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**

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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

	S61	hazardous waste 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

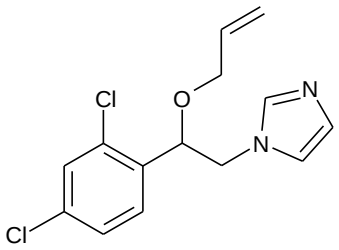
JUSTIFICATION

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.

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.

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.

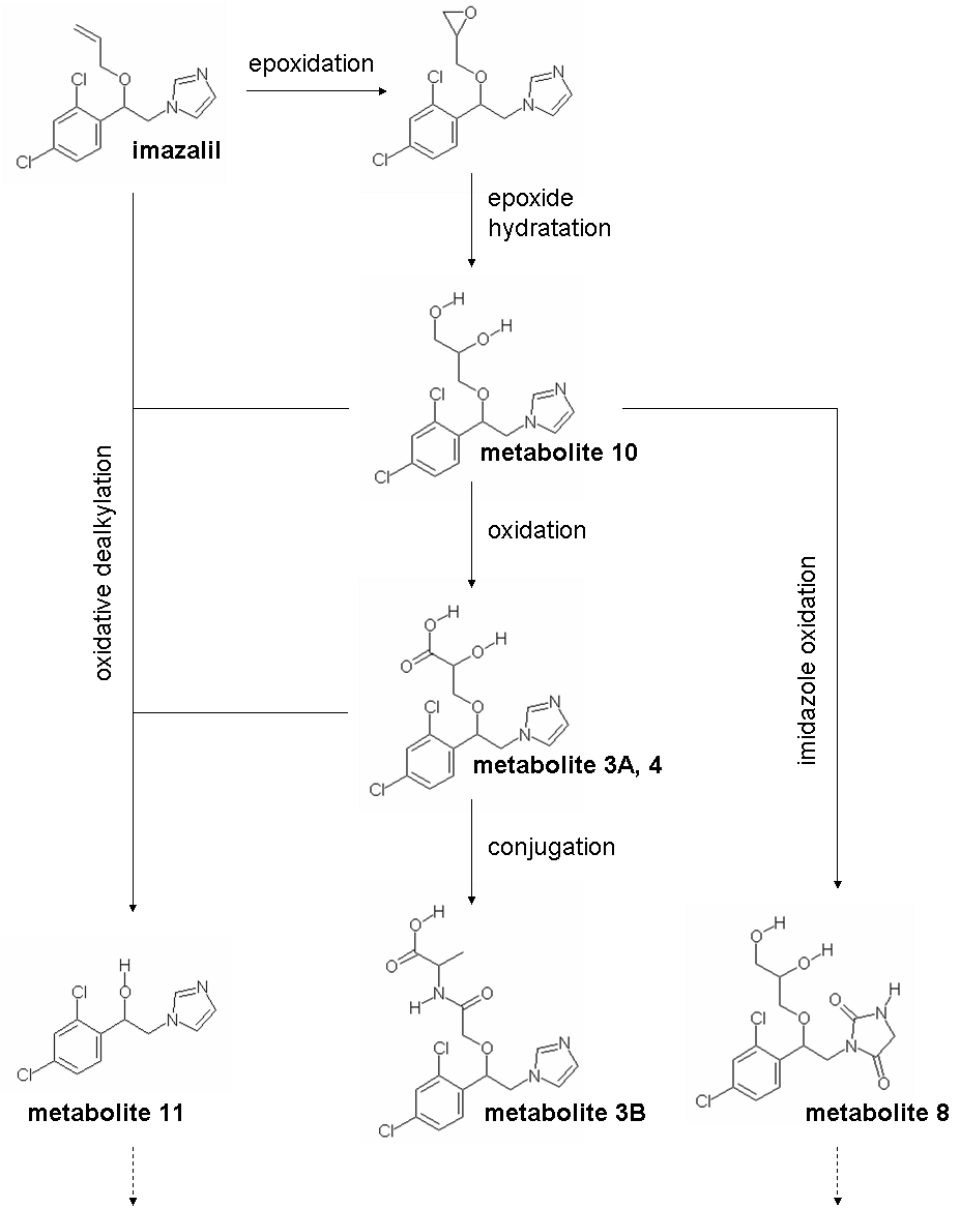


Figure 1: Major metabolic pathways of imazalil in the rat

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).

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

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.

5.5.2 Respiratory system

No data available

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.

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 reports 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 moderate. 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.

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.

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)”.

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), oral (gavage) d6-18	None	None (one split vertebra with extra rib within historic control range)
Gillardin et al., 1988,	Sim. to OECD 414	0-40-80-120 mg/kg bw/d	≥ 40 mg/kg: reduced bw and food consumption during	≥ 80 mg/kg: reduced live

Janssen Report R 27180 Exp. No. 2003/88-05	Rat, Sprague Dawley (24 F)	imazalil equivalents (imazalil sulphate), oral (gavage) d6-16	dosing ≥ 120 mg/kg: reduced bw and bw gain at delivery	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.

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 control 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)

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
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)

Animal species & strain	Number of animals	Doses, vehicle, duration	Result	Reference
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)
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

Animal species & strain	Number of animals	Doses, vehicle, duration	Result	Reference
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
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

Animal species & strain	Number of animals	Doses, vehicle, duration	Result	Reference
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
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).

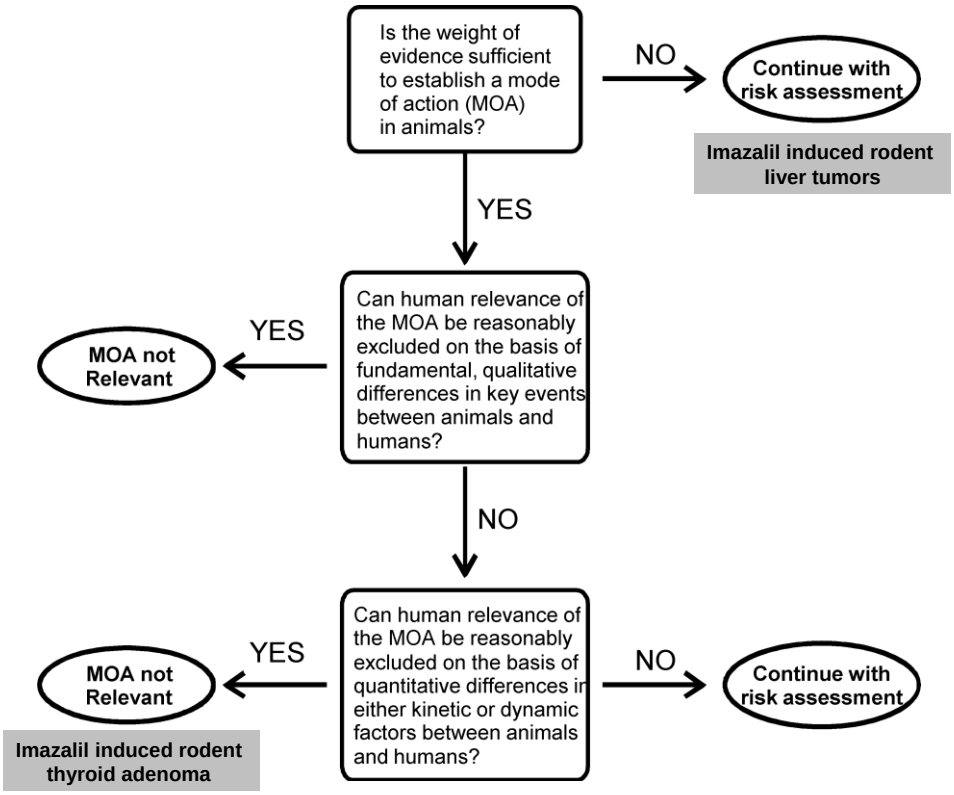


Figure 2: Human Relevance Framework (from Boobis et al., 2006) with conclusions for imazalil induced rodent tumours

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 mecha-

nism 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.

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