Committee for Risk Assessment

RAC

Annex 1

Background document

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

Imazalil (ISO)

EC number: 252-615-0

CAS number: 35554-44-0

CLH-O-0000002720-08-03/A1

The background document is a compilation of information considered relevant by the dossier submitter or by RAC for the proposed classification. It includes the proposal of the dossier submitter and the conclusion of RAC. It is based on the official CLH report submitted to public consultation. RAC has not changed the text of this CLH report but inserted text which is specifically marked as 'RAC evaluation'. Only the RAC text reflects the view of RAC.

Adopted

4 June 2013

Annex VI Report

PROPOSAL FOR HARMONISED CLASSIFICATION AND LABELLING

Substance Name: **Imazalil**

EC Number: **252-615-0**

CAS Number: **35554-44-0**

Index Number: **613-042-00-5**

Submitted by: **BAuA**
Federal Institute for Occupational Safety and Health
Federal Office for Chemicals
Friedrich-Henkel-Weg 1-25
D-44149 Dortmund, Germany

Version: **May 2012 (post ACCheck)**

CONTENTS

LIST OF TABLES

LIST OF FIGURES

PROPOSAL FOR HARMONISED CLASSIFICATION AND LABELLING

1 PROPOSAL FOR HARMONISED CLASSIFICATION AND LABELLING

1.1 Substance

Table 1: Substance identity

Substance name:	Imazalil
EC number:	252-615-0
CAS number:	35554-44-0 (unstated stereochemistry)
Annex VI Index number:	613-042-00-5
Degree of purity:	minimum 950 g/kg
Impurities:	confidential

Registration dossiers available:

None

1.2 Harmonised classification and labelling proposal

Table 2: The current Annex VI entry and the proposed harmonised classification

	Regulation (EC) No 1272/2008 (2nd ATP)	**Directive 67/548/EEC**
Current entry in Annex VI CLP Regulation	Acute Tox. 4; H302 Acute Tox. 4; H332 Eye Dam. 1; H318 Aquatic Acute 1; H400 Aquatic Chronic 1; H410	Xn; R20/22 Xi; R41 N; R50-53
Current proposal for consideration by RAC	Acute Tox. 3; H301 Carc. 2; H351 Aquatic Chronic 1; H410 M=10	Carc. Cat. 3; R40 N; R51-53 (SCL: N; R51/53: C≥ 25%; R52/53: 2.5% ≤C< 25%)
Resulting harmonised classification (future entry in Annex VI of CLP Regulation)	Carc. 2; H351 Acute Tox. 3; H301 Acute Tox. 4; H332 Eye Dam. 1; H318 Aquatic Chronic 1; H410 M=10	Carc. Cat. 3; R40 Xn; R20/22 Xi; R41 N; R51-53 (SCL: N; R51/53: C≥ 25%; R52/53: 2.5% ≤C< 25%)

1.3 Proposed harmonised classification and labelling based on CLP Regulation and/or DSD criteria

Table 3: Proposed classification according to the CLP Regulation

CLP Annex I ref	Hazard class	Proposed classification	Proposed SCLs and/or M-factors	Current classification [1)]	Reason for no classification [2)]
2.1.	Explosives				Data lacking
2.2.	Flammable gases				Data lacking
2.3.	Flammable aerosols				Data lacking
2.4.	Oxidising gases				Data lacking
2.5.	Gases under pressure				Data lacking
2.6.	Flammable liquids				Data lacking
2.7.	Flammable solids				Data lacking
2.8.	Self-reactive substances and mixtures				Data lacking
2.9.	Pyrophoric liquids				Data lacking
2.10.	Pyrophoric solids				Data lacking
2.11.	Self-heating substances and mixtures				Data lacking
2.12.	Substances and mixtures which in contact with water emit flammable gases				Data lacking
2.13.	Oxidising liquids				Data lacking
2.14.	Oxidising solids				Data lacking
2.15.	Organic peroxides				Data lacking
2.16.	Substance and mixtures corrosive to metals				Data lacking
3.1.	Acute toxicity - oral	Acute Tox. 3; H301		Acute Tox. 4; H302	
	Acute toxicity - dermal				Data lacking
	Acute toxicity - inhalation	Acute Tox. 4; H332		Acute Tox. 4; H332	
3.2.	Skin corrosion / irritation				Data lacking
3.3.	Serious eye damage / eye irritation	Eye dam. 1; H318		Eye dam. 1; H318	
3.4.	Respiratory sensitisation				Data lacking
3.4.	Skin sensitisation				Data lacking
3.5.	Germ cell mutagenicity				Data lacking
3.6.	Carcinogenicity	Carc. 2; H351		none	
3.7.	Reproductive toxicity				Data lacking
3.8.	Specific target organ toxicity –single exposure				Data lacking
3.9.	Specific target organ toxicity – repeated exposure				Data lacking
3.10.	Aspiration hazard				Data lacking

4.1.	Hazardous to the aquatic environment	Aquatic Chronic 1; H410	M-factor:10	Aquatic Acute 1; H400 Aquatic Chronic 1; H410	
5.1.	Hazardous to the ozone layer				Data lacking

[1)] Including specific concentration limits (SCLs) and M-factors
[2)] Data lacking, inconclusive, or conclusive but not sufficient for classification

Table 4: Proposed labelling according to the CLP Regulation

	Labelling	**Wording**
Pictograms	GHS05 GHS06 GHS08 GHS09	
Signal Word	Danger	
Hazard statements	H351 H301 H332 H318 H410	Suspected of causing cancer Toxic if swallowed Harmful if inhaled Causes serious eye damage Very toxic to aquatic life with long lasting effects
Precautionary statements	(P102) P271 P273 P281 P305+P351+P338 P308+P313 P363 P391 P405 P501	(Keep out of reach of children) Use only outdoors or in a well-ventilated area. Avoid release to the environment. Use personal protective equipment as required. IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses if present and easy to do – continue rinsing IF exposed or concerned: Get medical advice/attention. Wash contaminated clothing before reuse. Collect spillage. Store locked up. Dispose of contents/container to …

Table 5: Proposed classification according to DSD

Hazardous property	Proposed classification	Proposed SCLs	Current classification [1)]	Reason for no classification [2)]
Explosiveness				Data lacking
Oxidising properties				Data lacking
Flammability				Data lacking
Other physico-chemical properties				Data lacking
Thermal stability				Data lacking
Acute toxicity	Xn; R20/22		Xn; R20/22	
Acute toxicity – irreversible damage after single exposure				Data lacking
Repeated dose toxicity				Data lacking
Irritation / Corrosion	Xi; R41		Xi; R41	
Sensitisation				Data lacking
Carcinogenicity	Carc. Cat. 3; R40		none	
Mutagenicity – Genetic toxicity				Data lacking
Toxicity to reproduction – fertility				Data lacking
Toxicity to reproduction – development				Data lacking
Toxicity to reproduction – breastfed babies. Effects on or via lactation				Data lacking
Environment	N; R51/53	N; R51/53: C≥ 25%; R52/53: 2.5% ≤C< 25%	N; R50-53	

[1)] Including SCLs
[2)] Data lacking, inconclusive, or conclusive but not sufficient for classification

Table 6: Proposed labelling according to DSD

	Labelling	Wording
Hazard Symbols, Indications of danger	Xn N	Harmful Dangerous for the environment
R-phrases	R20/22 R40 R41 R51/53	Harmful by inhalation and if swallowed Limited evidence of a carcinogenic effect Risk of serious damage to eyes Toxic to aquatic organisms, may cause long-term adverse effects in the aquatic environment
S-phrases	(S2) S26 S36/37/39 S60	Keep out of the reach of children In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. Wear suitable protective clothing, gloves and eye/face protection. This material and its container must be disposed of as hazardous waste

	S61	Avoid release to the environment. Refer to special instructions/safety data sheet

Specific concentration limits based on Directive 67/548/EEC:

Concentration	Classification
C≥ 25%	N; R51/53
2.5% ≤C< 25%	R52/53

Where C is the concentration of imazalil in the mixture.

M-factor based on Regulation Regulation (EC) No 286/2011 criteria (2nd ATP to the CLP-Regulation)

For chronic toxicity an M-factor of 10 is assigned by using the reported NOEC value of < 0.01 mg/L. obtained for *Daphnia magna* in a 21d semi-static study.

Proposed notes (if any):

None

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.

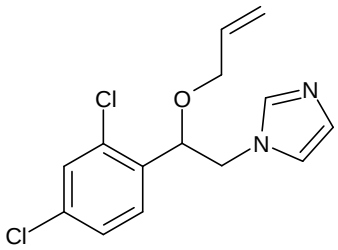
JUSTIFICATION

1 IDENTITY OF THE SUBSTANCE AND PHYSICAL AND CHEMICAL PROPERTIES

1.1 Name and other identifiers of the substance

Chemical Name:	(±)-1-[2-(2,4-dichlorophenyl)-2-(2-propenyloxy)ethyl]-1*H*-imidazole
EC Name:	not allocated
CAS Number:	35554-44-0 (unstated stereochemistry)
IUPAC Name:	(±)-1-(β-allyloxy-2,4-dichlorophenylethyl) imidazole
	or (±)-allyl 1-(2,4-dichlorphenyl)-2-imidazol-1-ylethyl ether

1.2 Composition of the substance

Chemical Name:	(±)-1-[2-(2,4-dichlorophenyl)-2-(2-propenyloxy)ethyl]-1*H*-imidazole
EC Number:	252-615-0
CAS Number:	35554-440 (unstated stereochemistry);
IUPAC Name:	(±)-1-(β-allyloxy-2,4-dichlorophenylethyl) imidazole or (±)-allyl 1-(2,4-dichlorphenyl)-2-imidazol-1-ylethyl ether
Molecular Formula:	$C_{14}H_{14}Cl_2N_2O$
Structural Formula:	
Molecular Weight:	297.18 g/mol
Typical concentration (% w/w):	confidential information
Concentration range (% w/w):	> 950 g/kg

1.3 Physico-chemical properties

All data are given for Imazalil as a racemic mixture of isomers

Table 7: Summary of physico-chemical properties

REACH ref Annex, §	Property	IUCLID section	Value	
VII, 7.1	Physical state at 20°C and 101.3 KPa	3.1	yellow to brown crystal-line solid (purity 99.5 %)	Draft Assessment report Monograph EFSA conclusions
VII, 7.2	Melting/freezing point	3.2	51.5 °C (purity 99.99 %)	
VII, 7.3	Boiling point	3.3	not observed, decomposition starts at 260 °C.	
VII, 7.4	Relative density	3.4 density	1.348 g/cm^3 at 26 °C (purity 98.3 %) 1.255 g/cm^3 at 24 °C (purity 97.5 %)	
VII, 7.5	Vapour pressure	3.6	1.58 x 10^{-4} Pa at 25 °C (purity 99.7 %)	
VII, 7.6	Surface tension	3.10	46.6 mN/m at 20 °C (90 % saturated solution in water)	
VII, 7.7	Water solubility	3.8	0.184 g/L at 20 °C and pH 7.6 (purity > 99.9 %)	
VII, 7.8	Partition coefficient n-octanol/water (log value)	3.7 partition coefficient	log $P_{O/W}$ = 2.63 at pH 5 log $P_{O/W}$ = 3.66 at pH 7 log $P_{O/W}$ = 3.82 at pH 9	
VII, 7.9	Flash point	3.11	not relevant	
VII, 7.10	Flammability	3.13	not highly flammable no gas evolution nor ignition in contact with water (purity 97.0 %)	
VII, 7.11	Explosive properties	3.14	not explosive (based on the chemical structure)	
VII, 7.12	Self-ignition temperature		No self-ignition up to the melting point (purity 97.0 %)	
VII, 7.13	Oxidising properties	3.15	no oxidising properties (based on the chemical structure)	
VII, 7.14	Granulometry	3.5	not relevant	
XI, 7.15	Stability in organic solvents and identity of relevant degradation products	3.17	not relevant	
XI, 7.16	Dissociation constant	3.21	pKa = 6.49	
XI, 7.17,	Viscosity	3.22	not relevant	
	Auto flammability	3.12	no self-ignition up to the melting point	
	Reactivity towards container material	3.18	not determined	
	Thermal stability	3.19	decomposition starts at 260 °C	

2 MANUFACTURE AND USES

2.1 Manufacture

There are several manufactures of imazalil.

2.2 Identified uses

Post-harvest use on fruits.

2.3 Uses advised against

fungitoxic and fungistatic action.

3 CLASSIFICATION AND LABELLING

Table 8: Current classification in Annex VI, Table 3.1 in the CLP Regulation

Index Number 613-042-00-5	Classification	Wording
Hazard classes, Hazard categories	Acute Tox. 4 Acute Tox. 4 Eye Dam. 1 Aquatic Acute 1 Aquatic Chronic 1	
Hazard statements	H332 H302 H318 H400 H410	Harmful if inhaled Harmful if swallowed Causes serious eye damage Very toxic to aquatic life Very toxic to aquatic life with long lasting effects.

Table 9: Current labelling in Annex VI, Table 3.1 in the CLP Regulation

Index Number 613-042-00-5	Labelling	Wording
Pictograms	GHS05 GHS07 GHS09	
Signal Word	Danger	
Hazard statements	H332 H302 H318 H410	Harmful if inhaled Harmful if swallowed Causes serious eye damage Very toxic to aquatic life with long lasting effects
Precautionary statements	-	-

Specific concentration limits are not set.

Table 10: Current classification in Annex VI, Table 3.2 in the CLP Regulation

Index Number 613-042-00-5	Classification	Wording
Hazard Symbols, Indications of danger	Xn Xi N	Harmful Irritant Dangerous for the environment

R-phrases	R20/22 R41 R50-53	Harmful by inhalation and if swallowed Risk of serious damage to eyes Very toxic to aquatic organisms, may cause long-term adverse effects in the aquatic environment

Table 11: Current labelling in Annex VI, Table 3.2 in the CLP Regulation

Index Number 613-042-00-5	Labelling	Wording
Hazard Symbols, Indications of danger	Xn N	Harmful Dangerous for the environment
R-phrases	R20/22 R41 R50/53	Harmful by inhalation and if swallowed Risk of serious damage to eyes Very toxic to aquatic organisms, may cause long-term adverse effects in the aquatic environment
S-phrases	(S2) S26 S39 S60 S61	Keep out of the reach of children In case of contact with eyes, rinse immediately with plenty of water and seek medical advice Wear eye/face protection This material and its container must be disposed of as hazardous waste Avoid release to the environment. Refer to special instructions/Safety data sheet.

3.1 Self classification(s)

As in Annex I of 67/548/EEC.

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.

Additional key elements
According to the DAR (2009), Imazalil does not have explosive or oxidising properties and is not auto-flammable or (highly) flammable.

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

Supplemental information - In depth analyses by RAC
-

4 ENVIRONMENTAL FATE PROPERTIES

The environmental fate properties assessment for imazalil is based on the Draft Re-Assessment Report and the Proposed Decision of Netherlands prepared in the context of the renewal of the inclusion of imazalil in Annex I of Council Directive 91/414/EEC (revised DRAR September 2009, RMS Netherlands). Some studies have been assessed in the Draft Assessment Report prepared in the context of the first inclusion of imazalil in Annex I of Council Directive 91/414/EEC (DAR July 1996, RMS Luxembourg).

General

Imazalil has one chiral centre and as a result is a racemic mixture of two enantiomers: the A (+) isomer (R) and B (-) isomer (S). The the R-enantiomer is more potent than the S-enantiomer, depending on the test organism. Since all ecotoxicity tests are performed with the racemic mixture imazalil, and since it is not to be expected that the enantiomer distribution in a synthesised racemic mixture will change under different environmental circumstances, RMS con-siders that no further separate consideration of the enantiomers is necessary for the eco-toxicological risk assessment. It is however to be confirmed by the notifier that the enantio-mer distribution in the synthesised racemic mixture will not change under different environ-mental circumstances. This can be addressed via a scientifically reasoned case, based on the existing data set.

4.1 Degradation

4.1.1 Stability

Hydrolysis

- Van Leemput, L. Heykants, J. 1982, Report No. R023979/L1

Aqueos imazalil solution of 20 mg/L were incubated at pH 5.7 and 9 at 25 °C in the dark for periods up to 61 days. All of imazalil was recovered at the end of the incubation time. No alteration product was detected. Imazalil is hydrolytically stable at pH 5 – 9.

Photolysis in water

- Adam, D. 2008, Report No.: B78153, AGR 3856

An interim report of a new photolysis study was presented in the revised DRAR. The interim report was accepted for the rate of the photolytic degradation of imazalil. The final report will be submitted as soon as the identity of all metabolites is known.

Photodegradation of [2-etyhl-14C]-labelled imazalil (Batch 2213, radiochemical purity 100 %, 2.09 GBq/mmol corresponding to 7.03 MBq/mg considering a molecular weight of 297.18 g/mol for the unlabelled test item) was studied in sterile phosphate aqueous buffer solution at pH 7 under artificial light using xenon lamps that had a spectral energy distribution similar to that of natural sunlight.

The half-lifes for the decline of imazalil were calculated of 36.1 h (pH 7), 18.15 h (surface water; pH 7.5) and 3.2 h (with 2 % acetone as photosensitizer). Under suntest conditions (continuous irradiation) a DT_{50} of 6.1 d and a DT_{90} of 20.2 d was assessed.

Environmentally relevant degradation times for Central Europe were calculated for 50 degree of latitude with a DT_{50} of 11.6 d and a DT_{90} of 38.6 d and for 30-40 degree of latitude with a DT_{50} of 11.1 d and a DT_{90} of 37.0 d.

Imazalil undergoes continuous photolysis in the aquatic environment.

4.1.2 Biodegradation

4.1.2.1 Biodegradation estimation

4.1.2.2 Screening tests

Readily biodegradability

- Koyasu, J. 2002, Report No.: A020224

The ready biodegradability of imazalil was studied in a 28-day biodegradation test by following the Biological Oxygen Demand (BOD) (measured by a closed system oxygen consumption measuring apparatus). In addition Dissolved organic carbon (DOC) and imazalil (HPLC) were measured after 28 days. The test was stated to be performed according to OECD 301C.

Test solutions (300 mL, triplicate) containing imazalil (100 mg/L) and activated sludge inoculum (30 mg/L) were incubated in airtight flasks in the dark for 28 days at 25 °C. Single flasks for inoculum blank control (inoculum, no test substance), reference substance (aniline, 100 mg/L) and abiotic control (imazalil, 100 mg/L in purified water) were included.

BOD in the inoculum controls (0 mg after 28 days) satisfied the validity criterion of OECD 301C (≤60 mg/L). The pass level for the reference substance (40 % degradation after 7 days and 65 % after 14 days) was partially reached: 54 % after 7 days but only 58 % after 14 days. After 28 days, the BOD in the flasks with imazalil was 0.0, 0.5 and 1.4 mg (0, 1 and 2 % biodegradability), indicating that imazalil was not readily biodegradable in this test. DOC, measured at day 28 was 56.6–58.1 mg/L and 56.3 mg/L in the abiotic control, indicating no loss of DOC from the test system during incubation. HPLC measured imazalil concentrations were 100.4–100.6 mg/L in the test solutions and 101.0 mg/L in the abiotic control, indicating that imazalil did not degrade under the test conditions.

Despite the pass level for the reference substance only partially being fulfilled, it can be concluded that imazalil was not readily biodegradable in a biodegradation test (based on BOD, DOC and HPLC measurements) according to OECD 301C.

4.1.2.3 Simulation tests

Biodegradation in water/sediment systems

- Mamouni, A., 2008, Report No.: B72360, AGR 3854

The behaviour of [^{14}C]-labelled imazalil, uniformly labelled on C adjacent to phenyl ring was studied in two water/sediment systems, in the River Rhine system and in the Froeschweiher pond in Switzerland over a period of 152 days. Duplicate flasks and traps were analysed at 0, 1, 7, 14, 28, 56, 100 and 152 days after treatment.

Table 12: Physico-chemical properties of the two water/sediment-systems

Parameter	River Rhine, Switzerland		Fröschweiher Pond, Switzerland	
	Water	Sediment	Water	Sediment
Textural class (USDA)	-	Loamy sand	-	Silt loam
% sand/silt/clay (USDA)	-	81/12/7	-	21/54/25
TOC (mg/L water, % sediment)	1.28	0.70	4.52	4.22
pH (medium not specified)	7.91	7.38	7.75	7.07
microbial biomass [µg C/g] (start)	-	497	-	1281
microbial biomass [µg C/g] (end)	-	584	-	2277

Level P-I and P-II DT_{50} values of imazalil were calculated following the recommendations and procedures of the "Guidance document on estimating persistence and degradation kinetics from Environmental Fate studies on pesticides in EU registration" (SANCO/10058/2005) (SFO = single first-order, FOMC = first-order multi-compartment, DFOP = double first-order parallel model, HS = hockey stick). All calculations were performed with ModelMaker v 4.0 software. Because of discrepancies on the P-II calculations and because the 10 % level was not reached during the study; the level P-I values were used for persistence and modelling according to FOCUS.

The radioactivity level in water decreased to <10% AR on day 14 and was 1.7–4.6 % AR after 152 days. Sediment radioactivity reached a maximum after 56 days (92–96 % AR) and decreased to 85–91 % AR at the end of the study. The non-extractable fraction in the sediment increased to a maximum of 35–46% AR at study end. CO_2 was 2.9–3.9 % AR at study end and no other volatiles were produced. The level of imazalil in water was 4.5–8.0 % AR on day 14 and ≤ 2.1 % AR at study end. The levels of imazalil reached a maximum in sediment of 62-69% AR on day 14 and were 37–40 % AR on day 100. Metabolites in water were ≤ 2.2 % AR. The most important metabolite in sediment was maximum 9.9 % AR (day 28). Other sediment metabolites were always ≤ 5.0 % AR. One metabolite was identified as R014821 (maximum 0.7 % AR in water and 5.0 % AR in sediment).

Table 13: Dissipation times of ^{14}C-imazalil in two water/sediment systems

System	Parameter for	Kinetics	DT50 (d)	DT90 (d)
Rhine River water	Persistence (level P-I dissipation)	SFO	3.17 (P)	10.5 (P)
Rhine River sediment	Persistence (level P-I dissipation)	SFO	159 (P)	527 (P)
Rhine River total	Persistence, Modelling (level P-I degradation)	DFOP	97.4 (P) 165 (M)	544 (P)
Froeschweiher pond water	Persistence (level P-I dissipation)	SFO	2.35 (P)	7.82 (P)
Froeschweiher pond sediment	Persistence (level P-I dissipation)	SFO	187 (P)	623 (P)
Froeschweiher pond total	Persistence, Modelling (level P-I degradation)	DFOP	79.6 (P) 161 (M)	453 (P)

For persistence, the following level P-I endpoints are estimated: Total system $DegT_{50}$: 97.4 and 79.6 days, water column DT_{50}: 3.17 and 2.35 days and sediment DT_{50}: 159 and 187 days. For modelling the following level P-I endpoints were estimated: Total system $DegT_{50}$: 165 and 161 days.

4.1.3 Summary and discussion of persistence

Biodegradation in water

Imazalil was found to be not readily biodegradable in the available study.

In water/sediment systems imazalil was metabolised at a rate with $DegT_{50}$ values of 79.6 days and 94.7 days.

Based on the findings from screening test on ready biodegradability and water/sediment simulation test imazalil appears to be susceptible for primary degradation and not ultimate mineralisation. Considering the results of the test on ready biodegradability and levels of mineralisation in the simulation study, imazalil is considered not readily/ rapidly biodegradable (a degradation > 70 % within 28 days) for purposes of classification and labelling.

4.2 Environmental distribution

Not relevant for this dossier.

4.3 Bioaccumulation

4.3.1 Aquatic bioaccumulation

4.3.1.1 Bioaccumulation estimation

Imazalil has a log Kow of 3.66 (pH 7) and 3.82 (pH 9).

4.3.1.2 Measured bioaccumulation data

A bioconcentration study with imazalil and Rainbow trout under flow-through conditions (Weytjens D. *et al.*, 1995) produced a BCF for imazalil ranging between 48.7- 63.8 L/kg ww. The clearance time CT_{50} was 27.3 – 43.3 hours.

Environmental Assessment Report - Revised version: The bioaccumulation of Imazalil (R 23979) in the Rainbow trout (*Salmo gairdneri*). (Weytjens D. *et al.*, 1995)

Guidelines :

The flow-through test for the investigation of bioaccumulation of substances in fish, OECD No. 305E

GLP : GLP study.

Material and Methods :

This study was performed to determine the BCF, depuration rate constants and uptake rate constants. 96 Rainbow fish (*Salmo gairdneri*) were exposed during 11 days to 2 concentrations (0.025 and 0.25 mg/l + one control) of imazalil technical grade (base + sulphate; Because the solubility of the imazalil base was too low to prepare the required stock solutions, a sufficient amount was transformed to the sulphate salt). (Equivalence of imazalil base and its salts was discussed by Van Leemput, 1987)

The experimental data were processed accordingly with the appropriate kinetic equations, i.e. sum of two first-order equations (two first-order depletion phases α and β)

Findings :

Table 14:

Initial imazalil concentration	0.025 mg/l	0.25 mg/l
Depuration rate constants (1/hour)	α = 0.5303 ± 0.2525 β = 0.0254 ± 0.0023	α = 0.149 ± 0.0121 β = 0.016 ± 0.0026
Half-lives (hours)	T1/2 α = 1.4 T1/2 β = 27.7	T1/2 α = 4.7 T1/2 β = 43.3
Uptake rate constant (1/hour)	14.3	5.5
BCF = concentration in fish at t = 168 h /median concentration in medium	1.050/0.01645 = 63.8	10.0/0.2055 = 48.7

Conclusions :

BCF of imazalil in the Rainbow trout was in the range of 48.7 to 63.8 L/kg ww. This was far below the values predicted by the equations based on the octanol-water coefficient. Imazalil was rapidly eliminated and/or transformed by the fish. Terminal elimination half-life was in the range 27.3 - 43.3 hours. Fish eliminated and transformed imazalil which prevented a build-up in the tissues.

4.3.2 Terrestrial bioaccumulation

4.3.3 Summary and discussion of bioaccumulation

Imazalil has a log Kow of 3.66 (pH 7) and 3.82 (pH 9). The experimentally derived steady state BCF of 63.8 L/kg ww was obtained based on plateau concentration of substance in whole fish and average concentration of substance in water. The BCF is not above the trigger of 100 (criterion for bioaccumulating potential conform Directive 67/548/EEC) and also not above the trigger of 500 (criterion for bioaccumulating potential conform Regulation EC 1272/2008).

4.4 Secondary poisoning

Not relevant for this dossier.

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).

Additional key elements

The dossier submitter provided more details on the two chronic invertebrate studies.

Study 1: 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 *D. 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 actual 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 (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. For the other tested concentrations, 20 and 25% reductions were found at 0.023 and 0.08 mg/l, respectively. 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).

Study 2: 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 *D. 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 actual measured concentrations were 90-114% during the test. 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 at the other tested concentration of 0.25 mg/l. The reproduction reduction of 12% at 0.025 mg/l Imazalil was not significant. Therefore a 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 (*D. magna*) was already determined.

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.

5 HUMAN HEALTH HAZARD ASSESSMENT

5.1 Toxicokinetics (absorption, metabolism, distribution and elimination)

The toxicokinetics of imazalil sulphate salt was assessed in male and female Wistar rats with the active substance radioactively labeled at position 2 of its enoxyethyl moiety (Mannens et al., 1993) and compared between single oral low dose (1.25 mg/kg bw), high dose (20 mg/kg bw), and repeated (14 d) low dose application following a protocol similar to OECD TG 417. In addition, systemic bioavailability after oral dosing of 5.3 mg/kg bw imazalil sulphate was determined in cattle by comparison of the respective AUC values to those achieved after i.v. administration of the same dose (Heykants et al., 1982).

Based on excretion of radioactive metabolites in rat, the oral absorption of imazalil sulphate can be considered to be complete (100 %) at the tested doses. Plasma levels of the intact active substance after i.v. and oral administration to cattle, however, indicated limited systemic availability due to an extensive first-pass extraction in the liver. Excretion of radioactivity in the rat amounted to 50 % or more within 1 day, suggesting an elimination half-life of less than 24 h. Data from cattle resulting in a terminal plasma $t_{1/2}$ of 11 hours support this conclusion. The amount of radioactivity excreted in the urine of rats was slightly higher than that found in faeces (46-60 % vs. 32-48 %), and the amount excreted with faeces by females was slightly lower than by male animals (-7.5 %). Excretion of radioactivity in air was not measured, but may account for some of the material not recovered (2-11 %). Residual radioactivity 96 h after administration of ^{14}C-imazalil sulphate amounted to 0.8 - 1.2 % and was mainly found in liver (0.5 %) and carcass (0.4 %). Overall, there was no major difference in excretion pattern between the dose groups. Similarly, metabolism was extensive for all dosing schemes, with more than 25 metabolites detected. Major metabolites included M3 (5.9-12.6 %), M4 (4.4-7.7 %), M8 (3.9-7.6 %), M10 (1.9-12 %) and M11 (0.4-1.2 %). M10 was identified as the product resulting from epoxidation of imazalil at its propenyl moiety and consecutive epoxide hydratation (Figure 1). M10 was apparently further oxidized at the propanediole moiety to form the corresponding carboxylic acid isomers M3A (fraction A of M3) and M4. Conjugation of M3A and M4 with alanine resulted in fraction B of M3 (M3B). Alternatively, oxidation of the imidazole converted M10 into M8. M11 may be generated from parent compound, M10 or M 3A and M4 by oxidative dealkylation to substract the propenyl group. Although data on the toxicokinetics of imazalil base is not available, it can be assumed, that once absorbed into the systemic circulation, the parent compound will be metabolized and excreted as its sulphate salt.

Dermal absorption of imazalil was measured in rat at various time points after application of ^{14}C-labelled imazalil base emulsifiable concentrate corresponding to a dose of 4 mg/cm^2 and 3 serial dilutions thereof following a protocol similar to OECD TG 427 (van Beijsterveldt, 1993). In absence of data for the fate of imazalil present within the skin at the end of the application period and in accordance with the OECD Guidance Document on Dermal Absorption (Sanco/222/2000), this fraction was included for calculation of the absorbed dose. Absorption within the most relevant exposure period of 10 hours increased from 34 and 25 % for doses of 4 and 0.4 mg/cm^2, respectively, to 45 and 68 % for doses of 0.04 and 0.004 mg/cm^2. This increase can not easily be attributed to imazalil concentration as the lower doses were achieved by application of dilutions of the emulsifiable concentrate in water and changes in composition of the formulation may also be a cause of this difference. Plasma levels of unlabelled imazalil were determined after topical application of imazalil spray at a dose of 4 mg/kg bw in cattle and compared to those observed after intravenous and oral administration of an equivalent dose of imazalil sulphate (Heykants et al., 1982). Peak plasma levels of

49 ng/mL imazalil were observed within 1 hour. Based on the AUC (0-48 h), an absolute systemic bioavailability of 5.1 % was calculated for dermal application in cattle. Data on the dose fraction absorbed was not provided.

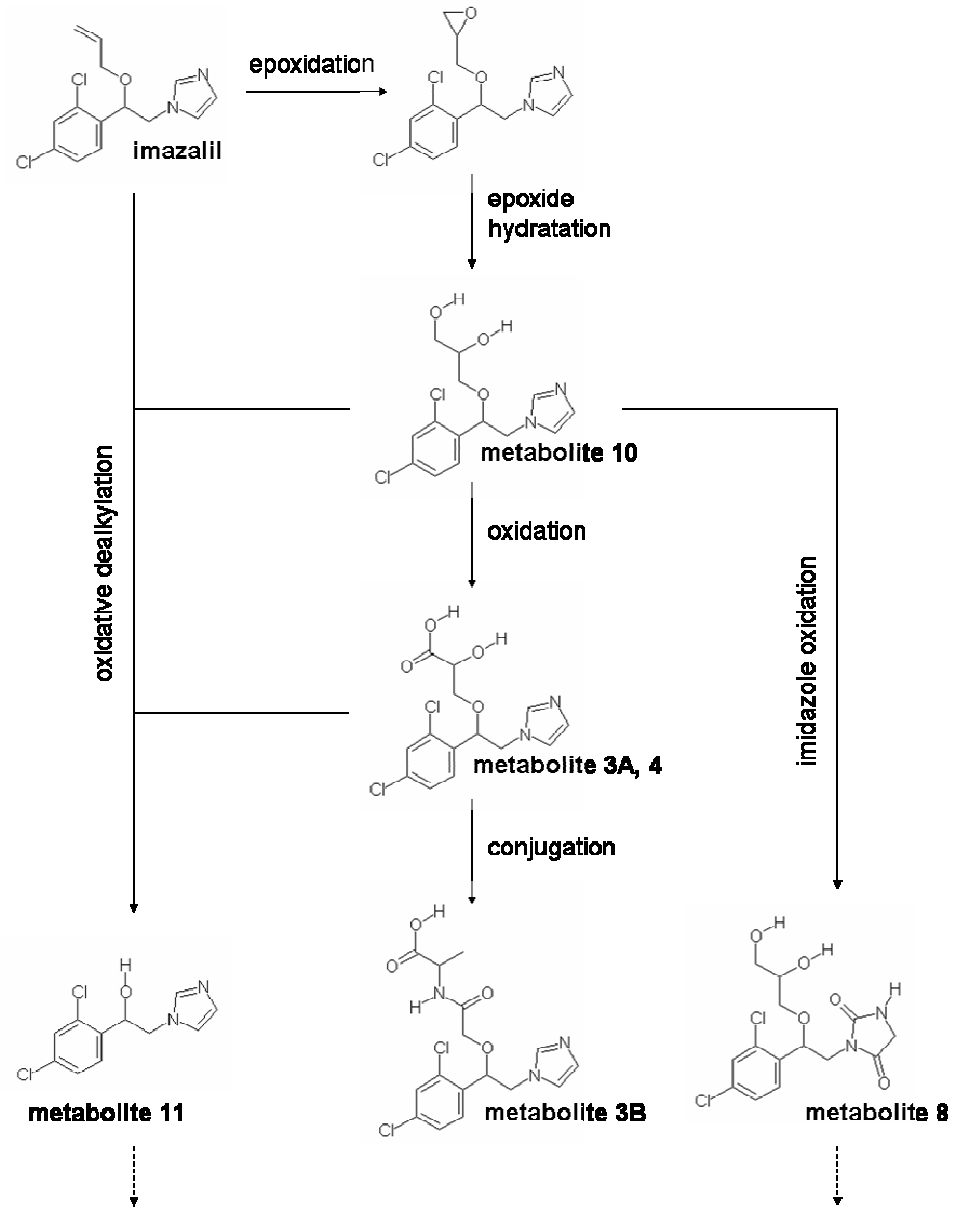


Figure 1: Major metabolic pathways of imazalil in the rat

5.2 Acute toxicity

Acute toxicity of imazalil was assessed in rats after oral, intraperitoneal and dermal application (Goodwine, 1990a; Niemegeers, 1977; Teuns et al., 1990a). An inhalation study conducted with imazalil smoke was not found suitable for evaluation of imazalil toxicity due to severe deficiencies in methodology and reporting (Appelman & Woutersen, 1983). Additional information on the inhalation toxicity of the a.s. in rats was provided by a pesticide assessment report on imazalil (Pesticide Safety Directorate/ECCO-Team, 1996).

5.2.1 Acute toxicity: oral

Oral dosing of rats with 160 mg/kg bw or more caused clinical symptoms including ataxia, piloerection, hypotonia, hypothermia, exophthalmia, tremors, excitation. From 320 mg/kg bw, this was accompanied by salivation, lacrimation, diuresis, diarrhoea, palpebral ptosis, loss of the righting reflex, hyperaemia, gastrointestinal lesions/bleeding and significant mortality. The oral LD_{50} was determined as 343 and 227 mg/kg bw for male and female rats, respectively (Goodwine, 1990a). This study was performed according to a protocol similar to OECD TG 401.

Table 15: Summary of acute oral toxicity

Animal species & strain	Number of animals per dose level	Doses, route of administration, vehicle	LD_{50} (mg/kg bw), Clinical signs	Reference year
Rat, Wistar	10 M + 10 F	160-320-640 mg/kg bw, oral (gavage) in aq. suspension	LD_{50}: 343/227 mg/kg bw (M/F); Clinical signs: ≥ 160 mg/kg: ataxia, piloerection, hypotonia, hypothermia, exophthalmia, tremors, excitation ≥ 320 mg/kg: salivation, lacrimation, diuresis, diarrhoea, palpebral ptosis, loss of the righting reflex, hyperemia, gastrointestinal lesions and bleeding	Goodwine WR, 1990a, Janssen Report No. R23979/15

5.2.2 Acute toxicity: inhalation

When imazalil was administered as dust in concentrations of 1.97 mg/L and more over 4 hours, the resulting clinical signs resembled those seen after oral administration in many regards. In addition, local effects including decreased and laboured respiration were noted. Necropsy showed severe lesions of the lungs and the eyes, pale livers and intestinal haemorrhage. The latter may have been the result of gastrointestinal intake of deposited material. Acute inhalative LC_{50} values of 2.88 and 1.84 mg/L were calculated for male or females, respectively (Pesticide Safety Directorate/ECCO-Team, 1996). Based on physiological default values and a respiratory fraction of approx. 35 % at doses close to the LC_{50}, corresponding inhaled systemic doses of 169 (M) and 110 (F) mg/kg bw can be derived, which are within the same order of magnitude as the reported LD_{50} values after oral or intraperitoneal administration. While this study was similar to OECD TG 403, another study (Appelman & Woutersen, 1983) can not be regarded suitable for risk assessment due to the use of smoke and severe deficiencies in dose determination.

Table 16: Summary of acute inhalation toxicity

Animal species & strain	Number of animals per dose level	Doses, route of administration, vehicle	LC_{50} (mg/L); Clinical signs	Reference year

Rat, Sprague-Dawley	5 M + 5 F	1.97-3.15-4.57 mg/L x 4 h, dust	LC_{50}: 2.43/2.88/1.84 mg/L x 4 h (combined/M/F), corr. to 169/110 mg/kg bw Clinical signs: wet fur, decreased respiration, hunched posture, lethargy, piloerection, laboured respiration, ataxia, coma, red/brown stains around snouts and eyes, hypothermia, ptosis, loss of righting reflex; necropsy: dark abnormally red lungs, pale liver, intestinal heamorrhage, opaque cornea (4.57 mg/L); surviving animals appeared normal after day 6	Blagden SM, 1990, Safepharm Report No. 306-3/R-5740
Rat, Wistar	5 M + 5 F	Nominal dose: 2 mg/L x 4 h, smoke	LC_{50}: not determined, no mortality	Appelman LM, Woutersen RA, 1983, TNO Report No. V 83.308/230831

5.2.3 Acute toxicity: dermal

Single dermal application of 2000 mg/kg bw dry substance resulted in sedation of 6/10 animals and slight skin reactions. Other gross pathology or mortality was not observed (Teuns et al., 1990a). This study was compliant with OECD TG 402.

Table 17: Summary of acute dermal toxicity

Animal species & strain	Number of animals per dose level	Doses, route of administration, vehicle	LD_{50} (mg/L); Clinical signs	Reference year
Rabbit, New Zealand White	5 M + 5 F	0-2000 mg/kg bw, dermal	LD_{50}: > 2000 mg/kg bw Clinical signs: sedation (6/10) on day 1; very slight erythema (7/10), very slight oedema (3/10), slight to moderate (reversible) skin scaling and thickening	Teuns G, et al., 1990a, Janssen Report No. R23979 - Exp. No. 2344

5.2.4 Acute toxicity: other routes

Lethal doses and symptoms of intoxication following oral administration were confirmed by an earlier non-guideline study with intraperitoneal injection (Niemegeers, 1977). The corresponding LD_{50} values of 288 and 155 mg/kg bw in males and females were close to those obtained with oral administration.

Table 18: Summary of acute toxicity by other routes

Animal species & strain	Number of animals per dose level	Doses, route of administration, vehicle	Result	Reference year
Rat, Wistar	5 M + 5 F	80-160-320 mg/kg bw, intraperitoneal	LD_{50}: 288/155 mg/kg bw (M/F) Clinical signs: ≥ 80 mg/kg: Piloerection, hypotonia, hypertonia, ataxia ≥ 160 mg/kg: mortality (3/5 F), tremors, convulsions, ptosis, exophthalmia ≥ 320 mg/kg: mortality (4/5 M, 5/5 F), salivation, sedation, lung oedema, dyspnea	Niemegeers, 1977, Janssen Preclinical Research Report No. R 23979/7

5.2.5 Summary and discussion of acute toxicity

Acute exposure to imazalil can lead to mortality at moderate doses. Taking into account the classification limits of $200<LD_{50}\leq2000$ mg/kg bw**,** imazalil is classified acc. to 67/548/EEC as "Harmful if swallowed" (Xn, R22), based on an acute oral LD_{50} of 343/227 mg/kg bw in male/female rats and "Harmful by inhalation" (Xn, R20), based on an acute LC_{50} of 2.88/1.84 mg/L imazalil dust in male/female rats (classification limits: $1<LC_{50}\leq5$ mg/L/4h).

Taking into account the classification limits of $50<LD_{50}\leq300$ mg/kg bw under Regulation (EC) 1272/2008, Category 3 is proposed for acute oral toxicity based on an LD_{50} value of 227 mg/kg bw in female rats. If an average was estimated for male and female animals, the resulting value of 285 mg/kg bw would also be within the classification limits for Cat. 3. The reported LC_{50} values for imazalil dust result in classification into category 4 for acute inhalation toxicity (classification limits: $1<LC_{50}\leq5$ mg/L/4h). Following the criteria provided within Annex I of the Regulation (EC) 1272/2008 for Classification and Labelling, imazalil therefore, requires classification as follows: Acute Tox. 3; H301 (Toxic if swallowed) and Acute Tox. 4; H332 (Harmful if inhaled).

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.

5.3 Irritation

Imazalil dry substance was evaluated for irritation of the skin and the eyes in rabbits according to OECD TG 404 and 405 (Goodwine, 1990b; Teuns et al., 1990b).

5.3.1 Skin

No formation of erythema or oedema was observed following single application of 0.5 g dry powder for 4 hours to the skin of 3 rabbits (Goodwine, 1990b).

Table 19: Summary of skin irritation

Animal species & strain	Number of animals	Doses	Result	Reference

Rabbit, New Zealand White	3	0.5 g dry powder	Not irritating (all scores for erythema and oedema at 24, 48 and 72 h: 0)	Goodwine WR, 1990b, Janssen Report No. 1864

5.3.2 Eye

Administration of 0.1 g of the active substance to the rabbit eye resulted in opaque or translucent lesions of the cornea, redness of the conjunctiva, chemosis and changes in the iris. Corneal lesions persisted over at least 21 days and were described as opacities covering more than one quarter but less than one half of the area (Teuns et al., 1990b).

Table 20: Summary of eye irritation

Animal species & strain	Number of animals	Doses	Result	Reference
Rabbit, New Zealand White	3 F	0.1 g	Corneal opacity, not reversible in 2/3 animals by day 21 Irritation scores at 24/48/72 h: cornea 2/1.7/1.7, iris 0.3/1/0.7, conjunctiva 1/0.7/0.3, chemosis 1.3/0.7/0.7	Teuns G, et al., 1990b, Janssen Report No. R23979 - Exp. No. 2253

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.

5.3.3 Respiratory tract

No data available

5.3.4 Summary and discussion of irritation

Classified as "Risk of serious damage to eyes" (Xi; R41) based on persistent lesions of the

cornea acc. to 67/548/EEC and as "Eye Dam. 1; H318 (Causes serious eye damage)" according to Regulation (EC) No 1272/2008.

5.4 Corrosivity

No indication for corrosivity from physicochemical data or skin irritation studies (5.3.1).

5.5 Sensitisation

Imazalil was evaluated for skin sensitisation in the adjuvant Guinea Pig Maximisation Test of Magnusson and Klingman (GMPT) and in the non-adjuvant Buehler test performed acc. to OECD TG 406 or similar to OECD TG 406, respectively (Teuns et al., 1990c; Wnorowski, 1997).

5.5.1 Skin

Following challenge, one of 20 animals showed a mild reaction in the GMPT (Teuns et al., 1990c). The positive control DNCB produced a response rate of 100 %.

One of 10 animals developed a very faint, non-confluent erythema 24 h post-challenge in the Buehler test (Wnorowski, 1997). The positive control DNCB produced 3/10 moderate, 5/10 faint, and 2/10 very faint reactions in this test.

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.

5.5.2 Respiratory system

No data available

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.

5.5.3 Summary and discussion of sensitisation

Directive 67/548/EEC and Regulation (EC) 1272/2008 state that in a properly conducted test, response rates of at least 30 and 15 % are expected for mild/moderate sensitisers in adjuvant (GMPT) and non-adjuvant (Buehler) tests, respectively. Therefore, on the basis of the available animal data, imazalil in a non-irritant formulation does not meet the existing criteria for classification for sensitisation.

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.

5.6 Repeated dose toxicity

5.6.1 Repeated dose toxicity: oral

Subacute/subchronic toxicity of orally administered imazalil base was evaluated in a number of studies essentially following the recommendations of OECD TG 408/452 in rats, mice, and dogs with similar results: In the rat, doses of ≥ 32/38 mg/kg bw/d (M/F) applied with the diet over 3 months (van Deun et al., 1996), as well as an approximate dose of 20 mg/kg bw/d administered for the longer period of 6 months (Lina et al., 1983), resulted in increased liver weight and histological changes of the liver such as hepatocyte hypertrophy and fatty vacuolisation. These were accompanied by changes in corresponding serum parameters, namely LDH (increased), AST and ALT (decreased), and urea (decreased). Further deviations were decreased body weight, an increase in adrenal weight and adrenocortical cell swelling, as well as haematological abnormalities exemplified by decreased monocyte and increased red blood cell counts with concurrent changes in mean corpuscular volume (decreased) and mean corpuscular haemoglobin concentration (increased). Almost identical signs of hepatotoxicity were observed in mice fed a diet containing ≥ 200 ppm or ≥ 47/55 mg/kg bw/d (M/F) for 3 months (Verstraeten et al., 1993; van Deun et al., 1994). In the dog, imazalil administered daily for 1 year as capsule resulted in signs of beginning hepatotoxicity at the highest dose of 20 mg/kg bw/d as indicated by increased liver weight and elevated serum alkaline phosphatase activities. Other findings such as softened faeces, salivation, vomiting, decreased calcium concentration and lowered appetite as well as body weight gain are of unclear aetiology. Significant variations in haematological parameters (white blood cell counts, mean corpuscular haemoglobin concentration) were also noted, but regarded to be within the historical control range or borderline.

Table 21: Summary of oral RDT

Animal species & strain	Number of animals	Doses, vehicle, duration	Result	Reference
Rat, Wistar	10 M + 10 F	0-16/19-32/38-64/76 mg/kg bw/d (M/F), 3 months (dietary)	NOAEL: 16/19 mg/kg bw/d (M/F) ≥ 32/38 mg/kg bw/d: hepatocyte swelling (M, 1 mo) and vacuolisation (F, 1 mo), liver weight↑ (1 mo); adrenal weight↑, adrenocortical cell swelling (F, after 3 month) ≥ 64/76 mg/kg bw/d: AST↓, ALT↓, urea↓, MCV↓	Van Deun et al., 1996, Janssen Report R023979 Exp. No. 3514
Rat, Wistar	10 M + 10 F	0-64/79-129/150-181/236-252/333 mg/kg bw/d (M/F), 3 months (dietary)	NOAEL: N/A LOAEL: 64/79 mg/kg bw/d (M/F) ≥ 64/79 mg/kg bw/d: liver weight↑ (M), dark livers, hypertrophy, fatty vacuolisation, AST↓, ALT↓, urea↓; haematology: monocytes↓ (M), RBC↑ (F), MCV↓ (F), MCHC↑ (F)	Van Deun et al., 1996, Janssen Report R023979 Exp. No. 3672

Rat, Wistar	10 M + 10 F	approx. 0-1.25-5-20 mg/kg bw/d, 6 months (dietary)	NOAEL: ~5 mg/kg bw/d ~20 mg/kg bw/d: body weight↓ (M), LDH↑ (F), kidney and liver weight↑	Lina et al., 1983, Civo Instituts TNO Report No. V 83.186/220555
Mouse, Swiss Albino	25 M + 25 F (interim: 10 M + 10 F)	0-12/14-47/55-138/166 mg/kg bw/d (M/F), 3 months (dietary) with one month inter-im	NOAEL: 12/14 mg/kg bw/d (M/F) ≥ 47/55 mg/kg bw/d: hepatocyte vacuolisation, centrilobular clearing, liver weight↑ (M) ≥ 138/166 mg/kg bw/d: liver weight↑ (F), AP↑ (M, 1 mo)	Van Deun et al., 1994, Janssen Report R023979 Exp. No. 3140
Mouse, Swiss Albino	10 M + 10 F	0-200-400-800 ppm, 3 months (dietary)	NOAEL: N/A LOAEL: 200 ppm ≥ 200 ppm: hepatic vacuolar degeneration (M), AST↓ and cholesterol↓ (F) ≥ 400 ppm: vacuolar degen-eration and centrilobular swelling ≥ 800 ppm: swollen and dark livers, liver weight↑, body weight and bw gain↓ (F); Hct↑, Hb↑ (F)	Verstraeten et al., 1993, Janssen Report R 23979 Exp. No. 2020
Dog, Beagle	4 M + 4 F	0-1.25-2.5-20 mg/kg bw/d, 1 year, capsule	NOAEL: 2.5 mg/kg bw/d 20 mg/kg bw/d: AP↑, liver weight↑, softened faeces, salivation, vomiting, serum calcium↓, lowered appetite and bw gain, borderline haematological variations (WBC, MCHC)	Verstraeten et al., 1989, Janssen Report R 23979 Exp. No. 1899

5.6.2 Repeated dose toxicity: inhalation

No data available

5.6.3 Repeated dose toxicity: dermal

Dermal toxicity of imazalil after repeated exposure was assessed in a rabbit study performed to a protocol similar to OECD TG 410 (Teuns et al., 1991). In a preliminary study over 4 days, a dose of 250 mg/kg bw/d was found to induce slight hepatotoxicity and erythema which developed into severe skin lesions. Detailed information regarding the nature of the lesions and type and volume of vehicle use in the preliminary test was not provided. A more detailed evaluation of this range-finding study in the 2009 monograph under 91/414/EEC re-ports slight erythema (grade 1) and slight to moderate fissures and scaling (grade 1 and 2) towards the end of the exposure period (day 4) and post exposure (day 5 and 6). A higher dose of 1000 mg/kg bw/d increased the intensity of liver toxicity, which was then graded as mod-erate. No adverse reactions were observed at 63 mg/kg bw/d. In the main study, no relevant adverse effects (local or systemic) were reported after dermal administration of up to 160 mg/kg bw/d imazalil for 6 hours per day on 5 days per week over 3 weeks.

Table 22: Summary of dermal RDT

Animal species & strain	Number of animals	Doses, vehicle, duration	Result	Reference
Rabbit, New Zealand White	5 M + 5 F	63-250-1000 mg/kg bw/d in sesame oil 4 days	NOAEL: 63 mg/kg bw/d ≥ 250 mg/kg bw/d: slight hepatotoxicity, erythema developing to severe skin lesions at day 4 1000 mg/kg bw/d: moderate hepatotoxicity	Teuns et al., 1991, Janssen Report R 23979 Exp. No. 2418
Rabbit, New Zealand White	5 M + 5 F	0-10-40-160 mg/kg bw/d in sesame oil 6 h/d for 5 d/wk over 3 wks	NOAEL: 160 mg/kg bw/d No relevant adverse effects	Teuns et al., 1991, Janssen Report R 23979 Exp. No. 2418

5.6.4 Other relevant information

Experimental therapy with escalating topical and systemic doses of imazalil was performed in a patient suffering from fungal infection (alternariosis). Burning sensations and irritation was reported for concentrations of 5 % imazalil in PEG 400 or 0.4 % in saline when applied topically to mucosa or inflamed skin (Stiller & Stevens, 1986).

5.6.5 Summary and discussion of repeated dose toxicity:

According to Directive 67/548/EEC, substances should be classified and labelled if significant health effects are observed at levels ≤ 100 mg/kg bw/d after subchronic (90 day) dermal administration, or at levels ≤ 300 mg/kg bw/d after subacute exposure. Criteria of Annex I Regulation (EC) 1272/2008 would not require an equivalent classification as the effect level of 250 mg/kg bw/d was above the upper classification level of 200 mg/kg bw/d (dermal, rat or rabbit). Thus, no classification and labelling regarding skin irritation is proposed.

Hepatic and haematologic changes following repeated oral administration of doses ≤ 50 mg/kg bw/d are considered primarily adaptive and not of sufficient severity to require classification as R 48 (Danger of serious damage to health by prolonged exposure) according to the rules laid down in Directive 67/548/EEC chapter 3.2.4 and Annex I of Regulation (EC) 1272/2008.

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.

5.7 Mutagenicity

5.7.1 In vitro data

Imazalil was evaluated for mutagenicity in Salmonella typhimurium and Chinese hamster lung fibroblasts as well as for clastogenicity in human peripheral lymphocytes in absence and presence of S9 mix, and for induction of DNA repair in primary rat hepatocytes (Table 7). The concentration range tested included cytotoxic levels. There was no indication for genotoxicity in any of these in vitro systems.

Table 23: Summary of in vitro mutagenicity

Test system	Test object	Concentration	Results	Reference and year
Ames test, sim. to. OECD TG 471	*S. typhimurium* TA1535, TA1538, TA97, TA98, TA100	0-5-10-25-50-100-250-500 µg/plate	Non mutagenic, toxic at ≥ 250 µg/plate	Vanparys, Marsboom, 1988, Janssen Report R 23979 Exp. No. 1999
Mammalian chromosome aberration assay, sim. to OECD 471	Peripheral human lymphocytes	0-9-36-73-145 µg/mL	Non mutagenic, toxic at 145 µg/mL, reduction of mitotic index at 73 µg/mL (w/o S9 mix)	Lenaerts et al., 1990, Janssen Report R 23979 Exp. No. SCK 86/02D/R23979

Mammalian cell gene mutation test, sim. to OECD 476	Chinese hamster lung fibroblasts (V79)	0-20-60-65-70-80 µg/mL	Non mutagenic, toxic at ≥ 60 µg/mL	Van Gompel et al., 1995, Janssen Report R 023979 Exp. No. 3470
Unscheduled DNA synthesis, OECD 482	Primary rat hepatocytes (male) *in vitro*	0-0.09-0.3-0.9-3-9-30 µg/mL	Non mutagenic, toxic at ≥ 9 µg/mL	Fautz et al., 1990, Cytotest Cell Research GmbH Report No. 192600

5.7.2 In vivo data

Imazalil technical product did not show any potential for inducing micronuclei in the erythrocytes of male and female mice when given once orally at non-toxic and toxic doses (20-320 mg/kg bw) (Vanparys & Narsboom, 1988). Hence, imazalil is unlikely to cause chromosomal aberrations or to interfere with the mitotic spindle apparatus in the bone marrow in this species.

Table 24: Summary of in vivo mutagenicity

Test system	Method	Route of administration	Toxic dose	Result	Reference
Mouse, Swiss Albino (5 M + 5 F)	Micronucleus test, sim. to OECD 474	Oral, single application (0-20-80-320 mg/kg bw)	320 mg/kg bw: decreased bone marrow proliferation (48, 72 h)	No sign. increase in micronuclei at any dose and any sampling time (24, 48 and 72 h)	Vanparys, Marsboom, 1988, Janssen Report R 23979 Exp. No. 1911

5.7.3 Human data

No data available

5.7.4 Other relevant information

No other relevant data available

5.7.5 Summary and discussion of mutagenicity

Overall, there is no reason for concern regarding potential genotoxicity of imazalil based on the available in vitro and in vivo test results.

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.

5.8 Carcinogenicity

Carcinogenicity of imazalil after prolonged oral administration with the diet was investigated in rats and mice by Van Deun et al. (1999) and Verstraeten et al. (1993), respectively, according to protocols essentially following OECD TG 452. The rat study has, to the knowledge of the authors, not been available for earlier risk assessments of imazalil in the EU.

Another carcinogenicity study in rats can not be regarded suitable for risk assessment due to deficiencies in dose selection (Lina et al., 1984, Civo Instituts TNO Report No. V 84.140/220555). This study is therefore not considered here.

Data regarding the carcinogenicity of imazalil by inhalation or after dermal administration was not available.

5.8.1 Carcinogenicity: oral

Chronic toxicity and carcinogenicity of imazalil after prolonged oral administration with the diet was investigated in rats and mice. In addition, a one-year study with daily administration of imazalil containing capsules was performed in dogs (Verstraeten et al., 1989, Janssen Report R 23979 Exp. No. 1899).

In all three species, the liver was identified as the main target organ. Haematological parameters were affected in rats and mice with a higher sensitivity of female than male animals, while only males showed hypertrophic changes of the thyroid.

Later studies attributed the reduction in bodyweight to reduced food palatability and food intake with constant or slightly increased food conversion (Van Deun et al., 1999). In males, histopathology revealed hepatocyte vacuolisation and eosinophilic inclusions. Mean thrombocyte counts were increased in females, and males showed reduced plasma albumin values. Further effects on haematological and plasma parameters were observed in female rats fed 200 ppm over 24 months (Van Deun et al., 1999). There, haemoglobin values and red blood cell counts were increased for females, while mean corpuscular volume was reduced. Plasma

potassium, calcium, inorganic phosphate and urea nitrogen were also lower than in controls. At the same dose of 200 ppm, similar adverse effects were reported in mice treated for 23 months (Verstraeten et al., 1993), including increased haematocrit, haemoglobin and red blood cell count in females, as well as macroscopic and microscopic liver changes (vacuolisation, sinusoidal cell pigmentation and swelling) in males. In mice, feeding with 200 ppm of imazalil was sufficient to cause a significant increase in the frequency of hepatocytic carcinoma in males. With higher doses of 1200-2400 ppm in rats or 600 ppm in mice, adverse effects on the haematological system as well as the liver were enhanced and included the other sex. A higher incidence of hepatocytic neoplasms was also reported in female mice fed 600 ppm, thyroid and liver adenoma increased in male rats exposed to ≥ 1200 and 2400 ppm, respectively.

Additional observations with potential relevance to disruption of the hormonal homeostasis as a possible mode of action were made in female rats: exposure to 1200 ppm over 24 month stimulated the mammary glands and doubling of the dose led to a decreased incidence in mammary tumours (Van Deun et al., 1999).

A statistically significantly increased frequency of hepatocellular adenoma (mice and rats) as well as carcinoma (mice only) was observed at 160 mg/kg bw/d in male rat compared to the control group (13 *vs.* 2-4 in other dose groups; Van Deun et al., 1999) as well as historical controls (US EPA, 2002) and at ≥ 42 and 105 mg/kg bw/d in male and female mice, respectively (Verstraeten et al., 1993). In addition, the number of thyroid adenomas was elevated in male rats treated with ≥ 60 mg/kg bw/d imazalil, concurrent with swelling and weight increase of thyroids.

Based on further mechanistic studies, imazalil-induced thyroid adenomas in rat were regarded as not relevant to human health due to quantitative species differences as outlined in section 5.10.

In contrast, the currently available mechanistic information does not allow to exclude a relevance of hepatic neoplasms observed in rats and mice after chronic imazalil exposure to human health on the basis of quantitative or qualitative inter-species differences (see also 5.10). However, it could be concluded that the mechanism involved is most likely non-genotoxic and tumour-promoting with the existence of a practical threshold. An increase in liver carcinoma from 10 % in controls to 22 % at a dose of 105 mg/kg bw/d was observed in male mice. The lowest NOAEL for hepatic adenoma was 10 mg/kg bw/d, which is 4 times the NOAEL for chronic toxicity of 2.5 mg/kg bw/d derived from the one year dog study. Hence, the latter is expected to provide adequate protection.

Conclusion:

Chronic administration of imazalil in rats and mice confirmed the liver as main target organ. Haematological parameters were also affected in rats and mice with a higher sensitivity of female than male animals. In addition, males showed hypertrophic changes of the thyroid.

A statistically significantly increased frequency of hepatocellular adenoma (mice and rats) was observed

- at 120 mg/kg bw/d in the male rat compared to the control group (13 vs. 2-4 in other dose groups; Van Deun et al., 1999) as well as historical controls (US EPA, 2002), and
- at ≥ 33 and 131 mg/kg bw/d in male and female mice, respectively (Verstraeten et al., 1993).

Liver carcinoma increased from 10 % in controls to 22 % at a dose of 105 mg/kg bw/d for male mice.

In addition, the number of thyroid adenomas was elevated in male rats treated with ≥ 60 mg/kg bw/d imazalil, concurrent with swelling and weight increase of the thyroids.

Additional observations with potential relevance to disruption of the hormonal homeostasis as a possible mode of action were made in female rats. There, exposure to 80 mg/kg bw/d over 24 month stimulated the mammary glands and doubling of the dose led to a decreased incidence in mammary tumours (Van Deun et al., 1999).

Table 25: Summary of oral carcinogenicity

Animal species & strain	Number of animals	Doses, vehicle, duration	Result	Reference
Rat, Wistar-Hannover	50 M + 50 F	0-50-200-1200-2400 ppm 0-2.5/3.5-10/14-60/80-120/160 mg/kg bw/d (M/F), oral (dietary), 24 months	≥ 14 mg/kg (F only): Hb↑ , RBC↑, MCV↓, AST↓, ALT↓, K↓, Ca↓, P(i)↓, urea↓, urinary WBC ↑ ≥ 60/80 mg/kg: *Neoplastic:* thyroid adenoma ↑ (M) bw ↓, liver weight ↑, liver foci (M), dark livers (F), thyroid weight ↑ (M), swollen thyroid (M), pale/rough kidneys (F), MCV ↓ (M), MCH ↓ (M), MCH ↓ (F), AST ↓, ALT ↓, AP ↓, Ca ↓, urea ↓, protein ↓, glucose ↑, lipids ↓ (F) ≥ 120 mg/kg (M only): *Neoplastic:* hepatocellular adenoma ↑	Van Deun et al., 1999, Janssen Report R023979 Exp. No. 3817
Mouse, Swiss Albino	50 M + 50 F	0-50-200-600 ppm 0-8.1/9.9-33/42-105/131 mg/kg bw/d (M/F), oral (dietary), 23 months	≥ 33/42 mg/kg: *Neoplastic:* hepatocytic adenoma (M), hepatic neoplastic nodules (M), macroscopic liver changes/masses (M), liver foci (M), hepatocyte vacuolisation (M), sinusoidal cell pigmentation and swelling (M), Hct ↑ (F), Hb ↑ (F), RBC ↑ (F) ≥ 105/131 mg/kg: *Neoplastic:* hepatocytic adenoma (F) and carcinoma (M) absolute and rel. liver weight ↑, liver foci (F), hepatocyte vacuolisation (F), sinusoidal cell pigmentation and swelling (F), bw and bw gain ↓ (M)	Verstraeten et al., 1993, Janssen Report R 23979 Exp. No. 2194

5.8.2 Carcinogenicity: inhalation

No data available

5.8.3 Carcinogenicity: dermal

No data available

5.8.4 Carcinogenicity: human data

No data available

5.8.5 Other relevant information

Mechanistic studies are described in section 5.10.

5.8.6 Summary and discussion of carcinogenicity

Based on further mechanistic studies, imazalil-induced thyroid adenomas in rat can be regarded as not relevant to human health due to quantitative species differences (see 5.10).

At present, a total of fifteen mechanistic studies were submitted to elucidate the mode of action of imazalil on the induction of liver tumors. In chapter 5.10, the presented data and the potential modes of action for induction of hepatocellular neoplasia by imazalil are discussed.

In conclusion, a mode of action for the increased incidence of liver tumours in male rats and male and female mice exposed chronically to imazalil could not be established with certainty. Therefore, the currently available mechanistic information does not allow to exclude a relevance of hepatic neoplasms observed in rats and mice after chronic imazalil exposure to human health on the basis of quantitative or qualitative inter-species differences (see also 5.10), although the mechanism involved is most likely non-genotoxic with the existence of a threshold and an induction of a mixed type of microsomal enzymes.

With respect to the discussion of classification and labelling of phenobarbital, IARC (2001) states that there is *inadequate evidence* in humans for the carcinogenicity of phenobarbital but there is *sufficient evidence* in experimental animals for the carcinogenicity of phenobarbital.

The results of the mechanistic examinations and of the toxicological studies indicate that imazalil and phenobarbital may share some common mechanisms. A definite conclusion on the similarity of the mode of action of both substances cannot be established. Therefore, we conclude that imazalil may be of relevance to human health and we propose a classification for carcinogenicity for imazalil.

Accordingly, Directive 67/548/EEC requires classification of imazalil as “Carc. Cat. 3; R40 (Limited evidence of a carcinogenic effect)” based on the observation of an increased incidence of hepatic neoplasms in two animal species. Adoption of the criteria described in Annex I of the Regulation (EC) No 1272/2008 on C&L result in classification of imazalil as “Carc. 2; H351 (Suspected of causing cancer)”.

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.

Additional key elements

During public consultation, IND referred to three new mechanistic studies, which are

briefly summarised below.

In vivo study with Imazalil in wild type and humanized PXR/CAR mice (Elcombe, 2012a):
In order to investige whether Imazalil exhibits the properties of a typical CAR/PXR agonist, groups of 10 male wild type mice (C57Bl/6NTac) and mice humanised for CAR and PXR (hPXR/hCAR) were administered 0, 50, 200 or 600 ppm Imazalil or 1000 ppm phenobarbital (PB; positive control) via the diet for 7 days. The main results are given in the summary table below.

Summary of *In Vivo* Study with Imazalil

Parameter	Wild Type C57Bl/6				Humanised hPXR/hCAR			
	imazalil			PB	imazalil			PB
	50 ppm	200 ppm	600 ppm	1000 ppm	50 ppm	200 ppm	600 ppm	1000 ppm
Plasma:								
ALT	-	-	-	1.3x	-	-	-	2x
ALP	-	-	-	1.3x	-	-	-	2.2x
Liver:								
Absolute Wt	-	-	-	1.4x	-	-	-	1.4x
Relative Wt	-	-	-	1.4x	-	-	-	1.4x
Lipid perox.	-	-	-	-	-	-	-	-
PCO	0.8x	0.8x	0.8x	0.6x	-	0.9x	0.7x	0.8x
P450	-	1.5x	2.5x	3.7x	-	-	1.8x	3.8x
PROD	-	3.2x	6.7x	33x	-	0.5x	-	8.7x
BROD	1.9x	5.2x	6.3x	65x	0.6x	0.4x	-	10x
BQ	0.7x	-	1.9x	2.7x	-	0.6x	1.6x	3.9x
mRNA:								
Cyp2b10	-	4x	39x	154x	-	-	6x	93x
Cyp3a11	-	-	6x	5x	-	2x	3x	7x
Cyp4a10	-	-	-	0.2x	-	-	-	-
Cyp4a14	0.5x	0.4x	0.3x	0.1x	-	-	-	0.5x
Acox1	-	-	-	-	-	-	-	-
Gadd45β	-	-	4x	11x	-	--	4x	10x
Protein:								
Cyp2b10	+	++	+++	+++	-	-	++	+++
Cyp3a11	-	-	++	++	-	-	+	++
Cyp4a	-	-	-	-	-	-	-	-
Apoptosis[1]	nd	nd	+	++	nd	nd	+	++
Histopathology:								
Hypertrophy	-	-	-	++	-	-	+	+++
Mitosis	-	-	-	+	-	-	-	+
S-phase	6x	3x	4x	31x	3x	3x	3x	24x

Values are fold change of control values.
-, no change. [1] Caspase-3 cleavage. nd, not determined.

Imazalil was well tolerated at all doses levels (equal to 6.69, 29.5 and 95.8 mg/kg bw/d in wild type mice, and 7.19, 26.1 and 78.2 mg/kg bw/d in hPXR/hCAR mice) and had no effect on body weights or food consumption in both types of mice. No overt hepatotoxicity was evident as assessed by plasma clinical chemistry or histopathology (with only minimal to moderate vacuolation and minimal inflammatory and mononuclear infiltrates in most treated animals, and minimal to moderate hypertrophy only at 600 ppm in hPXR/hCAR mice). Liver weights were also not increased, and there was no evidence for

increased hepatic lipid peroxidation (indicative of oxidative stress).

Administration of Imazalil to wild type mice resulted in dose-dependent increases in total cytochrome P450 and associated monooxygenase activities, specifically pentoxyresorufin-*O*- depentylation (PROD; Cyp2b10), benzyloxyresorufin-*O*-debenzylation (BROD; Cyp2b10/ Cyp3a11) and benzyloxyquinoline-*O*-debenzylation (BQ; Cyp3a11). Consistent with the increased cytochrome P450 activities, marked dose-dependent increases were seen in the expression of mRNAs and proteins for these cytochromes. Little, if any, induction of monoxygenase activities was seen in hPXR/hCAR mice administered Imazalil. Similarly, only small increases in Cyp2b and Cyp3a mRNA and proteins were seen in these mice (mainly at 600 ppm).

Imazalil did not induce peroxisomal β-oxidation (palmitoyl-CoA oxidation, PCO) or microsomal Cyp4a, indicating lack of PPARα activation and peroxisome proliferation. Imazalil did however induce Gadd45β mRNA expression and caspase-3 cleavage products in both types of mice, indicative of apoptosis. Imazalil also increased hepatocellular DNA synthesis (S-phase) in both wild type and hPXR/hCAR mice. No dose-response relationship was however observed and the magnitude of the cell proliferation was lower than seen with phenobarbital. This positive control at 1000 ppm (equal to 155.3 and 127.8 mg/kg bw/d in wild type mice and hPXR/hCAR mice, respectively) in general induced similar, albeit more marked findings to those seen at high Imazalil doses.

It was concluded in the study report that Imazalil-mediated induction of Cyp2b10, and to a lesser degree Cyp3a11, in the presence of increased hepatocellular S-phase, strongly suggests that Imazalil is an activator of the nuclear receptors CAR and (probably) PXR, with a NOAEL of 50 ppm. Additionally, Imazalil appeared to be a more potent activator of mouse CAR than human CAR, as noted by generally smaller responses in humanised CAR mice when compared to the wild type mouse at comparable doses. According to the study author, the similarities to phenobarbital, a known non-genotoxic rodent liver carcinogen and CAR activator, further support the conclusion that liver effects seen with Imazalil are CAR-dependent.

In vitro study with cultured female human hepatocytes (Elcombe, 2012b):
Female human hepatocytes (cryopreserved, originating from one donor) were exposed to 0 (vehicle DMSO), 1, 3, 10 or 30 µM Imazalil for 96 h and evaluated for cytotoxicity, for CYP2B6 and CYP3A4 activity (by measuring PROD and BQ, respectively), and for cell proliferation (replicative DNA synthesis (S-phase); using epidermal growth factor (EGF) as positive control). Increased cell proliferation was not observed in human hepatocytes exposed to Imazalil at concentrations up to and beyond the toxicity threshold (see summary table on next page). Furthermore, treatment with Imazalil did not result in induction of CYP2B6 or CYP3A4.

Note: The study report mentions that earlier studies with phenobarbital in this test system (at doses up to 1000 µM) resulted in induction of CYP2B6 and CYP3A4 but not in cell proliferation.

In vitro study with cultured female mouse hepatocytes (Elcombe, 2012c):
The same experiment as described above was also performed with female CD-1 mouse hepatocytes, at concentrations of 0, 3, 10, 30 or 100 µM Imazalil. Treatment of mouse hepatocytes with Imazalil resulted in induction of Cyp 3a11, limited induction followed by dose-dependent inhibition of Cyp 2b10 and increased cell proliferation (see summary table below).

Summary of *In Vitro* Studies with Imazalil

Parameter	Mouse hepatocytes	Human hepatocytes

	3µM	10µM	30µM	100µM #	1µM	3µM	10µM	30µM #
Cytotoxicity	1.07	1.04	0.84*	0.03*	0.96	0.94*	0.77*	0.12*
Cyp 2b10:								
Activity (PROD)	1.17*	0.87	0.15*	-	0.76	0.76	0.72*	-
Cyp 3a11:								
Activity (BQ)	1.34*	2.00*	2.05*	-	1.07	1.27	0.23*	-
S-phase	1.07	1.79*	1.50*	-	0.93	0.67	0.78	-
	7.82* (EGF 25 ng/mL)				28.2* (EGF 25 ng/mL)			

Values are fold change from controls. *statistically significant change. #excessively cytotoxic dose

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 docu-

ment 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 in-

ducer, 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).

Supplemental information - In depth analyses by RAC

Incidence data (absolute) on the (non-)neoplastic lesions observed in the 2-yr mouse study (Verstraeten *et al*., 1993):

Table A6.7-2: Results of a carcinogenicity study in mice

Parameter	Control data				50 ppm		200 ppm		600 ppm	
	historical		study							
	m	f	m	f	m	f	m	f	m	f
Number of animals	-	-	50	50	50	50	50	50	50	50
Mortality (%)	-	-	30	54	50	56	34	52	44	64
Clinical signs	-	-	Bad condition, waste of food, abdominal distension (f), turbid eyes (m), ageing -related							
Body weight gain	-	-	-	-	-	-	↓ (week 7-15)	-	↓ (week 2-52)	-
Food consumption	-	-	Not affected (tendency for increased uptake noted was based on food wastage)							
Clinical chemistry	-	-	No changes							
Liver weight	-		-	-	-	-	-	-	↑ *(a+r)	↑ (a+r)
Liver	Non-neoplastic changes									
Focal cellular changes	-	-	-	-	-	-	10	-	-	-
Pigmentation of sinusoidal cells	-	-	-	-	-	-	-	-	20	-
Swelling of sinusoidal cells	-	-	-	-	-	-	37	-	-	-
Large vacuoles	-	-	-	-	-	-	8	-	9	-
Liver	Neoplastic changes									
Neoplasms	-	-	13	4	10	6	25	2	25	11
Neoplastic nodule	-	-	8	4	5	6	23	0	17	10
Carcinoma		-	5	0	7	1	6	2	11	2

*a - absolute
r - relative

Incidence data (absolute) on the (non-)neoplastic lesions observed in the 2-year rat study (Van Deun *et al*., 1999):

Table A6.7-1: Neoplastic and non-neoplastic lesions in Wistar (Hannover) rats (50 rats examined per dose and sex)

	Males					Females				
Dose level [ppm]	0	50	200	1200	2400	0	50	200	1200	2400
Neoplastic lesions										
Liver										
Hepatocellular adenoma	4	2	3	4	13[a]	2	1	2	1	2
Hepatocellular carcinoma	1	0	0	0	1	0	0	0	0	0
Thyroid										
Follicular cell neoplasia	4	8	6	11[a]	12[a]	7	5	4	4	1
Follicular cell adenoma	4	8	5	9	10	7	5	3	3	1
Follicular cell carcinoma	0	0	2	2	2	0	0	1	1	0
Non-neoplastic lesions										
Liver										
Centriacinar hepatocytic hypertrophy	4	6	8	36[c]	42[c]	2	6	5	14[b]	19[c]
Periacinar hepatocytic hypertrophy	7	8	13	3	3	6	6	7	24[c]	21[c]
Hepatocytic fatty vacuoles	0	0	1	12[c]	25[c]	1	1	1	6	3
Eosinphilic foci	20	19	22	28	37[b]	6	8	8	7	6
Pigment laden hepatocytes	0	0	0	2	3	2	0	10[a]	24[c]	29[c]
Focal cystic degeneration	0	2	2	3	7[a]	1	0	0	0	0
Thyroid										
Cystic follicular hyperplasia	5	11	9	13	17[b]	-	-	-	-	-

[a] $p<0.05$
[b] $p<0.01$
[c] $p<0.001$

Neoplastic incidence rates in rats and mice compared to historical control (HC) data:

	Dose (ppm)						**HC**
RAT 2-yr study	0	50	200		1200	2400	
♂ Hepatocellular adenoma carcinoma	8% 2%	4% 0%	6% 0%		8% 0%	26% * 2%	3.3-18% 0-6%
MOUSE 2-yr study	0	50	200	600			
♂ Hepatocellular adenoma carcinoma combined	16% 10% 26%	10% 14% 20%	46% * 12% 50% *	34% * 22% 50% *			6-34% 0-20% 6-40%
♀ Hepatocellular adenoma carcinoma combined	8% 0% 8%	12% 2% 12%	0% 4% 4%	20% 4% 22% *			0-6% 0-2% 0-8%

* Statistically significantly increased

5.9 Toxicity for reproduction

Reproductive toxicity of imazalil was addressed in a two-generation fertility study performed in rats using a protocol similar to OECD TG 416 and three developmental toxicity studies in

rats and rabbits using imazalil nitrate and imazalil sulphate following protocols similar to OECD TG 414 (Tables 10 and 11).

5.9.1 Effects on fertility

Reproductive toxicity of imazalil was evaluated in a full two-generation study in rats at nominal doses of 0, 5, 20 and 80 mg/kg bw/d (Dirkx et al., 1992). The highest dose caused parental toxicity as indicated by reduced body weight and body weight gain, increased incidence of pilo-erection, as well as vacuolisation of hepatocytes in P1 males. At this dose, females also showed a reduced gestation rate and an increased duration of gestation. The later was considered responsible for an increased rate of dystocia. Reproductive toxicity manifested at 80 mg/kg bw/d as a slightly reduced number of implantations, a reduced number of live pups and an increased number of stillborn pups. Reduced offspring survival was considered adverse only for the high dose group. Hence, the NOAEL for parental and reproductive toxicity was identical with 20 mg/kg bw/d (nominal). Teratogenic effects were not reported.

Table 26: Summary of effects on fertility

Animal species & strain	Number of animals	Doses, vehicle, duration	Result	Reference
Rat, Wistar	24 M + 24 F	0-5-20-80 mg/kg bw/d, oral (dietary) two-generation study	Parental NOAEL/LOAEL: 20/80 mg/kg bw/d Reproductive NOAEL/LOAEL: 20/80 mg/kg bw/d Offspring NOAEL/LOAEL: 20/80 mg/kg bw/d	Dirkx et al., 1992, Janssen Report R 23979 Exp. No. 2337

5.9.2 Developmental toxicity

Doses of imazalil nitrate, corresponding to a maximum of 4.1 mg/kg bw/d imazalil base, administered to rabbits from day 6 to 18 of gestation were insufficient to cause any detectable maternal or fetal toxicity (Dirkx & Marsboom, 1985). Imazalil sulphate resulted in reduced food consumption as well as body weight or body weight gain during the dosing period at or above the lowest tested dose of 40 mg/kg bw/d in rats (Gillardin et al., 1988) or at 20 mg/kg bw/d in rabbits (Dirkx, 1992). In rabbits, an increase in mortalities was also reported at this dose (8/15). A dose of 10 mg/kg bw/d was considered the NOAEL for maternal toxicity in rabbits. Developmental toxicity of imazalil sulphate included an increased number of resorptions and reduced numbers of offspring in both species. NOAELs for developmental toxicity were 40 mg/kg bw/d in the rat and 5 mg/kg bw/d in the rabbit. All malformations observed were similar to those seen in the controls and/or well within the historical range. It is concluded from the available data that imazalil is unlikely to be teratogenic.

Table 27: Summary for developmental toxicity

Reference	Protocol	Doses	Maternal effects	Developmental effects
Dirkx, Marsboom, 1985, Janssen Report 18531 Exp. No. 1482	Sim. to OECD 414 Rabbit, New Zealand White (15 F)	0-1-2.1-4.1 mg/kg bw/d imazalil equivalents (imazalil nitrate),	None	None (one split vertebra with extra rib within historic control range)

		oral (gavage) d6-18		
Gillardin et al., 1988, Janssen Report R 27180 Exp. No. 2003/88-05	Sim. to OECD 414 Rat, Sprague Dawley (24 F)	0-40-80-120 mg/kg bw/d imazalil equivalents (imazalil sulphate), oral (gavage) d6-16	≥ 40 mg/kg: reduced bw and food consumption during dosing ≥ 120 mg/kg: reduced bw and bw gain at delivery	≥ 80 mg/kg: reduced live pup weight ≥ 120 mg/kg bw/d: reduced no. of live foetuses, increased resorptions
Dirkx, 1992, Janssen Report R27180 Exp. No. 2615	Sim. to OECD 414 Rabbit, Albino (15 F)	0-5-10-20 mg/kg bw/d imazalil equivalents (imazalil sulphate), oral (gavage) d6-18	≥ 20 mg/kg: reduced bw and food consumption during dosing (only), increased mortality (8/15)	≥ 10 mg/kg: increased no. of resorptions and reduced no. of live foetuses

5.9.3 Human data

No data available

5.9.4 Other relevant information

None

5.9.5 Summary and discussion of reproductive toxicity

There was no indication for teratogenicity of imazalil. Other adverse effects on fertility or the foetus were associated with maternal toxicity or occurred at doses insignificantly below the maternal LOAEL. Therefore, classification and labelling for reproductive toxicity is not required.

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).

Additional key elements

In their response to comments, the dossier submitter provided more details on one of the developmental toxicity studies in rabbits (Dirkx, 1992):

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%)

In relation to these data, the dossier submitter stated that "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' a 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."

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.

5.10 Other effects

In an attempt to clarify the mechanisms by which imazalil causes rodent tumours and to better understand the relevance of the observations for human health, mechanistic toxicity studies were carried out in rats and mice.
Piccirillo (2000) and Verbeek et al. (2000) addressed changes in liver histopathology and thyroid hormone levels. Analysis of microsomal enzyme induction in liver and thyroid glands and analysis of liver cell proliferation in selected treatment groups was performed by Piccirillo (2000), Vermeir (2001) and Lawrence (2001). Liver cell proliferation was also studied in rats treated with imazalil over 7 days (Elmore et al., 2004) and in mice treated with imazalil over 4 days (Elmore, 2004), 2 or 13 weeks (Lawrence, 2001; O'Neill 2002; Piccirillo, 2002). Liver and thyroid epithelial cell proliferation, apoptosis, and oxidative stress dose response following 1, 2, 7, 14, or 28 consecutive days of dietary imazalil administration was reported by Mertens (2011). The results were summarized and the mode of action for imazalil induced liver tumors was evaluated by Piccirillo (2011). In addition, studies on the possible induction and/or inhibition of hepatic drug metabolizing enzymes in rats and mice was performed by Vermeir (1994, 1995, 1996) and Lavrijsen (1987) (Table 12):

Male Wistar rats of the Hannover substrain were exposed to 0, 41, 123 and 338 mg/kg bw/d imazalil with the diet for 1, 2 or 4 weeks followed by a recovery period of 4 and 9 weeks. A positive control group received phenobarbital at a dose of 126 mg/kg. No test-article related mortality was observed and no obvious signs of toxicity were noted. Clinical chemistry revealed a moderate decrease in aspartate aminotransferase which was within the historical control range at 123 mg/kg ppm but more pronounced at 338 mg/kg ppm imazalil. Endocrinological analysis showed an increase in TSH at weeks 1, 2 and 8 for all imazalil-treated groups similar to the phenobarbital group, although statistical significance was found only in week 8. Thyroxine (T4) levels exhibited a significant decrease at week 1, no significant differences at week 2 and elevated levels at week 4 at 123 and 338 mg/kg. T3 levels were slightly reduced at 338 mg/kg imazalil in week 4, and in weeks 1 to 4 at 126 mg/kg phenobarbital. Liver weights were increased during the dosing period in all groups receiving imazalil or phenobarbital. Thyroid weights were elevated at 338 mg/kg in weeks 2 and 4. Gross pathology revealed slightly swollen livers in all dosed groups. At 123 mg/kg imazalil and above, more pronounced lobulation and paleness were noted. Upon histopathological examination, centrilobular hypertrophy, periportal hypertrophy and vacuolisation at the higher doses tested were found. At the end of the recovery period, these alterations had disappeared (Verbeek et al., 2000).

Liver and thyroid samples were analysed for alterations in enzymes relevant to xenobiotic and thyroid hormone metabolism (Vermeir, 2001). The P-450 content of liver microsomes was significantly increased in rats during treatment ≥ 41 mg/kg bw/d imazalil or the positive con-

trol phenobarbital and returned to normal levels after 4 weeks of recovery. Affected activities included aniline hydroxylase, N-ethylmorphine N-demethylase and 7-ethoxyresorufin O-deethylase. A stronger inductive effect was found for 7-pentoxyresorufin O-dealkylase activity, which, nevertheless, remained at least one magnitude weaker than that of the positive control phenobarbital. No substantial effect on lauric acid hydroxylation was observed. A decrease in 5'-monodeiodinase activity was found in the top dose imazalil group and the phenobarbital group after week 1, but not in the dosage groups after 2 or 4 weeks or after recovery. A significant induction of thyroxine glucuronyltransferase activity was noted in both the imazalil- and phenobarbital-treated groups of week 1, as well as in the 338 mg/kg imazalil group of week 2 and 4, but not after recovery. Microsomal thyroid peroxidase activity varied inconsistently.

In similar studies, liver microsomes of male and female rats and mice, which had been dosed with imazalil for different time periods (one and three months, 7 days) were assayed for microsomal protein, cytochrome P-450 contents, and some other enzyme activities in order to investigate possible induction and/or inhibition of drug metabolizing enzymes by imazalil given orally at doses of 200, 400, 800 ppm (Vermeir, 1995), 800, 1600, 2400 and 3200 ppm (Vermeir, 1996), and 50, 200 and 600 ppm in mice (Vermeir, 1994).

After one-month of dosing (Vermeir, 1995), relative liver weights were increased in male rats at all dose levels, but not in females. Likewise, statistically significant increases of the microsomal protein content were observed in males at all dose levels, in females at the highest dose (800 ppm). The hepatic cytochrome P-450 content was raised at the 800 ppm-level in both gender. Alterations in the cytochrome P-450 isoenzyme pattern occurred mainly with respect to increases of the UDP-glucuronosyltransferase activities.

After three months of dosing (Vermeir, 1995), no effect could be observed anymore on the relative liver weights in male and female rats. The effect of three-month dosing with imazalil on the microsomal enzyme activities in male rat livers was more or less identical to that of the one-month treatment. In livers of female rats treatment for three months with imazalil resulted in a significant increase of the cytochrome P-450 content, and a supplementary induction of the aniline hydroxylase, 7-ethoxyresorufin *O*-deethylase, lauric acid hydroxylase and UDP-glucuronosyltransferase activities

Likewise, after three months of dosing (Vermeir, 1996), the microsomal protein content was increased in males and females at dose levels of 1600, 2400, and 3200 ppm. The hepatic cytochrome P-450 content was raised in males at all dose levels, the *N*-ethylmorphine *N*-demethylase, 7-ethoxyresorufin-, 7-pentoxyresorufin- and 7-ethoxycoumarin *O*-dealkylase activities were increased in males and females mainly at 2400 and 3200 ppm. In females, the aniline hydroxylase- and in males the lauric acid hydroxylase activities were increased at the highest dose levels of 2400 and 3200 ppm.

In mice, the relative liver weight, the hepatic protein and cytochrome P-450 contents were significantly increased in mice after one- and three months of dosing with 200 and 600 ppm imazalil (Vermeir, 1994). Dosing with imazalil for one month induced certain enzymatic activities, but also had inhibitory effects on other metabolic processes. In general, monooxygenase activities tended to be higher in female mice than in male mice. After three months of dosing enzymatic activities - with the exception of the induction of 7-ethoxycoumarin *O*-deethylase activity in a dose-dependent way- were either unaffected or inhibited as result of treatment.

Liver microsomal protein and cytochrome P-450 content, activities of NADPH-cytochrome c-reductase, aniline hydroxylase, ethoxycoumarin *O*-deethylase and ethoxyresorufin *O*-

deethylase activities were determined in male rats treated with 10 and 40 mg/kg bw/d imazalil for 7 days. The animals were kept for a recovery period of one week. A comparison was made with the effects of subacute administration of phenobarbital, 3-methylcholanthrene and dexamethasone. The treatment with 10 mg/kg bw/d had no significant effects on the parameters under evaluation. At 40 mg/kg bw/d, cytochrome P-450 content and *O*-deethylase activities were increased.

The results of the 1- and 3-month studies indicate a mixed type of induction in rats. Imazalil has a very weak phenobarbital-type induction potential, as shown by the results of a 7-day study. The increase in cytochrome P-450 content and certain enzyme activities is fully reversible.

In mice, imazalil admixed in the food and dosed for one or three months, significantly induces cytochrome P-450. When cytochrome-dependent enzymatic activities were measured, some activities were induced but the majority were inhibited. This effect might be due to residual imazalil in the liver microsomes, as the fungicide is known to have potent inhibitory properties.

In male Wistar rats, quantitative proliferating cell nuclear antigen (PCNA) analysis of cell proliferation revealed no significant differences between the imazalil-treated groups and vehicle group, nor the phenobarbital-treated control group (Lawrence, 2001). This was confirmed in a three-month oral study in mice (Lawrence, 2001).

In the absence of significant dose-dependent BrdU labelling, it was concluded that imazalil did not induce liver cell proliferation in male rats after oral administration of imazalil up to 4 weeks (Verbeek et al., 2000). This was supported by a similar study in male CD-1 mice receiving 100-1200 ppm imazalil for up to 13 weeks (Piccirillo, 2002) as well as in male CD-1 mice receiving 100, 200, 400, 600 and 1200 ppm for 2 or 13 weeks, including recovery periods after 2 and 13 weeks of dosing (O'Neill, 2002). Neither BrdU labelling nor PCNA staining suggested an increase in hepatocyte proliferation in comparison to vehicle control at the interim sacrifice at 2 weeks nor at 13 weeks, as well as after the recovery periods.

Hepatocyte and thyroid epithelial cell proliferation, apoptosis (caspase immunohistochemistry) and oxidative stress (4-hydroxy-2-nonenal (4-HNE) immunohistochemistry) dose response following 1, 2, 7, 14 or 28 consecutive days of imazalil administration were evaluated in rats. A positive control group was offered 1200 ppm of phenobarbital. BrdU uptake, the induction of CYP2B1/2 and UDP Glucuronyltransferase activities were also evaluated and a quantitative analysis of phenobarbital induced genes (*cyp2b1, cyp3a1, cyp3a2, gadd45b*) was performed. At dose levels of 1200 and 2400 ppm imazalil alterations in hepatocellular staining affinity (increased cytoplasmic homogeneity) at study day 1, higher liver weights at study days 14 and 28, an increased rate of BrdU incorporation in the thyroid gland at study day 14, and significant dose- and time-dependent increases in CYP2B1/2 and UGT1A activities and mRNA levels of *cyp2b1, cyp3a1, cyp3a2* and *gadd45b* were noted. Phenobarbital induced similar findings but they tended to occur earlier and with a greater magnitude. Phenobarbital also increased BrdU incorporation in the liver along with higher alanine aminotransferase and sorbitol dehydrogenase levels (Mertens et al., 2011).

Twenty-one blocks from a previously conducted rat study were immuno-histochemically stained with BrdU. Blocks were received from control animals or from rats that were treated for one week with imazalil or phenobarbital. No statistically significant differences were seen in the labeling index (LI) between these groups and it was concluded that high dose imazalil treatment does not influence hepatic proliferation at 7 days of treatment. It remained unknown

if imazalil induced hepatic replication at any other time point during the study (Elmore, 2004).

In another cell proliferation study, male mice were dosed with 1200 ppm imazalil in the feed for 4 days (Elmore 2004). Survival was not affected, the mean terminal body weight was reduced, ALT values and relative liver weights were increased, centrilobular hypertrophy and hepatocyte necrosis occurred in imazalil-treated mice. There was a statistically significant increase in BrdU labeling index of treated mice. These data indicate a mitogenic response in the mouse liver following oral imazalil administration.

Piccirillo (2011) summarized the mode of action for imazalil induced liver tumors based on the available studies of various durations with special attention to the study of Mertens et al. (2011). The main conclusions are:

- Imazalil is a microsomal enzyme inducer.
- Imazalil induces dose responsive liver effects in studies of various durations; for example: increased liver weight, hypertrophy of hepatocytes, mitogenic liver effects (reversible), benign adenomas in male rats and mice.
- Imazalil is a non-genotoxic agent.

Liver tumors were noted in the long-term studies in male mice and male rats at high dose levels were microsomal enzyme induction, increased liver weights and hypertrophy occurred in addition.
Phenobarbital as a nongenotoxic liver tumor promoter induces the same liver findings and tumors.
The differences of effects are considered a reflection of potency between PB and imazalil. Evaluation of apoptosis and of oxidative stress as possible mode-of-action for induction of liver tumors showed no effects on the measured parameters for either imazalil or PB. The induction of mRNA levels of *cyp2b1, cyp3a1, cyp3a2* and *gadd45b* were considered as key event in the mode-of-action for imazalil tumors.
The results of this study are summarized in the following table.

Table 28:

Parameter or Treatment	Imazalil (1200 and 2400 ppm)	Phenobarbital (1200 ppm)
Liver Weight	↑	↑↑
Higher ALT	No	Yes
Higher Sorbital Dehydrogenase	No	Yes
Cytoplasmic Homogeneity	↑	↑↑
Single-Cell Necrosis	No	Yes
Centrilobular Hepatocellular Hypertrophy	↑	↑↑
Higher Hepatic BrdU Hypertrophy	No	Yes
CYPB1/2 Induction	↑	↑↑↑
UGT1A Induction	↑	↑↑
Apoptosis (Caspase-3 Labeling)	No	No
Oxidative Stress (4-HNE Labeling)	No	No

Parameter or Treatment	Imazalil (1200 and 2400 ppm)	Phenobarbital (1200 ppm)
cyp2b1 mRNA	↑	↑↑
CAR (*NR1I3*)	No	No
cyp3a1 mRNA	↑	↑↑
cyp3a2 mRNA	↑	↑↑
gadd45b mRNA	↑	↑↑

Yes = Parameter difference present; No = Parameter difference absent;

↑, ↑↑, and ↑↑↑ = Qualitative indication of level of increase relative to control values

Table 29: Summary for mechanistic studies

Animal species & strain	Number of animals	Doses, vehicle, duration	Result	Reference
Rat, Wistar Hannover	10 M	0-41-123-338 mg/kg bw/d, oral (dietary), 1, 2, 4 wk and 4 or 9 wk recovery	≥ 41 mg/kg: liver and thyroid weight↑, liver swelling; T4 decreased in wk 1, normal in wk 2, elevated after 4 wks recovery; TSH elevated in wk 1, 2 (not significant) and after 4 wks recovery (significant) ≥ 123 mg/kg: bw↓, hepatic centrilobular (wk 1, 2, 4) and periportal (wk 2, 4) hypertrophy, liver paleness, lobulation and darkening, AST↓; T4 also elevated in wk 4 ≥ 338 mg/kg: hepatic periportal hypertrophy also in wk 1, hepatic vacuolisation, yellow liver foci; T3↓ (wk 4) Positive control (126 mg/kg phenobarbital): similar effect pattern, hepatic proliferation not affected	Verbeek et al., 2000, Janssen Report R023979/R 000524 Exp. No. 5009 (Appendix 1 of Piccirillo, 2000, VJP Project No. 5452-00-1)
Rat, Wistar Hannover	5 M	0-41-123-338 mg/kg bw/d, oral (dietary), 1, 2, 4 wks and 4 or 9 wks recovery	≥ 41 mg/kg: CytP450↑, aniline hydroxylase↑, N-ethylmorphine N-demethylase↑, 7-Pentoxyresorufin O-dealkylase↑↑, 7-ethoxyresorufin O-deethylase↑, thyroxine glucoronyltransferase (wk 1 only)↑ ≥ 338 mg/kg: 5-monodeiodonase↓, thyroxine glucoronyltransferase↑↑ Reversal to normal activity levels during recovery Positive control (126 mg/kg phenobarbital): similar effect pattern with stronger effect on PROD and weaker effect on EROD	Vermeir, 2001, Janssen Protocol No. R023979/FK3378 (ass. study to Verbeek et al., 2000) (Appendix 2 of Piccirillo, 2000, VJP Project No. 5452-00-1)

Animal species & strain	Number of animals	Doses, vehicle, duration	Result	Reference
Rat, Wistar Hannover strain	10 M	0-41-123-338 mg/kg bw/d, oral (dietary), 4 wks or 4 wks plus 4 wks recovery	No indication for induction of hepatic cell proliferation as examined by PCNA staining	Lawrence, 2001, Huntington Report No. JPA 077/012131
Rat Wistar	10 M	400- 1200-3200 ppm Positive control phenobarbital 1200 ppm oral (diet) 4 wks plus 4 wks and 9 wks recovery	See Appendix 1: Verbeek et al., 2000, Janssen Report R023979/R000524 Exp. No. 5009 and Appendix 2: Vermeir, 2001, Janssen Protocol No. R023979/FK3378 The thyroid effects are related to imazalil's influence on the activities of hepatic and thyroid enzymes involved in synthesis and metabolism/excretion of T4. This influence results in major fluctuations in thyroid peroxidase and thyroxine glucoronyltransferase activities resulting in similar fluctuations in T4 and TSH. The changes were reversible.	04: Piccirillo, 2000, VJP Project No. 5452-00-1 (add. Study to 24-mo combined chronic toxicity and carcinogenicity study)
Mice, CD-1	10 M	0-100-200-400-600-1200 ppm, oral (dietary), 2, 2+2, 13, 13+4 wks (+: recovery)	No indication for induction of hepatic cell proliferation by 100 to 1200 ppm over 2 or 13 weeks as examined by BrdU labelling and PCNA staining, No effect with positive control	05: Piccirillo, 2002, VJP Project No. 5452-02-1
Rat SPF Wistar	4 M, 4 F	0-200-400-800 ppm (20-40-80 mg/kg bw/d) oral (dietary), 1- (interim sacrifice) and 3-months (Livers were obtained from a 3-mo oral dose range finding study (Exp. No. 3514)	After 1-mo of dosing relative liver weights and microsomal protein content was increased in males at all dose levels and in female rat livers at the highest dose level. After 3-mo of dosing no statistically significant effect was observed anymore in male and female rat livers. The microsomal enzyme activities in males and females were more or less similar to that of the 1-mo treatment. The results indicate a mixed type of induction.	**06:** Vermeir (1995) Report No: R023979/FK1960
Rat SPF Wistar	4 M, 4 F	0-800-1600-2400-3200 ppm (80-160-240-320 mg/kg bw/d) oral (dietary), (Livers were obtained from a 3-mo oral dose range finding study (Exp. No. 3672)	After 3-mo of dosing relative liver weights, the microsomal protein content and several enzyme activities were increased in male and female rat livers at various dose levels. The results indicate a mixed type of induction.	**07:** Vermeir (1996) Report No: R023979/FK2060

Animal species & strain	Number of animals	Doses, vehicle, duration	Result	Reference
Mice SPF Albino Swiss	10 M, 10 F	0-50-200-600 ppm (10-40-120 mg/kg bw/d) (Livers were obtained from a 3-mo oral mechanistic toxicity study (Lawrence (2001) Exp. No. 3140) with one-month interim sacrifice)	After 1- and 3-mo of dosing relative liver weights, the microsomal protein content, cytochrome P-450 content and some enzymatic activities were increased as well as inhibited in male and female mice livers mainly of the highest dose. Imazalil levels in mouse liver microsomes increased in a dose-dependent way which might be responsible for the inhibitory effects.	**08:** Vermeir (1994) Report No: R023979/FK1600
Rat Wistar	10 M	0-10-40 mg/kg bw/d oral gavage for 7 days 7 days recovery period	At 40 mg/kg bw/d some cytochrome P-450 dependent enzyme activities were slightly increased. Imazalil has a very weak induction potential and the changes were fully reversible.	**09:** Lavrijsen et al. (1987)
Mice SPF Albino Swiss	25 M, 25 F	0-50-200-600 ppm 3-months oral mechanistic toxicity study with one-month interim sacrifice (PCNA Quantitative Analysis of Livers)	For both sexes, no statistically significant results between dosed groups and vehicle groups were found.	**10:** Lawrence (2001) Janssen Exp. No: E3140 Huntington Report No. JPA 076/012132
Mice Crl:CD-1 (ICR) BR	40-20-20-20-20-40 M	0-100-200-400-600-1200 ppm approx. 0-20-38-71-111-252 mg/kg/d) 2-wk and 13 wk dosing 10 mice of control and high dose group had a 2-wk and 4-wk recovery period Oral (diet)	The dose levels correspond with those concentrations in which liver tumors were observed in the chronic mouse study. NOAEL : 100 ppm No evidence of BrdU staining (Hepatocyte Proliferation Assay) of the hepatocytes in any treatment group.	**11:** O'Neill (2002) Study No. WIL-436001
Mice Crl:CD-1 (ICR) BR	6 M	0-1200 ppm (0-6.6 mg imazalil / day) 4 days Oral (diet)	The labeling index (LI) of BrdU-stained slides were increased in the 1200 ppm group. There was a strong mitogenic response in the mouse liver following acute administration of imazalil.	**12:** Elmore (2004) Study No. C131-001
Rat	21 paraffine blocks from a previously conducted study in rats	Control rats (5 blocks) – low dose imazalil (3 blocks) – mid dose imazalil (3 blocks) - high dose imazalil (5 blocks) - phenobarbital dosed rats (5 blocks)	No statistical significant differences were seen in the LI of these groups. Large individual variability among rats. Imazalil treatment did not influence hepatic proliferation at 7 days of treatment.	**13:** Elmore et al. (2004) ILS Project No. C131

Animal species & strain	Number of animals	Doses, vehicle, duration	Result	Reference
Rat Crl:WI(Han) Wistar	25/30 M	0-200-1200-2400 ppm pos. control group 1200 ppm phenobarbital 1, 2, 7, 14 or 28 days Oral (diet)	At 1200 and 2400 ppm: alterations in hepatocellular staining affinity, higher liver weights, increased rate of BrdU incorporation in the thyroid gland, increases in enzyme activities. Phenobarbital induces similar findings.	**14:** Mertens et al., 2011
Rat Crl:WI(Han) Wistar	The study results of Mertens et al. (2011) were summarised and a mode of action for imazalil induced liver tumors is proposed. In conclusion, imazalil has a PB-like nongenotoxic mode of action in the induction of liver tumors and the induction of these tumors is not relevant to humans.			**15:** Piccirillo, 2011

Conclusions regarding the relevance of rodent tumours induced by Imazalil for human health

According to the IPCS Human Relevance Framework (Boobis et al., 2006), conclusions with regard to the relevance of observations of tumours in laboratory animal tests may be gained by considering the "Cancer Mode of Action". This IPCS Framework publication is part of a larger project on the harmonisation of approaches for risk assessment and represents an update of an earlier publication by Sonich-Mullin et al. (2001) which had been integrated in the Technical Guidance Document on Risk Assessment (EUR 20418 EN/1).

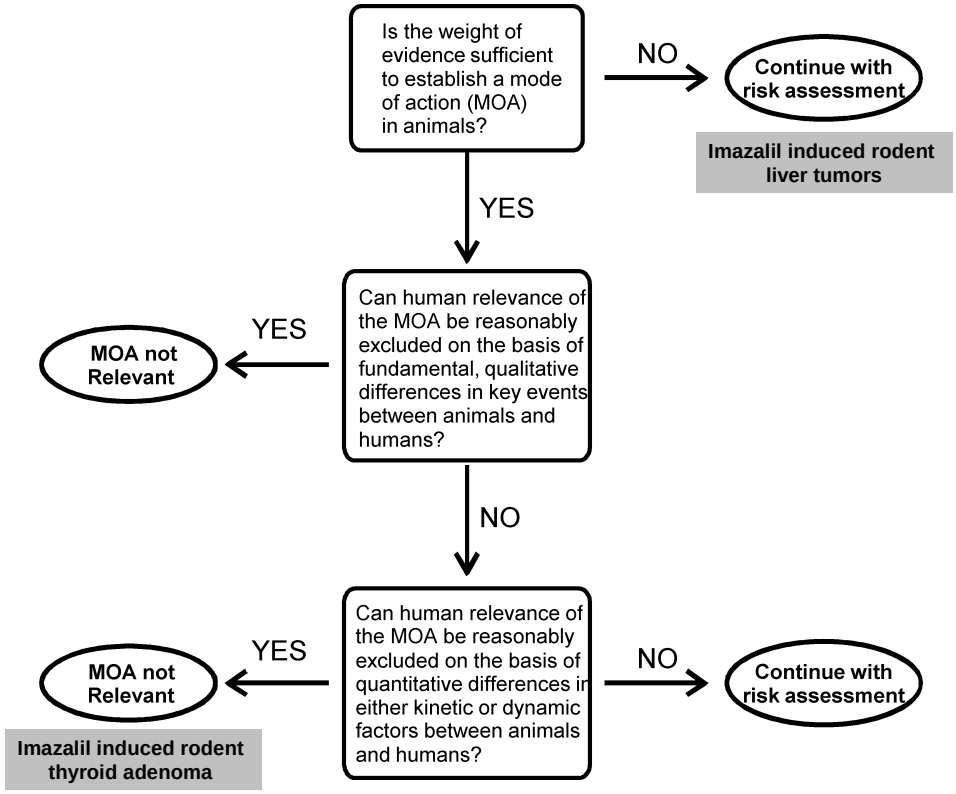


Figure 2: Human Relevance Framework (from Boobis et al., 2006) with conclusions for imazalil induced rodent tumours

Thyroid tumours

An increased incidence of follicular cell adenoma of the thyroids was reported in male Wistar rats fed ≥ 1200 ppm imazalil (≥ 60 mg/kg bw/d) in the diet over 2 years (Van Deun et al., 1999). At the same dose, weights of thyroid and liver were increased, thyroids were swollen and livers showed darkening and foci. A mechanistic study performed also in Wistar rats with 1, 2 or 4 weeks treatment followed by 4 or 9 weeks recovery confirmed effects on organ weight (Verbeek et al., 2000). In addition, deregulation of thyroid hormone homeostasis was observed from 400 ppm in the diet (41 mg/kg bw/d) with a temporary decrease in T4 levels in week 1, normal T4 levels in week 2 and elevated T4 levels after discontinuation of dosing. TSH levels were increased during dosing. Based on the work of Vermeir (2001), it can be concluded that the decrease in T4 levels was due to induction of an array of liver enzymes, including thyroxine glucoronyltransferase, which mediates the initial step in elimination of T4. Ultimately, T3 – which is generated from T4 in tissues by enzymatic deiodonation – is also affected, although Verbeek et al. (2000) reported only minor changes. It is well established that reduced levels of T4 and T3 result in a loss of feedback inhibition of the pituitary, resulting in a compensatory increase in TSH production and release. Swollen thyroids following chronic exposure of male rats to ≥ 123 mg/kg imazalil suggests that the increase in TSH levels was not transient but maintained over prolonged periods (Van Deun et al., 1999). Studies on the effect of iodine deficiency, partial thyroidectomy and transplantation of TSH-secreting tumours provide good evidence, that in rodents, direct or indirect stimulation of TSH levels alone leads to tumour formation (IARC, 1999). Each of these regimes induced thyroid tumours in rodents without the use of any other agent. Direct or indirect elevation of TSH through the pituitary-thyroid feedback mechanism has been identified as the common pathway for non-genotoxic rodent carcinogens causing thyroid tumours.

In humans, high circulating levels of TSH, as caused by congenital disorders or low iodine intake, are associated with an increased incidence of thyroid tumours of the follicular type (IARC, 1999). However, substantial species differences suggest, that the biochemical effects of imazalil in the rat will not cause similar hormonal imbalances with elevated TSH levels in humans: Thyroxine-binding globulin (TBG) is a plasma protein in humans with high affinity to T4, but is lacking in rats. The rat also exhibits enhanced thyroid hormone elimination with less efficient enterohepatic recirculation. Consequently, the half-life of thyroxine is 12 h in the rat, but 5–9 days in humans. Probably to compensate this, serum level of TSH are 25 or more times higher in the rodent than in humans. The histology of the resting rodent thyroid is similar to that of the stimulated human gland. It is therefore generally accepted, that the rapid turnover of T4 and the significantly higher level of activity of rodent thyroid gland make the rat significantly more sensitive to thyroid tumour induction due to hormonal imbalances than humans.

Applying the IPCS Human Relevance Framework (Boobis et al., 2006), it can be concluded that the MOA of thyroid tumour induction by imazalil in rats could be established (question 1), and although human relevance may not be excluded on the basis of fundamental, qualitative differences in key events between rats and humans (question 2), this can be done on basis of quantitiative differences in dynamic factors. Similar conclusions have been recently drawn for thiazopyr which operates by the same mode of action (Dellarco et al., 2006). Therefore, the observation of an increased incidence of thyroid tumours in rats following chronic exposure to imazalil is not considered relevant to human health.

Hepatic tumours

The liver represents the primary target organ of imazalil following repeated oral exposure in rats, mice and dogs. Typical organ and histopathologic changes included increased organ

weight, darkening of the liver, hepatocyte hypertrophy accompanied by a decrease in serum albumin, urea, AST or ALT. While the latter changes were regarded as adaptive, fatty or eosinophilic pigmented vacuolisation and focal cystic degeneration were considered as adverse. An increase in serum markers such as LDH and AP was seen only occasionally. Reported LOAELs were similar with values of 32/38 (3 mo) or approx. 20 mg/kg bw/d (18 mo) in rats, 47/55 (3 mo) or approx. 42/33 (23 mo) in mice, and 20 mg/kg bw/d in dogs. Increased incidences of hepatocellular adenoma werer reported in male rats at an approx. 4-fold higher dose of 120 mg/kg bw/d fed over 24 month and from the lower dose of 42 mg/hg bw/d (23 mo) in male mice (105 mg/kg bw/d for female mice). Liver carcinomas were increased in male mice at 131 mg/kg bw/d.

Genotoxicity was evaluated in 4 *in vitro* assays with and without metabolic activation by S9 mix and 1 *in vivo* micronucleus test. Although metabolism studies suggest the formation of an imazalil epoxide intermediate which is hydrated into the corresponding diol – only the latter could be detected as metabolite M10 – there was no indication that imazalil and its S9 metabolites induce mutations in *S. typhimurium* or Chinese hamster ovary cells, result in chromosomal aberration in human lymphocytes, or induce DNA repair in rat hepatocytes. Therefore, a non-genotoxic mode of action for hepatic tumour induction is concluded.

A total of 15 mechanistic studies were submitted and evaluated.

In a mechanistic study, a daily dose of 41 mg/kg bw imazalil was sufficient to induce liver weight increase, liver swelling (Verbeek et al., 2000; Mertens 2011).

Marked induction of various cytochrome P450 isoenzymes was seen within one week in male rats (Vermeir, 2001) and after one-month of 800 ppm dosing in both gender (Vermeir, 1995, 1996).

Phenobarbital (126 mg/kg) induced 7-pentoxyresorufin-O-delakylases more strongly than 41-338 mg/kg imazalil (1.2 vs. 0.06-0.07 nmol/min/mg) and imazalil had a stronger effect on 7-ethoxyresorufin-O-dealkylase (0.15-0.22 vs. 0.12 nmol/min/mg).

An increase in dose to 123 mg/kg bw/d was required to produce hepatic centrilobular and periportal hypertrophy, and vacuolisation appeared at a dose of 338 mg/kg and 1200/2400 ppm imazalil, respectively (Mertens et al., 2011). Upon discontinuation of treatment after 4 weeks, all signs subsided.

Analysis of cell proliferation by BrdU incorporation and PCNA staining did not reveal accelerated liver cell cycling in male rats following dosing at 41-338 mg/kg bw/d for 4 weeks, nor after an additional recovery period of 4 weeks (Verbeek et al., 2000; Vermeir, 2001; Lawrence 2001). Likewise, BrdU incorporation was not observed after 1200/2400 ppm imazalil, but in phenobarbital treated rats (Mertens et al. 2011). In a study of Elmore 2004, a statistically significant increase in BrdU labeling index of imazalil treated mice was noted. These data indicate a mitogenic response in the mouse liver following oral imazalil administration.

Absence of proliferative responses was also reported for male mice receiving 100 to 1200 ppm (approx. 25 to 300 mg/kg bw/d) imazalil in the diet over 2 or 13 weeks (Piccirillo, 2002). At a dose level of 10 mg/kg bw/d applied over 3 days, induction of CytP450 enzymes of families CYP1, 2 and 3 was also noted in male mice (Muto et al., 1997). A slight CYPB1/2 and UGT1A induction was noted in imazalil treated rats; the response after 1200 ppm phenobarbital was two- to three-fold stronger (Mertens et al., 2011).

Summarising the presented data, potential non-genotoxic modes of action for induction of hepatocellular neoplasia by imazalil can be discussed as follows:

- The experimental evidence does not indicate that the neoplastic mode of action of imazalil is based on induction of compensatory proliferation following massive liver cell death.
- Although published data suggests activation of transcription factors of the PPAR family (peroxisome proliferator activated receptors) *in vitro* (Takeuchi et al., 2006), this mechanism is unlikely to be relevant *in vivo* as the submitted data demonstrates the absence of a mitogenic response *in vivo* and the substance failed to induce biomarkers of PPAR activation in mice.
- The available histological information does also not support a mechanism involving chronic hepatic inflammation.
- It has further been postulated, that CytP450 induction as described above may lead to enhanced toxification of carcinogens or generation of ROS (Klauning et al., 2000). Hasegawa and Ito (1992) reported twofold increases in N,N-diethylnitrosamine (DEN, 200 mg/kg bw) induced preneoplastic changes in male F344 rats when treated with 1000 ppm imazalil in the diet. For activation, DEN requires metabolism by cytochrome P450 enzymes, predominantly CYP2E1 and CYP2B2, and their selective induction enhances carcinogenicity of DEN in the rat (Mori et al., 2002). CYP2B2 belongs to the (sub)family induced in rat liver by relevant doses of imazalil (Vermeir, 2001). Other studies show that CytP450 induction by imazalil does also occur in mice (Muto et al., 1997) and is possible in humans (Lemaire et al., 2006). Such a mechanism would, in principle, be of relevance to humans and is not necessarily limited to the liver.
- Some similarities in the pattern of metabolic enzyme induction and histopathological changes in the liver by imazalil and the rodent hepatocarcinogen phenobarbital were observed (predominantly CYP2B induction, liver weight increase, hepatocyte hypertrophy). Epidemiological studies for phenobarbital failed to show an increased risk for hepatic cancer in patients exposed to high doses over years (Andrews, 2005). However, extrapolation of the situation to imazalil appears pre-mature, especially because the molecular target(s) of imazalil remain(s) unidentified and may include PXR (Lemaire et al., 2006) rather than CAR which is thought to be the primary target of phenobarbital (Holsapple et al., 2006; Kodama and Negishi, 2006). This would be in agreement with difference in the magnitude of induction of individual CytP450 isoenzymes. In addition, the precise neoplastic mode of action of phenobarbital remains unclear and may involve interference with control of apoptosis or cell-cell communication (Oliver and Roberts, 2002). Phenobarbital itself is classified as possibly carcinogenic to humans (IARC group 2B) (WHO, 2001).

In conclusion, a mode of action for the increased incidence of liver tumours in male rats and male and female mice exposed chronically to imazalil could not be established with certainty.

Also with the additionally submitted recent study of Mertens et al. (2011) a definite conclusion cannot be established. In this study the number and type of parameters investigated are considered not adequate to draw a conclusion on the mode of action of imazalil. A low density array should have been performed which is capable to detect a greater number of genes. In addition, the parameters investigated do not indicate a similar mode of action with phenobarbital, since the results are mainly opposed. Only the induction of mRNA levels of *cyp2b1*, *cyp3a1*, *cyp3a2* and *gadd45b* were positive for imazalil and phenobarbital, although with higher potency for phenobarbital. These changes were considered by the author as key event

in the mode-of-action for imazalil tumors, i.e imazalil liver tumors in rodents are induced through a PB-like nongenotoxic mode of action. We consider the results of this study and mainly the induction of the mRNA levels of a low number of genes as not sufficient to agree to the conclusion of the author.

In contrast to the postulated mode of action of imazalil and to the discussion of classification and labelling, IARC (2001) stated that there is *inadequate evidence* in humans for the carcinogenicity of phenobarbital but there is *sufficient evidence* in experimental animals for the carcinogenicity of phenobarbital. Therefore IARC came to the overall evaluation that phenobarbital is *possibly carcinogenic to humans (Group 2B).*

We agree that the mode of action of imazalil will most likely involve non-genotoxic mechanisms, that imazalil induces a mixed type of microsomal enzymes and induces dose responsive liver and thyroid gland effects in studies of various durations and that imazalil and phenobarbital may share some common mechanisms. Based on the statement of IARC and on the results of the toxicological studies with imazalil and phenobarbital we conclude that imazalil may be of relevance to human health and propose a classification with carc. Cat 2 for imazalil.

5.11 Derivation of DNEL(s) or other quantitative or qualitative measure for dose response

Not relevant for this type of dossier.

6 HUMAN HEALTH HAZARD ASSESSMENT OF PHYSICO-CHEMICAL PROPERTIES

6.1 Explosivity

Imazalil is not explosive.

6.2 Flammability

Imazalil is not highly flammable.

6.3 Oxidising potential

Imazalil has no oxidising properties.

7 ENVIRONMENTAL HAZARD ASSESSMENT

The environmental hazard assessment for imazalil is based on the Draft Re-Assessment Report and Proposed Decision of the Netherlands prepared in the context of the possible inclusion of imazalil in Annex I of Council Directive 91/414/EEC (DRAR May 2009 and September 2009, RMS the Netherlands). As the available data set does not justify the current harmonised classification, a revision is proposed. There is no information on the basis or justification of the current harmonised classification listed in Annex VI to the CLP-Regulation.

7.1 Aquatic compartment (including sediment)

7.1.1 Toxicity test results

7.1.1.1 Fish

Short-term toxicity to fish

The acute toxicity of imazalil to fish is summarised in Table 30.

Table 30: Acute toxicity of imazalil to fish

Guideline/ Test method	Species	Exposure		Results		Reference
		Design	Duration (h)	Endpoint	Value (mg/L)	
OECD 203	*Onchorynchus mykiss*	flow through	96	LC_{50}	1.48 m.m.	Weytjens and Wils (1989)
OECD 203	*Brachydanio rerio*	Semi-static	96	LC_{50}	2.75 m.m.	Weytjens and Wils (1988)

m.m.: mean measured

Long-term toxicity to fish

The long term toxicity of imazalil to fish is summarised in Table 31.

Table 31: Long-term toxicity of imazalil to fish

Guideline/ Test method	Species	Exposure		Results		Reference
		Design	Duration (d)	Endpoint	Value (mg/L)	
OECD 204	*Oncorhynchus mykiss*	flow through	28	NOEC	0.225 m.m.	Weytjens, D. (1989)

m.m.: mean measured

The effects of imazalil on rainbow trout fish were tested in a 28 day prolonged flow-through toxicity test. Ten young rainbow trout (*Oncorhynchus mykiss*) per test concentration were

exposed to five concentration levels of 0.01, 0.03, 0.10, 0.30 and 1.0 mg imazalil/L and a control.
Fish were inspected daily for mortality and any adverse behaviour different from the control group. At the end of the test period, surviving fish were blotted dry, weighed and body length was measured.
Water samples were sampled daily from all aquaria during the entire test period for analytical measurement of the test substance concentration. Oxygen concentration, pH and temperature were measured three times weekly.
As the test substance concentration was < 80 % of the nominal concentration, the effect values are based on mean measured concentrations. A NOEC of 0.225 mg/l related to both mortality and behaviour was determined. Growth could not be evaluated, as there is no information in the study report on weight and length of the fish at test start. Therefore, this test is only a prolonged toxicity test, as no sensitive sublethal endpoints were examined.

7.1.1.2 Aquatic invertebrates

Short-term toxicity to aquatic invertebrates

The acute toxicity of imazalil to invertebrates is summarised in Table 32.

Table 32: Acute toxicity of imazalil to invertebrates

Guideline/ Test method	Species	Exposure		Results		Reference
		Design	Duration (d)	Endpoint	Value (mg/L)	
OECD 202	*Daphnia magna*	static	48 h	EC_{50}	3.5 nom.	Weytjens and Wils (1990)

Long-term toxicity to aquatic invertebrates

Two long-term studies investigating the effects of imazalil on reproduction and survival of *Daphnia magna* are summarized in Table 33.

Table 33: Long-term toxicity of imazalil to invertebrates

Guideline/ Test method	Species	Exposure		Results		Reference
		Design	Duration (d)	Endpoint	Value (mg/L)	
OECD 202, part II	*Daphnia magna*	Semi-static	21	NOEC reproduction	< 0.01 nom. (< 0.0071 m.m.)	Weytjens, D. (1989)
OECD 211	*Daphnia magna*	Semi-static	21	NOEC reproduction	0.025 nom.	Kuhl, R., Wydra, V. (2008)

m.m: mean measured

In the first study according to OECD 202 (Weytjens 1989), first-instar daphnids were exposed in a 21-day study to six concentrations of imazalil (0.01 – 3 mg/L nominal, 0.0071 to 2.5 mg/l mean measured) and a control under semi-static test conditions. Four replicated each containing 10 adult daphnids were introduced. Effects on reproduction were already found at the

lowest test concentration. Therefore, no discrete NOEC could be determined (NOEC < 0.01mg/L).
A further long-term reproduction study with *Daphnia magna* according to OECD 211 was provided (Kuhl, Wydra 2008). In this study, the toxicity of imazalil was tested in a semi-static reproduction test with *Daphnia magna* over a period of 21 days. Five concentrations between 0.008 to 0.80 mg imazalil/L were chosen. Measured concentrations were between 90 and 114 % of nominal, thus the effect values are based on nominal concentrations. Ten organisms per test concentration were exposed individually to the test item and a control. A NOEC for reproduction of 0.025 mg/L was derived from the study.

7.1.1.3 Algae and aquatic plants

The toxicity of imazilil to algae and aquatic plants is summarised in Table 34.

Table 34: Short-term toxicity of imazalil to algae and aquatic plants

Guideline/ Test method	Species	Exposure		Results		Reference
		Design	Duration (d)	Endpoint	Value (mg/L)	
OECD 201	*Selenastrum capricornutum*	static	3	E_bC_{50} E_rC_{50} NOEC	0.87 m.m. 1.20 m.m. 0.457 m.m.	Van Ginneken, I. (1996)

m.m: mean measured

The study with algae can be regarded as the key study for the aquatic toxicity of imazalil and hence for classification and labeling. Therefore the study is presented in more detail below:

The effect of Imazalil (R 23979) on the growth of the unicellular green algae *Selenastrum capricornutum*. (I. Van Ginneken, 1996)

Guidelines : Study in compliance with OECD guideline 201

GLP : Yes

Material and methods :

Unicellular algae *Selenastrum capricornutum* were exposed to 5 concentrations (0.3, 0.6, 1.2, 2.4 and 4.8 mg/l + one control) of imazalil technical (purity 97.0%) during 72 hours. All concentrations and control in triplicate. Measured concentrations were 0.234, 0.457, 0.940, 1.485, 3.303 mg/l. Test under continuous illumination at 25 ± 1 °C.

Findings :

E_bC_{50} - 72 h (EC_{50} based on growth) = 0.87 mg/l (based on measured concentrations)

E_rC_{50} - 72 h (EC_{50} based on growth rate) = 1.20 mg/l (based on measured concentrations)

NOEC - 72 h = 0.457 mg/l (based on measured concentrations)

Conclusions :

The study is conform. Imazalil is very toxic to algae *Selenastrum capricornutum.*Sediment organisms

The toxicity of imazalil to sediment organisms is summarised in Table 35.

Table 35: Toxicity of imazalil to sediment organisms

Guideline/ Test method	Species	Exposure		Results		Reference
		Design	Duration (d)	Endpoint	Value (mg/L)	
ASTM E1706	*Chironomus riparius*	flow through	17 d	NOEC	27.5 mg/kg sediment m.m. (0.178 mg/ L)	Wyness, L.E. (1996)

m.m: mean measured

7.1.1.4 Other aquatic organisms

7.1.2 Calculation of Predicted No Effect Concentration (PNEC)

Not relevant for this type of dossier.

7.2 Terrestrial compartment

Not relevant for this type of dossier.

7.3 Atmospheric compartment

Not relevant for this type of dossier.

7.4 Microbiological activity in sewage treatment systems

Not relevant for this type of dossier.

7.5 Calculation of Predicted No Effect Concentration for secondary poisoning (PNEC_oral)

Not relevant for this type of dossier.

7.6 Conclusion on the environmental classification and labelling

Imazalil is hydrolytically stable at pH 5 – 9. Imazalil was found to be not readily biodegradable within 28 days in the Modified MITI test (OECD guideline 301C).

Imazalil has a log Kow of 3.66 (pH 7) and 3.82 (pH 9). In a BCF study, a steady state BCF value of 63.8 L/ kg ww was obtained based on plateau concentration of substance in whole fish and average concentration of substance in water. The BCF is not above the trigger of 100/ 500 for not readily biodegradable substances.

The acute toxicity of imazalil to fish and invertebrates is in the mg/L range with a toxicity of LC_{50} = 1.48 mg/L to fish and of EC_{50} = 3.5 mg/L to aquatic invertebrates.

Imazalil shows also a high toxicity to algae (ErC_{50} = 1.2 mg/L, NOEC = 0.457 mg/L). The lowest endpoints in long- term studies were observed with fish (28-d prolonged study NOEC = 0.225 mg/L) and aquatic invertebrates (21-d reproduction study NOEC ≤ 0.01 mg/L).

Conclusion of environmental classification according to Directive 67/548/EEC

In aquatic toxicity studies, ErC_{50} values for algae, acute LC_{50} value for fish and EC_{50} value for invertebrates were obtained at imazalil concentrations > 1 mg/L and < 10 mg/L.
Imazalil is not readily biodegradable according to the Modified MITI test (OECD 301C).
In a BCF study, a steady state BCF value of 63.8 L/kg ww (without lipid normalization) was obtained.
In long- term toxicity studies NOEC < 1 mg/L for invertebrates and fish were determined.

Imazalil therefore fulfils the criteria for classification with N; R51/53.

Based on the toxicity data for *Selenastrum capricornutum* (ErC50 1.2 mg/L) the following specific concentration limits should be applied:

Concentration	Classification
C ≥ 25%	N; R51/53
2.5 % ≤ C < 25 %	R52/53

where C is the concentration of imazalil in the mixture.

Conclusion of environmental classification according to Regulation EC 1272/2008 (2nd ATP to the CLP-Regulation)

In aquatic toxicity studies, ErC_{50} values for algae, acute LC_{50} value for fish and EC_{50} value for invertebrates were obtained at imazalil concentrations > 1 mg/L and < 10 mg/L.
Imazalil therefore does not fulfil the criteria for classification as aquatic environmental hazard acute category 1, H400.
Imazalil is not readily biodegradable according to the Modified MITI test (OECD 301C).
In a BCF study, a steady state BCF value of 63.8 L/kg ww (without lipid normalization) was obtained.
There are adequate chronic toxicity data available for all three trophic levels. In the long- term toxicity studies NOEC < 0.1 mg/L for invertebrates and NOEC < 1 for fish and algae were determined. Imazalil therefore fulfils the criteria for classification as aquatic environmental hazard chronic category 1, H410.
The chronic M-factor is 10, based on the lowest chronic toxicity data for *Daphnia magna* (NOEC < 0.01 mg/L) in a 21d- semi-static study.

JUSTIFICATION THAT ACTION IS REQUIRED ON A COMMUNITY-WIDE BASIS

Imazalil is an active substance in the meaning of Directive 91/414/EEC and therefore subject to harmonised classification and labelling (Regulation EC 1272/2008 article 36.2).

OTHER INFORMATION

This proposal for harmonised classification and labelling is based on the data provided for the registration of the active substance imazalil according to Directive 91/414/EEC. The summaries included in this proposal are partly copied from the DRAR. Some details of the summaries were not included when considered not relevant for a decision on the classification and labelling of this substance. For more details the reader is referred to the DRAR.

REFERENCES

Adam, D. (2008): 14C-IMAZALIL-Aqueous Photolysis and Determination of the Quantum Yield. – Interim Report, Janssen Report B78153, AGR 3856

Andrew, D. (2005): PSD Guidance Document: Interpretation of liver enlargement in regulatory toxicity studies. York, England, Pesticides Safety Directorate

Anonymous (2009): European Commission. Draft Re-Assessment Report Imazalil, Volume 3 Annex B prepared by the Netherlands

Appelman, L.M.; Woutersen, R.A. (1983): Acute inhalation toxicity of an Imazalil containing smoke, developed by a smoke generator, in rats. TNO Report No. V 83.308/230831
http://www.pesticides.gov.uk/uploadedfiles/Liver%20paper%20post%20ACP(1).doc

Appelman, L. M.; Woutersen, R. A. (1983): Acute inhalation toxicity study of an Imazalil containing smoke, developed by a smoke-generator, in rats. TNO Report No. V83.308/230831

Boobis, A.R. ; Cohen, S.M.; Dellarco, V.; McGregor, D.; Meek, M.E.; Vickers, C.; Willcocks, D.; Farland, W. (2006): IPCS framework for analyzing the relevance of a cancer mode of action for humans. Critical reviews in toxicology 36(10):781-792

Dellarco, V.L.; McGregor, D.; Berry, S.C.; Cohen, S.M.; Boobis, A.R. (2006): Thiazopyr and thyroid disruption: case study within the context of the 2006 IPCS Human Relevance Framework for analysis of a cancer mode of action. Critical reviews in toxicology 36(10):793-801

Dirkx, P.; Lampo, A.; Vandenberghe, J.; Coussement, W.; van Cauteren, H. (1992): 2-generation reproduction study with 1 litter per generation in Wistar rats; administration: orally through the diet. Janssen Report R 23979 Exp. No. 2337

Dirkx, P.; Marsboom, R. (1985): Oral embryotoxicity and teratogenicity study in New Zealand white rabbits (Segment II). Janssen Report 18531 Exp. No. 1482

Dirkx, P. (1992): Embryotoxicity and teratogenicity study in albino rabbits (Segment II). Janssen Report R27180 Exp. No. 2615

Elmore, A.R. (2004): Cell proliferation study in mice following dietary Imazalil administration. Integrated Laboratory Systems (ILS), Durham, NC, USA unpublished report C131-001

Elmore, A.R. (2004): BrdU Assessment of rat livers following Imazalil exposure. Integrated Laboratory Systems (ILS), Durham, NC, USA unpublished report C131-002

European Commission (2003): Technical Guidance Document on Risk Assessment. EUR 20418 EN/1

Fautz, R.; Miltenberger, H.G.; Völkner, W. (1990): Unscheduled DNA synthesis in primary hepatocytes of male rats in vitro with Imazalil. Cytotest Cell Research GmbH Report No. 192600

Gillardin, J.M.; Van Cauteren, H.; Sanz, G.; Marsboom, R. (1988): Embryotoxicity and teratogenicity study in sprague-dawley rats. Janssen Report R 27180 Exp. No. 2003/88-05

Goodwine, W.R. (1990a): Comparative acute oral toxicity studies of the different salts of

Imazalil in rats. Janssen Report No. R23979/15

Goodwine, W.R. (1990b): Primary dermal irritation study in rabbits. Janssen Report No. 1864

Hasegawa, R.; Ito, N. (1992): Liver medium-term bioassay in rats for screening of carcinogens and modifying factors in hepatocarcinogenesis. Food and chemical toxicology 30(11):979-992

Heykants, J.; WoestenborRegulation (EC) 1272/2008, R.; Meuldermans, W.; Desplenter, L. (1982): The bioavailability of enilconazole in cattle after oral and topical doses of 4 mg/kg in comparison with an equivalent intravenous dose. Janssen Preclinical Research, Report No. R 23979/34

Holsapple, M.P.; Pitot, H.C.; Cohen, S.M.; Boobism, A.R.; Klaunig, J.E.; Pastoor, T.; Dellarco, V.L.; Dragan, Y.P. (2006): Mode of action in relevance of rodent liver tumors to human cancer risk. Toxicological Sciences 89(1):51-6

IARC (1999): Some Thyrotropic Agents. IARC Monographs on the Evaluation of Carcinogenic Risks to Humans 79:161-164

Klauning, J.E. ; Kamendulis, L.M.; Xu, Y. (2000): Epigenetic mechanisms of chemical carcinogenesis. Human & experimental toxicology 19(10):543-55

Kodama, S.; Negishi, M. (2006): Phenobarbital confers its diverse effects by activating the orphan nuclear receptor CAR. Drug metabolism reviews 38(1-2):75-87

Koyasu, J. (2002): , Ready biodegradability test of Imazalil. Mitsubishu Report No. A020224.

Lavrijsen, K., Van Houdt, J., Van Dijck, D., Meuldermans, W. and Heykants, J. (1987): Study on the induction and/or inhibition potential of Imazalil towards drug metabolizing enzymes in rat liver. Janssen Report R23979/56

Lawrence, A.C. (2001): Three month oral mechanistic toxicity study with one month interim sacrifices in Albino Swiss mice (PCNA quantitative analysis of livers). Huntington Report No. JPA 076/012132

Lawrence, A. (2001): 1-Month repeated dose oral toxicity study in the wistar rat with 1,2 and 4 weeks interim sacrifice and with 4 and 9 week recovery period (PCNA quantitative analysis of livers). Huntington Report No. JPA 077/012131 Exp. No. 5009

Lemaire, G.; Mnif, W.; Pascussi, J.M.; Pillon, A.; Rabenoelina, F.; Fenet, H.; Gomez, E.; Casellas, C.; Nicolas, J.C.; Cavaillès, V.; Duchesne, M.J.; Balaguer, P. (2006): Identification of new human pregnane X receptor ligands among pesticides using a stable reporter cell system. Toxicological Sciences 91(2):501-509

Van Leemput, L., Heykants, J. (1982): Hydrolysis as a possible mechanism of dissipation of imazalil (R 23979) from aqueous environments. Janssen Report No. R023979/L1

Lenaerts, P.; Deknudt, G.; Vanparys, P.; Marsboom, R. (1990): In vitro chromosome aberration assay on human lymphocytes. Janssen Report R 23979 Exp. No. SCK 86/02D/R23979

Lina, B. A. R.; Til, H. P.; Van Nesselrooij, J. H. J.; et al. (1984): Eighteen-month oral toxicity study with imazalil base-R 23979 in rats. Civo Instituts TNO Report No. V 84.140/220555

Lina, B.A.; Til, H.; van Nesselrooij, J.H.; Kuper, C.F.; Falke, H.E. (1983): Six-Month oral toxicity study with Imazalil BASE-R 23979 in rats. Civo Instituts TNO Report No. V 83.186/220555

Mannens, G.; Van Leemput, L.; Heykants, J. (1993): General metabolism of Imazalil in the rat. Janssen Report No. R 23979/FK1116

Mamouni, A. (2008): 14C-Imazalil – Route and rate of degradation in aerobic aquatic sediment systems. RCC AG Report No. B72360, AGR 3854.

Mertens, J.J.W.M. (2011): Cell proliferation study in male Wistar rats after administration of Imazalil in the diet for 1, 2, 7, 14, or 28 days. WIL Research Laboratories, Inc., Ashland OH, USA. unpublished report WIL-436011

Mori, Y.; Koide, A.; Kobayashi, Y.; Morimura, K.; Kaneko, M.; Fukushima, S. (2002): Effect of ethanol treatment on metabolic activation and detoxification of esophagus carcinogenic N-nitrosamines in rat liver. Mutagenesis 17(3):251-256

Muto, N.; Hirai, H.; Tanaka, T.; Itoh, N.; Tanaka, K. (1997): Induction and inhibition of cytochrome P450 isoforms by imazalil, a food contaminant, in mouse small intestine and liver. Xenobiotica 27(12):1215-1223

Niemegeers, C.J.E. (1977): Acute intraperitoneal toxicity of R 23 979 in wistar rats. Janssen Preclinical Research Report No. R 23979/7

OECD (2004): Guidance Document on Dermal Absorption Sanco/222/2000 rev.7, 19 March 2004

Oliver, J.D.; Roberts, R.A. (2002): Receptor-mediated hepatocarcinogenesis: role of hepatocyte proliferation and apoptosis. Pharmacology & Toxicology 91(1):1-7

O'Neill, T.P. (2002): Cell proliferation study in male CD-1 mice after administered Imazalil in the diet for 2 or 13 weeks. WIL Research Laboratories, Inc., Ashland, OH, USA unpublished report WIL-436001

Pesticide Safety Directorate/ECCO-Team (1996): European Commission Peer Review Programme IMAZAIL. 5008/ECCO/PSD/96

Piccirillo, V.J. (2000): Imazalil: One-month repeated dose oral toxicity study with 1 and 2 week interim sacrifices and 4 and 9 week recovery periods to evaluate thyroid effects. VJP 5452-00-1

Piccirillo, V.J. (2002): Overview of liver effects and cell cycle changes in male cd-1 mice after dietary administration of Imazalil for 2 or 13 weeks. VJP Project No. 5452-02-1

Picirillo, V.J. (2011): Summary and evaluation of the mode of action for Imazalil induced liver tumors including analysis of study result from cell proliferation study in male Wistar rats after administration of Imazalil in the diet for 1, 2, 7, 14 or 28 days. VJP Project No. 5452-02-1

Sonich-Mullin, C.; Fielder, R.; Wiltse, J.; Baetcke, K.; Dempsey, J.; Fenner-Crisp, P.; Grant, D.; Hartley, M.; Knaap, A.; Kroese, D.; Mangelsdorf, I.; Meek, E.; Rice, J.M.; Younes, M. (2001): International Programme on Chemical Safety, IPCS conceptual framework for evaluating a mode of action for chemical carcinogenesis. Regulatory toxicology and pharmacology 34(2):146-152

Stiller, R.L.; Stevens, D.A (1986): Studies with a plant fungicide, imazalil, with vapor-phase activity, in the therapy of human alternariosis. Mycopatholigia 93: 169-172

Takeuchi, S.; Matsuda, T.; Kobayashi, S.; Takahashi, T.; Kojima, H. (2006): In vitro screen-

ing of 200 pesticides for agonistic activity via mouse peroxisome proliferator-activated receptor (PPAR)alpha and PPARgamma and quantitative analysis of in vivo induction pathway. Toxicology and applied pharmacology 217(3):235-44

Teuns, G. ; Ligtvoet, T.; Coussement, W.; Lampo, A.; van Cauteren, R.; Marsboom, R. (1990a): Study of the acute dermal toxicity of Imazalil technical in New Zealand white rabbits. Janssen Report No. R23979 - Exp. No. 2344

Teuns, G.; Coussement, W. ; Van Cauteren, R.; Marsboom, R. (1990c): Imazalil: R 23979 technical – dermal sensitization study according to the Magnusson Guinea-Pig Maximization Test. Janssen Exp. No. 2417

Teuns, G.; Peeters, V.; Coussement, W.; Lampo, A.; Van Cauteren, R.; Marsboom, R. (1990b): Imazalil: R 23979 technical grade – primary eye irritation study in New Zealand white rabbits. Janssen Report No. R23979 - Exp. No. 2253

Teuns, G. (1991). Reproduction study in Mallard Ducks. Janssen Exp. No. 2288

Teuns, G.; Vandenberghe, J.; Coussement, W.; Lampo, A.; Van Cauteren, H. (1991): Repeated dose dermal toxicity study in New Zealand white rabbits (21 days). Janssen Report R 23979 Exp. No. 2418

Van Beijsterveldt, L. (1993): Dermal Absorption of 14C-Imazalil in male rats after topical application of its Fungaflor 500 EC formulation. Janssen Report No. R 23979/FK1326

Van Deun, K.; et al., (1999): Combined oral chronic toxicity / carcinogenicity study in the SPF wistar rat. Janssen Report R023979 Exp. No. 3817

Van Deun, K.; Lammens, L.; Vandenberghe, J.; Benze, J.; Lampo, A.; Coussement, W.; van Cauteren, H. (1996): Three-Month oral dose range finding and mechanistic toxicity study with one month interim sacrifice in SPF wistar rats. Janssen Report R023979 Exp. No. 3514

Van Deun, K.; Lammens, L.; Vandenberghe, J.; Benze, J.; Lampo, A.; Coussement, W.; van Cauteren, H. (1996): 3-Month dose range finding and mechanistic toxicity study in SPF wistar rats. Janssen Report R023979 Exp. No. 3672

Van Deun, K.; Vandenberghe, J.; Lammens, L.; Lampo, A.; Coussement, W.; van Cauteren, H. (1994): Three-Month oral mechanistic toxicity study with one month interim sacrifice in SPF albino swiss mice. Janssen Report R023979 Exp. No. 3140

Van Ginneken, I. (1996). The effect of Imazalil (R 23979) on the growth of the unicellular green alga *Selenastrum capricornutum*. From Janssen Pharmaceutica N.V. Company file No.: AASc/0034

Van Gompel, J.; Vanparys, P.; Van Cauteren, H. (1995): In vitro mammalian gene mutation assay. Janssen Report R 023979 Exp. No. 3470

Vanparys, P.; Marsboom, R. (1988): Ames reverse mutation test with salmonella typhimurium; administration: incubation with or without a metabolic activation system. Janssen Report R 23979 Exp. No. 1999

Vanparys, P.; Marsboom, R. (1988): Micronucleus test in mice. Janssen Report R 23979 Exp. No. 1911

Verbeek, J.; Verstynen, B.; Vandenberghe, J.; Vynckier, A.; De Coster, R.; Lampo, A.; Jansen, T.; Coussement, W. (2000): One-Month repeated dose oral toxicity study in the wistar rat

with 1 and 2 week interim sacrifice and with 4 and 9 weeks of recovery. Janssen Report No. R023979/R000524 Exp. No. 5009

Vermeir, M., Lavrijsen, K., Van Leemput, L. (1994): Study on the possible induction and/or inhibition of hepatic drug metabolizing enzymes by Imazalil in male and female SPF Albino Swiss mice, after oral administration through the diet for one and three consecutive months. Janssen Report FK1600.

M. Vermeir, M., Lavrijsen, K., Meuldermans, W. (1995): Study on the possible induction and/or inhibition of hepatic drug metabolizing enzymes by Imazalil in male and female SPF Wistar rats, after oral administration through the diet for one or three months at levels of 200, 400 and 800 ppm. Janssen Report FK1960

Vermeir, M., Lavrijsen, K. (1996): Study on the possible induction and/or inhibition of hepatic drug metabolizing enzymes by Imazalil in male and female SPF Wistar rats, after oral administration through the diet for three months at levels of 800, 1,600 and 2,400 and 3,200 ppm. Janssen Report FK2060

Vermeir, M. (2001): Study on the possible induction and/or inhibition of hepatic drug metabolizing enzymes and of the hepatic 5´-Monodeiodinase and thyroid Peroxidase activities by Imazalil in male SPF wistar rats, after oral administration through the diet for one, two and four weeks at levels of 400, 1200 and 3200 ppm. Janssen Protocol No. R023979/FK3378

Verstraeten, A.; Lampo, A.; Coussement, W. (1993): Imazalil Base; carcinogenicity study in swiss mice; administration through the diet for 2 years. Janssen Report R 23979 Exp. No. 2194

Verstraeten, A.; Teuns, G.; Van Cauteren, H.; Vandenberghe, J.; Marsboom, R. (1989): Imazalil Base: Chronic toxicity study in beagle dogs (repeated dosage for 12 month by oral administration). Janssen Report R 23979 Exp. No. 1899

Verstraeten, A.; Vandenberghe, J.; Lampo, A.; Coussement, W.; van Cauteren, H. (1993): Three-Month oral toxicity study in albino swiss mice. Janssen Report R 23979 Exp. No. 2020

Weytjens, D. and Wils, R. (1988). The acute toxicity of Imazalil (R 23979) for the Zebra fish (*Brachydanio rerio*). Janssen Pharmaceutica N.V., Company file No. : R 23979/AF/Br/5

Weytjens, D. and Wils, R. (1990). The acute toxicity of Imazalil (R 23979) in the water-flea (*Daphnia magna*). Janssen Pharmaceutica N.V., Company file No. : R 23979/AD/K6

Weytjens, D. and Wils, R. (1989). The acute toxicity of imazalil for the rainbow trout *(Salmo gairdneri).* Janssen Pharmaceutica N.V., Company file No. : R 23979/AF/Sg

Weytjens, D. (1989) Prolonged toxicity test with Imazalil (R 23979) in the Rainbow trout *(Salmo gairdneri).* Janssen Pharmaceutica N.V., Company file No. : R 23979/PF/Sg

Weytjens, D. (1989) Daphnia reproduction test with Imazalil (R 23979). Janssen Pharmaceutica N.V., Company file No. : R 23979/RD/K6

Kuhl, R., Wydra, V. (2008) Influence of Imazalil technical to Daphnia magna in a Reproduction test. Janssen Pharmaceutica N.V., Report No. : AGR4026

Weytjens, D. et al. (1995). Environmental Assessment Report - Revised version: The bioaccumulation of Imazalil (R 23979) in the Rainbow trout (*Salmo gairdneri*). Janssen Pharmaceutica N.V., Company file No. : R 23979/BF/Sg

WHO (2001): IARC Monographs on the Evaluation of Carcinogenic Risks to Humans, Vol-

ume 79: 161-164

Wnorowski, G. (1997): Dermal sensitization test – Buehler Method. Janssen psl project no. 5337

Wyness, L.E. (1996). Imazalil : Chronic sediment toxicity test using an infaunal insect *Chironomus riparius*. Janssen Pharmaceutica N.V. 1073/2-1018