtained in a bioaccumulation study. The BCF value is not above the trigger of 500 (criterion for bioaccumulating potential under CLP) and also not above the trigger of 100 (criterion for bioaccumulating potential under DSD). The RAC agrees with the dossier submitter that Imazalil does not meet the criteria for a bioaccumulative substance.

Acute toxicity - CLP
Aquatic acute toxicity studies are available for all trophic levels. The lowest L(E)C_{50} value obtained was 1.20 mg/l for growth rate in algae (*S. capricornutum*).
This lowest E_rC_{50} of 1.20 mg/l is above the cut-off value of 1 mg/l, therefore Imazalil does not fulfil the criteria for aquatic acute 1 (H400).

Chronic toxicity - CLP
The RAC concluded that the long term fish test provided does not give sufficient detail on sublethal effects to be used for chronic toxicity classification purposes. It should be considered a prolonged toxicity test, not a chronic toxicity test. As no chronic tests are available for all three trophic levels, the most stringent outcome of table 4.1.0 (b)i and 4.1.0(b)iii should be considered, taking into account the chronic toxicity values for *Daphnia* and algae (< 0.1 mg/L and > 0.1 to 1 mg/l, respectively) and the acute value for fish (> 1 to 10 mg/l). The lowest NOEC value (*D. magna* NOEC < 0.01 mg/l) was used by the dossier submitter in the classification for long-term environmental hazard. In principle, the RAC agrees with the use of this key study for classification and labelling purposes. The RAC however does not agree with the reporting of the most appropriate toxicity value and the value for setting the M-factor (see below), although in the end this does not result in a classification proposal different from that of the dossier submitter.

Reporting of nominal concentrations/mean measured concentration
Daphnia were exposed to Imazalil with nominal concentrations of 0.01, 0.03, 0.1, 0.3, 1.0 and 3.0 mg/l. The measured concentrations were 0.007, 0.023, 0.08, 0.262, 0.763 and 2.481 mg/l, respectively. The measured concentrations were between 70% - 87% of the nominal concentrations at the start of the study. These values fall below 80% of the nominal concentration, therefore the biological response should be expressed based on measured concentration. In the background document the dossier submitter reports a NOEC of 0.01 mg/l whilst in his response to comments received during public consultation it is reported "*that a no discrete NOEC could be determined (NOEC < 0.007 mg/l*)." The RAC considers the value of < 0.007 mg/l based on mean measured concentration as the most appropriate toxicity value. This value should also be used for deriving the chronic M-factor and not 0.01 mg/l. Having said this, using either the nominal or measured value does not change the proposed M-factor of 10.

NOEC/LOEC toxicity value
The use of NOEC instead of LOEC for effects on reproduction is reported. According to the additional detailed information provided by the DS:

- *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).*

The NOEC for reproduction could not be determined. Therefore reporting the result as LOEC (≤ 0.007 mg/l) is more appropriate. A distinct or individual NOEC could not be determined, only a "less than" value. This poses a problem in setting the M-factor for chronic toxicity because this is dependent on a NOEC or EC_{10} value that is fixed. Based on the available data, it can only be concluded that the LOEC and the NOEC for algae are below 0.007 mg/l. Due to the lack of a fixed NOEC value, the chronic M-factor will be determined using the 'less than' value of 0.007 and taking into account that the substance is not rapidly degradable. The resulting M factor is M=10 based on 0.001 < LOEC ≤ 0.01 for not rapidly degradable substances. It is noted that this M-factor does not necessarily represent the most stringent M-factor for Imazalil because the actual NOEC value is not known for the study and may be lower than 0.001 mg/l.
In conclusion, Imazalil fulfils the criteria for classification as Aquatic Chronic 1 (H410) under CLP with an M-factor of 10, taking into account the LOEC value ≤ 0.007 mg/l and the fact that the substance does not rapidly degrade.

Aquatic toxicity - DSD
The lowest L(E)C_{50} value obtained was 1.20 mg/l for growth rate in algae (*S. capricornutum*). This value is > 1 mg/L and ≤ 10 mg/l. Imazalil is considered not readily degradable. Thus, Imazalil fulfils the criteria for classification with N; R51-53. Concentration limits for substances classified

as N; R51-53 are not included in Annex VI. Therefore, the specific concentration limits as proposed by the dossier submitter are not necessary.

The RAC supported the environmental classification proposed by the dossier submitter for both acute and chronic aquatic toxicity, aside from the inclusion of specific concentration limits.

ANNEXES:

Annex 1 Background Document (BD) gives the detailed scientific grounds for the opinion. It is based on the CLH report prepared by the dossier submitter; the evaluation performed by the RAC is contained in RAC boxes.

Annex 2 Comments received on the CLH report, response to comments provided by the dossier submitter and the RAC (excl. confidential information).