# CLH report

# Proposal for Harmonised Classification and Labelling

**Based on Regulation (EC) No 1272/2008 (CLP Regulation),
Annex VI, Part 2**

# Substance Name: IPCONAZOLE

**EC Number:** **Not allocated**

**CAS Number:** **Not included**

**Index Number:** **Not allocated**

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# Part A.



# PART B.

# Part A.

## 1 PROPOSAL FOR HARMONISED CLASSIFICATION AND LABELLING

### 1.1 Substance

**Table 1: Substance identity**

| | |
|---|---|
| **Substance name:** | ***Ipconazole*** |
| **EC number:** | ***Not allocated*** |
| **CAS number:** | ***Refer to table 4 in Part B*** |
| **Annex VI Index number:** | ***Not allocated*** |
| **Degree of purity:** | ***≥ 95.5%***<br>***[87.5-93% ipconazole cc (cis-cis) racemate and 6.5-9.5 ipconazole ct (cis-trans) racemate]*** |
| **Impurities:** | ***There are a number of process impurities which have been taken into account and are not considered to contribute to the classification and labelling.*** |

### 1.2 Harmonised classification and labelling proposal

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

| | **CLP Regulation** |
|---|---|
| **Current entry in Annex VI, CLP Regulation** | Not currently listed |
| **Current proposal for consideration by RAC** | Acute Tox 4; H302 – Harmful if swallowed<br>STOT-RE 2; H373 – May cause damage to organs (eyes, skin, liver and gastrointestinal tract) through prolonged or repeated exposure<br>Repr 2; H361d – Suspected of damaging the unborn child<br>Aquatic Chronic 1; H410 – Very toxic to aquatic life with long lasting effects<br>M = 100 |
| **Resulting harmonised classification (future entry in Annex VI, CLP** | Acute Tox 4; H302 – Harmful if swallowed<br>STOT-RE 2; H373 – May cause damage to organs |

| | |
|---|---|
| **Regulation)** | (eyes, skin, liver and gastrointestinal tract) through prolonged or repeated exposure<br><br>Repr 2; H361d – Suspected of damaging the unborn child<br><br>Aquatic Chronic 1; H410 – Very toxic to aquatic life with long lasting effects<br><br>M = 100 |

## 1.3 Proposed harmonised classification and labelling based on CLP Regulation

**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 | Not classified | Not applicable | Not classified | conclusive but not sufficient for classification |
| **2.2.** | Flammable gases | Not classified | Not applicable | Not classified | conclusive but not sufficient for classification |
| **2.3.** | Flammable aerosols | Not classified | Not applicable | Not classified | conclusive but not sufficient for classification |
| **2.4.** | Oxidising gases | Not classified | Not applicable | Not classified | conclusive but not sufficient for classification |
| **2.5.** | Gases under pressure | Not classified | Not applicable | Not classified | conclusive but not sufficient for classification |
| **2.6.** | Flammable liquids | Not classified | Not applicable | Not classified | conclusive but not sufficient for classification |
| **2.7.** | Flammable solids | Not classified | Not applicable | Not classified | conclusive but not sufficient for classification |
| **2.8.** | Self-reactive substances and mixtures | Not classified | Not applicable | Not classified | conclusive but not sufficient for classification |
| **2.9.** | Pyrophoric liquids | Not classified | Not applicable | Not classified | conclusive but not sufficient for classification |
| **2.10.** | Pyrophoric solids | Not classified | Not applicable | Not classified | conclusive but not sufficient for classification |
| **2.11.** | Self-heating substances and mixtures | Not classified | Not applicable | Not classified | conclusive but not sufficient for classification |
| **2.12.** | Substances and mixtures which in contact with water emit flammable gases | Not classified | Not applicable | Not classified | conclusive but not sufficient for classification |
| **2.13.** | Oxidising liquids | Not classified | Not applicable | Not classified | conclusive but not sufficient for classification |

| | | | | | |
|---|---|---|---|---|---|
| **2.14.** | Oxidising solids | Not classified | Not applicable | Not classified | conclusive but not sufficient for classification |
| **2.15.** | Organic peroxides | Not classified | Not applicable | Not classified | conclusive but not sufficient for classification |
| **2.16.** | Substance and mixtures corrosive to metals | Not classified | Not applicable | Not classified | conclusive but not sufficient for classification |
| **3.1.** | Acute toxicity - oral | **Acute Tox 4; H302 – Harmful if swallowed** | None | Not classified | - |
| | Acute toxicity - dermal | Not classified | Not applicable | Not classified | conclusive but not sufficient for classification |
| | Acute toxicity - inhalation | Not classified | Not applicable | Not classified | conclusive but not sufficient for classification |
| **3.2.** | Skin corrosion / irritation | Not classified | Not applicable | Not classified | conclusive but not sufficient for classification |
| **3.3.** | Serious eye damage / eye irritation | Not classified | Not applicable | Not classified | conclusive but not sufficient for classification |
| **3.4.** | Respiratory sensitisation | Not classified | Not applicable | Not classified | Data lacking |
| **3.4.** | Skin sensitisation | Not classified | Not applicable | Not classified | conclusive but not sufficient for classification |
| **3.5.** | Germ cell mutagenicity | Not classified | Not applicable | Not classified | conclusive but not sufficient for classification |
| **3.6.** | Carcinogenicity | Not classified | Not applicable | Not classified | conclusive but not sufficient for classification |
| **3.7.** | Reproductive toxicity | **Repr 2; H361d – Suspected of damaging the unborn child** | None | Not classified | - |
| **3.8.** | Specific target organ toxicity –single exposure | Not classified | Not applicable | Not classified | conclusive but not sufficient for classification |

| | | | | | |
|---|---|---|---|---|---|
| **3.9.** | Specific target organ toxicity – repeated exposure | **STOT-RE 2 – H373 – May cause damage to organs (eyes, skin, liver and gastrointestinal tract) through prolonged or repeated exposure.** | None | Not classified | - |
| **3.10.** | Aspiration hazard | Not classified | Not applicable | Not classified | conclusive but not sufficient for classification |
| **4.1.** | Hazardous to the aquatic environment | **Aquatic Chronic 1; H410 – Very toxic to aquatic life with long lasting effects** | **M = 100** | Not classified | - |
| **5.1.** | Hazardous to the ozone layer | Not classified | Not applicable | Not classified | conclusive but not sufficient for classification - |

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

## Labelling:

Pictogram(s): GHS07, GHS08, GHS09

Signal word: Warning

Hazard statements: H302 - Harmful if swallowed
H361d - Suspected of damaging the unborn child
H373 - May cause damage to organs (eyes, skin, liver, gastrointestinal tract) through prolonged or repeated exposure
H410 – Very toxic to aquatic life with long lasting effects

Precautionary statements: Not included in Annex VI

**Proposed notes assigned to an entry:** None

# 2 BACKGROUND TO THE CLH PROPOSAL

## 2.1 History of the previous classification and labelling

Ipconazole has not previously been reviewed for harmonised classification and labelling in the EU.

At the time of writing, no REACH registration dossiers have been submitted for this substance.

## 2.2 Short summary of the scientific justification for the CLH proposal

## 2.3 Current harmonised classification and labelling

Ipconazole was approved for Annex I listing as a 3A review substance under Council Directive 91/414/EEC, with the UK as Rapporteur Member State. The EFSA conclusion (EFSA Journal 2013;11(4):3181) [74] was that ipconazole was harmful if swallowed (H302), may cause damage to organs through prolonged or repeated exposure (H373) and was suspected of damaging the unborn child (H361d). It also considered very toxic to aquatic life with long lasting effects (H410).

Ipconazole does not meet the criteria for classification for physical hazards.

Ipconazole is classified as harmful for acute toxicity via the oral route as the ATE values ranged from 468 – 1338 mg/kg. **Acute Tox 4; H302 – Harmful if swallowed** is proposed. The ATEs via the inhalation and dermal routes were above the values for classification. Acute exposure to ipconazole did not result in toxicity to specific organs or produce narcotic effects. There were some signs of respiratory tract irritation following acute inhalation exposure in animals, but taken into consideration with the observations in the repeated-dose and skin and eye irritation studies it is concluded that they are insufficient to classify. Therefore, it is not proposed to classify for STOT-SE. Signs of mild irritation were observed in the skin and eye irritations studies, but all scores were below the values for classification. Ipconazole gave a negative result in a Guinea Pig maximisation test and it is not proposed to classify for sensitisation.

Repeated-dose administration of ipconazole resulted in a number of adverse effects in rats, mice and dogs at doses below the relevant guidance values for classification. This included ocular effects (potentially related to a decrease in plasma cholesterol), hepatocyte necrosis, lesions in the oesophagus, pharynx, larynx and hard palate, fatty deposits in the liver and fatty vacuolation in the adrenal glands. A further systemic finding of concern was skin reddening in dogs following oral (capsule) administration. Effects at doses relevant for classification were noted after oral, dermal and inhalation exposure. Therefore, it is proposed to classify ipconazole with **STOT-RE 2; H373 – May cause damage to organs (eyes, skin, liver and gastrointestinal tract) through prolonged or repeated exposure.**

There was no indication that ipconazole has a mutagenic effect on somatic or germ cells and no classification for germ cell mutagenicity is proposed.

From studies in the rat and mouse, there was no indication that administration of ipconazole caused an increased incidence of tumours. Therefore, no classification for carcinogenicity is proposed.

There were marginal and inconsistent reductions in some reproductive parameters (reductions in total litter size (F2 only) and live birth index (F1 only)) in a two-generation study in rats.

However, as these were only marginally below the historical control ranges, were inconsistent across the generations and did not show statistical significant differences from the controls, they are not considered to provide evidence of a specific treatment related effect and therefore, no classification for effects on fertility is proposed.

There are four developmental studies (two in rats and two in rabbits) in which several findings indicative of developmental toxicity were observed. These included, microphthalmia and short/kinky tails in both rats and rabbits along with visceral defects (e.g., abnormalities of major blood vessels associated with the aortic arch and increased incidence of left umbilical artery in rats). There were also increases in fetal resorptions/deaths resulting in reduced live fetuses per litter in rats and rabbits. It is noted that signs of maternal toxicity were observed and the majority of findings were only reported in preliminary studies. Litter effects were evident in the rat preliminary study and the number of fetuses with malformation tended to be clustered in a few litters rather than being more evenly distributed. Overall, it is proposed to classify ipconazole with **Repr 2; H361d – Suspected of damaging the unborn child.**

Ipconazole is hydrolytically stable and is considered to be 'not rapidly degradable' for the purposes of hazard classification. Despite a log $K_{ow}$ of 4.28-4.65, the substance has a low potential for bioaccumulation as the steady-state BCF has been experimentally determined in fish to be 225-283 l/kg. This is less than the CLP cut-off value of 500 l/kg. Acute and Chronic aquatic toxicity data are available for fish, invertebrates and algae. In acute studies, the most sensitive species is the fish, but the L(E)C50 is above the range for classification. Therefore, not classification for aquatic acute toxicity is proposed. The lowest chronic NOEC is the 28-day mean measured value of 0.00044 mg/l for *Pimephales promelas* from a reliable fish early-life stage study. These data indicate that the substance should be classified as **Chronic Category 1; H410 – Very toxic to aquatic life with long lasting effects, with an M factor of 100.**

## 2.4 Current self-classification and labelling

### 2.4.1 Current self-classification and labelling based on the CLP Regulation criteria

The following self-classifications have been notified to the Classification and Labelling Inventory:

Acute Tox. 4 (H302), Aquatic Acute 1 (H400), Aquatic Chronic 1 (H410);

Acute Tox 4 (H302, H332), Aquatic Acute 1 (H400), Aquatic Chronic 1 (H410);

Acute Tox 4 (H332);

Acute Tox 4 (H302), Repr. 2 (H361), Aquatic Chronic 2 (H411).

# 3 JUSTIFICATION THAT ACTION IS NEEDED AT COMMUNITY LEVEL

Ipconazole is a systemic fungicide for the seed treatment of wheat and barley that inhibits 14-C demethylation in the ergosterol biosynthesis pathway of plant disease fungi. The target site for ipconazole is reported to be the enzyme cytochrome P-450, which catalyses the 14-C demethylation in this pathway. In 2013, it was approved for Annex I listing as a 3A review substance under Council Directive 91/414/EEC, with the UK as Rapporteur Member State. In accordance with Article 36 (2) of the CLP Regulation, ipconazole should now be considered for harmonised classification and labelling. Therefore, this proposal considers all human health and environmental hazard classes. This CLH dossier presents a classification and labelling proposal based mainly on the information presented in the assessment of ipconazole under Directive 91/414/EEC. The assessment made under that Directive is attached to the IUCLID dossier. The EFSA conclusion (EFSA Journal 2013;11(4):3181) [74] was that ipconazole was harmful if swallowed (H302), may cause damage to organs through prolonged or repeated exposure (H373) and was suspected of damaging the unborn child (H361d).

# Part B.

## SCIENTIFIC EVALUATION OF THE DATA

### 1 IDENTITY OF THE SUBSTANCE

#### 1.1 Name and other identifiers of the substance

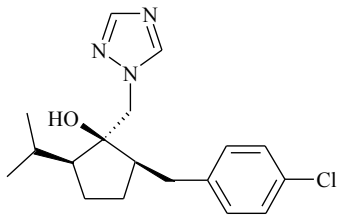
**Table 4: Substance identity**

| | |
|---|---|
| **EC number:** | Not allocated |
| **EC name:** | Not allocated |
| **CAS number (EC inventory):** | Not listed |
| **CAS number:** | Related CAS identifiers<br>125225-28-7* (all stereoisomers)<br>115850-69-6 cis-cis (cc) racemate<br>115937-89-8 cist-trans (ct) racemate |
| **CAS name:** | - |
| **IUPAC name:** | (1*RS*,2*SR*,5*RS*;1*RS*,2*SR*,5*SR*)-2-(4-chlorobenzyl)-5-isopropyl-1-(1*H*-1,2,4-triazol-1-ylmethyl) cyclopentanol* |
| **CLP Annex VI Index number:** | Not listed |
| **Molecular formula:** | $C_{18}H_{24}ClN_3O$ |
| **Molecular weight:** | 333.9 g/mol |

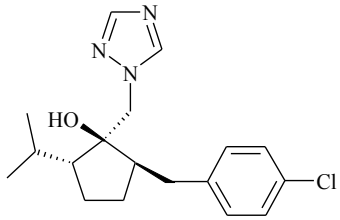
* As included in the Draft Assessment Report and EFSA conclusion [74]. It should, however, be noted that the CAS number 125225-28-7 is generic and covers all possible stereoisomers.

**Structural formula:**

**Ipconazole cc (cis-cis) racemate**
(1*RS*,2*SR*,5*RS*)-2-(4-chlorobenzyl)-5-isopropyl-1-(1*H*-1,2,4-triazol-1-ylmethyl) cyclopentanol
CAS: 115850-69-6




**Ipconazole ct (cis-trans) racemate**
(1*RS*,2*SR*,5*SR*)-2-(4-chlorobenzyl)-5-isopropyl-1-(1*H*-1,2,4-triazol-1-ylmethyl) cyclopentanol
CAS: 115937-89-8




## 1.2 Composition of the substance

**Table 5: Constituents (non-confidential information)**

| Constituent | Typical concentration | Concentration range | Remarks |
|---|---|---|---|
| Ipconazole (1*RS*,2*SR*,5*RS*;1*RS*,2*SR*,5*SR*)-2-(4-chlorobenzyl)-5-isopropyl-1-(1*H*-1,2,4-triazol-1-ylmethyl) cyclopentanol | - | ≥ 95.5% | |
| cc - racemate | 90.1% | 87.5-93% | |
| ct - racemate | 7.3% | 6.5-9.5% | |

Current Annex VI entry: not listed.

The active substance, is approximately a 12:1 ratio of the cc and ct racemates (i.e., 90.1% ipconazole *cc* and 7.3% ipconazole ct). Further details on the composition are provided in the technical dossier.

Whilst it is noted that the substance contains > 80% of ipconazole-cc racemate (90.1% typical), the ISO name Ipconazole is associated with the IUPAC name (1RS,2SR,5RS;1RS,2SR,5SR)-2-(4-chlorobenzyl)-5-isopropyl-1-(1H-1,2,4-triazol-1-ylmethyl)cyclopentanol. The substance is therefore identified as such in the CLH report for consistency. It is proposed to include the CAS numbers 125225-28-7, 115850-69-6 and 115937-89-8 in the description of the substance as related chemical identifiers:

| Index No | International Chemical Identification | EC No | CAS No |
|---|---|---|---|
| 603-RST-VW-Y | ipconazole (ISO); (1RS,2SR,5RS;1RS,2SR,5SR)-2-(4-chlorobenzyl)-5-isopropyl-1-(1H-1,2,4-triazol-1-ylmethyl)cyclopentanol<br>[CAS No. 125225-28-7 (all stereoisomers);<br>CAS No. 115850-69-6 (cis-cis racemate);<br>CAS No. 115937-89-8 (cis-trans racemate)] | - | - |

**Table 6: Impurities (non-confidential information)**

| Impurity | Typical concentration | Concentration range | Remarks |
|---|---|---|---|
| Confidential | | | |

There are a number of process impurities in the substance. These have been taken into consideration and are not considered to impact on the classification proposed in this dossier. Further information on the impurities is considered to be confidential but full details are provided in the technical dossier.

Current Annex VI entry: Not listed

**Table 7: Additives (non-confidential information)**

| Additive | Function | Typical concentration | Concentration range | Remarks |
|---|---|---|---|---|
| None | | | | |

Current Annex VI entry: Not applicable

### 1.2.1 Composition of test material

The composition of the tested material is considered to meet the specification outlined above.

## 1.3 Physico-chemical properties

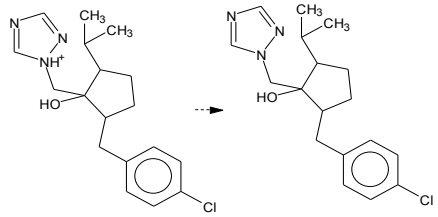
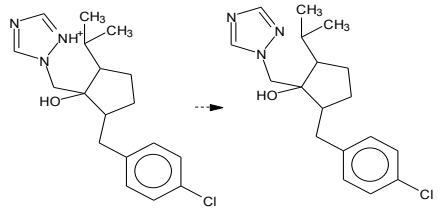
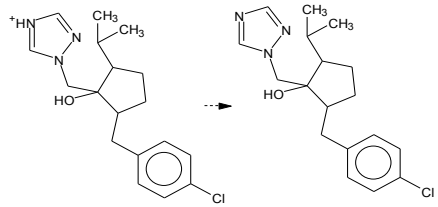
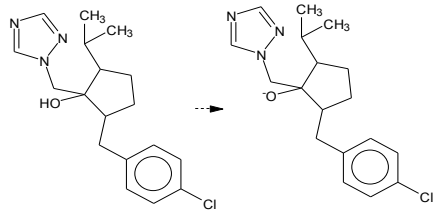
References are taken from the Draft Assessment Report (DAR) - Ipconazole - Volume 3, Annex B.2: Physical and Chemical properties – November 2011 and Addendum 5 to the DAR – Volume 3, Annex B.2: Physical and Chemical properties - November 2012

All studies were conducted to GLP and were considered acceptable during the review of the active substance. The studies were conducted on ipconazole (pure or technical) or the ipconazole cc and ct racemates. Where ipconazole cc or ct were tested this is noted in the table.

**Table 8: Summary of physico - chemical properties**

| Property | Value | Reference | Comment (e.g. measured or estimated) |
|---|---|---|---|
| State of the substance at 20°C and 101,3 kPa | White powder | Comb, A.L., 2005a, b, c [1], [2], [3]<br>DAR B.2.1.7 and DAR B.2.1.8 | Visual inspection<br>98.1% (technical), 99.3% (cc) and 98.3% (ct) |
| Melting/freezing point | 81-89 $^{o}$C<br><br>85.5-88 $^{o}$C | Comb, A.L, 2007<br>[4]<br>Riggs, A.S, 2001a<br>[5]<br>DAR B.2.1.1 | EEC A1 (capillary method)<br>99.7% (pure)<br><br>98.4% (technical) |
| Boiling point | > 400 $^{o}$C at 102 kPa | Woolley, S.M & Mullee, D. M., 2000a and 2000b<br>[6][7]<br>DAR B.2.1.2 | EEC A2 (DSC)<br>99.7% (cc) and 99.7% (ct) |
| Relative density | 1.23 at 20 $^{o}$C | Woolley, S.M & Mullee, D. M., 2000g and 2000h<br>[8][9]<br>DAR B.2.1.4 | EEC A3 (pycnometer method)<br>99.7% (cc) and 99.7% (ct) |
| Vapour pressure | 3 x $10^{-6}$ Pa at 25 $^{o}$C | Comb, A.L., 2007<br>[4]<br>DAR B.2.1.5 | EEC A4 (vapour pressure balance method)<br>99.7% pure |
| Surface tension | 56.6 mN/m at 20 $^{o}$C | Comb, A.L., 2005a<br>[1]<br>DAR B.2.1.24 | OECD 115<br>98.1% (technical) |

| | | | |
|---|---|---|---|
| Water solubility | 11.0 mg/L in pure water<br>9.05 mg/L in pH 5 buffer<br>10.4 mg/L in pH 7 buffer<br>10.4 mg/L in pH 9 buffer | Comb, A.L, 2007<br>[4]<br>DAR B.2.1.11 | EEC A6 (column elution method)<br>99.7% (pure) |
| | 4.97 mg/L in pure water<br>5.79 mg/L in pH 5 buffer<br>4.60 mg/L in pH 7 buffer<br>4.71 mg/L in pH 9 buffer<br><br>9.34 mg/L in pure water<br>9.86 mg/L in pH 5 buffer<br>8.68 mg/L in pH 7 buffer<br>9.13 mg/L in pH 9 buffer | Riggs, A.S., 2001b<br>[10]<br>DAR B.2.1.11 | EEC A6(flask method)<br>99% (ct)<br><br><br><br>99.2% (ct) |
| Partition coefficient n-octanol/water | Log Pow = 4.65 (effect of pH not considered)<br><br>Log Pow = 4.44 (effect of pH not considered) | Riggs, A. S., 2001d<br>[11]<br>DAR B.2.1.13 | OECD 117 (HPLC)<br>100% (cc)<br><br>100% (ct) |
| | Log Pow = 4.49 at 20 °C (effect of pH not considered)<br><br>Log Pow = 4.28 at 20 °C (effect on pH not considered) | Comb, A.L., 2012a and b<br>[12][13]<br>Addendum 5<br>DAR B.2.1.13 | EEC A8 (Shake flask)<br>99.6% (cc)<br><br>100% (ct) |
| Flash point | Not applicable (solid wit melting point > 80°C) | | |
| Flammability | Not flammable; melted and burned but did not sustain a flame on removal of ignition source. Flame did not propagate along the pile.<br><br>Experience in handling and use indicates the substance is not pyrophoric and does not emit flammable gases on contact with water. | Comb, A.L., 2005a<br>[1]<br>DAR B.2.1.20 | EEC A10<br>98.1% (technical) |
| Explosive properties | Not explosive in response to heat, shock or friction. | Comb, A.L., 2005a<br>[1]<br>DAR B.2.1.22 | EEC A14<br>98.1% (technical) |
| Self-ignition temperature | No auto-ignition below 400 °C | Comb, A.L., 2005a<br>[1]<br>DAR B.2.1.20 | EEC A15<br>98.1% (technical) |
| Oxidising properties | Not oxidising | Comb, A.L., 2005a<br>[1]<br>DAR B.2.1.23 | EEC A17<br>98.1% (technical) |
| Granulometry | No data | | |

| | | | |
|---|---|---|---|
| Stability in organic solvents and identity of relevant degradation products | No data | | |
| Dissociation constant | Potential dissociated species:<br>pKa = -5.43<br>  <br>pKa = -2.42<br>  <br>pKa = 2.32<br>  <br>pKa = 17.34<br>  | Yu, W.S., (2001b)<br>[14]<br>DAR B.2.1.18 | Estimated (Sparc Online Calculator) |
| Viscosity | Not applicable | | |

# 2 MANUFACTURE AND USES

## 2.1 Manufacture

Ipconazole is manufactured outside of the EU.

## 2.2 Identified uses

Ipconazole is used as a fungicidal seed treatment in agricultural applications within the EU.

# 3 CLASSIFICATION FOR PHYSICO-CHEMICAL PROPERTIES

**Table 9: Summary table for relevant physico-chemical studies**

| Method | Results | Remarks | Reference |
|---|---|---|---|
| Refer to table 8 | | | |

## 3.1 Physico-chemical properties

### 3.1.1 Summary and discussion of physico-chemical properties

In a standard study (EEC A10, Comb, A. 2005(a) DAR B.2.1.20), ipconazole melted and burned but did not sustain combustion on removal of the ignition source. Consequently, it does not meet the criteria for classification as a flammable solid. In addition, experience in handling and use indicates that the substance is not pyrophoric and does not emit flammable gases on contact with water.

In a standard study (EEC A14, Comb, A. 2005(a) DAR B.2.1.22), ipconazole was not found to be sensitive to the effects of heat, shock or friction. Consequently, it does not meet the criteria for classification as an explosive substance.

In a standard study (EEC A17, Comb, A. 2005(a) DAR B.2.1.23), ipconazole was not found to be oxidising. Consequently, it does not meet the criteria for classification as an oxidising solid.

### 3.1.2 Conclusions on classification and labelling

**Not classified – conclusive but not sufficient for classification**

# 4 HUMAN HEALTH HAZARD ASSESSMENT

This assessment is largely based on the information provided in the Draft Assessment Report for ipconazole, Volume 3 Annex B.6 (November 2011).

## 4.1 Toxicokinetics (absorption, metabolism, distribution and elimination)

The following summary is derived from the assessment made for the review under Directive 91/414/EEC.

### 4.1.1 Non-human information

The toxicokinetics and metabolism of ipconazole have been investigated in rats following oral administration (single high and low doses, plus repeated (14 days) dosing). After a single dose, ipconazole was extensively absorbed (> 90%) at the low dose level (2 mg/kg). The majority (> 80%) of a high (100 mg/kg) or low single dose was excreted within 48 hours, mainly via the biliary route (> 50% of the applied dose), with very little retention of material after five days. Systemic exposure ($C_{max}$ and AUC values) was proportionally higher at the high dose than the difference in doses would indicate, suggesting non-linear kinetics. On repeated dosing, the $T_{max}$ was shortened, the AUC values increased and the half-life extended. Increased retention of radiolabelled material in the carcass was also indicated on repeated dosing, but remained relatively low (< 1.4% of the administered dose). The highest residues were detected in the liver after both single (low- and high-dose) and repeated administration, but, overall, levels retained in tissues were low at 120 hours after the (final) dose (total < 0.7% of administered single dose; ≤ 1.4% of a repeated, daily dose). Ipconazole was extensively metabolised [15], with a maximum of 2.2% of the administered dose excreted unchanged. Large numbers of metabolite fractions occurred at low levels (each < 4% of the dose). All metabolites that occurred at ≥ 6% of the administered dose were identified. Hydroxylation and conjugation were the major metabolic pathways, with the ring structure remaining largely intact. The only notable exception was a small amount (up to 6.9% of the dose) of free triazole (1,2,4-triazole) detected in urine.

During the evaluation under Directive 91/414/EEC, questions were raised over the isomer-specific toxicity of ipconazole. Specifically, there was a concern over the possibility that the toxicity of the substance was owing to one or more metabolites derived from ipconazole ct; the possibility was raised that a small difference in the metabolism of the ct stereoisomers by humans compared with laboratory animals could affect the ability to predict the toxicity of ipconazole (ISO) to humans from the available data. The following case was presented by industry to support its assertion that 'the possibility of the formation of metabolites in humans that are specific to the ipconazole ct stereoisomers, and their subsequent responsibility for toxic effects, is remote':

- theoretical and experimental evidence to indicate that interconversion between ipconazole cc and ct is unlikely in the mammalian body;
- although the theoretical considerations suggest likely differences in the metabolism of ipconazole cc and ct, experimental data from rats and goats indicate that there is no significant formation of ct-specific metabolites;

- no evidence for major species-specific differences in metabolism based on the similar metabolism and kinetics of ipconazole in rats and goats. In particular the metabolite profile in faeces, a major route of excretion in both species, showed no major differences (qualitative or quantitative) of particular concern, i.e. the profiles should be considered broadly similar;
- no evidence from acute and genotoxicity studies for the ct being of more toxicological concern than the  isomer.

The RMS accepted the evidence for the lack of interconversion of ipconazole cc and ct, but concluded that, notwithstanding the data on rats and goats, there was some remaining uncertainty surrounding the comparative animal versus human metabolism of ipconazole.

No marked differences in toxicokinetics between males and females were observed.

### 4.1.2 Human information

None available.

### 4.1.3 Summary and discussion of toxicokinetics

Ipconazole is extensively absorbed after oral administration in rodents, is widely distributed and extensively metabolised and excreted.

## 4.2 Acute toxicity

Acute toxicity has been investigated by the oral, inhalation and dermal routes in rats and mice.

**Table 10: Summary table of relevant acute toxicity studies**

| **Acute Oral** | | |
|---|---|---|
| **Method** | **$LD_{50}$** | **Observations and remarks** |
| Rats, Sprague-Dawley, 5/sex/group<br>Males: 300, 420, 600, 850, 1200, 1700, 2400, 3400 mg/kg<br>Females: 600, 850, 1200, 1700, 2400 mg/kg<br>Administered in olive oil<br>14-day observation period<br>87.27% cc : 10.23% ct, purity 97.5%<br>Japanese guideline (no deviations from OECD 401), GLP<br>Kureha Corporation (1989)[16] | Males = 1338 mg/kg<br>Females = 888 mg/kg | Deaths occurred at ≥ 1200 mg/kg in males and at ≥ 850 mg/kg in females; most deaths occurred between 1 and 4 days after dosing.<br>Clinical signs appeared from 3 hours after dosing and included decreased locomotor activity, loose stool, lateral position, lacrimation, reddish tear, reddish nasal discharge, tip toe gait, ptosis and loss of hair. Skin reddening was observed in one high-dose male.<br>Body weight losses were recorded in male survivors at ≥ 1200 mg/kg and reduced body weight gains were recorded at day 3 at 850 mg/kg. Body weight losses were recorded in female survivors at ≥ 850 mg/kg.<br>In males that died, the predominant macroscopic findings were in the stomach: distension, thinning of the stomach wall, multiple white patches in the glandular stomach, no stomach content and sporadic perforation of the stomach. In females that died, the most common finding was atrophy of the spleen. |
| Mice, CD-1, 5/sex/group<br>300, 420, 600, 850, 1200 mg/kg in olive oil<br>14-day observation period<br>87.27% cc : 10.23% ct, purity 97.5%<br>Japanese guideline (no deviations from OECD 401), GLP<br>Kureha Corporation (1989) [17] | Males = 537 mg/kg<br>Females = 468 mg/kg | Deaths occurred at ≥ 420 mg/kg, on days 2 or 3.<br>Body weight losses (≥ 420 mg/kg) or reduced body weight gains (300 mg/kg) were initially recorded in males.<br>The predominant findings at necropsy of mice that died were in the gastrointestinal tract, most commonly reddish change or distension in the duodenum. Many animals that died showed no macroscopic changes. |
| **Acute Inhalation** | | |
| **Method** | **LC50** | **Observations and remarks** |
| Nose-only exposure for 4 hours<br>Rats, Sprague-Dawley, 5/sex<br>Administered undiluted as a dust, mean test atmosphere concentration = 1.88 mg test substance / litre air, average mass median aerodynamic diameter (MMAD) = 3.9 µm<br>14-day observation period<br>OECD 403, GLP<br>Kureha Corporation (2003) [18] | > 1.88 mg/l | There were no deaths and no clinical signs of toxicity during the period of exposure.<br>During the two hours after removal from the chamber, clinical signs included excessive lacrimation, salivation and red nasal discharge.<br>During the 14-day observation period, clinical signs included clear and red nasal discharge, chromodacryorrhoea, yellow anal-genital staining and decreased faecal volume.<br>Most females did not gain weight or showed a small loss in weight during the first week of observations.<br>There were no abnormal findings at necropsy, but the extent of internal examination of the respiratory tract was unclear. |

| | | |
|---|---|---|
| Whole-body exposure for 4 hours<br>Rats, Sprague-Dawley, 5/sex/group<br>Administered as a mixture of 70% ipconazole with a carrier (white carbon / kaolin 1:1). Atmospheric dust concentrations of ipconazole were 2.19 and 3.53 mg/l. MMAD = 5.3 µm.<br>14-day observation period<br>cc:ct content and purity not reported.<br>OECD 403, GLP<br>Kureha Corporation (1991) [19] | > 3.53 mg/l | There were no deaths.<br>Clinical signs of toxicity included salivation, slight nasal discharge (reddish) and urine incontinence, all of which had resolved one hour after exposure. Salivation in some animals of both groups re-occurred from 5 to 8 days after exposure.<br>Body weights were unaffected.<br>No adverse macroscopic findings were recorded, but the extent to which the respiratory tract was examined was unclear. |
| **Acute Dermal** | | |
| **Method** | **LD50** | **Observations and remarks** |
| Rats, Sprague-Dawley, 5/sex/group<br>2000 mg/kg, semi-occlusive, exposure for 24 hours<br>14-day observation period<br>87.27% cc : 10.23% ct, purity 97.5%<br>Japanese guideline (no deviation from OECD 402), GLP<br>Kureha Corporation (1989) [20] | > 2000 mg/kg | There were no deaths, no clinical signs of toxicity or abnormal gross necropsy findings. |

### 4.2.1 Non-human information

#### 4.2.1.1 Acute toxicity: oral

Two acute oral studies have been conducted, one in rats and one in mice. $LD_{50}$ values of 1338 (male rats), 888 (female rats), 537 (male mice) and 468 (female mice) were obtained.

#### 4.2.1.2 Acute toxicity: inhalation

Two acute inhalation studies with ipconazole administered as a dust undiluted or with a carrier have been conducted. The 4-hour $LC_{50}$ values were > 1.88 mg/l and > 3.53 mg/l.

#### 4.2.1.3 Acute toxicity: dermal

The dermal $LD_{50}$ in a limit-dose test in rats was > 2000 mg/kg.

#### 4.2.1.4 Acute toxicity: other routes

No information.

#### 4.2.2 Human information

No information.

#### 4.2.3 Summary and discussion of acute toxicity

See section 4.2.4.

#### 4.2.4 Comparison with criteria

In two acute oral studies, conducted in rats and mice, $LD_{50}$ values ranged from 468 (female mice) to 1338 (male rats). These values fall with the range for acute oral toxicity category 4 under CLP ( > 300 mg/kg, ≤ 2000 mg/kg). It is therefore proposed that ipconazole should be classified as Acute Tox 4 – H302.

In two acute inhalation studies, concentrations of ipconazole of up to 3.53 mg/l did not result in any deaths. No classification is proposed for acute inhalation toxicity.

The $LD_{50}$ value obtained from an acute dermal study was above the classification cut-off (≤ 2000 mg/kg) in the CLP Regulation. No classification is proposed for acute dermal toxicity.

#### 4.2.5 Conclusions on classification and labelling

| |
|---|
| **Acute Tox 4; H302 – Harmful if swallowed** |

### 4.3 Specific target organ toxicity – single exposure (STOT SE)

#### 4.3.1 Summary and discussion of Specific target organ toxicity – single exposure

The information gained from the acute oral and dermal toxicity studies (section 4.2) did not indicate that ipconazole resulted in toxicity to specific organs after a single exposure.

There were some clinical signs indicative of respiratory tract irritation upon acute inhalation exposure, which included red followed by clear nasal discharge and moist rales. Data from medical surveillance of manufacturing plant personnel and clinical cases / poisoning incidents did not report respiratory irritation in humans.

#### 4.3.2 Comparison with criteria

STOT-SE is divided into three categories. Categories 1 and 2 are assigned on the basis of significant or severe toxicity that is non-lethal and their criteria include guidance cut-off values. From the available acute studies, there was no evidence that a single exposure to ipconazole resulted in toxicity to specific organs, and thus a classification in category 1 or 2 is not appropriate.

STOT-SE 3 is reserved for transient target organ effects, which are limited to respiratory tract irritation and narcotic effects. There were no indications of ipconazole having a narcotic effect in the available acute studies. The criteria for respiratory tract irritation are largely based on human data; such data was limited but did not raise concern for respiratory irritation. In a nose-only inhalation study in rats, clinical signs included a red nasal discharge for several days followed by a clear discharge and rales, whereas in a whole-body study in rats the only sign of respiratory tract

irritation was a slight nasal discharge immediately following exposure. Dyspnoea and rhinitis, which are given as clinical signs of irritation in the CLP criteria, were not reported. There were no histopathology investigations to support these findings. In a 28-day repeated-dose inhalation study in which ipconazole was administered at concentrations up to 1 mg/l (section 4.7.1.2.), clinical signs of respiratory tract irritation were not reported. In this study, histopathological changes were noted in the larynx of animals after 2 to 4 weeks of exposure, but it is not known if they were the result of acute or only repeated exposure. Upon repeated-dose administration by the oral and dermal routes (section 4.7.1.), indications of gastro-intestinal irritation were apparent (those observed in the dermal study being attributed to oral ingestion), but again it was not possible to conclude if these were due to acute or repeated exposure. In the acute oral studies (section 4.2), the predominant finding in animals that died after a single exposure was gastrointestinal pathology, although this appeared to be of a different nature from that observed in the repeated-dose studies. Furthermore, ipconazole did not meet the criteria for classification for skin or eye irritation (section 4.4). Overall, it is concluded that there is insufficient evidence for respiratory tract irritation following a single exposure, and so classification as STOT-SE Category 3 is not appropriate.

### 4.3.3 Conclusions on classification and labelling

**Not classified (conclusive but not sufficient for classification)**

## 4.4 Irritation

### 4.4.1 Skin irritation

The potential of ipconazole to cause skin irritation has been tested in rabbits.

**Table 11: Summary table of relevant skin irritation studies**

| Method | Results | Remarks | Reference |
|---|---|---|---|
| Rabbit, Japanese white, 6 males.<br>4-hour exposure of ipconazole as supplied under a semi-occlusive dressing.<br>88.5% cc : 9.1% ct, purity 97.6%.<br>Comparable to OECD 404, but six animals used. GLP. | Very slight erythema (score 1) was recorded in two animals, one hour after patch removal; reversed by 24 hours after patch removal. No other reactions were observed. | Not irritant | Kureha Corporation (1997) [21] |

#### 4.4.1.1 Non-human information

One skin irritation study has been conducted, in rabbits, in accordance with a Japanese test method guideline published in 1984. Skin effects were graded based on Japanese Guidelines on Agricultural Chemicals at 1, 24, 48 and 72 hours after patch removal. Only minimal effects were observed in two animals (score 1 = barely perceptible), with no reactions in any other animals. The effects were rapidly reversible. The primary cutaneous irritation score was 0.1 (not irritating).

#### 4.4.1.2 Human information

No information.

#### 4.4.1.3 Summary and discussion of skin irritation

See section 4.4.1.4.

#### 4.4.1.4 Comparison with criteria

In the one available study, skin reactions in rabbits were scored in accordance with a Japanese guideline. The only reaction observed was a very slight erythema in 2/6 animals at one hour after removal of the patch (primary cutaneous irritation score = 0.1). For a study conducted with six animals, two approaches can be used to evaluate the data in the context of the CLP criteria: the overall average for all animals; or the average score per animal (Skin Irritant Category 2 is applied if 4/6 rabbits show a mean score of 2.3 or above). Ipconazole did not, therefore, meet the criteria for classification as a skin irritant under the CLP Regulation.

#### 4.4.1.5 Conclusions on classification and labelling

**Not classified (conclusive but not sufficient for classification)**

### 4.4.2 Eye irritation

The potential of ipconazole to cause eye irritation has been tested in rabbits.

**Table 12: Summary table of relevant eye irritation studies**

| Method | Results | Remarks | Reference |
|---|---|---|---|
| Rabbits, Japanese white, 9 males.<br>In one group (3 animals), treated eyes were washed with purified water 2 minutes after application. In the second group (6 animals), the treated eye remained unwashed for 24 hours after treatment. Ipconazole was applied as supplied.<br>88.5% cc : 9.1% ct, purity 97.6%.<br>Comparable to OECD 405, but included a treatment group where treated eyes were washed shortly after treatment. GLP. | *Group 1: washed eyes*<br>Mean scores for 24, 48 and 72 hours for each of the 3 animals were: corneal opacity (0.33, 0.67, 0.67); iris lesion (0, 0, 0); conjunctival redness (1, 1, 1); chemosis (0.3, 0.3, 0.3).<br>*Group 2: unwashed eyes*<br>The mean scores (6 animals) for corneal opacity, iris lesions and redness of the conjunctiva and chemosis at 24, 48 and 72 hours were 0.4, 0, 0.9 and 0.3. | Reactions were fully reversible in all animals by 7 days. | Kureha Corporation (1997) [22] |

#### 4.4.2.1 Non-human information

One eye irritation study has been conducted, in rabbits, in accordance with a Japanese test method guideline published in 1984. Two groups of animals were included, one in which the eyes were rinsed 2 minutes after application of the test substance, and one in which the eyes were unwashed for 24 hours after application. Irritant effects were graded based on the Draize method at 1, 24, 48 and 72 hours and 4 and 7 days after instillation of the test substance. In the group with unwashed eyes, corneal opacity (mainly grade 0-1, but grade 2 in one animal at 24 hours) was reported in 4/6 animals. Conjunctival redness and chemosis (grade 1) and discharge (grade 1-3) occurred in all animals. Congestion of the iris (grade 1) was observed in one animal. The scores obtained in the

unwashed eyes were similar to those recorded in the washed eyes. All reactions had fully resolved by day 7.

#### 4.4.2.2 Human information

No information.

#### 4.4.2.3 Summary and discussion of eye irritation

See section 4.4.2.4.

#### 4.4.2.4 Comparison with criteria

The guidance on the application of the CLP criteria recommends that, for a study on 6 animals, classification for eye irritation Category 2 is appropriate if at least 4 out of 6 rabbits show a mean score of ≥ 1 for corneal opacity; and/or ≥ 1 for iris lesions; and/or ≥ 2 for conjunctival erythema; and/or ≥ 2 for conjunctival swelling. These classification criteria were not met. Therefore, classification for eye irritation is not appropriate.

#### 4.4.2.5 Conclusions on classification and labelling

**Not classified (conclusive but not sufficient for classification)**

### 4.4.3 Respiratory tract irritation

See section 4.3.

## 4.5 Corrosivity

Ipconazole was not corrosive when tested for skin and eye irritation [21, 22].

### 4.5.1 Conclusions on classification and labelling

**Not classified (conclusive but not sufficient for classification)**

## 4.6 Sensitisation

### 4.6.1 Skin sensitisation

The potential of ipconazole to induce skin sensitisation has been investigated in one guinea pig study.

**Table 13: Summary table of relevant skin sensitisation studies**

| Species/Method | Doses | No. sensitised/total no. | Result | Reference |
|---|---|---|---|---|
| Guinea pigs, Dunkin-Hartley, 20 in test groups, 10 in controls.<br>Magnusson and Kingman maximisation test.<br>88.5% cc: 9.1% ct, purity 97.6%.<br>OECD 406, GLP | Induction:<br>- Intradermal: 2.5% in paraffin oil<br>- Topical: 50% in white petrolatum<br>Challenge: 50% in white petrolatum | Test: 0/20<br>Ipconazole negative control: 0/20<br>Positive control: 10/10 animals had grade 3 reactions to 2,4-dinitrochlorobenzene (DNCB)<br>DNCB negative control: no reactions (5/10) or discrete or patchy erythema (grade 1, 5/10). | Negative | Kureha Corporation (1997) [23] |

#### 4.6.1.1 Non-human information

The potential of ipconazole to induce skin sensitisation has been investigated in one guinea pig adjuvant method, the maximisation test. No dermal responses were recorded in any of the test animals, whereas the positive control animals all reacted strongly.

#### 4.6.1.2 Human information

No information.

#### 4.6.1.3 Summary and discussion of skin sensitisation

Information obtained from the one available study indicated that ipconazole was not a skin sensitiser.

#### 4.6.1.4 Comparison with criteria

Ipconazole did not meet the criteria (≥ 30% of animals responding in an adjuvant assay) for classification for skin sensitisation.

#### 4.6.1.5 Conclusions on classification and labelling

**Not classified (conclusive but not sufficient for classification)**

### 4.6.2 Respiratory sensitisation

There is no information available on the potential of ipconazole to induce respiratory sensitisation.

#### 4.6.2.1 Conclusions on classification and labelling

| |
|---|
| **Not classified (data lacking)** |

## 4.7 Repeated dose toxicity

The repeated-dose toxicity of ipconazole has been investigated in rats, mice and dogs by the oral, dermal and inhalation routes.

### 4.7.1 Non-human information

#### 4.7.1.1 Repeated-dose toxicity: oral

##### 4.7.1.1.1 Rat

A 28-day and a 90-day repeated-dose study by the oral route are available in the rat.

**Table 14.1: Summary table of relevant repeated-dose toxicity studies in the rat (oral)**

| Method | Dose Levels | Observations and Remarks |
|---|---|---|
| Oral (dietary) for 28 days<br>Rats, Han Wistar, 5/sex/group<br>91.7% cc : 6.7% ct, purity 98.4%<br>OECD 407, GLP<br>Kureha Corporation (2003) [24] | 0, 300, 1000, 3000 ppm, equivalent to males = 0, 30.5, 94.5, 208-273 mg/kg/d<br>Females = 0, 31.3, 91.0, 164-236mg/kg/d<br>The 3000 ppm group was terminated after 3 weeks because of excessive toxicity. | *3000 ppm*<br>Animals showed marked weight loss or low weight gain, reduced food consumption and indications of the test diet being unpalatable. Females were affected to a greater extent than males; clinical signs in females included thin build, piloerection, excessive chewing, salivation, hunched posture, reddening of the buccal cavity and forepaws, exfoliation on the hind paws and hair loss of the head and forelimbs. Signs in males were restricted to reddening and encrustations in the buccal cavity. Other findings included changes in haematology and clinical chemistry parameters, increased liver and spleen weights, decreased thymus and uterus weight, minimal adipose tissue, moderate/marked myometrial atrophy in the uterus (5/5 animals) and changes in the non-glandular region of the stomach (fore-stomach) (marked epithelial hyperplasia and hyperkeratosis, moderate erosion, slight to moderate ulceration and slight sub-epithelial inflammation in females). This group was terminated at the end of week 3.<br>*300, 1000 ppm*<br>Clinical signs were restricted to females at 1000 ppm and included hair loss (head and dorsal body surface), encrustations in the buccal cavity, exfoliation on the forelimbs/dorsal body surface and individual instances of thin build, piloerection or reddening in the buccal cavity.<br>In females at 300 ppm and both sexes at 1000 ppm, body weights were reduced compared with controls (reduced body weight gain or body weight loss over the first week) and food consumption was lower.<br>Haematology investigations showed the presence of hyperchromatic erythrocytes in all females and 1 male at 1000 ppm and 2 females at 300 ppm. Lymphocyte counts were slightly higher than controls in females at<br>1000 ppm. There were some blood chemistry findings in females at 1000 ppm (increased aspartate aminotransferase (AST), γ-glutamyl transpeptidase (GGT) and cholesterol, decreased urea, low plasma albumin:globulin ratio). Urinalysis showed low urine volume, low electrolyte levels (both sexes at 1000 ppm plus females at 300 ppm) and high urinary pH (females at 1000 ppm).<br>The only notable macroscopic findings were in females at 1000 ppm and comprised thin appearance (2/5), marked hair loss (1/5) and depressions in the stomach (3/5). Changes in organ weights were also restricted to females at 1000 ppm and consisted of increased relative liver (15%* higher than controls), spleen (34%** ↑) and thyroid (40% ↑) weights.<br>Histopathology revealed findings indicative of an irritant effect in the oesophagus (hyperkeratosis**, all animals at 1000 ppm, no cases in other groups) and non-glandular region of the stomach (epithelial hyperplasia, hyperkeratosis, erosion, ulceration, sub-epithelial inflammation in males at 1000 ppm*, females at 300* and 1000** ppm). All females at 1000 ppm had prominent sinusoidal lining cells in the adrenals.<br>The NOAEL in males was 300 ppm (30.5 mg/kg/d); a NOAEL was not identified in females. |

| Method | Dose Levels | Observations and Remarks |
|---|---|---|
| Oral (dietary) for 90 days<br>Rats, Han Wistar, 16/sex/group<br>91.7% cc : 6.7% ct, purity 98.4%<br>OECD 408 & 424, GLP<br>Kureha Corporation (2006) [25] | 0, 30, 70, 150, 300 ppm, equivalent to<br>males = 0, 2.5, 5.8, 12.6, 25.9 mg/kg/d<br>females = 0, 3.1, 7.0, 15.4, 33.2 mg/kg/d<br>A further group of 16 males received 600 ppm (= 52.2 mg/kg/d) | There were no deaths or clinical signs of toxicity.<br>There was an overall reduction in body weight gain at 600 ppm in males (14%*) and 300 ppm in females (17%*) that was not associated with a sustained decrease in food intake.<br>There were no treatment-related changes in ophthalmological, haematology, blood chemistry nor urinalysis parameters.<br>The main macroscopic finding was a thickened area in the stomach of 1 male (150 ppm) and 1 female (300 ppm). This female also had a depression in the wall of the stomach. In 300 ppm females, the absolute weight of the adrenals was 19% lower than controls and the relative weight 11% lower; also, the absolute weight of uterus and cervix was 33% lower, relative weight 28% lower. In males at 600 ppm, absolute thymus weight was reduced by 24% and relative weight by 13%.<br>Upon histopathology, substance-related effects were observed in both males and females. Epithelial hyperplasia of the non-glandular fore-stomach was observed in males at ≥ 150 ppm (up to 4/10 animals at 600 ppm) and females at 300 ppm (5/10*); in the high-dose females there was also one case of ulceration of the fore-stomach and one of hyperkeratosis. There were no substance-related lesions of the glandular stomach or oesophagus. Kidney findings were restricted to females: ↑ incidence of corticomedullary mineralisation (total 0/10, 2/10, 3/10, 6/10*, 9/10* at 0, 30, 70, 150 and 300 ppm, graded minimal or slight) and cortical scarring only at 300 ppm (3/10). Other findings in 300 ppm females were ↓ incidence of luminal dilatation of the uterus (1/10 compared with 4/10 of controls) and ↓ incidence of epithelial keratinisation of the vagina (3/10 compared with 8/10 of controls). In males at 600 ppm, incidences of focal inflammation in the liver (4/10 compared with 1/10 of controls), developmental cysts in the pituitary and inflammation of the prostate were ↑.<br>Neurotoxicological investigations were negative and neurohistopathology of 6 animals/sex from the control and high-dose groups did not reveal treatment-related changes. Brain weights and anatomical measurements were unaffected.<br>The NOAELs were set at 70 ppm (7.0 mg/kg/d) for females and 300 ppm (25.9 mg/kg/d) for males. |

*N.B. The values for NOAEL are provided for information only; they have been agreed by a PRAPeR Expert Meeting. * Statistically significant at p ≤ 0.05. ** Statistically significant at p ≤ 0.01*

The major adverse effects in the two repeated-dose oral studies in the rat were on the non-glandular region of the stomach (fore-stomach) and comprised epithelial hyperplasia, hyperkeratosis, ulceration and erosion. These adverse effects occurred mainly from 300 ppm (approximately 30 mg/kg/d) in both studies, and were recorded from three weeks of exposure. Hyperkeratosis of the oesophagus was also reported at 1000 ppm (94.5 / 91.0 mg/kg/d) in the 28-day study. The study authors attributed these lesions to an irritant effect of ipconazole.

The other major target organ was the kidney: in the 90-day study, a statistically significant increase in the incidence of corticomedullary mineralisation (graded minimal or slight) occurred from 150 ppm (15.4 mg/kg/d) and a non-statistically significant increase in the incidence of cortical scarring occurred at 300 ppm (33.2 mg/kg/d), both in females. The study author proposed that these changes were related to non-specific toxicity. Historical control data provided by the applicant under Directive 91/414/EEC in the same rat strain and with dietary exposure indicated a maximum incidence per study for slight corticomedullary mineralisation of 1/10 females. In comparison, the incidence of these lesions graded as slight in the present study was 0/10, 0/10, 0/10, 1/10, 4/10 at 0, 30, 70, 150, 300 ppm; thus only the increase in the high-dose group is considered in this report to be treatment-related. Although there were some changes in urinary parameters in the 28-day study, there were no associated gross or histopathological findings in the kidney or bladder in that study.

Other findings included effects on the uterus (reduction in the incidence of luminal dilatation and uterine/cervix weight at 300 ppm / 33.2 mg/kg/d for 90 days, myometrial atrophy at 3000 ppm / 164-236 mg/kg/d for 21 days) and vagina (reduction in the incidence of epithelial keratinisation at 300 ppm / 33.2 mg/kg/d for 90 days). The review under Directive 91/414/EEC concluded that the uterus findings in the 90-day study (organ weight and pathology) were incidental, being associated with differences in the predominant stage of the oestrus cycle in the different dose groups, and not substance-related. These findings are thus not considered further in this classification report. There were some changes in organ weights without concomitant histopathological changes, the exception being the adrenals (prominent sinusoidal lining cells at 1000 ppm / 91 mg/kg/d in females).

#### 4.7.1.1.2 Mouse

A 28-day and a 90-day repeated-dose study by the oral route are available in the mouse.

**Table 14.2: Summary table of relevant repeated-dose toxicity studies in the mouse (oral)**

| Method | Dose Levels | Observations and Remarks |
|---|---|---|
| Oral (dietary) for 28 days<br>Mouse, CD-1, 6/sex/group<br>91.7% cc : 6.7% ct, purity 98.4%<br>OECD 407, GLP<br>Kureha Corporation (2006) [26] | 0, 250, 500, 1000 ppm,<br>equivalent to<br>males: 0, 44.8, 88.1, 151.8 mg/kg/d<br>females: 0, 53.4, 95.9, 193.8 mg/kg/d<br>A further group received 2000 ppm (157.9 mg/kg/d in males, 294.7 mg/kg/d in females during week 1) but was terminated after 10 days owing to excessive toxicity. | *2000 ppm*<br>This group was terminated on day 10. There were indications that the treated diet was unpalatable, and there was marked weight loss and low food consumption. Gross findings were pale livers in males, thickened stomach in females and congested lungs and bronchi in both sexes. At histopathology, there was epithelial hyperplasia and sub-epithelial inflammation in the fore-stomach and oesophagus in both sexes, plus hyperkeratosis in the oesophagus. Centrilobular hepatocyte hypertrophy and centrilobular hepatocyte vacuolation were identified in the livers of males and females, together with the presence of hepatocyte fat. One female had a moderate area of hepatocyte necrosis. There was a slight decrease in cellularity in red cell pulp in the spleen in all animals.<br>*250, 500, 1000 ppm*<br>There were no deaths or clinical signs of toxicity.<br>Over the first 3 days, animals of both sexes at 1000 ppm lost weight and males at 500 ppm had a low weight gain; thereafter, weight gain amongst all groups was normal. Food consumption was reduced in males and females at 500 and 1000 ppm.<br>There were no dose-related effects on haematology parameters.<br>Total cholesterol was markedly reduced: 3.39, 2.46**, 1.83**, 0.74** mmol/l in males and 2.27, 1.93, 1.39**, 0.51** mmol/l in females at 0, 250, 500, 1000 ppm. Triglyceride concentrations were not affected. Alkaline phosphatase (ALP), alanine aminotransferase (ALT) and AST levels were raised in both sexes at 100 ppm. Females at 1000 ppm showed low albumin concentrations and males at 500 or 1000 ppm showed low total protein concentrations.<br>Relative liver and spleen weights were higher than controls at 500 and 1000 ppm, associated with enlarged spleen and pale liver at the same doses. One female at 1000 ppm had thickening of the limiting ridge in the stomach.<br>Findings at histopathology were recorded in the liver, oesophagus and fore-stomach and are summarised below. There were no adverse microscopic findings in the spleen. The thyroid, thymus, adrenals and uterus were not examined microscopically. |

<table>
<tr><th>Method</th><th>Dose Levels</th><th>Observations and Remarks</th></tr>
<tr><td></td><td></td><td>
<u>Summary of histopathological findings</u>
<table>
<tr><th></th><th colspan="4">Males</th><th colspan="4">Females</th></tr>
<tr><td>ppm<br>mg/kg/d</td><td>0<br>0</td><td>250<br>44.8</td><td>500<br>88.1</td><td>1000<br>151.8</td><td>0<br>0</td><td>250<br>53.4</td><td>500<br>95.9</td><td>1000<br>193.8</td></tr>
<tr><td>n</td><td>6</td><td>6</td><td>6</td><td>6</td><td>6</td><td>6</td><td>6</td><td>6</td></tr>
<tr><td><u>Liver</u></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td></tr>
<tr><td>focal hepatocyte necrosis</td><td>0</td><td>0</td><td>0</td><td>2</td><td>0</td><td>0</td><td>1</td><td>1</td></tr>
<tr><td>centrilobular hepatocyte hypertrophy</td><td>0</td><td>1</td><td>6**</td><td>6**</td><td>0</td><td>0</td><td>0</td><td>6**</td></tr>
<tr><td>panacinar fat</td><td>0</td><td>1</td><td>2</td><td>5</td><td>0</td><td>2</td><td>6**</td><td>6**</td></tr>
<tr><td>centrilobular hepatocyte vacuolation</td><td>0</td><td>2</td><td>4</td><td>6**</td><td>0</td><td>4</td><td>6**</td><td>6**</td></tr>
<tr><td><u>Oesophagus</u></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td></tr>
<tr><td>epithelial hyperplasia</td><td>0</td><td>0</td><td>0</td><td>5*</td><td>0</td><td>0</td><td>0</td><td>3</td></tr>
<tr><td>sub-epithelial inflammation</td><td>0</td><td>0</td><td>0</td><td>5*</td><td>0</td><td>0</td><td>0</td><td>3</td></tr>
<tr><td><u>Fore-stomach</u></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td></tr>
<tr><td>epithelial hyperplasia</td><td>0</td><td>0</td><td>0</td><td>4</td><td>0</td><td>0</td><td>2</td><td>3</td></tr>
<tr><td>sub-epithelial inflammation</td><td>0</td><td>0</td><td>0</td><td>0</td><td>0</td><td>0</td><td>2</td><td>3</td></tr>
</table>
A NOAEL was not identified.
</td></tr>
<tr><td>Oral (dietary) for 90 days<br>Mouse, CD-1, 9/sex/group<br>OECD 408, GLP<br>91.7% cc : 6.7% ct, purity 98.4%<br>Kureha Corporation (2005) [27]</td><td>0, 30, 150, 500 ppm, equivalent to<br>males: 0, 4.4, 20.2, 69.7 mg/kg/d<br>females: 0, 5.1, 25.4, 90.8 mg/kg/d</td><td>
There were no substance-related deaths or clinical signs of toxicity.<br><br>
At 500 ppm, body weight gains were mainly affected during week 1. Overall body weight gains of males were reduced by 53% and of females by 22%. Reductions in food consumption occurred in weeks 1 and 2. There were no clear dose-related effects on body weight gains at lower doses.<br><br>
Total white blood cell counts were reduced by 32-33% in females at 150 and 500 ppm. Blood chemistry investigations showed that AST was slightly raised in females at 500 ppm. Plasma cholesterol concentrations were reduced (3.7, 3.41, 2.87*, 1.71** mmol/l in males and 2.08, 2.46, 1.73, 1.24** mmol/l in females at 0, 30, 150, 500 ppm). Plasma triglyceride levels were unaffected. Total protein and albumin concentrations in females at 500 ppm were reduced.<br><br>
Absolute (females, by 6%) liver weights and those relative to body weight (both sexes, by 24-29%) were increased at 500 ppm. Thymus weight was reduced in females at 500 ppm (absolute by 20%, relative by 14%). At 500 ppm, uterus + cervix weight was decreased (absolute by 32%, relative to body weight by 26%), but for only one of these females was the absolute uterus + cervix weight below the historical control range.
</td></tr>
</table>

| Method | Dose Levels | Observations and Remarks |
|---|---|---|

Upon gross examination, 1 male and 1 female at 500 ppm had pale livers. There were no macroscopic effects on the lens (ophthalmological examination was not performed).

Findings at histopathology were recorded in the liver, fore-stomach and adrenals and are summarised below. There were no substance-related findings in the glandular stomach or oesophagus (although the latter observation was based on only one section per mouse).

| | Males | | | | Females | | | |
|---|---|---|---|---|---|---|---|---|
| **ppm** | 0 | 30 | 150 | 500 | 0 | 30 | 150 | 500 |
| **mg/kg/d** | 0 | 4.4 | 20.2 | 69.7 | 0 | 5.1 | 25.4 | 90.8 |
| n | 9 | 9 | 9 | 9 | 9 | 9 | 9 | 9 |
| Liver | | | | | | | | |
| generalised fatty deposits | 1 | 0 | 2 | 5 | 0 | 1 | 2 | 5* |
| centrilobular fatty deposits | 3 | 1 | 1 | 1 | 0 | 0 | 1 | 3 |
| centrilobular hepatocyte hypertrophy | 7 | 7 | 7 | 8 | 0 | 0 | 1 | 0 |
| hepatocyte vacuolation | 1 | 0 | 6 | 9** | 0 | 0 | 1 | 9** |
| Adrenal gland | | | | | | | | |
| vacuolation of X-zone | 0 | 0 | 0 | 0 | 6 | 9 | 9 | 9 |
| cortical vacuolation | 1 | 0 | 0 | 4 | 0 | 0 | 0 | 0 |
| Fore-stomach | | | | | | | | |
| focal epithelial hyperplasia | 0 | 0 | 0 | 2 | 0 | 0 | 0 | 0 |
| sub-epithelial inflammation | 1 | 0 | 0 | 4 | 0 | 0 | 0 | 1 |

The NOAEL was 30 ppm (4.4 mg/kg in males, 5.1 mg/kg/d in females).

*N.B. The values for NOAEL are provided for information only; they have been agreed by a PRAPeR Expert Meeting. * Statistically significant at $p \leq 0.05$. ** Statistically significant at $p \leq 0.01$*

The major target organs in the mouse were the liver and the non-glandular region of the stomach (fore-stomach) after administration of ipconazole for 28 and 90 days.

The effects on the liver comprised increased organ weights, macroscopic effects (pale liver) and microscopic changes (hepatocellular hypertrophy, necrosis, fatty deposits and hepatocyte vacuolation) mainly from 500 ppm (88.1 mg/kg/d in males, 95.9 mg/kg/d in females) in the 28-day study and from 150 ppm (20.2 mg/kg/d in males, 25.4 mg/kg/d in females) in the 90-day study.

Increased liver weight of the extent seen (absolute = 6%) and hepatocellular hypertrophy are regarded as adaptive changes and thus not relevant to classification. The other microscopic changes are adverse and thus potentially relevant to classification. Increased incidences of hepatocyte vacuolation (from 20.2 mg/kg/d in the 90-day study) and fatty liver (at 69.7 / 90.8 mg/kg/d in the 90-day study) were reported in both studies. The author of the 90-day study noted that hepatocyte fatty vacuolation is a toxic response to disruption of the triglyceride cycle, and considered that it was responsible for changes in clinical chemistry (decreased plasma cholesterol, observed in both studies) and pale livers (observed in both studies).

Lesions were recorded in the fore-stomach in both studies and comprised epithelial hyperplasia and sub-epithelial inflammation at 69.7 mg/kg/d in males of the 90-day study. The same lesions were also observed in the oesophagus in the 28-day study at 1000 ppm (151.8 / 193.8 mg/kg/d). These findings were consistent with an irritant effect of ipconazole.

Adrenal gland changes were reported in the 90-day study. An increased incidence of X-zone vacuolation was observed in all female treatment groups. However, historical control data provided by the applicant under Directive 91/414/EEC indicated that this is a common finding in CD-1 mice (incidences of 0-100%, mean 58% in 13-week studies) and so it will not be considered further in this report. Adrenal cortical vacuolation occurred in 4/9 male mice at 500 ppm (69.7 mg/kg/d) and is considered to be of toxicological relevance.

Slight effects on red blood cell parameters (28-day study) and plasma protein levels (28-day and 90-day studies) might have been related to poor nutrition (decreased food consumption and body weight loss or low body weight gain). The decrease in uterus plus cervix weight might have been accounted for by general toxicity; additionally, most individual values were within the historical control range, and so the toxicological relevance of this effect is uncertain.

#### 4.7.1.1.3 Dog

Oral repeated-dose studies are available in the dog with durations of 22 days to one year.

**Table 14.3: Summary table of relevant repeated-dose toxicity studies in the dog (oral)**

| Method | Dose Levels | Observations and Remarks |
|---|---|---|
| Oral (capsule) for up to 22 days<br>Dog, beagle, 1/sex/group<br>91.7% cc: 6.7% ct, purity 98.4%<br>Kureha Corporation (2005) [28] | Phase I: escalating dose of 10, 20, 40, 80, 120, 160 mg/kg/d for 3, 4, 3, 5, 5, 2 days, respectively<br>Phase II: 160 mg/kg/d for 14 days | Maximum tolerated dose study.<br>*Phase 1: escalating-dose phase*<br>There were no adverse effects at 10 or 20 mg/kg/d. At 40 mg/kg/d, one male had liquid faeces. At 80 mg/kg/d, grey-coloured loose faeces occurred in both dogs and from this dose there was weight loss and low food consumption. Reddening of the inside pinna and gums was recorded at 120 mg/kg/d, together with reddened skin around the eyes and yellow discharge from the eyes at 160 mg/kg/d. High liver and kidney weights were recorded in both animals, also pale liver and congestion on the mucosa of the caecum and colon; the female had a mottled liver.<br>*Phase 2: constant-dose phase (160 mg/kg/d)*<br>Effects comprised: loose/liquid faeces and emesis from day 1, occasional under-activity/unsteady gait, reddening of the skin, ocular discharge body weight loss, slightly high neutrophil, haematocrit, haemoglobin and RBC counts. Blood chemistry at termination revealed increased ALPalkaline phosphatase, ALT and GGT. Cholesterol and triglycerides were low in both animals, with slightly raised protein in females and slightly lower albumin in males. Liver (both animals) and kidney (male) weights were increased. The liver of the male was enlarged and that of the female was pale and mottled. Both animals had a congested area in the mucosa of the caecum and colon. |
| Oral (capsule) for 28 days<br>Dog, beagle, 2/sex/group<br>OECD 409 used as a general guide, GLP<br>91.7% cc: 6.7% ct, purity 98.4%<br>Kureha Corporation (2005) [29] | 0, 24, 60, 150 mg/kg/d | Range-finding study.<br>1/2 males (day 22) and 2/2 females (day 24) at 150 mg/kg/d were sacrificed, having shown poor food consumption, body weight loss or absence of weight gain, poor condition associated with ocular discharge (from day 20).<br>Clinical signs at 150 mg/kg/d comprised inappetance, loose-liquid faeces, reddening of the skin (inside the ears, gums, lips, abdomen and around the eyes), swollen eyelids and evidence of pain occurred from day 22. At 60 mg/kg/d, there were occasional instances of loose/liquid faeces and inappetance; reddening of the gums, lips, ear and muzzle and ocular discharge were recorded in both sexes in the final week of the study. The only clinical sign at 24 mg/kg/d was reddening inside the ears on days 27 and 28.<br>Reduced weight gain or body weight loss was apparent in all treatment groups.<br>In the 150 mg/kg/d group, ophthalmological examination in week 4 could only be conducted on the one surviving male; this animal had moderate mucoid discharge, hyperaemia and congested blood vessel in the conjunctiva in both eyes and slight blepharoedema of both eyelids. The same findings occurred in one male (mucoid discharge classed as severe) and one female (without blepharoedema) at 60 mg/kg/d. The animals sacrificed early all showed opacities in the eyes upon necropsy.<br>In the animals sacrificed early, the haematology findings were: slightly increased haematocrit and haemoglobin concentration, markedly increased white blood cells, increased platelet counts and shorter activated partial thromboplastin time (all in males only). There were no toxicologically |

| Method | Dose Levels | Observations and Remarks |
|---|---|---|
| | | relevant haematology findings in the animals that survived to week 4.<br><br>In the animals sacrificed early, the blood chemistry findings were: increased ALT, AST, total bilirubin, urea, triglycerides, total protein; decreased creatine phosphokinase, cholesterol, sodium, chloride and albumin:globulin ratio. In the animals that survived to week 4, there was increased ALT activity from 24 mg/kg/d, increased ALPalkaline phosphatase ≥ 60 mg/kg/d, and increased GGT, urea, creatinine and total bilirubin.<br><br>Relative weights of the liver (also in the females), kidney and adrenal glands of the early-sacrificed male were high and the relative thymus weight reduced. The male at 150 mg/kg/d that survived to 4 weeks had an increased relative adrenal weight (78% ↑) and reduced relative thymus weight (58% ↓) but no effect on liver or kidney weights. Relative liver and kidney weights were also increased at 24 mg/kg/d. Reduced thymus weights were observed in animals from 24 mg/kg/d.<br><br>Additional findings at necropsy of the early-sacrificed animals were moist fur around the eyes, yellow colouration of several tissues, haemorrhage in the femoro-tibial joints, abnormal gastrointestinal tract contents and depressions/dark areas in the liver, gall bladder and stomach. The urinary bladder contents were an abnormal colour (dark orange). In the 150 mg/kg/d male that survived to week 4, abnormal findings were noted in the stomach, ileum, caecum, liver and gall bladder. At 60 mg/kg/d, swollen eyelids and scabbing or hair-loss around the eyes and isolated incidences of abnormal findings in the gall bladder or stomach and enlarged or pale liver were observed.<br><br>Histopathological examination of the early-sacrificed animals revealed effects in the liver (bile duct proliferation, slight focal necrosis, prominent inflammatory cells in the portal area, moderate multi-focal pigment-laden hepatocytes and pigment-laden Kupffer cells), thymus (marked thymic cortical atrophy in the male) and lungs (slight or moderate multi-focal interstitial pneumonitis in both sexes). In the remaining animals, the same histopathological effects were also observed in the liver (from 24 mg/kg/d), lung (from 60 mg/kg/d) and thymus (at 150 mg/kg/d).<br><br>A NOAEL was not established. |

<table>
<tr>
<td>Oral (capsule) for 90 days<br>Dog, beagle, 4/sex/group<br>OECD 409, GLP<br>91.7% cc: 6.7% ct, purity 98.4%<br>Kureha Corporation (2006) [30]</td>
<td>0, 2, 10, 40 mg/kg/d</td>
<td>
There were no unscheduled deaths. Clinical signs comprised reddening of the skin (typically the ears, eyes, muzzle, neck and gums) from 10 mg/kg/d, and, at 40 mg/kg/d, occasional ocular discharge (2 females from week 2, 1 male from week 4) and hair-loss/thinning around the eye and muzzle (3 males, 3 females). Additionally, one male at 40 mg/kg/d was reported to be thin.<br><br>
Overall body weight gain was significantly reduced at 40 mg/kg/d (males 52%** ↓; females 50%** ↓, largely attributable to 2 females). Food consumption of this dose group was significantly reduced.<br><br>
The following ophthalmological findings, conducted in week 13, were reported in the 40 mg/kg/d group (no such findings reported in any other group):
<table>
<tr><th>40 mg/kg/d</th><th>Males</th><th>Females</th></tr>
<tr><td>n</td><td>4</td><td>4</td></tr>
<tr><td>Cataracts (total)</td><td>3</td><td>4</td></tr>
<tr><td>of posterior capsule</td><td>2</td><td>3</td></tr>
<tr><td>of posterior + anterior capsule</td><td>1</td><td></td></tr>
<tr><td>complete cataract</td><td></td><td>1</td></tr>
<tr><td>Peri-orbital alopecia</td><td>2 (slight)</td><td>3 (slight)</td></tr>
<tr><td>Blepharo-oedema</td><td>1 (slight)</td><td>1 (very slight)</td></tr>
<tr><td>Episcleral blood vessel congestion, vascularisation on dorsal limbus of eye</td><td>1 (1 eye)</td><td></td></tr>
</table>
<br>
There were no notable haematology findings. Investigation of blood chemistry showed that at 40 mg/kg/d there was increased activity of alkaline phosphatase, alanine aminotransferase, aspartate aminotransferase and gamma-glutamyltransferase. Bilirubin was increased in one male at this dose. At 40 mg/kg/d, plasma cholesterol was decreased in males (43%** ↓) and females (50%** ↓). Calcium concentrations were reduced in both sexes in the high-dose group. There were slight reductions in mean triglyceride values at all dose levels in females, but with individual inconsistencies.<br><br>
Urinalysis showed that, at 40 mg/kg/d, there was decreased urine volume, increased specific gravity and increased protein concentration in males; also the urine tended to be darker than that of controls. Bilirubin was present in the urine of one male.<br><br>
The substance-related macroscopic findings of note are summarised below;
</td>
</tr>
</table>

*Summary of macroscopic findings*

| | Males | | | | Females | | | |
|---|---|---|---|---|---|---|---|---|
| **mg/kg/d** | 0 | 2 | 10 | 40 | 0 | 2 | 10 | 40 |
| n | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 |
| Liver | | | | | | | | |
| weight (%) | | | 13 ↑ | 23 ↑ | | | | 10 ↑ |
| rel weight (%) | | | | 23 ↑ | | | | |
| enlargement | | | | 1 | | | | |
| pale colour | | | | 1 | | | | 1 |
| Thymus | | | | | | | | |
| weight (%) | | | | 63 ↓ | | | | 41 ↓ |
| rel weight (%) | | | | 57 ↓ | | | | 28 ↓ |
| ↓ size | | | | 2 | | | | 1 |
| Kidney | | | | | | | | |
| weight (%) | | 14 ↑ | 16 ↑ | 23 ↑ | | | | |
| rel weight (%) | | 14 ↑ | 16 ↑ | 26 ↑ | | | | |
| Dark/black or gelatinous particles in gall bladder | | | 1 | 4 | | | | 1 |
| Hair loss | | | | 3 | | | | 3 |
| Scabbing | | | | 2 | | | | |
| Congested areas in urinary bladder | | | | 1 | | | | 1 |

The substance-related histopathological findings of note are summarised below:

*Summary of histopathological findings:*

| | Males | | | | Females | | | |
|---|---|---|---|---|---|---|---|---|
| **mg/kg/d** | 0 | 2 | 10 | 40 | 0 | 2 | 10 | 40 |
| n | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 |
| Liver | | | | | | | | |
| bile duct proliferation | 0 | 0 | 0 | 2 | 0 | 0 | 0 | 3 |
| centrilobular hypertrophy | 0 | 0 | 3 | 3 | 0 | 0 | 1 | 3 |
| pigment-laden Kupffer cells | 0 | 1 | 1 | 3 | 0 | 0 | 0 | 3 |
| focal necrosis (moderate) | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 |
| Thymus | | | | | | | | |
| reduced cellularity of cortex | 0 | 0 | 0 | 3 | 0 | 0 | 0 | 2 |
| Eyes | | | | | | | | |
| lenticular degeneration | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 |
| anomaly of lenticular fibres | 0 | 0 | 2 | 4 | 0 | 0 | 0 | 0 |
| Adrenals | | | | | | | | |
| cortical vacuolation | 0 | 1 | 1 | 3 | 1 | 1 | 2 | 0 |

The NOAEL was 10 mg/kg/d for males and females.

<table>
<tr>
<td>Oral (capsule) for 1 year<br><br>Dog, beagle, 4/sex/group<br><br>OECD 452, GLP<br><br>91.2% cc: 6.9% ct, purity 98.1%<br><br>Kureha Corporation (2007) [31]</td>
<td>0, 1.5, 5, 20 mg/kg/d</td>
<td>
There were no substance-related deaths, although one male in the 1.5 mg/kg/d group died in week 30.<br><br>
The only clinical sign of note was skin reddening (pinnae, abdomen, tail, legs/paws, muzzle) in 2 males and 1 female at 5 mg/kg/d and all animals at 20 mg/kg/d. This was first observed in weeks 2-3 and, particularly in the high-dose animals, eventually progressed over the whole body.<br><br>
The overall body weight gain of females at 20 mg/kg/d was reduced by 20% (no effect in males). Food consumption was not adversely affected.<br><br>
Ophthalmological examination revealed lenticular opacities in 2 males and 1 female at 20 mg/kg/d (none in any other groups), consisting of a very faint opacity on the posterior capsule of 1 male, a posterior and anterior capsular opacity in the other male and a posterior suture line opacity in the left eye of the female.<br><br>
There were no adverse effects on haematology or urinalysis parameters. At 20 mg/kg/d, blood chemistry showed reduced cholesterol (approximately 20%) in both sexes and reduced calcium in males.<br><br>
Liver weights relative to body weight were increased at 20 mg/kg/d in males (26%**) and females (34%**). Adjusted kidney weights were also reduced in males (12%*, 19%**, 26%** at 1.5, 5, 20 mg/kg/d) and females (21%** at 20 mg/kg/d). There were no clear treatment-related macroscopic findings.<br><br>
The substance-related histopathological findings of note are summarised below:<br><br>
<table>
<tr><td></td><td colspan="4">Males</td><td colspan="4">Females</td></tr>
<tr><td>mg/kg/d</td><td>0</td><td>1.5</td><td>5</td><td>20</td><td>0</td><td>1.5</td><td>5</td><td>20</td></tr>
<tr><td>n</td><td>4</td><td>3</td><td>4</td><td>4</td><td>4</td><td>4</td><td>4</td><td>4</td></tr>
<tr><td><u>Liver</u></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td></tr>
<tr><td>bile duct proliferation</td><td>1</td><td>0</td><td>2</td><td>2</td><td>2</td><td>1</td><td>0</td><td>2</td></tr>
<tr><td>centrilobular hypertrophy</td><td>1</td><td>2</td><td>4</td><td>3</td><td>1</td><td>0</td><td>2</td><td>3</td></tr>
<tr><td><u>Thymus</u></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td></tr>
<tr><td>reduced cellularity of cortex</td><td>0</td><td>0</td><td>0</td><td>2</td><td>0</td><td>1</td><td>0</td><td>1</td></tr>
<tr><td><u>Eyes</u></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td></tr>
<tr><td>lenticular degeneration</td><td>0</td><td>0</td><td>0</td><td>1</td><td>0</td><td>0</td><td>0</td><td>0</td></tr>
<tr><td><u>Adrenals</u></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td></tr>
<tr><td>cortical fatty vacuolation</td><td>1</td><td>1</td><td>1</td><td>4</td><td>0</td><td>1</td><td>1</td><td>4</td></tr>
<tr><td><u>Urinary bladder</u></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td></tr>
<tr><td>inflammatory cell infiltration</td><td>0</td><td>0</td><td>0</td><td>0</td><td>0</td><td>0</td><td>0</td><td>1</td></tr>
<tr><td>transitional cell hyperplasia</td><td>0</td><td>0</td><td>0</td><td>0</td><td>0</td><td>0</td><td>0</td><td>1</td></tr>
</table>
<br>
The NOAEL was 1.5 mg/kg/d for males and females.
</td>
</tr>
</table>

*N.B. The values for NOAEL are provided for information only; they have been agreed by a PRAPeR Expert Meeting. * Statistically significant at p ≤ 0.05. ** Statistically significant at p ≤ 0.01*

After administration of ipconazole to dogs for three weeks to one year, the main target organs were the liver, eyes (lens), thymus and adrenals. Other observations included reddening of the skin and evidence for effects on the kidneys and urinary bladder.

Increased weight, macroscopic and microscopic changes were consistently recorded in the liver in the studies. Many of the blood chemistry findings also related to the liver. The increased plasma alkaline phosphatase and high bilirubin (recorded in the 28-day and 90-day studies) were attributable to the findings in the bile duct. High plasma transaminase and gamma-glutamyltransferase were likely to be related to the centrilobular hypertrophy and, together with the increased weight and hypertrophy, are considered to be an adaptive rather than adverse response. However, there were also clearly adverse effects in the liver, including consistent gross and histopathological changes. Enlarged and/or pale liver was observed at 60 mg/kg/d in the 28-day range-finding study, with isolated incidences also at 40 mg/kg/d in the 90-day study. Adverse histopathological changes were recorded from 24 mg/kg/d in the 28-day range-finding study and at 40 mg/kg/d in the 90-day study (bile duct proliferation, pigment-laden Kupffer cells, moderate focal necrosis in 1/4 females). Macroscopic (depressions/dark areas, dark or gelatinous particles) changes were recorded in the gall bladder in the 28-day and 90-day studies; these were possibly linked to the extensive biliary excretion of ipconazole and its metabolites (section 4.1).

A notable finding in the dog studies was the ocular effects. In the 28-day range-finding study, adverse ophthalmological effects were noted from 60 mg/kg/d and all the animals sacrificed during the third week showed opacities in the eyes. In the 90-day study, all the animals at 40 mg/kg/d had cataracts. Additionally, lenticular degeneration was recorded in 2/4 females at 40 mg/kg/d; in one of these females it was also associated with keratitis, iritis and oedema of the iris. Anomaly of the lenticular fibres occurred in 2/4 males at 10 mg/kg/d and 4/4 males at 40 mg/kg/d. Lenticular opacities and degeneration were also reported in the one-year study, both at 20 mg/kg/d. Lens opacity and lenticular degeneration are possibly linked to the reduced plasma cholesterol that was recorded in these studies, which is consistent with ipconazole's mode of action.

Another effect that was consistent with ipconazole's mode of action was the cortical (fatty) vacuolation of the adrenals that was observed in the 90-day and one-year studies, which suggested an effect on steroid hormone synthesis by the adrenal gland. Increased adrenal weights in the 28-day range-finding study were not further investigated and did not occur in the subsequent studies.

The study authors attributed the effects on the thymus (reduced weight, microscopic evidence of cortical atrophy, reduced cellularity of the cortex) to stress. The cause of interstitial pneumonitis in the range-finding study was not identified.

Increases in kidney weight in the 90-day and one-year studies were considered to be substance-related, but in the absence of any evidence of kidney damage the study authors considered them to be an adaptive response to urinary excretion of ipconazole and/or its metabolites (although it is noted in section 4.1 that urinary excretion is a minor route of excretion). Individual high-dose animals in the 90-day and one-year studies showed effects on the urinary bladder that appeared to be linked to an irritant effect of the substance.

Skin reddening (observed in all studies and occurring from 10 mg/kg/d in the 90-day study) also appeared to be indicative of irritation, but the effect was purely systemic, as there was no source of topical exposure (the substance was administered in capsules). Furthermore, there was no associated histopathological change in the skin nor an effect on leucocyte numbers in the peripheral blood that would suggest a widespread inflammatory change. The effect worsened on continued exposure so that towards the end of the one-year study, the whole body was affected in 2/7 animals at 5 mg/kg/d and 8/8 animals at 20 mg/kg/d. Reddening of the gums, ocular discharge or hair loss around the

eye/muzzle was noted in the 90-day study (40 mg/kg/d) but not in the one-year study, and the study author postulated that a possible explanation for the effects in these areas was ipconazole or a metabolite in saliva and/or tears (although analysis of these secretions was not conducted).

#### 4.7.1.2 Repeated-dose toxicity: inhalation

A repeated-dose study by the inhalation route is available in rats.

**Table 14.4: Summary table of relevant repeated-dose toxicity studies (inhalation)**

| Method | Dose Levels | Observations and Remarks |
|---|---|---|
| Inhalation (nose-only) for 28 days<br>Rats, Sprague-Dawley, 10/sex/group<br>OECD 412, GLP<br>91.2% cc : 6.9% ct, purity 98.1%<br>Kureha Corporation (2006) [32] | 0, 30, 100, 300, 1000 mg/m$^3$ for 6 hrs/day, 5 days/week<br>Administered as a dust. Mass median aerodynamic diameter 1.9 – 3.8 (respirable) | Three males and two females at 1000 mg/m$^3$ were sacrificed in poor condition after 2 weeks of exposure. Exposure of this group was discontinued after 2 weeks but the surviving animals were retained until scheduled sacrifice. Clinical signs in this group were anogenital staining, swollen extremities, irregular gait and hunched appearance. Findings in the animals that were sacrificed early included hair loss that correlated with epithelial hyperkeratosis and hyperplasia; pallor of tissues, particularly the GI tract and spleen; thickening (correlated with hyperkeratosis) of the oesophagus; decreased spleen size correlated with decreased lymphoid tissue and increased apoptosis; hyperplasia of the epithelium of the hard palate, squamous/squamoid metaplasia of the cuboidal/columnar epithelium overlying the ventral sero-mucous glands in the larynx; hyperplasia in the fore-stomach; and minimal-slight centrilobular hypertrophy in the liver.<br>There were no deaths at ≤ 300 mg/m$^3$. The only clinical sign was anogenital staining in females at 300 mg/m$^3$ in the final week.<br>Reduced body weight gain or body weight loss was recorded at 1000 mg/m$^3$ during the first two weeks but recovered once the treatment had been stopped. Body weight gains were reduced only in males at 100 and 300 mg/m$^3$ (up to 36%**), without a change in food consumption.<br>There were no treatment-related ophthalmological findings.<br>Haematology showed that lymphocytes were decreased in all treated male groups but without an effect on females. Neutrophils were increased in females at 300 and 1000 mg/m$^3$.<br>Blood chemistry investigations showed increased ALT and AST in females at 300 mg/m$^3$. At 1000 mg/m$^3$ there was decreased glucose (males), increased phosphorus and increased total protein/globulin (females), which correlated with increased globulin in females at 300 mg/m$^3$.<br>In females at 300 mg/m$^3$, absolute and relative liver weights (20-22%** ↑) and adrenal weights (17-19%* ↑) were increased, whilst relative thymus weight was decreased (23%* ↓).<br>Gross findings in the animals that survived to the end of the study were limited to enlarged adrenals (1 female at 100 mg/m$^3$, 2 females at 300 mg/m$^3$) and hair loss at the extremities (2 females at 300 mg/m$^3$).<br>The histopathology findings in the animals that were exposed for the full study duration are summarised below. |

*Summary of histopathological findings:*

| | Males | | | | Females | | | |
|---|---|---|---|---|---|---|---|---|
| **mg/m³** | 0 | 30 | 100 | 300 | 0 | 30 | 100 | 300 |
| Liver n | 10 | 0 | 1[a] | 10 | 10 | 10 | 10 | 10 |
| centrilobular hypertrophy | 0 | | 0 | 0 | 0 | 0 | 1 | 4 |
| Oesophagus & hard palate n | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 |
| oesophagus - hyperkeratosis | 0 | 0 | 0 | 10 | 0 | 0 | 3 | 10 |
| hard palate – epithelium hyperplasia | 1 | 3 | 4 | 5 | 1 | 1 | 5 | 6 |
| Larynx n | 10 | 9 | 10 | 10 | 9 | 10 | 8 | 9 |
| squamous/ squamoid metaplasia | 1 | 5 | 6 | 7 | 0 | 9 | 6 | 8 |
| Skin n[a] | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 2 |
| hyperkeratosis | | | | | | 0 | | 2 |
| Adrenals n | 10 | 0 | 0 | 10 | 10 | 10 | 10 | 10 |
| cortical hypertrophy | 0 | | | 0 | 0 | 0 | 0 | 4 |

[a]: evaluated only in tissues with gross changes

Changes in the oesophagus, hard palate, larynx, skin and liver showed evidence of recovery in the 1000 mg/m³ animals that were maintained for the 10-11 day recovery period.

The systemic NOAEL was 30 mg/m³.

*N.B. The values for NOAEL are provided for information only; they have been agreed by a PRAPeR Expert Meeting. * Statistically significant at p ≤ 0.05. ** Statistically significant at p ≤ 0.01*

Irritant effects on the hard palate and larynx were noted at all dose levels. Irritant effects were also observed on the skin, which the study authors suggested were caused by transfer of the test substance from the oral cavity to the extremities. The organs targeted by systemic effects were the liver, adrenals and spleen. Excessive toxicity at 1000 mg/m³ resulted in discontinuation of this dose after two weeks of administration, but there was evidence of reversibility of effects in those animals that survived to the end of the study.

#### 4.7.1.3 Repeated-dose toxicity: dermal

A repeated-dose study by the dermal route is available in rats.

**Table 14.5: Summary table of relevant repeated-dose toxicity studies (dermal)**

| Method | Dose Levels | Observations and Remarks |
|---|---|---|
| Dermal for 28 days<br>Rats, Sprague Dawley, 10/sex/group<br>US EPA and Japanese guideline, GLP<br>90.7% cc: 6.7% ct, purity 97.4%<br>Kureha Corporation (2006) [33] | 0, 10, 150, 1000 mg/kg/d for 6 hours/day, 5 days/week | There were no deaths or clinical signs of systemic toxicity.<br>Skin erythema occurred in up to half the animals at 1000 mg/kg/d during weeks 3 and 4. Severity ranged from very slight to well-defined in most cases, with 1 male having a moderate to severe response in the final week. At 150 mg/kg/d, very slight erythema was recorded in 1 female during the final week and 2 females on day 3.<br>Terminal weight gain was reduced in both sexes by up to 9% at 1000 mg/kg/d. Food consumption was slightly decreased at 1000 mg/kg/d during week 2.<br>There were no treatment-related findings in ophthalmological, haematology or blood chemistry investigations.<br>In females of the 1000 mg/kg/d group, absolute and relative adrenal weights (44-52%*) and relative liver weight (12%**) were increased.<br>Gross findings were limited to minimal or mild thickening of the skin in 1 male and 1 female at 1000 mg/kg/d, mild red abnormal colouration of the glandular stomach in 1 female at 1000 mg/kg/d and moderate erosion/ulceration of the glandular stomach in 1 female at 150 mg/kg/d.<br>Histopathology revealed effects in the skin at all doses from day 18, consisting of minimal to mild epidermal hyperplasia (dose-response relationship) and hyperkeratosis (no clear dose-response relationship and no increase in severity with increasing dose). Minimal adrenal cortical hypertrophy/hyperplasia correlated with the adrenal weight changes but without a dose-response relationship. Minimal to moderate oesophageal, pharyngeal and laryngeal hyperkeratosis and minimal to mild hyperkeratosis in the fore-stomach were observed at all dose levels. In females at 1000 mg/kg/d, minimal renal pelvic (2/10) or renal tubular (5/10) mineralisation was recorded.<br>The NOAEL for systemic effects was 150 mg/kg/d. |

*N.B. The values for NOAEL are provided for information only; they have been agreed by a PRAPeR Expert Meeting. * Statistically significant at $p \leq 0.05$. ** Statistically significant at $p \leq 0.01$*

Repeated dermal exposure of rats to ipconazole resulted in irritant effects (erythema) after two weeks of exposure, mainly at 1000 mg/kg/d but with less severe effects at 150 mg/kg/d in a small number of animals. Histopathology of the skin revealed epidermal hyperkeratosis (often with hyperplasia) at all dose levels.

The study authors attributed the hyperkeratosis of the oesophagus, pharynx, larynx and fore-stomach to an irritation following oral ingestion of the substance, for example by grooming. Systemic effects were noted on food consumption, body weight, liver and adrenal weights (with histopathological effects) and mineralisation in the kidneys at 1000 mg/kg/d. The adrenal effects were not dose-related and the study authors attributed them to a stress response to the treatment procedure.

#### 4.7.1.4 Repeated-dose toxicity: other routes

No information.

### 4.7.2 Human information

No information.

### 4.7.3 Other relevant information

The chronic/carcinogenicity studies in rats and mice are presented in section 4.9. The non-tumour findings of note are summarised here.

Ipconazole was administered orally to rats at doses up to 13.3 mg/kg/d for one (toxicity phase) or two (carcinogenicity phase) years. The main findings were increased incidences of epithelial hyperplasia of the fore-stomach and centrilobular hepatocyte hypertrophy in the liver, both in males, from 9 mg/kg/d.

In mice, ipconazole was administered orally for 78 weeks at doses up to 57 mg/kg/d. The main target organs were again the liver and the fore-stomach. Hepatocyte vacuolation was recorded from 24.1 mg/kg/d in males and 2.3 mg/kg/d in females, whilst hepatocyte necrosis (mainly graded as minimal to slight) was increased in males at 45.3 mg/kg/d. In males, there were also increased incidences of parenchymal inflammatory cells from 24.1 mg/kg/d and karyomegaly (enlarged nuclei) at 45.3 mg/kg/d in the liver, but the incidences of these in the controls were also high. Fore-stomach lesions (minimal epithelial hyperplasia, hyperkeratosis) were increased in female mice in all dose groups, although without clear dose-response relationships. Alveolar haemorrhage in the lungs and dilated mucous glands in the larynx, both in males, were postulated by the study authors to be related to an irritant action of ipconazole.

### 4.7.4 Summary and discussion of repeated-dose toxicity relevant for classification as STOT RE according to CLP Regulation

The repeated-dose toxicity of ipconazole has been investigated by the oral, dermal and inhalation routes in rats, mice and dogs. In addition to excessive toxicity in some of the studies, leading to early termination of some dose groups, the major findings were fore-stomach lesions in rats and mice, eye effects in dogs, liver effects in rats, mice and dogs, and skin reddening in dogs. Other effects were reported in the adrenals, kidney, urinary bladder and thymus. The relevance of each of these effects to a possible classification for repeated-dose toxicity is discussed below.

*Excessive toxicity leading to early termination of dose groups*

Some high-dose groups of all the tested species were terminated early for humane reasons. The clinical signs that led to termination appeared to be related to poor palatability of the test diet rather than a specific toxic effect; hence this effect is not relevant for classification.

*Fore-stomach lesions in rats and mice*

Fore-stomach lesions were reported in rats and mice and comprised epithelial hyperplasia, hyperkeratosis, erosion, ulceration and sub-epithelial inflammation. Lesions were reported from approximately 30 mg/kg/d in rats (28-day and 90-day oral studies) and from 69.7 mg/kg/d in mice (28-day and 90-day oral studies). Inhalation and dermal exposure of rats for 28 days also resulted in fore-stomach lesions. There were no such lesions in the gastrointestinal tract of dogs.

The relevance of rodent fore-stomach lesions for human risk assessment has been reviewed by RIVM ([34]). The rodent stomach consists of two anatomically distinct parts: a non-glandular fore-stomach; and a glandular stomach. The rodent glandular stomach is structurally and functionally similar to the stomach of other mammalian non-rodent species, including humans. In contrast, the fore-stomach has no counterpart in humans (nor several test laboratory species, including dogs and rabbits). The fore-stomach forms a continuum with the oesophagus and is lined with keratinised, stratified squamous epithelium; humans have comparable squamous epithelial tissues in the oral cavity and upper two-thirds of the oesophagus. A difference between the rodent oesophagus and that of most mammals (including humans) is that the rodent oesophagus (and also the fore-stomach) does not contain mucosal glands that secrete mucus to aid the passage of food; however, the keratin coating of oesophageal epithelial cells is more pronounced in rodents than humans and provides further protection against chemical insults. Another difference between the species is that rodents lack a vomiting reflex, whereas humans are able to vomit to rid themselves of irritating substances. The main function of the fore-stomach is storage and triturition of food prior to digestion in the glandular stomach. Because of this storage function, the passage of food through the fore-stomach is much slower than through the human mouth and oesophagus; there is therefore a longer time for interaction of ingested chemicals and epithelial cells in the fore-stomach than in the human mouth and oesophagus. Although the epithelium of the rodent oesophagus and fore-stomach are morphologically identical and continuous, it is rare for oesophageal lesions to occur even if fore-stomach lesions are observed [34]; this could be explained by the rapid passage of ingested material through the oesophagus, and/or the difference in physiological conditions (e.g. pH, surface population of bacteria and yeast). Overall, RIVM's conclusion was that fore-stomach effects induced by non-genotoxic substances only after oral administration are not relevant for humans.

Ipconazole induced fore-stomach lesions in rats and mice by the oral route. Hyperkeratosis in the fore-stomach in the rat dermal study was attributed to oral ingestion through grooming; following inhalation exposure, fore-stomach lesions were induced only in high-dose animals that were sacrificed early. However, in considering the relevance of these findings to humans, it is notable that the same lesions that occurred in the fore-stomach were also observed in the oesophagus (oral 28-day studies, rats ≥ 91 mg/kg/d, mice ≥ 152 mg/kg/d; rat inhalation 28-day study at 300 mg/m$^3$; rat dermal study ≥ 10 mg/kg/d), larynx (inhalation study ≥ 30 mg/m$^3$, dermal study ≥ 10 mg/kg/d), pharynx (dermal study ≥ 10 mg/kg/d) and hard palate (inhalation study ≥ 100 mg/m$^3$). The lesions in areas other than the fore-stomach were not observed in 90-day studies when tested up to 52.2 mg/kg/d in rats or 91 mg/kg/d in mice, nor in two-year studies at doses ≤ 57 mg/kg/d (apart from a slight increase in the incidence of dilated mucous glands of the pharynx of male mice). This observation indicates that in the induction of this local, irritant effect, a high local concentration is likely to be more important than the total body dose on a mg/kg/d basis. The fore-stomach lesions tended to occur at lower doses, which is consistent with the fore-stomach being more sensitive because of the retention of ingested material there.

The fact that no oesophageal, pharyngeal or larynx lesions were observed in dogs might lessen the concern for humans. Overall, however, the occurrence of epithelial hyperplasia, hyperkeratosis and sub-epithelial inflammation in areas other than the fore-stomach (oesophagus, pharynx, larynx, hard palate) in several studies indicates that they were not specific to the rodent fore-stomach; thus they should be considered as potentially of relevance to humans. It is also noted that stomach ulcers and erosion were reported in a rabbit developmental toxicity study with ipconazole (section 4.10.2), which supports the latter position.

*Eye effects in dogs*

Ocular effects, in the form of opacities, cataracts, lenticular degeneration and anomaly of the lenticular fibres, were reported in the dog studies. These occurred at oral doses of ≥ 60 mg/kg/d for 28 days, 40 mg/kg/d for 90 days and 20 mg/kg/d for one year. Ocular effects were not reported in rats or mice when ipconazole was administered for up to two years (although ophthalmological examination was not performed on mice, as their eyes are usually considered to be too small for this investigation).

The applicant under Directive 91/414/EEC argued that some of the ocular effects observed in dogs were either artefacts or were not relevant to humans. The lenticular fibre anomaly reported in the 90-day study comprised disrupted and shattered fibres with intervening clean spaces. This finding was only reported in males and showed no correlation with ophthalmoscopic observations or blood cholesterol, nor did it occur in the one-year study. The fixative used, Davidson's fluid, has a relatively high alcohol and acetic acid concentration, and was reported to give relatively poor fixation of the lens, which could result in shattering. The applicant therefore concluded that the lenticular fibre anomaly was an artefact.

Lens fibre (lenticular) degeneration was reported in two females at 40 mg/kg/d in the 90-day study (one of these animals also had inflammatory lesions in the cornea and iris) and in one male at 20 mg/kg/d in the one-year study. The occurrence of this finding correlated, at a group level, with the presence of cataracts and reduced plasma cholesterol, although at the individual level there was not a correlation. The applicant reported that the lens contains high concentrations of ascorbic acid and glutathione, and postulated that these might be important to maintain the normal function of the lens. It was further proposed that oxidative damage plays an important role in the formation of steroid-induced cataracts; as dogs have lower levels of glutathione reductase activity and ascorbate than humans and some laboratory species, the applicant concluded that dogs were more susceptible than humans to oxidative damage of the lens.

The above observation might be consistent with the absence of ocular effects in rats and mice in the present studies, since they have higher glutathione reductase than dogs (although ascorbate is lower in rats than dogs, with levels in mice not reported). However, given that, in the 90-day dog study, 7 of the 8 dogs at 40 mg/kg/d showed cataracts, and there was a clear reduction in plasma cholesterol compared with controls (43-50% decrease) at this dose, the possibility of an association between reduction in cholesterol and the induction of ocular effects remains. Opacities together with reduced plasma cholesterol were also reported in the 28-day and one-year dog studies. Ipconazole inhibits sterol synthesis and thus reduces the plasma cholesterol concentration in animals. The membranes of the cells of the lens contain high levels of cholesterol, and other chemical substances that reduce cholesterol levels have also been reported to produce cataracts. Furthermore, it has been reported that 'the cell membrane of the human lens contains the highest relative concentration of cholesterol in nature…Because the lens must synthesize the cholesterol needed to support its life-long growth, an inhibition of lens cholesterol synthesis with drugs can produce cataracts in animals and humans' [35]. The absence of ocular effects in rats and mice after ipconazole administration does not contradict this supposition, since cholesterol levels were not reduced in the rat studies and, although they were reduced in mice, no ophthalmology or histopathology on mouse eyes was performed. It is thus concluded that the adverse ocular effects induced by ipconazole in dogs are potentially relevant to humans.

*Liver effects in rats, mice and dogs*

The liver was a target organ in rats, mice and dogs when ipconazole was administered by the oral, inhalation and dermal routes. The following adverse effects were reported:

- Rats, 90-day study, at 52.2 mg/kg/d: focal inflammation;
- Mice, 28-day study
  - ≥ 88.1 mg/kg/d: pale liver, centrilobular hepatocyte vacuolation, panacinar fat;
  - ≥ 151.8 mg/kg/d: focal hepatocyte necrosis;
- Mice, 90-day study
  - ≥ 20.2 mg/kg/d: hepatocyte vacuolation;
  - ≥ 69.7 mg/kg/d: fatty deposits, pale liver;
- Mice, 2-year study
  - ≥ 2.3 mg/kg/d: hepatocyte vacuolation
  - 45.3 mg/kg/d: hepatocyte necrosis (mainly graded as minimal to slight) in males;
- Dogs, 28-day study
  - ≥ 24 mg/kg/d: bile duct proliferation, pigment-laden Kupffer cells, slight focal necrosis, prominent inflammatory cells in the portal area;
  - ≥ 60 mg/kg/d: enlarged and/or pale liver, abnormal findings in the gall bladder;
- Dogs, 90-day study
  - 40 mg/kg/d: isolated incidences of enlarged and/or pale liver, bile duct proliferation, pigment-laden Kupffer cells, moderate focal necrosis in 1/4 females, dark or gelatinous particles in the gall bladder.

These effects are relevant to humans and will thus be considered further in deciding upon a classification for repeated-dose toxicity.

*Skin reddening in dogs*

An unusual finding was skin reddening (and hair loss) in dogs upon oral exposure of ipconazole. This was reported in all studies and occurred from 10 mg/kg/d in the 90-day study. The cause of the reddening and hair loss was not established by the studies, but since the substance was administered in capsules it appeared to be a systemic effect rather than local irritation. It was also notable that the severity worsened as the duration of exposure increased. Furthermore, ipconazole was not a skin irritant in a test for skin irritation (section 4.4.1.). Skin reddening and hair loss were occasionally reported in rats (for example, one male in an acute study, some animals exposed for 28 days) but not in mice after oral exposure. These effects were also recorded in rats after inhalation exposure, which might have been caused by transfer of the substance to the extremities and dermal exposure. Interestingly, histopathological changes in the skin of rats exposed dermally were first recorded after 18 days of administration, indicating that this was a repeated-dose and not an acute effect.

The adverse skin effects in dogs are regarded as being of relevance to humans.

*Other findings*

Changes in the kidney were reported in rats and comprised an increase in the incidence of corticomedullary mineralisation (graded minimal or slight) at 33.2 mg/kg/d for 90 days (females only). After dermal exposure of rats for 28 days, renal tubular mineralisation was recorded at 1000 mg/kg/d in females only. Increases in kidney weight in the 90-day and one-year dog studies were not supported by gross or histopathological findings, and so were considered by the study authors to be an adaptive response to urinary excretion of ipconazole and/or its metabolites. No adverse kidney effects were reported in mice. The adverse effect in rats is possibly relevant for classification.

In rats, a change in adrenal gland weight was associated with prominent sinusoidal lining cells at 91 mg/kg/d in females exposed for 28 days, but was not observed in the 90-day or chronic studies. Adrenal cortical vacuolation occurred in 4/9 male mice at 69.7 mg/kg/d in the 90-day study but not after chronic exposure. Cortical (fatty) vacuolation of the adrenals was observed in dogs after exposure for 90 days (at 40 mg/kg/d) and one year (at 20 mg/kg/d). The nature of the vacuolation reported in mice was not investigated by specific staining, but was possibly also fatty and related to ipconazole's mode of action (action on steroid hormone synthesis by the adrenal gland).

The study authors attributed the effects on the thymus in dogs (reduced weight, microscopic evidence of cortical atrophy, reduced cellularity of the cortex) to a secondary, stress-related finding. As there were no gross or histopathology changes in the thymus of rats or mice, the finding in dogs will not be considered further.

Myometrial atrophy in the uterus was reported in 5/5 rats in a 28-day oral study rats at 236 mg/kg/d, but at this dose the animals were sacrificed early because of excessive toxicity. It was not reported at the next dose of 91 mg/kg/d, nor in any other study or species. Therefore, this finding is not considered relevant for classification for repeated-dose toxicity.

### 4.7.5 Comparison with criteria of repeated-dose toxicity findings relevant for classification as STOT-RE

Under CLP, STOT-RE is assigned on the basis of a substance demonstrating evidence of significant or severe toxicity, generally at or below the oral guidance value of 100 mg/kg/d (for a classification in category 2) obtained in a 90-day rat study. The oral guidance value for a classification in category 1 is ≤ 10 mg/kg/d. The equivalent guidance values for a 28-day study are ≤ 300 mg/kg/d and ≤ 30 mg/kg/d, respectively; for a one-year study, they are ≤ 25 mg/kg/d and 2.5 mg/kg/d, respectively. For inhalation exposure to a dust, the guidance value is ≤ 0.2 mg/L/6h/d in a 90-day rat study, whilst that for dermal exposure is ≤ 200 mg/kg/d in rats or rabbits. 'Significant' toxicity is taken to mean changes that clearly indicate functional disturbance or morphological changes that are toxicologically relevant. 'Severe' toxicity is considered to be more profound or serious and indicates changes that are of a considerably adverse nature with a significant impact on health.

Some of the effects observed in the repeated-dose toxicity studies with ipconazole were attributed to irritation. This was not an acute effect, since the standard tests for skin and eye irritation indicated that ipconazole did not meet the criteria for classification for these end-points (section 4.4). Moreover, skin effects in the 28-day dermal study did not manifest until 18 days of exposure, and the skin effects in dogs were systemic, not local. It is therefore appropriate that the effects attributed to irritation in the repeated-dose studies should be considered under the classification of STOT-RE.

The effects that were potentially relevant to humans and occurred below the guidance cut-off values outlined above are summarised in table 14.6.

**Table 14.6: Summary table of findings at doses relevant to STOT-RE classification for ipconazole**

| Study design | Guidance cut-off value (mg/kg/d) | Findings at dose relevant to STOT-RE Cat 1 | Findings at dose relevant to STOT-RE Cat 2 |
|---|---|---|---|
| Rat 28-day dietary | Cat 1: 30<br>Cat 2: 300 | Lowest dose 30.5 mg/kg/d: no adverse effects in males | 31.3 mg/kg/d: reduced body weight and food consumption in females<br>91 mg/kg/d: adrenal glands (prominent sinusoidal lining cells), females; lesions of the oesophagus*.<br>164-236 mg/kg/d: severe toxicity led to early termination |
| Rat 90-day dietary | Cat 1: 10<br>Cat 2: 100 | 5.8-7 mg/kg/d: no adverse effects | 33.2 mg/kg/d: kidney effects (corticomedullary mineralisation, minimal or slight, females)<br>52.2 mg/kg/d: liver (focal inflammation) |
| Rat 2-year dietary | Cat 1: 1.25<br>Cat 2: 12.5 | 1.6 / 1.2 mg/kg/d: no adverse effects | > 13.3 mg/kg/d: liver (centrilobular hepatocyte hypertrophy, males only) |
| Rat 28-day inhalation | Cat 1: 0.06 mg/L/6h/d<br>Cat 2: 0.6 mg/L/6h/d | 0.03 mg/L/6h/d: no adverse systemic effects; local effects on hard palate and larynx* | ≥ 0.1 mg/L/6h/d: local effects on oesophagus, hard palate, larynx |
| Rat 28-day dermal | Cat 1: 60<br>Cat 2: 600 | 10 mg/kg/d: no adverse effects | 150 mg/kg/d: no adverse effects<br>Next dose 1000 mg/kg/d |
| Mouse 28-day dietary | Cat 1: 30<br>Cat 2: 300 | Lowest dose 44.8 / 53.4 mg/kg/d | ≥ 44.8 / 53.4 mg/kg/d: liver effects<br>≥ 152 mg/kg/d: lesions in the oesophagus*; focal hepatocyte necrosis |
| Mouse 90-day dietary | Cat 1: 10<br>Cat 2: 100 | 4.4 / 5.1 mg/kg/d: no adverse effects | ≥ 20 mg/kg/d: liver (hepatocyte vacuolation)<br>≥ 70 mg/kg/d: adrenal gland (cortical vacuolation) |
| Mouse 18-month dietary | Cat 1: 1.9<br>Cat 2: 19 | 1.9 / 2.3 mg/kg/d: centrilobular hepatocyte vacuolation (females only) | ≥ 24 mg/kg/d: liver (generalised hepatocyte vacuolation, parenchymal inflammatory cells) |
| Dog 28-day oral (capsule) | Guidance values based on rat study | Lowest dose 24 mg/kg/d<br>≥ 24 mg/kg/d: liver effects<br>≥ 60 mg/kg/d: ocular effects (opacities, cataracts, lenticular degeneration) | |
| Dog 90-day oral (capsule) | Guidance values based on rat study | 2 mg/kg/d: no adverse effects<br>≥ 10 mg/kg/d: skin reddening and hair loss<br>40 mg/kg/d: ocular effects (opacities, cataracts, lenticular degeneration); adrenal glands (fatty vacuolation); liver effects | |
| Dog 1-year oral (capsule) | Guidance values based on rat study | 1.5 mg/kg/d: no adverse effects<br>≥ 5 mg/kg/d: skin reddening<br>20 mg/kg/d: ocular effects (opacities, cataracts, lenticular degeneration); adrenal glands (fatty vacuolation) | |

* This was a local irritant effect rather than systemic, specific target-organ toxicity, and as such the local concentration is more important than the total body dose on a mg/kg/d basis. It is thus not appropriate to adjust the guidance value to take account of the exposure duration.

Repeated-dose administration of ipconazole therefore resulted in a number of adverse effects in rats, mice and dogs. When compared with the classification criteria, the significant findings can be summarised as:

- *significant organ damage noted at necropsy and/or subsequently confirmed at microscopic examination:* ocular effects that were potentially related to a decrease in plasma cholesterol;
- *multi-focal or diffuse necrosis, fibrosis or granuloma formation in vital organs with regenerative capacity:* hepatocyte necrosis, although this was focal and generally graded as minimal to slight;
- *morphological changes that are potentially reversible but provide clear evidence of marked organ dysfunction*: lesions in the oesophagus, pharynx, larynx and hard palate (although it is recognised that these effects resulted from local irritation, not specific target-organ toxicity); fatty deposits in the liver; fatty vacuolation in the adrenal glands.

A further systemic finding of concern to support classification was skin reddening in dogs following oral administration in a capsule. The effects noted above, summarised in table 14.6, largely occurred at doses that were consistent with a classification in STOT-RE category 2; there were no findings that indicated that category 1 would be a more appropriate classification. Since adverse effects were recorded after oral, dermal and inhalation exposure, it is not proposed to specify a route of exposure. The primary target organs of specific toxicity were the eyes (although possibly secondary to lowering of plasma cholesterol), skin, liver and gastrointestinal tract.

#### 4.7.6 Conclusions on classification and labelling of repeated-dose toxicity findings relevant for classification as STOT RE

**STOT-RE 2; H373 – May cause damage to organs (eyes, skin, liver, gastrointestinal tract) through prolonged or repeated exposure**

## 4.8 Germ cell mutagenicity (Mutagenicity)

The genotoxic potential of ipconazole has been investigated in several *in vitro* studies and one *in vivo* study.

**Table 15: Summary table of relevant *in vitro* and *in vivo* mutagenicity studies**

| ***In vitro* data** | | | |
|---|---|---|---|
| **Method** | **Organism/strain** | **Concentrations tested** | **Result** |
| Bacterial reverse mutation assay (Ames)<br>87.27% cc: 10.23% ct, purity 97.51%<br>Japanese guideline, similar to OECD 471 but with duplicate plates instead of triplicate. GLP.<br>Nishitomi (1999) [36] | TA100, TA1535, TA1537, TA98, WP2*uvrA* | With and without S9: 0 – 5000 µg/plate | **Negative** ± metabolic activation |
| Mammalian chromosomal aberration assay<br>Main deviation from OECD 473: cytotoxicity was not determined in the main experiment. GLP.<br>87.27% cc: 10.23% ct, purity 97.51%<br>Nishitomi (1989)[37] | Chinese hamster lung cells (CHL/IU cell line) | With and without S9: 0 – 70 µg/plate | **Negative** ± metabolic activation but noted that there was insufficient evidence of cytotoxicity in the main experiment to indicate that the dose levels were high enough. |
| Mammalian cell gene mutation assay<br>US EPA guideline, similar to OECD 476. GLP.<br>91.2% cc: 6.9% ct, purity 98.1%<br>Cifone (2001) [38] | Chinese hamster ovary (CHO) cells, HGPRT locus | With S9: 0 – 70 µg/ml<br>Without S9: 0 – 50 µg/ml | **Negative** ± metabolic activation |
| Bacterial DNA repair assay<br>87.27% cc: 10.23% ct, purity 97.51%<br>No OECD guideline available, GLP.<br>Nishitomi (1989) [39] | *Bacillus subtilis* strains H17 ($rec^+$) and M45 ($rec^-$) | Up to 20 µg/disk (no further details available) | **Negative** ± metabolic activation |

| *In vivo* data | | | |
|---|---|---|---|
| **Method** | **Organism/strain** | **Concentrations tested** | **Result** |
| Micronucleus test (oral gavage)<br>91.7% cc:6.7% ct, purity 98.4%<br>OECD 474, GLP.<br>Kureha Corporation (2005) [40] | CD-1 mice, 5/sex/dose with 2 extra at the high doses to ensure 5/dose at the time of sacrifice | Males: 0, 250, 500, 1000 mg/kg/d<br>Females: 0, 375, 750, 1500 mg/kg/d<br>Administered on two consecutive days, 24 hours apart.<br>Femoral bone marrow was collected 24 hours after the second application. | **Negative**<br>Clinical signs of toxicity were observed at 500 & 1000 mg/kg/d in males and 750 & 1500 mg/kg/d in females (6 animals of the latter group sacrificed *in extremis* so not available for mutagenicity analysis). The PCE/NCE ratio was decreased at all dose levels. |

### 4.8.1 Non-human information

#### 4.8.1.1 *In vitro* data

Ipconazole was negative in four *in vitro* genotoxicity assays that investigated bacterial mutation, mammalian cell mutation, mammalian chromosome aberration and DNA repair.

#### 4.8.1.2 *In vivo* data

Ipconazole was negative in a guideline-compliant micronucleus assay in which evidence of bone marrow toxicity was demonstrated, indicating that sufficiently high doses were administered.

### 4.8.2 Human information

No information.

### 4.8.3 Other relevant information

No information.

### 4.8.4 Summary and discussion of mutagenicity

Ipconazole was negative in all the available tests for genotoxicity.

### 4.8.5 Comparison with criteria

There was no indication that ipconazole has a mutagenic effect on somatic or germ cells in several *in vitro* assays and an *in vivo* micronucleus assay. The criteria for classification for mutagenicity were not met.

### 4.8.6 Conclusions on classification and labelling

**Not classified (conclusive but not sufficient for classification)**

## 4.9 Carcinogenicity

The chronic toxicity and carcinogenic potential of ipconazole have been investigated in rats and mice by the oral route.

**Table 16: Summary table of relevant carcinogenicity studies**

| Method | Dose levels | Observations and remarks<br><br>(effects of major toxicological significance) |
|---|---|---|
| Oral (dietary) for 2 years<br><br>Rat, Han Wistar, 20/sex/group for 52 weeks, additional 50/sex/group for 104 weeks<br><br>OECD 453, GLP<br><br>91.7 cc : 6.7% ct, purity 98.1-98.4%<br><br>Kureha Corporation (2006) [41] | Males: 0, 30, 80, 200, 300 ppm equivalent to:<br><br>toxicity phase 0, 1.6, 4.2, 10.9, 15.9 mg/kg/d<br><br>carcinogenicity phase 0, 1.3, 3.6, 9.0, 13.3 mg/kg/d<br><br>Females: 0, 30, 80, 200, 300 ppm initially, then 0, 30, 80, 120, 200 ppm from week 2, equivalent to:<br><br>toxicity phase 0, 2.2, 5.9, 9.1, 15.0 mg/kg/d<br><br>carcinogenicity phase 0, 1.9, 4.9, 7.3, 12.6 mg/kg/d | Treatment did not affect survival rates, which were > 60% in all groups except for the female 80 ppm group (56%). There were no treatment-related clinical signs, persistent effects on body weight gain nor food consumption.<br><br>*Non-tumour findings*<br><br>There were no substance-related ophthalmological, haematology or blood chemistry findings.<br><br>The only change in organ weights noted at 52 and 104 weeks was a non-statistically significant increase in absolute and relative ovary weight (21% and 30%, respectively) at 200 ppm. There were no substance-related macroscopic changes.<br><br>Histopathology at 52 weeks did not reveal any adverse effects of treatment. Substance-related non-tumour histopathology findings at 104 weeks were found in the fore-stomach (epithelial hyperplasia in males: 0%, 4%, 2%, 10%, 8% at 0, 30, 80, 200, 300 ppm), liver (centrilobular hepatocyte hypertrophy in males: 14%, 18%, 14%, 14%, 26% at 0, 30, 80, 200, 300 ppm) and urinary bladder (oedema: 16%, 15%, 9%, 9%, 29% in males at 0, 30, 80, 200, 300 ppm; 8%, 17%, 9%, 22%, 14% in females at 0, 30, 80, 200/120, 300/200 ppm). Additional findings in females at 300/200 ppm were epithelial keratinisation of the vagina (33% compared with 16% in controls, both above the historical control range of 0-6%) and interstitial cell hyperplasia of the ovary (12% compared with 4% in controls, historical control range 0-10%).<br><br>There was no consistent effect of treatment on sensory reactivity, grip strength and motor activity (measured during week 50).<br><br>*Tumour findings*<br><br>Thyroid: the incidence of follicular adenoma was slightly increased in the high-dose groups (males: 4%, 4%, 5%, 10%, 10% at 0, 30, 80, 200, 300 ppm; females: 0%, 6%, 3%, 0%, 8%* at 0, 30, 80, 200/120, 300/200 ppm). The historical control range (same strain and laboratory) for males was 4-17% (mean 8.4%) and for females 0-10.2% (8 studies). There was no increase in the incidence of cystic follicular cell hyperplasia, follicular cell carcinoma nor substance-related increase in the incidence of c-cell hyperplasia.<br><br>The NOAELs of relevance for human risk assessment were 300 ppm (13.3 mg/kg/d) for males, 200 ppm (12.6 mg/kg/d) for females. |

| | | |
|---|---|---|
| Oral (dietary), 78 weeks<br>Mouse, CD-1, 5/sex/group<br>OECD 451, GLP<br>91.7 cc : 6.7% ct, purity 98.1-98.4% Kureha Corporation (2007) [42] | 0, 15, 175, 350 ppm, equivalent to<br>males: 0, 1.9, 24.1, 45.3 mg/kg/d<br>females: 0, 2.3, 26, 57 mg/kg/d | Treatment did not affect survival rates, which were ≥ 69% in all groups. There were no clinical signs of toxicity.<br>*Non-tumour findings*<br>In females at 350 ppm there was an overall reduction in body weight gain of 14%. In these animals food consumption was reduced during week 1.<br>Liver weight was increased in males at 350 ppm (16%** ↑ relative weight). Adrenal weight was increased in females at 350 ppm (14% ↑ relative weight).<br>At 350 ppm, an increased incidence of pallor of the liver (males: 29%* compared with 12% in controls) and enlarged adrenals (females: 20% compared with 8% in controls) were noted. There was no effect on eye opacity, although a specialised ophthalmological examination was not undertaken.<br>Non-tumour histopathology findings are summarised below. |

| | **Males** | | | | **Females** | | | |
|---|---|---|---|---|---|---|---|---|
| **ppm** | 0 | 15 | 175 | 350 | 0 | 15 | 175 | 350 |
| Liver | | | | | | | | |
| hepatocyte necrosis | 2% | 10% | 6% | 20%** | 10% | 10% | 4% | 4% |
| hepatocyte vacuolation (centrilobular) | 57% | 59% | 80%** | 78%* | 6% | 25%** | 61%** | 55%** |
| hepatocyte vacuolation (generalised) | 8% | 8% | 22%* | 41%** | 20% | 18% | 25% | 61%** |
| parenchymal inflammatory cells | 73% | 80% | 94%** | 84% | 67% | 78% | 71% | 86%* |
| karyomegaly (enlarged nuclei) | 45% | 41% | 43% | 65%* | 4% | 8% | 6% | 2% |
| Larynx | | | | | | | | |
| dilated mucous glands | 18% | 25% | 7% | 35%* | 10% | 0% | 0% | 10% |
| Fore-stomach | | | | | | | | |
| epithelial hyperplasia | 24% | 12% | 31% | 22% | 8% | 16% | 39%** | 27%** |
| hyperkeratosis | 80% | 88% | 90% | 88% | 75% | 94%** | 86% | 92%* |
| Lungs | | | | | | | | |
| alveolar haemorrhage | 12% | 12% | 16% | 24% | 4% | 14% | 12% | 14% |

*Tumour findings*

Haemopoietic system: histiocytic sarcomas were recorded in 0, 1, 0, 2 males and 0, 1, 1, 3 females at 0, 15, 175 and 350 ppm, respectively. The incidences in the high-dose groups (4% males, 6% females) were within the historical control range of 6 studies conducted at the same test laboratory and same time period (0-4% males, mean 1.3%; 0-10% females, mean 4.8%). They were also within the range (0-8% males, 0-18% females) reported for US-bred CD-1 mice from 51 studies initiated between 1987 and 1996 (Ref. 29 (2000)).

The NOAEL was 15 ppm (1.9 mg/kg/d in males, 2.3 mg/kg/d in females).

*N.B. The values for NOAEL are provided for information only; they have been agreed by a PRAPeR Expert Meeting. * Statistically significant at p ≤ 0.05. ** Statistically significant at p ≤ 0.01*

### 4.9.1 Non-human information

#### 4.9.1.1 Carcinogenicity: oral

In rats, increases in histopathological findings in the fore-stomach, livers and urinary bladder in the higher dose groups were slight and not statistically significant. Furthermore, the incidences of oedema in the urinary bladder of both the concurrent controls and treated groups were much higher than the historical control ranges (males 0-1.7%, females 0-2%), prompting the study author to conclude that this finding was an artefact of the tissue processing. Vaginal and ovary findings in females of the high-dose group were also slightly increased compared with controls, but when the historical control ranges were considered they did not provide convincing evidence of substance-related adverse effects. Although there was a statistically significant increase in the incidence of follicular adenomas in females at 300/200 ppm (without a dose-response relationship at the lower doses), this was within the historical control range and so is not considered to be treatment related. There were no other neoplastic findings of note.

In mice exposed for 78 weeks, the main target organs were the liver and the non-glandular fore-stomach. Liver effects included hepatocyte necrosis, showing an increased incidence in males (at 350 ppm, graded: minimal 6, slight 2, moderate 2), and increased incidence of karyomegaly (enlarged nuclei), also in males at 350 ppm (45.3 mg/kg/d). The nature of the hepatocyte vacuolation was not investigated, but taking into consideration the findings of the 90-day mouse study, they were likely to be fatty vacuolation, related to ipconazole's mode of action. Changes in the fore-stomach were predominantly observed in females but without clear dose-response relationships. Almost all cases of epithelial hyperplasia of the fore-stomach were graded as minimal. Effects on the lung and larynx in males could have been related to irritancy. A slight increase in the incidence of histiocytic sarcomas was within the historical control range; it is thus concluded that there was no substance-related increase in tumours.

#### 4.9.1.2 Carcinogenicity: inhalation

No information.

#### 4.9.1.3 Carcinogenicity: dermal

No information.

### 4.9.2 Human information

No information.

### 4.9.3 Other relevant information

Ipconazole was negative in four *in vitro* assays and an *in vivo* micronucleus test (section 4.8).

### 4.9.4 Summary and discussion of carcinogenicity

Information on the carcinogenic potential of ipconazole is provided by two carcinogenicity studies, one in rats and one in mice. In these studies, increased incidences of two tumour types were observed: thyroid follicular cell adenomas in female rats and histiocytic sarcomas of the haemopoietic system in mice. The only statistically significant increase was in the thyroid follicular cell adenomas in high-dose female rats, but there was not a clear dose-response relationship (0%, 6%, 3%, 0%, 8% at 0, 30, 80, 200/120, 300/200 ppm). Incidences of both tumour types were within the historical control ranges for the same laboratory and animal strain. Overall, they are not considered to provide evidence of carcinogenicity.

### 4.9.5 Comparison with criteria

There were no increased incidences of tumours that could be attributed to administration of ipconazole. Thus, the substance does not meet the criteria for classification for carcinogenicity. Furthermore, it was negative in a series of *in vitro* and *in vivo* genotoxicity tests. Classification for carcinogenicity is not proposed.

### 4.9.6 Conclusions on classification and labelling

**Not classified (conclusive but not sufficient for classification)**

## 4.10 Toxicity for reproduction

The reproductive toxicity of ipconazole has been investigated in a preliminary one-generation study, a two-generation study in rats and in developmental toxicity studies in rats and rabbits.

### 4.10.1 Effects on fertility

**Table 17: Summary table of relevant reproductive toxicity studies - Fertility**

| Method | Dose levels | Observations and remarks<br><br>(effects of major toxicological significance) |
|---|---|---|
| Oral (dietary)<br><br>Two-generation<br><br>Rats, Han Wistar, 32/sex/group F0, 28/sex/group F1<br><br>OECD 416, GLP<br><br>91.7% cc: 6.7% ct, purity 98.1-98.4%<br><br>Kureha Corporation (2006) [43] | 0, 30, 100, 300 ppm<br><br>In males, equivalent to 2.2, 7.2, 22 mg/kg/d in F0, 2.8, 9.3, 29.1 mg/kg/d in F1<br><br>In females, equivalent to 2.6-5, 8.4-16, 25.5-48 mg/kg/d in F0, 3-5, 9.9-17, 31.6-48 mg/kg/d in F1<br><br>Administered for 10 weeks before pairing, during pairing, gestation and lactation until termination in F0 and F1 generations. F2 offspring received diet until termination on day 25 of age. | Histological investigations were conducted only on adults; reproductive organs and pituitary were examined in controls and high-dose groups, as well as any gross pathological findings in other animals.<br><br>*P0 parental toxicity*<br><br>There were no treatment-related deaths or clinical signs of toxicity.<br><br>During gestation, body weight gains of females at 300 ppm were slightly but statistically significantly reduced (↓ 8%*). Male food consumption was unaffected. Female food consumption was statistically significantly reduced at 100 and 300 ppm during the first week of treatment (↓ 8%*), days 0-12 of gestation (↓ 5* - 10%*) and between days 4-13 of lactation (↓ 7* - 13%**).<br><br>Treatment did not have any effects on oestrus cycles, mating performance, fertility, gestation duration nor gestation index (which was 100% in all groups).<br><br>Small (5-9%) differences in weights of the spleen (increased in males), kidneys (increased in males) and liver (increased in females) were recorded at 300 ppm.<br><br>At 300 ppm there was a reduction in caudal epididymal sperm concentration (↓ 9%*).<br><br>There were no treatment-related gross or histopathological findings.<br><br>*Offspring toxicity*<br><br>Treatment did not result in any changes in the incidence of total litter loss, clinical signs, sex ratios, macroscopic or histopathological findings in either the F1 or F2 generation. There were no effects on sperm parameters, oestrus cycles, mating performance, fertility, gestation length nor gestation index in the F1 generation.<br><br>Slight reductions in the mean number of implantation sites and offspring survival in some of the exposed groups are summarised below: |

*Summary of implantation sites and offspring survival*

| ppm | 0 | 30 | 100 | 300 |
|---|---|---|---|---|
| | **F1 litters (produced by F0 parents)** | | | |
| mean number of implantation sites | 12.8 | 13.3 | 12.0 | 12.0 |
| total litter size day 1 | 11.9 | 12.3 | 11.5 | 10.9 |
| live birth index day 1 | 97.0% | 96.3% | 98.6% | 96.1% |
| viability index day 4 | 99.3% | 99.3% | 99.3% | 96.5% |
| lactation index day 21 | 99.3% | 97.4% | 98.9% | 95.5% |
| | **F2 litters (produced by F1 parents)** | | | |
| mean number of implantation sites | 12.4 | 13.1 | 12.8 | 11.5 |
| total litter size day 1 | 11.5 | 12.6 | 12.1 | 10.3 |
| live birth index day 1 | 98.9% | 99.5% | 98.4% | 97.6% |
| viability index day 4 | 100.0% | 100.0% | 99.7% | 99.7% |
| lactation index day 21 | 99.7% | 98.8% | 99.6% | 99.6% |

Historical control data from four studies conducted at the same laboratory with the same strain of rat (2002-2006) are presented below:

| HCD | mean | min | max |
|---|---|---|---|
| | **F1 litters (produced by F0 parents)** | | |
| number of implantation sites | 12.3 | 11.4 | 12.8 |
| total litter size day 1 | 11.4 | 10.8 | 12.0 |
| live birth index day 1 | 98.8% | 97.7% | 99.5% |
| viability index day 4 | 98.9% | 98.3% | 99.4% |
| lactation index day 21 | 97.7% | 91.6% | 100.0% |
| | **F2 litters (produced by F1 parents)** | | |
| number of implantation sites | 12.2 | 11.5 | 12.8 |
| total litter size day 1 | 11.3 | 10.5 | 11.8 |
| live birth index day 1 | 98.2% | 95.1% | 99.7% |
| viability index day 4 | 99.4% | 98.9% | 100.0% |
| lactation index day 21 | 95.1% | 90.9% | 100.0% |

Mean body weights of F1 and F2 offspring on postnatal day 1 were similar in all groups. However, treatment-related effects on body weight gain were noted during the lactational and post-weaning periods. In the F1 generation, body weight gain to day 14 was significantly lower at 100 ppm (↓ 7%*) and 300 ppm (10-13%**). Subsequent body weight gains between days 14 and 21 were unaffected by exposure. Body weight gain was reduced in the post-weaning period to day 25 at 100 ppm (↓ 10-11%*) and 300 ppm (32-34%**). Overall body weight gain (day 1-25) was clearly reduced at 300 ppm (13-15%).

In the F2 generation at 300 ppm, body weight gains from day 1-14 and 14-21 were reduced (↓ 12-13%**), as they were from days 21-25 (↓ 19-24%**).

| | | |
|---|---|---|
| | | Overall body weight gain (day 1-25) was clearly reduced at 300 ppm (15-16%).<br><br>Spleen weights relative to body weight were higher than controls in the F2 generation at 300 ppm (↑ 10-11%*); other organ weight changes in the F1 and F2 pups were attributed to the differences in body weight.<br><br>A delay in the sexual maturation of F1 females (vaginal opening) was attributed to the delayed physical development of these females as a result of lower body weights. There were no effects on the sexual maturation of males.<br><br>The NOAEL for reproductive performance was 300 ppm (22-26 mg/kg/d). The NOAEL for general parental toxicity was 100 ppm (9-10 mg/kg/d) and that for offspring toxicity was also 100 ppm (8 mg/kg/d). |

*N.B. The values for NOAEL are provided for information only; they have been agreed by a PRAPeR Expert Meeting. * Statistically significant at p ≤ 0.05. ** Statistically significant at p ≤ 0.01*

#### 4.10.1.1 Non-human information

The doses used in the two-generation study in rats were based upon a preliminary, one-generation study, in which Wistar rats (8/sex/group F0, 12/sex/group F1) were administered 0, 30, 150 or 300 ppm ipconazole for 29 days before mating, during pairing, gestation and lactation until termination; the F1 animals were then exposed from weaning until termination at approximately 8 weeks of age [43]. The only treatment-related observations were some changes in the weights of female reproductive organs (uterus and oviduct decreased in all dose groups, ovary increased at 300 ppm).

In the main study, sperm concentrations in the caudal epididymides in F0 males were reduced, but in the absence of consistent changes in the F1 generation, these minor differences were considered not to be of toxicological significance. Minimal reductions in the number of implantation sites (not statistically significant) and litter sizes on post-natal days 1, 4 and 21 were generally within relevant historical control data. Overall, fertility parameters were unaffected in this study. Offspring toxicity was evident in the reduced body weight gains in the mid- and high-dose groups, but there was no indication of specific developmental toxicity. Some changes in the weights of female reproductive organs in the preliminary study were not confirmed in the two-generation study.

#### 4.10.1.2 Human information

No information available.

### 4.10.2 Developmental toxicity

**Table 18: Summary table of relevant reproductive toxicity studies - Development**

<table>
<tr><th>Method</th><th>Dose levels</th><th>Observations and remarks<br><br>(effects of major toxicological significance)</th></tr>
<tr><td>Oral (gavage)<br><br>Rats, Sprague-Dawley, 6 or 7 mated females/ group<br><br>No guideline. GLP.<br><br>89.09% cc:10.20% ct, purity 99.29%<br><br>Kureha Corporation (1990) [44]</td><td>0, 1, 10, 50, 100, 250, 500 mg/kg/d<br><br>in 1% aqueous sodium carboxy-methylcellulose<br><br>from gestation days 6-15<br><br>Dams were sacrificed on gestation day 20</td><td>This was a preliminary study. Investigations in the dams: gross pathology, ovaries & uterine contents examined, gravid uterine weights, corpora lutea and implantations determined. Investigations in the fetuses: number, sex & weights of live fetuses & placentae, incidences of resorptions and fetal deaths; live fetuses examined for external abnormalities. There was no examination for visceral or skeletal malformations and variations.<br><br>Maternal toxicity<br><br>Deaths occurred in 6/7 and 7/7 females at 250 and 500 mg/kg/d, respectively, between gestation days 8-10.<br><br>Clinical signs occurred at ≥ 100 mg/kg/d and included red discharge from the vagina (3/7), soiled fur (1/7), eye discharge (1/7) and loose faeces (1/7). Clinical signs were not reported in 3/7 dams.<br><br>At 100 mg/kg/d dams lost weight up to day 10 (up to 13% decrease); overall there was a marked reduction in body weight gain (↓ 85% days 0-15, ↓ 52% day 0-20), statistically significant. Body weight gain was also reduced at 50 mg/kg/d (↓ 24% days 0-15, ↓ 14% days 0-20) but not with statistical significance. The maternal body weights adjusted for uterine contents were only slightly reduced at 50 mg/kg/d (see table below under developmental toxicity).<br><br>Food consumption of the 50 mg/kg/d (statistically significant gestation days 6-9) and 100 mg/kg/d groups (statistically significant gestation days 6-9 and 9-12) was lower than controls during the dosing period.<br><br>There were no treatment-related gross findings in the survivors. In those that died before scheduled sacrifice, the main finding was distended stomach with watery contents.<br><br>Developmental toxicity<br><br>Ipconazole did not affect the number of corpora lutea or implantation sites when administered in doses ≤ 100 mg/kg/d. In the one dam at 250 mg/kg/d that survived, there was 100% fetal resorption/death. At 100 mg/kg/d, there was a reduction in the number of live fetuses per litter, an increase in the % of fetal resorptions and deaths, and decreased fetal weight (all statistically significant). The same parameters were affected at 50 mg/kg/d, but without statistical significance; see table below. Placental weight was increased at 100 mg/kg/d.<br><br>
<table>
<tr><th>mg/kg/d</th><th>0</th><th>1</th><th>10</th><th>50</th><th>100</th></tr>
<tr><td>Maternal body weight (g) day 20</td><td>405</td><td>416</td><td>408</td><td>386<br>-5%</td><td>336<br>-17%</td></tr>
<tr><td>Gravid uterine weight (g)</td><td>74</td><td>84</td><td>73</td><td>64<br>-14%</td><td>26**<br>-65%</td></tr>
<tr><td>Adjusted maternal body weight (g)</td><td>330</td><td>332</td><td>335</td><td>322<br>-2%</td><td>310<br>-6%</td></tr>
<tr><td>Live fetuses/litter</td><td>13.4</td><td>14.7</td><td>13.2</td><td>12.3</td><td>4.9**</td></tr>
<tr><td>% fetal resorption/deaths</td><td>7.7%</td><td>5.8%</td><td>13.6%</td><td>19.7%</td><td>69.3%**</td></tr>
<tr><td>Mean fetal weight males (mg)</td><td>3453</td><td>3589</td><td>3532</td><td>2926<br>-15%</td><td>2026**<br>-41.3%</td></tr>
<tr><td>Mean fetal weight females (mg)</td><td>3299</td><td>3365</td><td>3185</td><td>2895<br>-12%</td><td>2181**<br>-34%</td></tr>
</table>
</td></tr>
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<table>
<tr>
<td></td>
<td></td>
<td>The following external abnormalities were recorded in the pups:<br><br>
<table>
<tr><th>mg/kg/d</th><th>0</th><th>1</th><th>10</th><th>50</th><th>100</th></tr>
<tr><td>No. fetuses/litters</td><td>94/7</td><td>88/6</td><td>79/6</td><td>86/7</td><td>34/6</td></tr>
<tr><td>Meningoencephalocoele</td><td>0</td><td>0</td><td>0</td><td>0</td><td>1</td></tr>
<tr><td>Exencephaly</td><td>0</td><td>1</td><td>0</td><td>0</td><td>0</td></tr>
<tr><td>Microphthalmia</td><td>0</td><td>1</td><td>0</td><td>2<br>1 litter</td><td>7***<br>4 litters</td></tr>
<tr><td>Open eyelid</td><td>0</td><td>1</td><td>0</td><td>0</td><td>0</td></tr>
<tr><td>Micrognathia</td><td>0</td><td>0</td><td>0</td><td>0</td><td>1</td></tr>
<tr><td>Omphalocoele</td><td>0</td><td>0</td><td>0</td><td>0</td><td>1</td></tr>
<tr><td>Kinky and/or short tail</td><td>0</td><td>0</td><td>0</td><td>1<br>(kinky)</td><td>7***<br>short=2<br>kinky=6<br>2 litters</td></tr>
<tr><td>Total no. fetuses with abnormalities/litters with affected fetuses[a]</td><td>0</td><td>2/2</td><td>0</td><td>3/1</td><td>11***<br>4* litters</td></tr>
</table>
<br>A NOAEL was not identified (range-finding study).</td>
</tr>
<tr>
<td>Oral (gavage)<br><br>Rats, Sprague-Dawley, 24 females/group<br><br>OECD 414 (1981), GLP.<br><br>89.09% cc:10.20% ct, purity 99.29%<br><br>Kureha Corporation (1990) [45]</td>
<td>0, 3, 10, 30 mg/kg/d<br><br>in 1% aqueous sodium carboxymethylcellulose<br><br>from gestation days 6 to 15<br><br>Dams were sacrificed on gestation day 20</td>
<td>The study differs from the current guideline (2001) in that the litter was not used as the unit for the analysis of fetal effects; instead, analysis was based solely on the fetal incidences. However, litter incidence data were reported in the study report. The period of dosing is less than is currently preferred (normally from GD 5 in rodents).<br><br>Maternal toxicity<br><br>There were no maternal deaths or clinical signs of toxicity at any dose.<br><br>At 30 mg/kg/d: maternal body weights were lower than controls from gestation day 7 to 20, although no significant differences were noted for body weight adjusted for gravid uterine weight. Reductions in weight gain by 17-24% were recorded during the dosing period but there was no change over days 0-20. Reduction in food consumption was recorded at certain points during gestation in this group, but was comparable with controls during the post-dosing period.<br><br>There were no treatment-related gross findings.<br><br>Developmental toxicity<br><br>Gravid uterine weights, numbers of corpora lutea, implants, live fetuses and sex ratio were unaffected.<br><br>The incidence of fetal resorptions and deaths was 3.9%, 3.8%, 7.2%, 7.6% at 0, 3, 10, 30 mg/kg/d, respectively. The incidences in the mid- and high-dose groups were within the laboratory’s historical control range (4.5-8.5% for 6 studies conducted 1988-1992; 4.5-9.3% for 10 studies 1987-1993). The incidences in the control and low-dose groups were lower than the historical control range.<br><br>Fetal body weight was reduced at 30 mg/kg/d in both sexes (↓ 6-7%*) but not at 3 and 10 mg/kg/d.</td>
</tr>
</table>

The findings of note from fetal examination are summarised below:

| mg/kg/d | 0 | 3 | 10 | 30 |
|---|---|---|---|---|
| **External malformations** | | | | |
| Number of fetuses | 324 | 352 | 339 | 354 |
| Number of litters | 23 | 23 | 24 | 23 |
| Microphthalmia | 0 | 0 | 0 | 2 (0.6%) 2 litters |
| Cleft lip | 0 | 0 | 0 | 1 (0.3%) |
| Cleft palate | 0 | 0 | 0 | 1 (0.3%) |
| Vestigial tail | 0 | 0 | 1 (0.3%) | 0 |
| Total number of affected fetuses/litters | 0 | 0 | 0 | 2/2 |
| **Visceral malformations** | | | | |
| Number of fetuses | 155 | 169 | 163 | 168 |
| Number of litters | 23 | 23 | 24 | 23 |
| Double aortic arch | 0 | 0 | 0 | 1 (0.6%) |
| Coarctation of the aorta | 0 | 0 | 0 | 1 (0.6%) |
| Right aortic arch | 0 | 0 | 1 (0.6%) | 0 |
| Aberrant right subclavian artery | 0 | 0 | 0 | 1 (0.6%) |
| Agenesis of the spleen | 0 | 0 | 0 | 1 (0.6%) |
| Total number of affected fetuses/litters | 0 | 0 | 1 | 2/2 |
| **Visceral variations** | | | | |
| Number of fetuses | 155 | 169 | 163 | 168 |
| Number of litters | 23 | 23 | 24 | 23 |
| Left umbilical artery | 1 (0.6%) | 2 (1.2%) 2 litters | 2 (1.2%) 2 litters | 7* (4.2%) 6 litters |
| **Skeletal variations** | | | | |
| Number of fetuses | 169 | 183 | 176 | 186 |
| Number of litters | 23 | 23 | 24 | 23 |
| Supernumerary ribs | 3 (1.8%) 3 litters | 1 | 8 (4.5%) 6 litters | 13* (7%) 7 litters |

Historical control data for microphthalmia: studies conducted in the same laboratory and rat strain (conducted 1985-1995) indicated that microphthalmia was observed in 3/20 studies, with fetal/litter incidences of 0.64%/4.17% (1/1), 0.83%/4.55% (1/1) and 0.65%/9.52% (2/2). The overall mean incidence was 0.06% (4/6439 fetuses).

Historical control data for cardiovascular malformations: in 20 studies conducted in the same laboratory and rat strain (1985-1995) there were no incidences of double aortic arch or coarctation of the aorta. There was one case of right aortic arch in one study (1/163 fetuses = 0.61%). 1/121 pup with aberrant right subclavian artery was observed incidentally in a treatment group of one historical control study dated 1987-1988.

Historical control data for cleft lip and cleft palate: data gathered from Japanese laboratories from the same rat strain between 1994 and 2000 indicated a background incidence of cleft lip of 0.04% (range 0 – 0.3%) in one laboratory, with no cases in another 11 laboratories. Cleft palate was recorded in three laboratories, with incidences ranging from 0 – 0.4%.

<table>
<tr><td></td><td></td><td>Historical control data for supernumerary ribs: data from same laboratory and rat strain, 3 studies conducted 1987-1988: range 1.2-2.8%<br><br>The NOAEL for maternal and developmental toxicity was 10 mg/kg/d.</td></tr>
<tr><td>Oral (gavage)<br><br>Rabbits, Japanese White, 5 females/group<br><br>No guideline. GLP.<br><br>89.09% cc:10.20% ct, purity 99.29%<br><br>Kureha Corporation (1990) [46]</td><td>0, 10, 100, 300, 1000 mg/kg/d<br><br>in 1% aqueous sodium carboxymethylcellulose<br><br>from gestation days 6 to 18<br><br>Dams were sacrificed on gestation day 27</td><td>This was a preliminary study. Investigations in the dams: gross pathology, ovaries & uterine contents examined, gravid uterine weights, corpora lutea and implantations determined; investigation for very early resorptions when no uterine implants were grossly visible. Investigations in the fetuses: number, sex & weights of live fetuses & placentae, incidences of resorptions and fetal deaths; live fetuses examined for external abnormalities. There was no examination for visceral or skeletal malformations and variations.<br><br>Maternal toxicity<br><br>All dams at 300 and 1000 mg/kg/d died during the dosing period. Clinical signs of toxicity (discharge from the eyes and soiling of fur) were seen only in these dose groups.<br><br>At 100 mg/kg/d, dams lost 264g (up to 6%) from gestation days 6 to 18, compared with a gain of 53g for the controls and 66g for the 10 mg/kg/d group. Food consumption was reduced up to about gestation day 12 in the 100 mg/kg/d group but thereafter was comparable to or higher than the controls.<br><br>At gross necropsy of the animals that died before scheduled sacrifice, changes occurred in the stomach (ulcer/erosion and/or petechia) and liver (accentuated lobular pattern and pale colour). One animal at 100 mg/kg/d had hair loss, hydropericardium and erosion of the stomach.<br><br>Developmental toxicity<br><br>Ipconazole did not affect the number of corpora lutea or implantation sites when administered in doses ≤ 100 mg/kg/d. At 100 mg/kg/d there was a decrease in gravid uterine weight, the number of live fetuses, an increase in fetal resorptions/deaths, slightly reduced fetal weight and placental weight and a change to the fetal sex ratio (see table below).<br><br>
<table>
<tr><th>mg/kg/d</th><th>0</th><th>10</th><th>100</th></tr>
<tr><td>Maternal body weight (g) day 27</td><td>4296</td><td>4333</td><td>4364</td></tr>
<tr><td>Gravid uterine weight (g)</td><td>452</td><td>427<br>-6%</td><td>265<br>-41%</td></tr>
<tr><td>Adjusted maternal body weight (g)</td><td>3844</td><td>3906</td><td>4099</td></tr>
<tr><td>Live fetuses/litter</td><td>8.8</td><td>7.3</td><td>4.8</td></tr>
<tr><td>% fetal resorption/deaths</td><td>13.8%</td><td>4.8%[a]</td><td>58.5%*</td></tr>
<tr><td>Mean fetal weight males (g)</td><td>36.8</td><td>41.3</td><td>29.4</td></tr>
<tr><td>Mean fetal weight females (g)</td><td>37.2</td><td>37.8</td><td>31.4</td></tr>
<tr><td>Sex ratio (male/total)</td><td>0.477</td><td>0.591</td><td>0.750*</td></tr>
</table>
[a]One dam had implantation sites but no fetuses, and so was excluded from the analysis by the study authors. The study report did not provide the number of implantation sites in this animal, so it was not possible to determine the number of fetal resorptions.</td></tr>
</table>

<table>
<tr>
<td></td>
<td></td>
<td>The adverse findings from fetal external examination are shown below:<br><br>
<table>
<tr><th>mg/kg/d</th><th>0</th><th>10</th><th>100</th></tr>
<tr><td>No. fetuses/litters</td><td>44 / 5</td><td>22 / 3</td><td>24 / 4</td></tr>
<tr><td>Acephaly</td><td>0</td><td>0</td><td>1</td></tr>
<tr><td>Microphthalmia</td><td>0</td><td>0</td><td>1</td></tr>
<tr><td>General oedema</td><td>0</td><td>0</td><td>1</td></tr>
<tr><td>Vestigial tail</td><td>0</td><td>0</td><td>1</td></tr>
<tr><td>Short tail</td><td>0</td><td>0</td><td>3* / 3</td></tr>
<tr><td>Kinky tail</td><td>0</td><td>0</td><td>1</td></tr>
<tr><td>Total no. fetuses with abnormalities/litters with affected fetuses[b]</td><td>0</td><td>0</td><td>6** / 3*</td></tr>
</table>
<br>
Historical control data for microphthalmia: from studies conducted in the same laboratory and strain (2001-2010), the mean incidence was 0.24% (range 0 - 2.32%) from 13 studies (2262 fetuses) [47].<br><br>
Historical control data for short or absent tail: from studies conducted in the same laboratory and strain (2001-2010), the mean incidence of short tail was 0.07% (range 0 – 0.53%) from 13 studies (2262 fetuses). There were no cases of absent tail [47].<br><br>
The total number of fetuses with malformations in this historical control data was 6 / 2262 (0.27%).</td>
</tr>
<tr>
<td>Oral (gavage)<br><br>Rabbits, Japanese White (KBL:JW), 18 females/group<br><br>OECD 414 (1981). GLP.<br><br>89.09% cc:10.20% ct, purity 99.29%<br><br>Kureha Corporation (1990) [48]</td>
<td>0, 2, 10, 50 mg/kg/d<br><br>in 1% aqueous sodium carboxymethylcellulose<br><br>from gestation days 6 to 18<br><br>Dams were sacrificed on gestation day 27</td>
<td>The study differs from the current guideline (2001) in that the litter was not used as the unit for the analysis of fetal effects; instead, analysis was based solely on the fetal incidences. However, litter incidence data were reported in the study report, although there is not a listing of the abnormalities and other findings for each individual fetus. All fetal rabbit heads were examined internally by a single transverse section, which is considered to be adequate for the detection of hydrocephalus.<br><br>
Maternal toxicity<br><br>
There were no deaths or clinical signs of toxicity.<br><br>
Maternal body weights were reduced compared with controls at 50 mg/kg/d throughout the dosing period (by up to 106 g / 3%, not statistically significant). Mean body weight change of dams at this dose was reduced on several days during dosing (e.g., -73 g between days 6 and 15). At this dose, food consumption was slightly lower than controls (not statistically significant).<br><br>
Reproductive parameters (gravid uterine weights, numbers of corpora lutea, implants, live fetuses, resorptions/fetal deaths and sex ratios) were unaffected by treatment. There were no statistically significant effects on mean fetal body weight and placental weight.<br><br>
Developmental toxicity<br><br>
The only abnormality seen at external examination was one fetus with cleft palate in the 50 mg/kg/d group. There were no substance-related adverse findings upon visceral examination.</td>
</tr>
</table>

The findings of note upon skeletal examination are shown in the table below (there were several findings with single incidences in different treatment and control groups, not shown).

| **mg/kg/d** | **0** | **2** | **10** | **50** |
|---|---|---|---|---|
| Number of fetuses / litters | 111 / 16 | 163 / 17 | 137 / 16 | 155 /17 |
| **External findings** | | | | |
| Cleft palate | 0 | 0 | 0 | 1 (0.7%) |
| **Skeletal malformations** | | | | |
| Splitting of nasal bones | 0 | 0 | 1 (0.7%) | 1 (0.7%) |
| Hemi-vertebrae | 0 | 0 | 1 (0.7%) | 2 (1.3%) 2 litters |
| Bifurcation of the ribs | 0 | 0 | 0 | 2 (1.3%) 2 litters |
| Fusion of the sternebrae | 1 (0.9%) | 3 (1.8%) | 0 | 5 (3.2%) 3 litters |
| Number of affected fetuses / litters | 1 / 1 | 3 / 2 | 2 / 2 | 8 / 4 |
| **Skeletal variations** | | | | |
| Splitting of parietal bones | 2 (1.8%) | 1 (0.6%) | 2 (1.5%) | 20*** (12.9%) |
| Cervical ribs | 0 | 0 | 0 | 3 |
| Supernumerary ribs | 9 (8.1%) | 18 (11%) | 16 (11.7%) | 24 (15.5%) |
| Lumbarisation of the sacral vertebrae | 0 | 1 (0.6%) | 1 (0.7%) | 3 (1.9%) |
| Asymmetry of sternebrae | 0 | 1 (0.6%) | 1 (0.6%) | 4 (2.6%) |

Historical control data: Based on examination of 1116 fetuses from 150 litters, the historical control data from the same laboratory and test strain (1986-1989) were

- splitting of the parietal bones: fetal incidence 1.7%, range 0-14.3%
- supernumerary ribs: fetal incidence 21.8%, range 13.9-30.1%
- lumbarisation of the sacral vertebrae: fetal incidence 1.3%, range 0-4.9%
- asymmetry of the sternebrae: fetal incidence 0.3%, no value for range

Data from the same laboratory and test strain, 2001-2010 ([46]), 13 studies (2262 fetuses)

- cleft palate: no cases
- hemivertebrae: thoracic – mean 0.04, range 0-0.48; lumbar – mean 0.13, range 0-0.70
- bifurcation of the ribs: 0.12 (0-0.95)
- fusion of the sternebrae: 0.67 (0-2.57)

The NOAEL for maternal and developmental toxicity was 10 mg/kg/d.

*N.B. The values for NOAEL are provided for information only; they have been agreed by a PRAPeR Expert Meeting. * Statistically significant at p ≤ 0.05. ** Statistically significant at p ≤ 0.01. *** Statistically significant at p ≤ 0.001.*

[a] Statistical analysis of the number of fetuses in each litter with external malformations in the preliminary rat developmental toxicity study, undertaken by the dossier submitter, indicated that there was a pronounced litter effect (see Annex III); i.e., there was a statistically significant difference ($p < 0.0004$) between the analysis when the malformation data were grouped by litter and dose compared with grouping by dose alone. [b] A similar analysis for the rabbit preliminary developmental toxicity study indicated that there was not a litter effect for the external abnormalities.

#### 4.10.2.1 Non-human information

*External and visceral findings*

In a preliminary rat study, no fetuses were obtained at the highest doses of 250 and 500 mg/kg/d because of extensive maternal deaths. At the next doses of 50 and 100 mg/kg/d, there were increased incidences of microphthalmia and kinky/short tail (statistically significant at 100 mg/kg/d). There were also single-fetal incidences of meningoencephalocoele (protrusion of brain tissue through the skull), micrognathia (one jaw unusually small) and omphalocoele (umbilical hernia) at 100 mg/kg/d. Only external investigations were undertaken in this study, not visceral examinations; it is unclear how thoroughly the eye was examined, and hence if the reported finding of microphthalmia truly fitted the description of this malformation as given in the harmonised nomenclature for developmental toxicity (DevTox[1]: microphthalmia defined as small eye, eyeball or globe of eye upon visceral examination, compared with the external finding of small eye bulge, which 'may be associated with microphthalmia'). Overall, 11 fetuses of the 100 mg/kg/d group in the preliminary study were recorded to have malformations, in four litters. Six of these fetuses were in one litter (from dam number 1015, 6/8 fetuses affected: 2 with microphthalmia alone, two with kinky tail alone, one with short tail alone, one with microphthalmia, kinky tail, micrognathia and meningoencephalocele). Three were in another (dam number 1016, 3/5 fetuses affected: one with microphthalmia and kinky tail; one with microphthalmia and kinky plus short tail; one with kinky tail and omphalocele). The remaining two litters each had one fetus with microphthalmia (1/1 and 1/4 fetuses affected, respectively). The mean fetal weight was lower in the litters from dams 1015 (male mean 43% lower, female mean 17% lower) and 1016 (male mean 21% lower, female mean 26% lower) than in the other four litters obtained at this dose. Maternal toxicity in dams 1015 and 1016 was not, however, noticeably more marked than in the other dams of the group. The three fetuses with malformations in the 50 mg/kg/d group all occurred in the same litter.

In the main rat study, sections taken of the head did not include the eyes and so they were not subjected to histological examination. However, the eyes were examined for external alterations after removing the palpebral skin, enabling a close examination of the size of the eyeball. The microphthalmia observed at 30 mg/kg/d was unilateral, with one less than 2/3 the size of the other. The two cases occurred in two separate litters. One of the fetuses with microphthalmia also had a cleft lip and cleft palate. One rabbit fetus, from a dam dosed with 100 mg/kg/d ipconazole in the preliminary study, was recorded with microphthalmia. Cleft palate was recorded in one rabbit fetus dosed with 50 mg/kg/d in the main study. One rat fetus in the 10 mg/kg/d group of the main study had a vestigial tail, but this abnormality was not recorded at 30 mg/kg/d.

Malformations of major blood vessels associated with the aortic arch were recorded in two rat fetuses at 30 mg/kg/d, one with a double aortic arch and coarctation (narrowing) of the aorta, the other (from a different litter) with an aberrant right subclavian artery and also agenesis (absence) of the spleen. In the high-dose group there was also an increase in the incidence of left umbilical artery (in rats the umbilical artery is normally on the right side), which was seen in six litters and was outside the historical control range. The study authors were uncertain of the toxicological

[1] www.devtox.org/index.htm The objectives of the DevTox project are to: harmonise the nomenclature used to describe developmental anomalies in laboratory animals; to assist in the visual recognition of developmental anomalies with the aid of photographs; and, to provide a historical control database of developmental effects in laboratory animals. Project partners: Federal Institute for Risk Assessment (BfR), Fraunhofer Institute for Toxicology and Experimental Medicine (ITEM) and Institute of Clinical Pharmacology and Toxicology, Academical Medicine, Berlin, together with contributions from international scientists from research institutions, regulatory agencies and industry.

significance of this finding, and there is no description of a malpositioned or transposed umbilical artery in ECETOC [49] nor Makris [50]; in DevTox this finding is not classified as either a malformation or variation. Dilatation of the renal pelvis and/or ureter, classified as a visceral variation, was recorded in rat pups, but in the absence of a clear dose-response relationship (1, 7, 3, 8 at 0, 3, 10, 30 mg/kg/d) it will not be considered further. A predominant malformation in the rabbit preliminary study was the occurrence of short tails at a dose of 100 mg/kg/d, which occurred in 12.5% of the fetuses and three of the four litters. In this dose group, a kinky tail was also recorded in one fetus.

*Skeletal findings*

Several skeletal findings were recorded in the studies. These included low but increased incidences of skeletal malformations in rabbits from 10 mg/kg/d, but in the absence of concurrent historical control data these are difficult to interpret. Supernumerary ribs were recorded in both rat and rabbit fetuses, but uncertainty surrounds the developmental/teratogenic significance of such ribs, in particular their post-natal reversibility or otherwise. The presence of supernumerary ribs that are small in size may be considered to be less significant with respect to teratogenic potential than ribs that are more than half the size of a full rib, which are considered to be more likely to persist post-natally. Cervical ribs were also reported in the main rabbit study; these are not classified as either malformations or variations by DevTox, and are considered to be of low to moderate concern by ECETOC. Splitting of the parietal bone was recorded in almost 13% of the rabbit fetuses when ipconazole was administered at 50 mg/kg/d (compared with an incidence of 1.8% in controls). The head of the reproductive toxicology division of the laboratory where the study was conducted provided the following commentary:

> 'Splitting (or fissure) of the parietial bone' observed in alizarin red S-stained skeletal specimens of near term fetuses of Japanese White rabbits means an isolated site of ossification (bone fragment) which is mainly found in the anterior fontanel between the right and left parietal bones. This is classified as a skeletal malformation in our laboratories although it is not known whether the part of the fissure ossifies to fuse the fragment into the parietal bone with growth or not. The term splitting (or fissure) of the parietal bone has not been found in other literature. However, 'extra ossification site (bone island, fontanellar bone, or suture bone)' may correspond to this skeletal finding.'

It was also noted that the finding was not a malformation such as might adversely affect the survival of offspring, for example partial absence of the parietal bone, since an unstained part (fissure-like area) of the parietal bone is always filled with a translucent material, suggesting the existence of unstained bone. The UK Advisory Committee on Pesticides accepted the conclusion that the finding should be regarded as an unossified line and categorised as a variation. Furthermore, Makris *et al*. [50] note that a split parietal bone should not be confused with an unossified line. Splitting of the parietal bone has been recorded in primates, but was not categorised as either a malformation or variation by DevTox. Overall, for the purposes of classification this finding will be regarded as a variation rather than a malformation. Moreover, the incidence in the high-dose group was within the historical control range.

*Fetal resorptions and deaths*

Although there appeared to be a slight (not statistically significant) increase in fetal resorptions and deaths at 10 and 30 mg/kg/d in the main rat study, the increase was within the historical control data and was compounded by the atypically low incidences in the controls and low-dose group (which were below the historical control range). A reduction in the number of live fetuses per litter, an increase in fetal resorptions and deaths, and decreased fetal weight were also reported in the

preliminary study, at 50 mg/kg/d and 100 mg/kg/d (statistically significant in the latter and associated with maternal toxicity). These parameters were also affected in the rabbit preliminary study (100 mg/kg/d), as was the sex ratio, in association with maternal toxicity; there were no effects in the main study, in which the maximum dose was 50 mg/kg/d.

#### 4.10.2.2 Human information

No information available.

### 4.10.3 Other relevant information

### 4.10.4 Summary and discussion of reproductive toxicity

In a two-generation study in rats, some maternal toxicity was evident in the high-dose group (300 ppm, equivalent to 31.6-48 mg/kg/d in female F1) as a reduction in body weight gain and food consumption. Mating performance and fertility were unaffected. There were minor reductions in the total litter size (F2 only) and live birth index on day 1 (F1 only) at 300 ppm ipconazole; as these were only marginally below the historical control ranges, were inconsistent across generations and did not show statistically significant differences from the controls, they do not provide evidence of a specific, treatment-related effect on reproduction. A slight reduction in the viability index on day 4 (F1 only) at 300 ppm ipconazole was associated with a reduction in body weight gains in these groups, and is regarded as general offspring toxicity or an effect secondary to maternal toxicity.

Several findings that indicated a developmental toxicity (teratogenic) potential for ipconazole were reported in four developmental toxicity studies, two in rats and two in rabbits. Additionally, reductions in the number of live fetuses per litter and increases in fetal resorptions and deaths were recorded in the preliminary developmental toxicity studies. The relevance of these findings to classification is discussed below. More information on the findings (body weights, sex, external malformations) in individual pups in the mid- and high-dose groups of the rat studies is presented in Annex II.

*Microphthalmia in rats and rabbits*

Microphthalmia was reported in three of the four developmental toxicity studies. It was not reported in the two-generation study, but in this study histological investigations were not routinely conducted on the offspring, and the highest tested dose was lower than was employed in some of the developmental toxicity studies. Microphthalmia is classified as a malformation and hence is associated with a high level of concern. There was some uncertainty about how thoroughly this effect was investigated in the developmental toxicity studies and thus its interpretation as either an external or a visceral finding, but in the absence of further information it will be regarded as a malformation in this report.

The incidences of microphthalmia in the two rat developmental toxicity studies (a preliminary and a main study) are presented below.

**Table 18.1. Fetal and litter incidence of microphthalmia in the preliminary and main rat developmental toxicity studies**

| mg/kg/d | 0 | 0 | 1 | 3 | 10 | 10 | 30 | 50 | 100 |
|---|---|---|---|---|---|---|---|---|---|
| Study | Prelim | Main | Prelim | Main | Prelim | Main | Main | Prelim | Prelim |
| Number of fetuses examined | 94 | 324 | 88 | 352 | 79 | 339 | 354 | 86 | 34 |
| Number of fetuses affected | 0 | 0 | 1 (1.1%) | 0 | 0 | 0 | 2 (0.6%) | 2 (2.3%) | 7 (21%) |
| Number of litters affected | 0 | 0 | 1 | 0 | 0 | 0 | 2/23 (8.7%) | 1/7 (14%) | 4/6 (67%) |
| Maternal toxicity, adjusted maternal body weights | - | - | - | - | - | - | - | 2% ↓ adjusted bw | 6% ↓ adjusted bw cs |
| Historical control data (1985-1995): microphthalmia reported in 3/20 studies, with fetal/litter incidences of 0.64%/4.17% (1/1), 0.83%/4.55% (1/1) and 0.65%/9.52% (2/2). The overall mean incidence was 0.06% (4/6439 fetuses). | | | | | | | | | |

bw = body weight; cs = clinical signs (red discharge from the vagina, soiled fur, eye discharge and loose faeces)

The one case of microphthalmia in the 1 mg/kg/d group is considered to be an incidental finding because of the absence of this effect at 3 and 10 mg/kg/d. The incidence of this malformation increased in a dose-related manner at doses of ≥ 30 mg/kg/d. However, at 30 mg/kg/d, the litter and fetal incidences were within relevant historical control ranges (same strain, laboratory and conducted within five years either side of the ipconazole study); therefore the finding at this dose might have been an incidental finding. The incidence of microphthalmia did exceed the relevant historical control data at doses of ≥ 50 mg/kg/d and thus is concluded to be treatment-related at 50 and 100 mg/kg/d. The overall mean incidence of this malformation amongst the historical controls was low (0.06% in 20 studies).

At 100 mg/kg/d in the preliminary rat study, maternal toxicity was marked, comprising weight loss, an overall reduction in body-weight gain, red discharge from the vagina, soiled fur, eye discharge and loose faeces. Maternal toxicity at 50 mg/kg/d was minimal, with only a very slight (2%) reduction in body weight adjusted for uterine contents, and there were no clinical signs of toxicity. The body weights of affected fetuses were at least 25% lower than those of unaffected litter mates (see Annexes I and II).

Microphthalmia was also reported in 1/24 rabbit fetuses, at the dose of 100 mg/kg/d in the preliminary study; this dose resulted in severe maternal toxicity (loss of weight and decreased food consumption). This incidence (4%) was outside the available historical control data (range 0 – 2.32%, but conducted more than ten years later than the ipconazole study); however, as it was a preliminary study, the number of fetuses available for examination was small. Microphthalmia was not reported in the main rabbit study, in which ipconazole was administered in doses up to 50 mg/kg/d; at this dose, maternal toxicity was minimal.

*Short/kinky tails in rats and rabbits*

Short or kinky tails were observed in the preliminary rat study (50 and 100 mg/kg/d). The main study employed lower doses (tested up to 30 mg/kg/d); therefore the absence of this effect in the main study (apart from one affected fetus in the mid-dose group, which also had anal atresia) is not inconsistent with its occurrence in the preliminary study. Malformations were observed in a rabbit

preliminary study at a dose of 100 mg/kg/d, notably an increase in the incidence of short tails, which occurred in 12.5% of the fetuses and 3 of the 4 litters; the level of concern for such malformations is high. The fetal and litter incidences in rats and rabbits are summarised in the table below.

**Table 18.2. Fetal and litter incidence of tail effects in the rat and rabbit developmental toxicity studies**

| RATS | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| **mg/kg/d** | **0** | **0** | **1** | **3** | **10** | **10** | **30** | **50** | **100** |
| Study | Prelim | Main | Prelim | Main | Prelim | Main | Main | Prelim | Prelim |
| Number of fetuses examined | 94 | 324 | 88 | 352 | 79 | 339 | 354 | 86 | 34 |
| Kinky tail: number of fetuses affected | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 (1.2%) | 6 (17.6%) |
| Short tail: number of fetuses affected | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 (5.9%) |
| Vestigial tail: number of fetuses affected | 0 | 0 | 0 | 0 | 0 | 1 (0.3%) | 0 | 0 | 0 |
| Tail effects: number of litters affected | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 1/7 (14%) | 2/6 (33%) |
| Maternal toxicity, adjusted maternal body weights | - | - | - | - | - | - | - | 2% ↓ adjusted bw | 6% ↓ adjusted bw cs |
| Concurrent historical control data from the laboratory were not available. Data from 12 Japanese laboratories, 77 studies (1994-2000), same strain. Short tail reported by 2 laboratories: mean 0.03%, range 0-0.36%; mean 0.08%, range 0-0.42%. Kinky tail reported by 1 laboratory: mean 0.08%, range 0-0.3%. Rudimentary / vestigial tail reported by 2 laboratories: mean 0.05%, range 0-0.42%; mean 0.08%, range 0-0.39% [52]. | | | | | | | | | |

| RABBITS | | | | | | | |
|---|---|---|---|---|---|---|---|
| **mg/kg/d** | 0 | 0 | 2 | 10 | 10 | 50 | 100 |
| Study | Prelim | Main | Main | Prelim | Main | Main | Prelim |
| Number of fetuses examined | 44 | 111 | 163 | 22 | 137 | 155 | 24 |
| Absent/vestigial tail: number of fetuses affected | 0 | 0 | 0 | 0 | 0 | 0 | 1 (4.2%) |
| Short tail: number of fetuses affected | 0 | 0 | 0 | 0 | 0 | 0 | 3 (12.5%) |
| Kinky tail: number of fetuses affected | 0 | 0 | 0 | 0 | 0 | 0 | 1 (4.2%) |
| Tail effects: number of litters affected | 0 | 0 | 0 | 0 | 0 | 0 | 3/4 (75%) |
| Maternal toxicity, adjusted maternal body weights | - | - | - | - | - | - | Weight loss, ↓ fc |
| Concurrent historical control data from the same laboratory were not available. Data from 13 studies (2001-2010), same strain and laboratory: mean incidence of short tail 0.07% (range 0 – 0.53%) in 2262 fetuses. There were no cases of absent tail [47]. | | | | | | | |

bw = body weight; cs = clinical signs (red discharge from the vagina, soiled fur, eye discharge and loose faeces); fc = food consumption

Tail effects were therefore reported in the rat and rabbit preliminary studies with incidences that were outside the available historical control ranges (not concurrent with the ipconazole studies), predominantly at the maternally toxic dose of 100 mg/kg/d; at this dose, the body weights of affected rat fetuses were lower than those of unaffected fetuses (see Annexes I and II). Data on the body weights of affected rabbit fetuses compared with their unaffected littermates was not available. There was one case of kinky tail in rats at 50 mg/kg/d, when maternal toxicity appeared to be minimal.

*Visceral malformations and variations in rats*

In the main rat study, two fetuses at 30 mg/kg/d had abnormalities of major blood vessels associated with the aortic arch (double aortic arch and coarctation (narrowing) of the aorta in one; aberrant right subclavian artery together with absence of the spleen in the other, from a different litter). Both these litters also contained the two pups with microphthalmia and cleft lip/cleft palate; unfortunately, the information in the study report does not allow one to determine if these were the same two pups with the blood vessel abnormalities. None of these observations was reported in the relevant historical control data, although one incidental occurrence of an aberrant subclavian artery was noted in a treatment group of a historical study. The study authors categorised these findings as malformations. At this dose, overall mean fetal body weight was 6-7% lower than the controls.

In the high-dose group there was also an increase in the incidence of left umbilical artery (statistically significant), which was seen in seven pups in six litters and was outside the historical control range. The toxicological significance of this finding is unclear, but the study authors categorised it as a variation and it has been reported to be a common spontaneous finding in Wistar Hannover rats [51].

Visceral malformations and variations were not investigated in the rat (nor rabbit) preliminary study, and so it is not known if such findings occurred at the higher doses employed in that study. No abnormalities of the major blood vessels were detected in the main rabbit study. Given that the malformations were single cases that occurred in pups with more than one malformation (double aortic arch with coarctation of the aorta; aberrant right subclavian artery with absence of spleen) and clustered in just two litters, it is concluded that they do not provide convincing evidence of specific developmental toxicity. The increase in the incidence of left umbilical artery in the high-dose group is concluded to be a treatment-related effect.

*Other malformations and variations*

In the main rabbit study, 4/17 litters contained fetuses with skeletal malformations at 50 mg/kg/d, notably splitting of the nasal bones, hemi-vertebrae, bifurcation (branching) of the ribs and fusion of the sternebrae. In the absence of concurrent historical control data for the skeletal malformations, some uncertainty surrounds the interpretation of these findings. However, splitting of the nasal bones occurred in only one fetus of each of the mid-and high-dose groups, in conjunction with other skeletal malformations in the same litter at 50 mg/kg/d; as there was no dose-response relationship and the incidence was low, this finding will be disregarded for the purposes of classification. The incidence of fusion of the sternebrae did not show a dose-response relationship (1, 3, 0, 5 at 0, 2, 10, 50 mg/kg/d) nor a statistically-significant trend and thus will also not be considered further. The incidence of hemi-vertebrae showed a slight dose-response relationship (0, 0, 1, 2 at 0, 2, 10, 50 mg/kg/d), with two litters being affected in the high-dose group, but did not show a statistically significant trend. One of the fetuses in the 50 mg/kg/d group with hemi-vertebrae also had bifurcation of the ribs. The other fetus with bifurcation of the ribs also occurred in a high-dose-group litter in which was reported hemi-vertebrae (and other skeletal effects), but from the available information it is not possible to determine if it was the same animal. It was also not possible to

evaluate individual affected pup weights compared with unaffected pups. Skeletal malformations were not observed in rats. Overall, the low incidences of skeletal malformations in rabbits alone do not provide sufficient evidence of a specific treatment-related effect.

Single incidences of cleft palates in the high-dose groups in the rat (30 mg/kg/d) and rabbit (50 mg/kg/d) main studies were reported. The affected rat pup also had a cleft lip and microphthalmia in addition to the cleft palate, which was within the available historical control range (not concurrent). The body weight of this pup was 42% lower than its litter-mates (1947 mg versus 3333 mg, standard deviation 323 mg); it is not possible to determine if this low body weight was a general manifestation of delayed development or was a consequence of a specific developmental effect, with the poor pup health being secondary to the malformations. Concurrent historical control data were also not available for rabbits. Individual rabbit fetus weights were not available, but the mean fetal weight of this litter was considerably lower than the overall group mean (57-59% reduction for females/males). No cleft lips or palates were reported at 100 mg/kg/d in the preliminary studies in either species, although it is recognised that the smaller group sizes would have reduced the sensitivity of those studies to detect rare malformations. As there were only single incidences, associated with low pup weight and (in the rat) other malformations, and no cases at the higher doses tested in the preliminary studies, the finding of cleft lip/cleft palate is insufficient evidence of a specific developmental effect.

The splitting of the parietal bone that was observed in rabbits is considered to be a variation (unossified line; unlikely to affect survival) and was within the relevant historical control range; therefore it does not represent evidence of a specific effect on development to support classification. The additional skeletal variations recorded in rabbits did not show a statistically significant increase in incidence and, where data were available, were within the relevant historical control ranges.

The supernumerary ribs in the main rat study (statistically significant at 30 mg/kg/d) may have been treatment-related, as there was a dose-related increase in incidence that exceeded the relevant but rather limited historical control data (three studies). There was no information on the size of the extra ribs; generally, however, variations of this nature are not used as evidence for classification, and they are considered to be of low to moderate concern.

*Reproductive parameters*

In the preliminary rat developmental toxicity study, marked and statistically significant reductions in live litter size (4.9 versus 13.4 in controls) and mean fetal weight (34% to 41.3% reduction) and an increase in fetal resorption/death (69.3% compared with 7.7% in controls) were recorded at 100 mg/kg/d; a litter effect for this finding was not evident. There were clinical signs of maternal toxicity in some animals at this dose. Changes in these parameters also occurred at 50 mg/kg/d and to some extent at 10 mg/kg/d, although they were not statistically significant. There was no evidence of a treatment-related effect in the main rat study when doses up to 30 mg/kg/d were employed, at which maternal toxicity was only indicated by some reduced adjusted body weights (not statistically significant) and intermittent reductions in food consumption.

In rabbits at 100 mg/kg/d, there was also a reduction in live fetuses per litter and a statistically significant increase in fetal resorption/death (58.5% compared with 13.8% in controls); at this dose, maternal toxicity was severe (weight loss and decreased food consumption). The numbers of live rabbit fetuses per litter and fetal deaths were not affected at doses up to and including 50 mg/kg/d in the main study, a dose at which reductions in maternal body weight and food consumption were not statistically significant.

It has been reported that a conceptus may die *in utero* as a result of a primary toxic effect or as a consequence of malformations that result from the teratogenic potential of a chemical substance. In rodents and rabbits, the dead conceptus is resorbed or it may be aborted in rabbits; consequently, inspection of the uterus at the end of developmental toxicity studies does not allow elucidation of the cause of death.

*Summary*

The following effects are relevant to the classification of ipconazole for developmental toxicity:

- dose-related increase in the incidence of microphthalmia in rats (2/86 pups (2.3%) at 50 mg/kg/d, 7/34 (21%) at 100 mg/kg/d compared with none in controls and outside the historical control range), preliminary study; 1/24 (4%) in rabbits at 100 mg/kg/d, outside the historical control range, preliminary study;

- dose-related increase in the incidence of tail malformations (kinky and/or short) in rats (1/86 pups (1.2%) at 50 mg/kg/d, 7/34 (21%) at 100 mg/kg/d, preliminary study; absent tail in 1/24 (4.2%) rabbit fetuses, short in 3/24 (12.5%), kinky in 1/24 (4.2%) at 100 mg/kg/d, preliminary study. All outside the available (non-concurrent) historical control range;

- left umbilical artery (variation) in 7/354 rats (4.2%) in 6/23 litters at 30 mg/kg/d in the main study, outside the historical control range;

- statistically significant increases in fetal resorptions/deaths, resulting in reduced live fetuses per litter, in rats and rabbits at 100 mg/kg/d, preliminary studies.

### 4.10.5 Comparison with criteria

*Sexual function and fertility*

The marginal and inconsistent reductions in a few reproductive parameters in the available two-generation study, conducted in rats, are not sufficient evidence that ipconazole affects fertility. There were no indications of an adverse effect on sexual function. No classification is proposed.

*Development of the offspring*

The classification of a substance in category 1A is largely based upon human evidence. As no such evidence exists for ipconazole, classification in this category is not appropriate.

For a classification in category 1B, there should be clear evidence (usually from animal studies) of an adverse effect on development in the absence of other toxic effects, or if occurring together with other toxic effects the developmental effect is considered not to be a secondary non-specific consequence of other toxic effects. Category 2 is considered to be appropriate when there is some evidence of an adverse effect on development, but it is not sufficiently convincing to place the substance in category 1. Deficiencies in the study that make the quality of evidence less convincing might lead to a classification in Category 2, as could mechanistic information that raises doubt about the relevance of the effect for humans.

Increases in fetal resorptions/deaths and malformations (microphthalmia and tail effects) were reported (above the historical control ranges) only in the preliminary studies (it is recognised, however, that the highest dose in the main rat study was below the dose at which malformations and fetal resorptions/deaths were reported in the preliminary study) and generally only at the same doses at which maternal toxicity was recorded, in the form of clinical signs and body weight changes. Moreover, litter effects in the incidence of external malformations were evident in the rat preliminary study; therefore, the number of rat fetuses with malformations tended to be clustered in a few litters rather than being more evenly distributed. This casts some doubt on the finding being caused by exposure to ipconazole, since it might have been linked to a genetic problem in the dams. There was also some uncertainty associated with the investigation of microphthalmia in these studies, although in the absence of further information it has been regarded as a malformation for the purposes of this report. Effects on the left umbilical artery in rats were categorised as variations by the study authors, which are normally regarded to be of lower concern than malformations.

There was no mechanistic information to indicate that the findings were not relevant to humans.

Other considerations were that small numbers of animals and more limited investigations are included in preliminary studies. Moreover, the doses in these studies are chosen to inform on the selection of doses for the main study so the test system is ‘pushed’, and both the rat and the rabbit ipconazole studies resulted in maternal toxicity. For a classification in Category 1B, the adverse reproductive effect should occur in the absence of other toxic effects, or if occurring together with other toxic effects the adverse effect is considered not to be a secondary, nonspecific consequence. It has been reported that maternal toxicity played a role in the aetiology of some malformations, including microphthalmia and short or absent tails, resorptions and fetal death [53], although other authors have concluded otherwise [54-57]. In the ipconazole studies, statistical significance for the developmental findings occurred exclusively in the preliminary studies at the highest, maternally toxic doses. A number of doubts were thus associated with the findings of developmental toxicity in rats and rabbits only in preliminary studies. The guidance on the application of the CLP criteria indicates that deficiencies in the study might warrant classification in category 2 rather than 1B. Overall, there are sufficient grounds to conclude that category 2 is more appropriate than category 1B.

Therefore, based on increased incidences of fetal resorptions/deaths, microphthalmia and tail malformations only at maternally-toxic doses in preliminary studies, it is concluded that ipconazole meets the criteria for classification for reproductive toxicity category 2 – H361d.

This position is consistent with the EFSA conclusion [74] of Repr. 2 – H361d published in 2013, which was based on the above findings; no additional data on ipconazole have become available since the EFSA review was concluded.

### 4.10.6 Conclusions on classification and labelling

| |
|---|
| **Repr. 2; H361d – Suspected of damaging the unborn child** |

## 4.11 Other effects

No further information of relevance to classification and labelling.

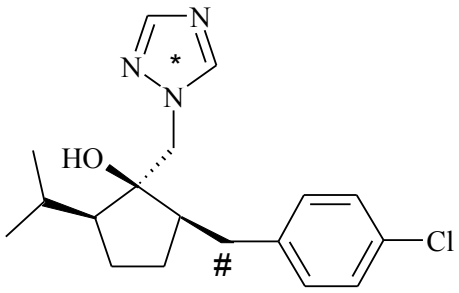
# 5 ENVIRONMENTAL HAZARD ASSESSMENT

## 5.1 Degradation

The ipconazole used in the environmental fate and behaviour studies consists of the ipconazole cc and ipconazole ct racemates. There was no evidence of a significant change in isomer ratio over the duration of any of the degradation studies. Radiolabel studies were carried out with [$^{14}$C-triazole] and/or [$^{14}$C-benzyl methylene] ipconazole with a radiochemical purity of greater than or equal to 97%. Radiolabelling positions are shown in Figure 1.

This assessment is largely based on the information provided in the Draft Assessment Report for ipconazole, Volume 3 Annex B.8 Environmental fate and behaviour and B.9 Ecotoxicology (November 2011) and Addenda 7 and 8 to the DAR – January 2013.

**Figure 1: Ipconazole structure and radiolabelling positions**




* Position of [$^{14}$C-triazole] radiolabel; # Position of [$^{14}$C-benzyl methylene] radiolabel

The relevant information on degradation is summarised in Table 19.

**Table 19: Summary of relevant information on degradation**

| Method | Results | Remarks | Reference |
|---|---|---|---|
| Aqueous hydrolysis (OECD 111) at pH 5, 7 and 9 | Hydrolytically stable (half-life >1 year at 25°C | GLP study | Hatzenbeler and Long, 2001a [58] |
| Soil photolysis (SETAC, 1995) | $DT_{50}$ ~241 days (expressed in terms of the equivalent number of days of summer sunlight at 40°N | GLP study | Shaw, 2005b [59] |
| Ready biodegradability - modified Sturm test (OECD 301B) | Not readily biodegradable | GLP study | Barnes, 2005 [60] |
| Degradation in aerobic water-sediment systems (OECD 308) | $DT_{50}$ ~241 days and 490 days for the total system in two water-sediment systems at 20°C | GLP study | Shaw, 2005d [61] |
| Degradation in soil (OECD 307) | $DT_{50}$ ~ 294 days at 20°C | GLP Study | Shaw, 2005a [62] |
| Degradation in soil (OECD 307) | $DT_{50}$ ~ 170, 184 and 225 days at 20°C in three soils | GLP study | Shaw, 2005c [63] |
| Anaerobic degradation in soil (OECD 307) | $DT_{50}$ ~ 779 days at 20°C | GLP study | Mellor, 2006 [64] |

### 5.1.1 Stability

Hydrolysis

The hydrolytic stability of ipconazole has been studied according to OECD 111 guideline [58]. The study was carried out according to GLP. The substance tested was radiolabelled, and both [$^{14}$C-triazole]- or [$^{14}$C-benzyl methylene]-ipconazole were tested.

The test was carried out using sterile aqueous buffers prepared in acetonitrile:water (2:1 v/v) containing 3 µg/ml of ipconazole. The buffers were incubated in the dark at 25±1°C for 30 days (pH 5, 7 and 9) or at 50°C±0.1°C for 7 days (pH 4, 7 and 9). The buffers were sampled on days 0, 1, 3, 7, 14, 21 and 30 (25°C experiment) and 0, 1, 3 and 7 days after application (50°C experiment). At each interval, duplicate samples were taken and analysed by Liquid Scintillation Counting (LSC) with a further sample also taken for parent compound analysis by high performance liquid chromatography (HPLC). All samples were analysed immediately after sampling. No traps for possible volatile products were included in the test design.

The measured concentrations of ipconazole in the buffers on day 0 were 2.67-2.91µg/ml, (mean 2.81 µg/ml). At pH5, 7 and 9 for the 25°C experiment 90.9-96.4% of the applied radioactivity (all attributed to parent compound) was recovered after 30 days. At pH5, 7 and 9 for the 50°C experiment 95.3-101.5% of the applied radioactivity (all attributed to parent compound) was recovered after 7 days. The average mass balance was 95.5% of the applied radioactivity at 25°C and 97.9% at 50°C. In all samples, unchanged ipconazole accounted for 99% of the radioactivity based on HPLC analysis.

Ipconazole was found to be hydrolytically stable in aqueous buffered solutions of pH 5, 7 and 9 at 25±1°C, and pH 4, 7 and 9 at 50±0.1°C, with a reported half-life greater than 1 year. No metabolites, degradation and reaction products were formed.

Photolysis

There are no data on aqueous photolysis. Aqueous photolysis is considered unlikely as there is no significant absorption at >290 nm (Draft Assessment Report (DAR) - Ipconazole - Volume 3, Annex B.2: Physical and Chemical properties – November 2011).

A soil photolysis study was conducted according to the SETAC guidelines (1995) and GLP (Shaw, 2005b [59]).

The test was carried out using [$^{14}$C-triazole]ipconazole (92.6% cc: 5.4% ct) and [$^{14}$C-benzyl methylene]ipconazole (95.6% cc:2.3% ct), applied at a nominal rate of 10.8 µg/10 cm$^2$ (calculated by the study author to be equivalent to an agricultural use rate of 108 g as./ha).

The soil used was a sandy loam consisting of 56.13% sand, 26.37% silt and 17.51% clay. The soil had an organic carbon content of 1.9% and a pH of 6. An aqueous slurry of 2 mm sieved soil was applied to glass plates, the thickness adjusted to 2 mm and allowed to air-dry overnight at approx 35°C. Stock solutions for each radiolabelled form were separately prepared in ethanol, and aliquots (100 µl organic solvent per soil layer) were applied evenly to the surface of 28 soil layers (i.e. 28 soil regions on 14 glass plates).

Of the 14 treated glass plates, 1 was taken for immediate (zero-time) analysis, 1 was taken for assessment of the distribution of radioactivity, 6 were placed in the Suntest apparatus for irradiation and 6 were placed in dark control incubation. Irradiated and dark control samples were incubated at

approx 20°C for periods up to 10 days under a stream of humidified air, which was then connected in series to VOC and $^{14}CO_2$ traps.

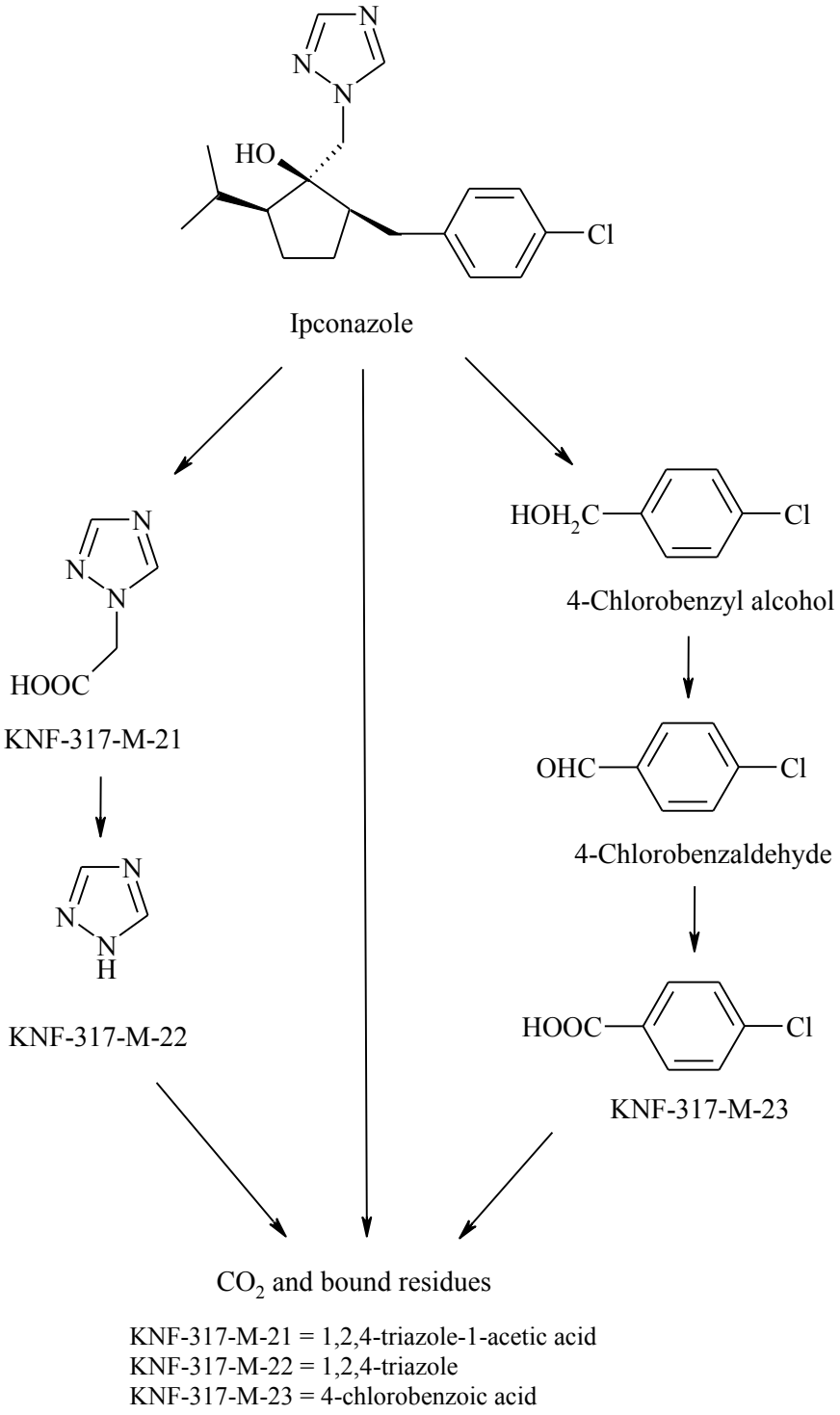
Irradiated samples were continuously irradiated using an artificial natural sunlight source (290-800 nm range) from a xenon arc light (wavelengths <290 nm were specifically excluded by filters). The mean irradiance from the lamp was 60.4 W/m$^2$ measured over the wavelength range 290–400 nm; this was thought to be equivalent to exposure to 40.64 days summer sunlight (assuming 12 hour day/night periods) at 40˚N.

For each radiolabelled form of [$^{14}$C]-ipconazole, duplicate soil layers (i.e. 1 glass plate) were taken for analysis after 2, 4, 6, 8 and 10 days of irradiation or dark control incubation. At each sampling occasion, trapping solutions were exchanged for fresh solutions and taken for analysis.

No significant degradation was seen in the dark control samples. In the exposed samples the amount of ipconazole, expressed as a % of the applied radioactivity declined to around 79% after 10 hours irradiation. There was no significant change in the isomer ratio. It was estimated that the $DT_{50}$ for the triazole-label ipconazole was around 241 days (expressed in terms of the equivalent number of days of summer sunlight at 40°N (southern Europe)).

A number of minor metabolites were seen in the illuminated samples, along with two more major metabolites. A major metabolite seen was 1,2,4-triazole, which was formed at a maximum of 10.4% of the applied radioactivity on day 8 (equivalent of 32.5 days 40˚N summer sunlight) of the study. In addition, 4-chlorobenzaldehyde was formed up to 6.3% of the applied radioactivity at day 8 of the study. The proposed route of photolytic degradation on soil is shown in Figure 2.

**Figure 2: Proposed route of photolytic degradation of ipconazole in soil**




KNF-317-M-21 = 1,2,4-triazole-1-acetic acid
KNF-317-M-22 = 1,2,4-triazole
KNF-317-M-23 = 4-chlorobenzoic acid

### 5.1.2 Biodegradation

#### 5.1.2.1 Biodegradation estimation

#### 5.1.2.2 Screening tests

Ready biodegradation of ipconazole was investigated using a modified Sturm test, according to the OECD 301B test guideline (Barnes, 2005[60]). The study was conducted in accordance with GLP, with minor deviations from the recommended temperature range of 22±2°C, (minimum and maximum 19.8 and 24.4°C, respectively), which were not considered to impact on the validity of the results.

The tests were carried out in amber glass culture (5 litre) bottles containing standard mineral medium. The bottles were inoculated with activated sewage sludge (30 mg/l) and aerated overnight with $CO_2$-free air prior to addition of the test substance (nominal concentration 10 mg C/l). Two control vessels were prepared, one containing inoculated mineral salts medium and the other inoculated mineral salts medium plus sodium benzoate (at a nominal concentration of 10 mg C/l). Additional mixtures were prepared of sodium benzoate and ipconazole (nominal 10 mg C/l each) to assess the potential inhibitory effect of test substance on activity of the microbial inoculum.

The degradation of sodium benzoate based on $CO_2$ production was 66% after 6 days and 93% after 29 days in the absence of ipconazole. In the presence of ipconazole, sodium benzoate biodegraded by 60% after 8 days, indicating, in the view of the study author, that ipconazole was not inhibitory to the activity of the microbial inoculum. Cumulative levels of $CO_2$ production in the controls after 29 days (77.0 and 78.7 mg $CO_2$) were within the acceptable range for this assay system, confirming that the inoculum was viable.

There was no evidence of $CO_2$ production by mixtures containing ipconazole after 29 days. As less than 60% of theoretical $CO_2$ production was reached within 29 days, ipconazole is considered to be not readily biodegradable.

#### 5.1.2.3 Simulation tests

Water-sediment systems

Aerobic water-sediment system degradation studies were conducted for ipconazole according to OECD guideline 308 (2002). The study was performed according to GLP (Shaw, 2005d[61]). The study was carried out at 20±2°C but a minor deviation outside of the temperature range occurred at the end of the acclimatisation period and beginning of the incubation period (range 16.1-26.1°C). This is not considered to affect the validity of the results.

The water and sediments used in the study were taken from two sources: a small pond (Bury Pond, Cambridgeshire, UK) and a large lake (Emperor Lake, Derbyshire, UK). The sediments were classified as a sandy loam clay (Bury Pond) and a loamy clay (Emperor Lake) and had an organic carbon content of 2.0% and 2.6% respectively and a pH of 7.7 and 6.9 respectively.

The substance used in the test was either [$^{14}$C-triazole] (96.2% cc: 5.4% ct) or [$^{14}$C-benzyl methylene]-labelled (95.6% cc: 2.3% ct) ipconazole at a nominal rate of 0.036 mg/l. Solutions of the [$^{14}$C]-ipconazole in ethanol were applied to the water phase of the sediment and the systems were incubated at 20°C in the dark for up to 100 days. The test vessels were connected to a series of

traps for collection of volatile organic compounds (one trap) and $^{14}CO_2$. Duplicate samples (one of each radiolabel) and trapping solutions were taken for analysis immediately after application and after 1, 2, 7, 14, 30, 59 and 100 days incubation. Additionally, samples of water were taken for analysis at 7, 14, 30, 44, 59, 73, 87 and 100 days. The $^{14}C$- present in the trapping solutions was analysed by LSC. The water and sediment samples were extracted and analysed by both LSC and HPLC methods. In addition the relative proportion of ipconazole cc and ipconazole ct were investigated in selected samples, and selected samples that had been treated with [$^{14}C$-triazole]ipconazole were analysed by thin layer chromatography (TLC) to investigate the presence of free triazole metabolites.

Redox potential and oxygen content measured in the water phase of both systems indicated aerobic conditions were maintained. In the sediment phase, the redox potential was generally <200 mV (and in the Bury Pond system was 14-46 mV over day 0-59), indicating reducing conditions. For both test systems, the range of pH in the water and sediment phases was 7.23-8.09 and 6.47-7.98, respectively.

The distribution of radioactivity in the two water-sediment systems determined during the study is shown in Figure 3 to Figure 6.

**Figure 3: Mass balance for [$^{14}C$-triazole]ipconazole in the Bury Pond water-sediment system**

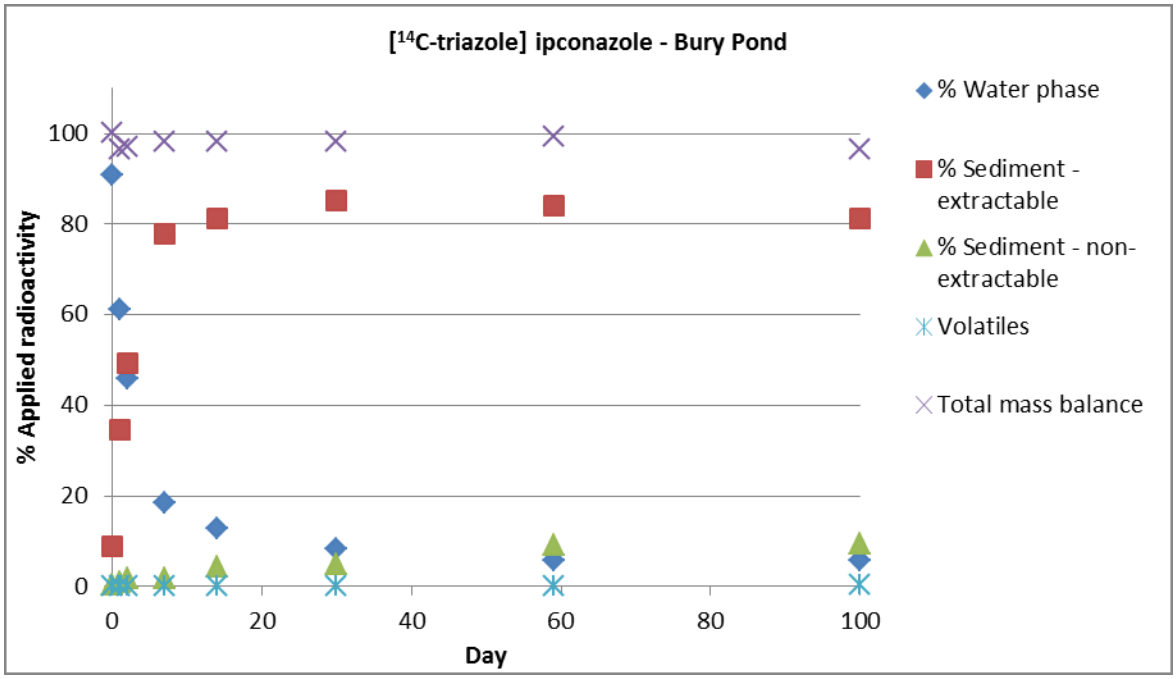


**Figure 4: Mass balance for [$^{14}$C-benzyl methylene]ipconazole in the Bury Pond water-sediment system**

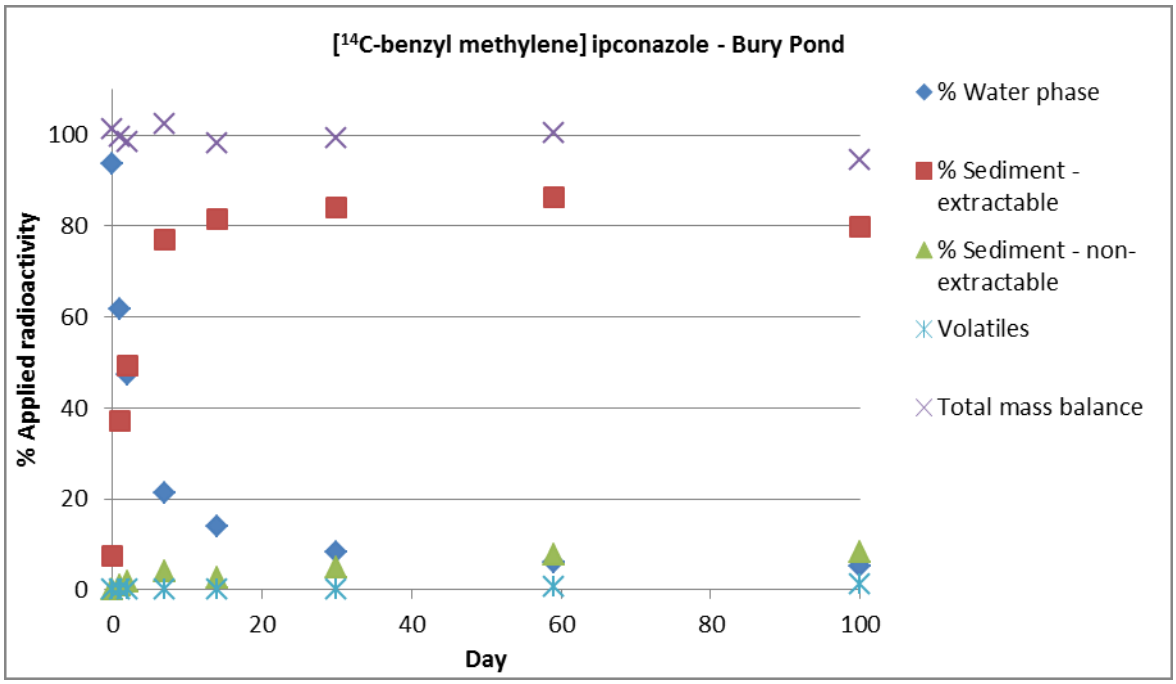



**Figure 5: Mass balance for [$^{14}$C-triazole]ipconazole in the Emperor Lake water-sediment system**

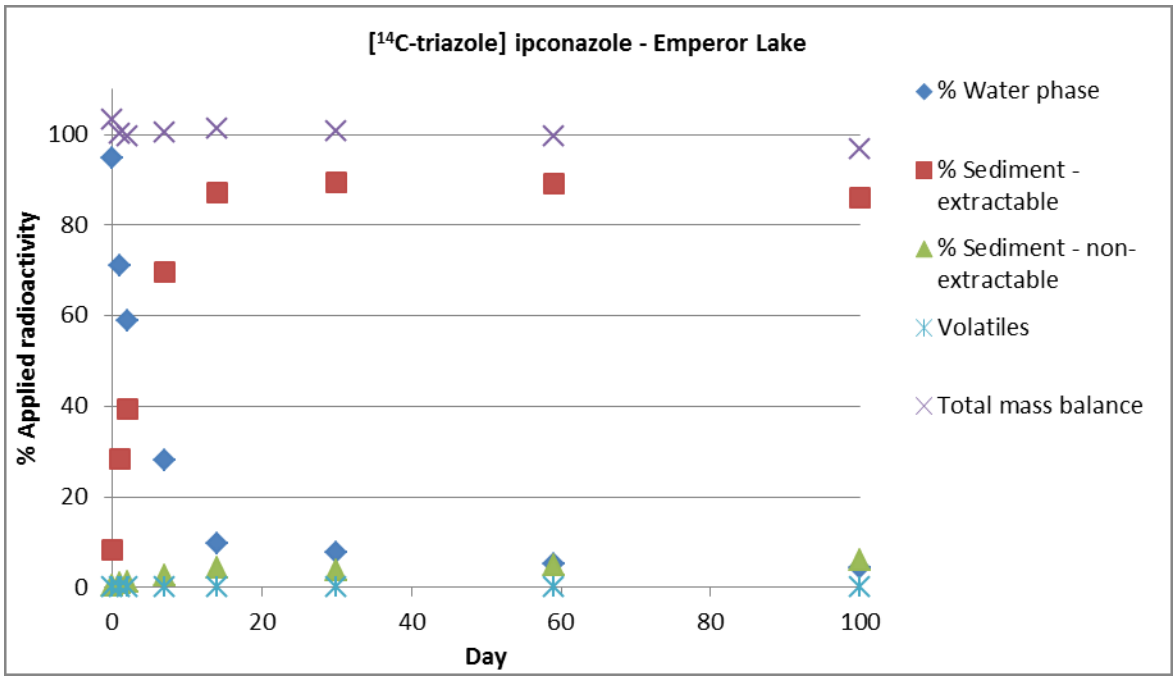
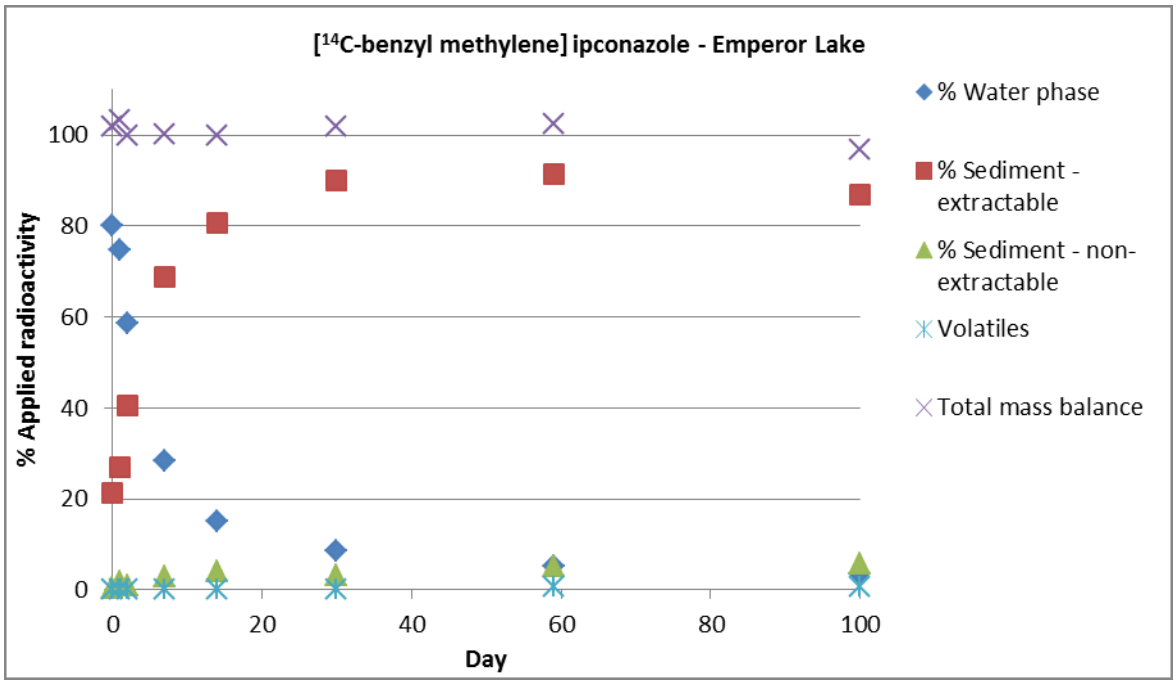


**Figure 6: Mass balance for [$^{14}$C-benzyl methylene]ipconazole in the Emperor Lake water-sediment system**




As can be seen above, the radioactivity partitioned from the water phase to the sediment phase, with over 80% of the radioactivity being present in the sediment phase after around 7-14 days incubation. Only a small proportion of the radiolabel was present as volatiles or $^{14}CO_2$ (1.1% or less), indicating that mineralisation was insignificant over the course of the study. Parent compound analysis indicated that, by day 100 of the study, around 67.7-75.2% (Bury Pond sediment) or 81.9-83.2% (Emperor Lake sediment) of the radiolabel present in the total system (water phase plus sediment phase) was as unchanged ipconazole (Table 20 and 21). There was no appreciable change in the relative ratio of isomers (cc to ct) present in samples analysed on day 0 and day 59 of the study, indicating that preferential degradation of one isomer over the other was not occurring in the study.

The time for 50% degradation ($DT_{50}$) for water, sediment and total water-sediment system are summarised in Table 22. The $DT_{50}$ in the total water-sediment system in this study is clearly >100 days (the study author estimated the $DT_{50}$ for the total water-sediment system to be around 241 days in the Bury Pond system and 490 days in the Emperor Lake system).

**Table 20: Characterisation of radioactivity from the Bury Pond water-sediment system after treatment with radiolabelled ipconazole (nominal rate of 0.036 mg/l) at 20°C.**

| Identity | % Applied radioactivity (days after application) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 0 | 1 | 2 | 7 | 14 | 30 | 59 | 100 |
| [$^{14}$C TRIAZOLE]-IPCONAZOLE – TOTAL SYSTEM | | | | | | | | |
| Ipconazole | 96.1 | 94.4 | 91.5 | 90.0 | 87.8 | 88.0 | 77.1 | 75.2 |
| Metabolite 1 | 1.3 | [a] | [a] | [a] | 0.4 | 0.5 | 1.0 | 2.7 |
| Metabolite 2 | 0.5 | 0.3 | [a] | [a] | [a] | 0.3 | 1.4 | 0.9 |
| Metabolite 3 | 0.4 | [a] | 0.4 | 0.4 | 1.3 | 0.5 | 1.5 | 4.1 |
| KNF-317-M-1 (see text) | 0.5 | [a] | 0.2 | 0.6 | 0.4 | 2.3 | 4.8 | 1.4 |
| Metabolite 5 | 0.9 | 0.4 | 0.5 | 0.8 | [a] | 0.3 | 1.5 | [a] |
| Others | [a] | 0.7 | 2.6 | 4.5 | 3.8 | 1.3 | 2.7 | 2.6 |
| [$^{14}$C BENZYL METHYLENE] IPCONAZOLE – TOTAL SYSTEM | | | | | | | | |
| Ipconazole | 95.7 | 94.6 | 92.9 | 92.9 | 89.3 | 86.7 | 79.8 | 67.7 |
| Metabolite 1 | 0.3 | [a] | [a] | [a] | 0.3 | 1.2 | 0.2 | 2.6 |
| Metabolite 2 | 0.3 | [a] | [a] | [a] | [a] | 1.8 | 1.3 | 3.6 |
| Metabolite 3 | 0.2 | 0.6 | 0.6 | 0.5 | 2.4 | 0.8 | 1.8 | 2.4 |
| KNF-317-M-1 (see text) | [a] | [a] | [a] | 0.8 | 0.8 | 0.9 | 6.3 | 5.7 |
| Metabolite 5 | 0.5 | 0.7 | 0.5 | 0.3 | [a] | 0.0 | 1.2 | 1.4 |
| Others | 4.3 | 2.7 | 2.7 | 3.9 | 2.5 | 2.8 | 1.6 | 1.8 |

Notes: a) Not apparent or below the LOD.
Others: Radioactivity not associated with specific components.

**Table 21: Characterisation of radioactivity from the Emperor Lake water-sediment system after treatment with radiolabelled ipconazole (nominal rate of 0.036 mg/l) at 20°C.**

| Identity | % Applied radioactivity (days after application) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 0 | 1 | 2 | 7 | 14 | 30 | 59 | 100 |
| [$^{14}$C TRIAZOLE]-IPCONAZOLE – TOTAL SYSTEM | | | | | | | | |
| Ipconazole | [b] | 97.9 | 93.7 | 92.5 | 92.4 | 92.7 | 86.7 | 83.2 |
| Metabolite 1 | [b] | 0.2 | 0.2 | [a] | 0.9 | 1.0 | 0.4 | 1.7 |
| Metabolite 2 | [b] | [a] | [a] | [a] | [a] | 0.2 | 0.2 | 0.4 |
| Metabolite 3 | [b] | [a] | 0.2 | [a] | 0.1 | 0.7 | 0.8 | 0.7 |
| KNF-317-M-1 | [b] | [a] | [a] | [a] | 0.3 | 1.0 | 1.4 | 1.6 |
| Metabolite 5 | [b] | 0.1 | 0.6 | 0.2 | 0.4 | 0.7 | 0.9 | [a] |
| Others | [b] | 1.1 | 3.7 | 5.1 | 2.8 | 0.7 | 4.1 | 2.7 |
| [$^{14}$C BENZYL METHYLENE] IPCONAZOLE – TOTAL SYSTEM | | | | | | | | |
| Ipconazole | 96.6* | 94.7 | 93.3 | 94.9 | 91.8 | 95.6 | 89.5 | 81.9 |
| Metabolite 1 | 0.1 | [a] | [a] | [a] | [a] | 0.8 | 0.1 | 2.3 |
| Metabolite 2 | 0.6 | 0.4 | 0.2 | [a] | 0.4 | 0.2 | 0.3 | 0.3 |
| Metabolite 3 | 0.5 | 0.1 | 0.8 | [a] | 0.8 | 0.2 | 1.2 | [a] |
| KNF-317-M-1 | [a] | 0.1 | 0.6 | [a] | 0.2 | 0.9 | 1.4 | 3.0 |
| Metabolite 5 | 0.6 | 0.3 | 0.6 | [a] | 0.4 | 0.4 | 0.8 | [a] |
| Others | 3.1 | 5.8 | 3.8 | 2.3 | 2.3 | 0.3 | 3.3 | 3.2 |

Notes: a) Not apparent or below the LOD.
b) The HPLC radiochromatogram of the sediment extracts in the day 0 sample was anomalous (a component, reported as an impurity not seen in other samples, eluted in addition to ipconazole).
Others: Radioactivity not associated with specific components.
* Sample values not included in kinetic modelling as they were reported to be outliers.

**Table 22: $DT_{50}$ of ipconazole in aerobic water-sediment systems**

| Phase | Assumed kinetics[a] | Time for 50% degradation - $DT_{50}$ (days) | | |
|---|---|---|---|---|
| | | Bury Pond | Emperor Lake | Geometric mean |
| Water | FOMC | 2 | 2.8 | 2.37 |
| | pseudo SFO | 5.3 | 5.8 | 5.54 |
| Sediment | SFO | 244[b] | 441[b] | 328[b] |
| Total system | SFO | 241[b] | 490[b] | 344[b] |

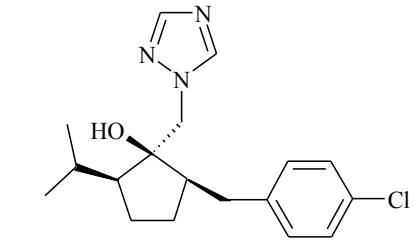
Notes: a) FOMC = first order multi-compartment; SFO = single first order.
b) Extrapolated beyond the study duration of 100 days.

No major metabolites (>10% of the applied radioactivity) were detected in water or sediment. One minor metabolite, labelled as KNF-317-M-1, peaked in the sediment after 59 days (around 6.3% of applied radioactivity in the total system) in Bury Pond sediment treated with [$^{14}$C-benzyl methylene]-ipconazole. With the [$^{14}$C-triazole]-ipconazole radiolabel and in the Emperor Lake system (both radiolabels), levels of KNF-317-M-1 were lower. At the study end, KNF-317-M-1 was still increasing in the Emperor Lake sediment but was declining at study end in the Bury Pond sediment. Four additional minor metabolites were detected at very low levels, each individually

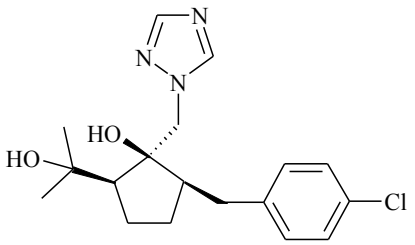
≤4.1% of the applied radioactivity (total system) in both systems. However, as degradation of ipconazole was minimal in both of the water-sediment systems, metabolites may not have had sufficient time to form in significant amounts by the study end. Therefore, the full route of degradation of ipconazole in such systems may not be fully defined.

The proposed metabolic pathway is outlined below in Figure 7 (UK, 2011; EFSA, 2013 [74]). The metabolite KNF-317-M-1 was thought to be formed by hydroxylation of the methylethyl moiety attached to the triazole ring in ipconazole. It is possible that some minor cleavage of the methylene bridge between the cyclopentane ring and phenyl ring moieties may occur, based on evidence of production of $^{14}CO_2$ (1.1% applied radioactivity) in aerobic aquatic systems treated with [$^{14}$C-benzyl methylene]-ipconazole.

**Figure 7: The metabolic pathway proposed by the applicant for ipconazole in water-sediment systems.**




Ipconazole




KNF-317-M-1

$CO_2$ and bound residues

Soil systems - aerobic

Study 1:
The degradation of ipconazole in aerobic soil was studied in a GLP study using the OECD 307 guideline (Shaw, 2005a[62]).

The test was carried out using both [$^{14}$C-triazole]ipconazole (cc 92.6%, ct 5.4%) and [$^{14}$C-benzyl methylene]ipconazole (cc 95.6%, ct 2.3%).

The soil used in the test was a sandy loam (56.13% sand, 26.37% silt and 17.51% clay) with an organic carbon content of 1.9% and a pH of 6. The soil was sieved (2 mm) and portions of the soil (equivalent to 200 g on a dry weight basis) were incubated in glass bottles within air flow-through systems, under a continuous humid air supply, in the dark at 20°C for one week prior to the start of the test. The soil moisture tension was adjusted to pF2[2] and maintained at this tension for the duration of the test. The test vessels were connected in series to one VOC trap and two $CO_2$ traps.

Stock solutions of the test substance were prepared in ethanol and/or water and aliquots were applied evenly to the soil surface (the volume of organic solvent added to the soil did not exceed 0.5% of the dry soil weight (v/w)). The soils were then mixed to ensure uniform incorporation of the test substance. The substances were applied to the soil at a nominal rate of 0.288 mg/kg. The rate was chosen by the applicant on the basis of an agricultural use rate of 108 g a.s./ha, assuming an incorporation depth of 2.5 cm, and a soil bulk density of 1.5 g/cm$^3$. In addition soil samples were also prepared at a higher nominal loading rate of 2.88 mg/kg in order to generate additional quantities of metabolites for identification, however none of these samples were utilised further.

The spiked soils were then incubated in the dark at 20°C for up to 230 days. At intervals during the study a single sample was taken for each radio-label for analysis; trapping solutions were also taken for analysis at the same time as for the soil samples. The $^{14}$C- present in the trapping solutions was analysed by LSC. The soil samples were extracted and analysed by both LSC and HPLC methods. In addition the relative proportion of cc and ct ipconazole were investigated in selected samples, and selected samples that had been treated with [$^{14}$C-triazole] ipconazole were analysed by thin layer chromatography (TLC) to investigate the presence of free triazole metabolites.

The results of the study are summarised in Table 23 and 24. By day 230 of the study 55.4% of the radiolabel from the [$^{14}$C-triazole] ipconazole was found to be parent ipconazole and 51.3% of the radiolabel from the [$^{14}$C-benzyl methylene] ipconazole was found to be parent ipconazole.

[2] Soil moisture content at field capacity of the soil.

**Table 23: Distribution of radioactivity from aerobic soil after treatment with [$^{14}$C] ipconazole at the rate of 0.288 mg/kg**

| Distribution | % Applied radioactivity (days after application) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | **0** | **3** | **7** | **14** | **30** | **60** | **90** | **122** | **230** |
| [$^{14}$C-TRIAZOLE] IPCONAZOLE | | | | | | | | | |
| Extractable soil residues | 95.9 | 96.8 | 93.9 | 92.4 | 93.3 | 88.3 | 82.3 | 83.6 | 68.4 |
| Unextractable soil residues | 2.8 | 4.2 | 4.7 | 6.6 | 8.2 | 12.2 | 14.0 | 14.2 | 17.8 |
| Volatiles $^{14}CO_2$ | NA | 0.2 | 0.2 | 5.9[a] | 2.3 | 2.3 | 3.2 | 4.3 | 9.3 |
| Organic | NA | ND | ND | ND | 0.1 | 0.1 | ND | 0.1 | 0.1 |
| Total | 98.7 | 101.2 | 98.8 | 104.9 | 103.9 | 102.9 | 99.5 | 102.2 | 95.6 |
| [$^{14}$C BENZYL METHYLENE] IPCONAZOLE | | | | | | | | | |
| Extractable soil residues | 95.7 | 92.5 | 92.6 | 88.8 | 91.4 | 86.7 | 79.5 | 78.8 | 62.2 |
| Unextractable soil residues | 2.2 | 3.6 | 5.3 | 6.9 | 7.6 | 10.0 | 11.0 | 13.8 | 14.6 |
| Volatiles $^{14}CO_2$ | NA | 0.6 | 1.3 | 2.3 | 3.5 | 5.7 | 8.2 | 9.8 | 16.0 |
| Organic | NA | ND | ND | ND | ND | ND | 0.1 | ND | ND |
| Total | 97.9 | 96.7 | 99.2 | 98.0 | 102.6 | 102.5 | 98.8 | 102.4 | 92.8 |

Notes: a) 1.7% of the radioactivity trapped in the KOH traps for this sample was not $CO_2$.
NA: Not applicable.
ND: Not detected.

**Table 24: Identification of radioactive components from aerobic soil after treatment with [$^{14}$C]-ipconazole at the rate of 0.288 mg/kg.**

| Identity | % Applied radioactivity (days after application) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | 0 | 3 | 7 | 14 | 30 | 60 | 90 | 122 | 230 |
| [$^{14}$C TRIAZOLE] IPCONAZOLE | | | | | | | | | |
| Ipconazole | 90.4 | 88.5 | 83.9 | 87.3 | 83.9 | 77.0 | 67.5 | 72.6 | 55.4 |
| Polar metabolites | 0.4 | 3.8 | 3.0 | 1.8 | 3.0 | 2.7 | 4.8 | 2.0 | 2.2 |
| Metabolite 1 | ND | ND | 3.2 | ND | ND | ND | 1.4 | 0.3 | 0.5 |
| Metabolite 2 | 0.3 | ND | 0.2 | ND | 0.2 | 0.5 | 0.6 | 0.3 | 0.5 |
| Metabolite 3 | 0.5 | 0.6 | 0.4 | 0.6 | 0.7 | 0.8 | 1.0 | 1.0 | 1.5 |
| Metabolite 4[a] | ND | 0.5 | 0.7 | 0.8 | 1.9 | 2.9 (1.8) | 3.5 | 3.1 (1.8) | 4.4 |
| Metabolite 5 | 0.7 | 0.6 | 0.5 | 0.4 | 0.4 | 0.7 | 0.4 | 0.5 | 0.3 |
| KNF-317-M-11 (see text) | 2.6 | 1.7 | 1.0 | 0.9 | 2.2 | 2.9 | 2.6 | 3.2 | 3.1 |
| Others | 1.0 | 1.1 | 1.0 | 0.6 | 1.0 | 0.8 | 0.5 | 0.6 | 0.5 |
| [$^{14}$C BENZYL METHYLENE] IPCONAZOLE | | | | | | | | | |
| Ipconazole | 90.0 | 85.7 | 86.7 | 84.4 | 83.2 | 79.6 | 69.1 | 68.9 | 51.3 |
| Polar metabolites | 1.2 | 1.9 | 0.9 | 0.3 | 1.9 | 0.3 | 0.6 | 0.2 | 0.8 |
| Metabolite 1 | ND | 0.3 | 1.7 | ND | 0.3 | 0.3 | 0.5 | 0.4 | 0.4 |
| Metabolite 2 | 0.4 | 0.4 | 0.4 | 0.3 | 0.4 | 0.4 | 0.6 | 0.4 | 0.6 |
| Metabolite 3 | 0.5 | 0.5 | 0.5 | 0.6 | 0.8 | 1.0 | 1.1 | 1.1 | 1.2 |
| Metabolite 4[a] | 0.1 | 0.6 | 0.5 | 0.9 | 1.7 | 2.5 (1.6) | 3.2 (2.2) | 3.8 | 3.7 |
| Metabolite 5 | 0.7 | 0.7 | 0.6 | 0.4 | 0.5 | 0.5 | 0.4 | 0.4 | 0.4 |
| KNF-317-M-11 (see text) | 1.4 | 1.4 | 0.6 | 0.9 | 1.6 | 1.4 | 3.2 | 3.0 | 2.6 |
| Others | 1.4 | 1.0 | 0.7 | 1.0 | 1.0 | 0.7 | 0.8 | 0.6 | 1.3 |

Notes: a) Metabolite 4 represents a zone of radioactivity that contained at least 3 components, 1 of which was identified as KNF-317-M-1 (see text). The proportion of KNF-317-M-1 in selected samples is stated in brackets alongside the value for the metabolite 4.

ND: Not detected.

Others: Radioactivity not associated with specific components.

The $DT_{50}$ (time for 50% degradation) of ipconazole was estimated to be 294 days (mean value for both radiolabels). The extent of mineralisation ($^{14}CO_2$ formation) was between 9.3 and 16% of the applied radioactivity after 230 days. The metabolites formed are considered further below.

Study 2:
A further aerobic soil degradation study was conducted according to the OECD 307 guideline and GLP (Shaw, 2005c[63]). The study was carried out using [$^{14}$C triazole] ipconazole in 3 soil types. The soils were a sandy clay loam (53.51% sand, 24.02% silt and 22.47% clay; 4.6% organic carbon content and pH 7.7), a silt loam (13.91% sand, 59.71% silt and 26.38% clay; 4.5% organic carbon content and pH 6.1) and a clay loam (39.19% sand, 29.09% silt, 31.72% clay; 2.0% organic carbon content and pH 7.1).

The test was carried out at 20°C but one soil was also tested at 10°C. The nominal application rate of the test substance was 0.288 mg/kg and the study methodology was essentially the same as the Shaw (2005a) study reported above, except that the study duration was 120-122 days rather than 230 days.

The results of the study are summarised in Table 25 and Table 26. At the end of the study, 55.1%-85.4% of the applied radioactivity remained as parent ipconazole and the $DT_{50}$ was determined to be 170 days at 20°C and 593 days at 10°C in the sandy clay loam soil, 225 days at 20°C in the silt loam soil and 184 days at 20°C in the clay loam soil. Isomer ratios were investigated at days 0 and 90, with isomer ratios remaining virtually unchanged at these three sample points, indicating that there was not preferential degradation of one isomer over another. Mineralisation ($^{14}CO_2$ formation) was between 0.2 and 3.9% of the applied radioactivity after 120 days at 20°C and around 0.1% of the applied radioactivity in the experiment at 10°C). The metabolites formed are considered further below.

**Table 25: Distribution of radioactivity from four aerobic soils after treatment with [$^{14}$C] ipconazole at a rate of 0.288 mg/kg and following incubation at 20°C and 10°C**

| **Distribution** | **% Applied radioactivity** | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| **Days after application** | **0** | **3** | **7** | **14** | **30** | **59** | **90** | **120** |
| Sandy clay loam soil incubated at 20°C | | | | | | | | |
| Extractable soil residues | 94.8 | 96.0 | 96.4 | 95.5 | 84.0 | 89.6 | 82.9 | 67.9 |
| Unextractable soil residues | 7.9 | 5.1 | 5.6 | 6.7 | 15.4 | 10.5 | 17.8 | 33.2 |
| Volatiles $^{14}CO_2$ | NA | ND | ND | ND | 0.1 | 0.4 | 0.7 | 1.0 |
| Organic | NA | ND | ND | ND | ND | ND | ND | ND |
| Total | 102.7 | 101.1 | 102.0 | 102.2 | 99.5 | 100.5 | 101.4 | 102.1 |
| Silty clay loam soil incubated at 20°C | | | | | | | | |
| **Days after application** | **0** | **3** | **7** | **14** | **32** | **60** | **90** | **122** |
| Extractable soil residues | 90.2 | 100.2 | 87.6 | 95.4 | 89.1 | 86.1 | 76.8 | 85.8 |
| Unextractable soil residues | 13.7 | 8.1 | 14.4 | 8.1 | 12.2 | 14.3 | 17.9 | 19.4 |
| Volatiles $^{14}CO_2$ | NA | ND | ND | ND | ND | 0.1 | ND | 0.2 |
| Organic | NA | ND | ND | ND | ND | ND | ND | ND |
| Total | 103.9 | 108.3 | 102.0 | 103.5 | 101.3 | 100.5 | 94.7 | 105.4 |
| Clay loam soil incubated at 20°C | | | | | | | | |
| **Days after application** | **0** | **3** | **7** | **14** | **32** | **60** | **90** | **122** |
| Extractable soil residues | 100.3 | 97.0 | 89.9 | 79.2 | 86.3 | 80.6 | 87.1 | 73.4 |
| Unextractable soil residues | 6.7 | 8.2 | 11.3 | 23.8 | 16.6 | 23.0 | 24.5 | 21.9 |
| Volatiles $^{14}CO_2$ | NA | ND | ND | ND | ND | 0.8 | 2.1 | 3.9 |
| Organic | NA | ND | ND | ND | ND | ND | ND | ND |
| Total | 107.0 | 105.2 | 101.2 | 103.0 | 102.9 | 104.4 | 113.7 | 99.2 |
| Sandy clay loam soil incubated at 10°C | | | | | | | | |
| **Days after application** | **0** | **3** | **7** | **14** | **30** | **59** | **90** | **120** |
| Extractable soil residues | 100.3 | 98.2 | 99.6 | 100.6 | 93.0 | 97.8 | 93.1 | 93.4 |
| Unextractable soil residues | 8.1 | 5.3 | 4.0 | 4.5 | 7.9 | 6.1 | 9.5 | 10.4 |
| Volatiles $^{14}CO_2$ | NA | ND | ND | ND | ND | ND | ND | 0.1 |
| Organic | NA | ND | ND | ND | ND | ND | ND | ND |
| Total | 108.4 | 103.5 | 103.6 | 105.1 | 100.9 | 103.9 | 102.6 | 103.9 |

Notes: NA: Not applicable.
ND: Not detected.

**Table 26: Identification of radioactive components from four aerobic soils after treatment with [$^{14}$C]-ipconazole at a rate of 0.288 mg/kg and following incubation at 20°C and 10°C.**

| Identity | % Applied radioactivity | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| **Days after application** | **0** | **3** | **7** | **14** | **30** | **59** | **90** | **120** |
| Sandy clay loam soil incubated at 20°C | | | | | | | | |
| Ipconazole | 91.6 | 93.3 | 94.5 | 91.8 | 78.5 | 80.9 | 70.0 | 55.1 |
| Polar metabolites | 0.9 | 0.5 | 0.6 | 0.3 | 0.6 | 1.3 | 1.8 | 2.2 |
| Metabolite 1 | ND | ND | ND | ND | ND | ND | 0.2 | 0.2 |
| Metabolite 2 | ND | ND | ND | ND | ND | ND | 0.4 | 0.3 |
| Metabolite 3 | ND | ND | ND | 0.3 | 0.3 | 0.9 | 1.7 | 1.5 |
| Metabolite 4[a] | ND | 0.7 | 0.2 | 1.2 | 2.0 | 3.9 (2.1) | 5.2 (1.9) | 6.0 (2.8) |
| Metabolite 5 | 0.3 | ND | 0.1 | 0.3 | 0.4 | 0.4 | 0.6 | 0.6 |
| KNF-317-M-11 (see text) | 0.1 | 0.6 | 0.4 | 1.3 | 1.5 | 1.9 | 2.6 | 1.6 |
| Others | 1.9 | 0.9 | 0.6 | 0.3 | 0.7 | 0.3 | 0.4 | 0.4 |
| Silty clay loam soil incubated at 20°C | | | | | | | | |
| **Days after application** | **0** | **3** | **7** | **14** | **32** | **60** | **90** | **122** |
| Ipconazole | 89.0 | 98.3 | 85.8 | 91.0 | 84.8 | 76.0 | 67.6 | 70.8 |
| Polar metabolites | 0.2 | 0.2 | 0.3 | 0.7 | 1.2 | 1.7 | 2.4 | 3.9 |
| Metabolite 1 | ND | ND | ND | ND | ND | ND | ND | 1.4 |
| Metabolite 2 | ND | ND | ND | ND | ND | 0.4 | 0.4 | 1.1 |
| Metabolite 3 | ND | ND | ND | 0.2 | 0.1 | 0.6 | 0.8 | 1.5 |
| Metabolite 4[a] | ND | 0.4 | 0.4 | 1.2 | 1.4 | 2.3 | 2.3 (1.0) | 3.9 (1.7) |
| Metabolite 5 | 0.2 | 0.4 | 0.2 | 0.2 | ND | 0.7 | 0.4 | 0.7 |
| KNF-317-M-11 (see text) | ND | 0.2 | 0.4 | 1.2 | 0.9 | 4.0 | 2.6 | 1.6 |
| Others | 0.8 | 0.7 | 0.5 | 0.9 | 0.7 | 0.4 | 0.3 | 0.9 |
| Clay loam soil incubated at 20°C | | | | | | | | |
| **Days after application** | **0** | **3** | **7** | **14** | **32** | **60** | **90** | **122** |
| Ipconazole | 99.3 | 95.0 | 86.7 | 76.3 | 79.1 | 68.9 | 70.4 | 58.1 |
| Polar metabolites | ND | 0.2 | 0.3 | 0.3 | 1.2 | 1.5 | 2.8 | 2.9 |
| Metabolite 1 | ND | ND | ND | ND | ND | 0.1 | 0.2 | 0.4 |
| Metabolite 2 | ND | ND | ND | ND | ND | 0.4 | 0.6 | 0.7 |
| Metabolite 3 | ND | ND | ND | ND | 0.7 | 1.1 | 1.5 | 1.6 |
| Metabolite 4[a] | 0.2 | 0.6 | 0.9 | 1.3 | 2.8 | 4.3 (2.5) | 6.0 (3.6) | 6.7 (2.9) |
| Metabolite 5 | ND | 0.4 | 0.4 | ND | 0.3 | 0.6 | 0.9 | 1.0 |
| KNF-317-M-11 (see text) | ND | 0.2 | 0.9 | 0.8 | 1.8 | 3.2 | 4.7 | 1.7 |
| Others | 0.8 | 0.6 | 0.7 | 0.5 | 0.4 | 0.5 | ND | 0.3 |

| Identity | % Applied radioactivity | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Sandy clay loam soil incubated at 10°C | | | | | | | | |
| **Days after application** | **0** | **3** | **7** | **14** | **30** | **59** | **90** | **120** |
| Ipconazole | 98.3 | 96.9 | 98.0 | 98.4 | 90.3 | 93.9 | 87.4 | 85.4 |
| Polar metabolites | 0.7 | 0.3 | 0.3 | 0.2 | 0.2 | 0.4 | 0.6 | 0.6 |
| Metabolite 1 | ND | ND | ND | ND | ND | ND | ND | ND |
| Metabolite 2 | ND | ND | 0.1 | ND | ND | ND | ND | 0.1 |
| Metabolite 3 | ND | ND | ND | ND | ND | 0.3 | 0.4 | 0.6 |
| Metabolite 4[a] | 0.2 | 0.1 | 0.3 | 0.3 | 0.8 | 1.5 | 2.0 (1.1) | 2.3 (1.2) |
| Metabolite 5 | 0.3 | ND | 0.2 | ND | ND | 0.2 | 0.3 | 0.4 |
| KNF-317-M-11 (see text) | ND | 0.3 | 0.4 | 0.7 | 0.8 | 1.2 | 2.0 | 3.9 |
| Others | 0.8 | 0.6 | 0.3 | 1.0 | 0.9 | 0.3 | 0.4 | 0.1 |

Notes: a) Metabolite 4 was a zone of radioactivity that contained at least 3 components, one of which was identified as KNF-317-M-1 (see text). The proportion of KNF-317-M-1 in selected samples is stated in brackets alongside the value for the Metabolite zone. Other individual components in this zone constituted not more than 2% of the applied radioactivity in any sample.

ND: Not detected.

Others: Radioactivity not associated with specific components.

Study 3:
The degradation of ipconazole in anaerobic soil has been investigated using the OECD 307 guideline in a GLP study (Mellor, 2006[64]). The study included an initial aerobic incubation period followed by an anaerobic incubation period. The test was carried out using both [$^{14}$C-triazole]ipconazole (cc 92.6%:ct 5.4%) and [$^{14}$C-benzyl methylene]ipconazole (cc 95.6%, ct 2.3%). The soil used in the test was a sandy loam consisting of 57.12% sand, 24.44% silt and 18.43% clay. The soil had an organic carbon content of 2.0% and a pH of 6.0.

The test design and methodology for the first 30 days, i.e. the aerobic incubation, was identical to the aerobic studies described above. After 30 days incubation the soil samples were flooded to a depth of 1-2 cm with oxygen-free distilled water, and the flow-through gas was changed to oxygen-free nitrogen (after anaerobic conditions had been established the gas flow was reduced to three periods of 20 minutes per day). The test system was then incubated under these conditions for a further 90 days (to give a total study duration of 120 days). Redox potential measurements indicate that anaerobic conditions developed from 30 days through to study end.

The results of the aerobic/anaerobic incubation are shown in Table 27 and Table 28.

**Table 27: Distribution of radioactivity from an anaerobic soil system after treatment with [$^{14}$C] ipconazole at the rate of 0.288 mg/kg**

| Distribution | % Applied radioactivity (days after application) | | | | | |
|---|---|---|---|---|---|---|
| | 0 | 30 | 37 | 60 | 91 | 120 |
| [$^{14}$C TRIAZOLE] IPCONAZOLE | | | | | | |
| Overlying water | NA | NA | 1.7 | 1.6 | 1.2 | 1.2 |
| Extractable soil residues | 96.7 | 93.5 | 92.6 | 89.6 | 88.6 | 88.3 |
| Unextractable soil residues | 3.5 | 2.9 | 7.4 | 9.8 | 12.5 | 12.3 |
| Volatiles $^{14}CO_2$ | NA | 0.6 | 0.6 | 0.6 | 0.5 | 0.6 |
| Organic | NA | ND | ND | ND | ND | ND |
| Total | 100.2 | 97.0 | 102.3 | 101.6 | 102.8 | 102.4 |
| [$^{14}$C BENZYL METHYLENE] IPCONAZOLE | | | | | | |
| Overlying water | NA | NA | 1.1 | 1.1 | 1.2 | 0.6 |
| Extractable soil residues | 98.3 | 90.4 | 90.1 | 88.1 | 86.8 | 86.0 |
| Unextractable soil residues | 3.5 | 3.4 | 7.6 | 9.0 | 11.4 | 11.7 |
| Volatiles $^{14}CO_2$ | NA | 3.3 | 3.9 | 3.6 | 3.6 | 3.8 |
| Organic | NA | ND | ND | ND | ND | ND |
| Total | 101.8 | 97.1 | 102.7 | 101.8 | 103.0 | 102.1 |

Notes: NA: Not applicable.
ND: Not detected.

There was no significant change in the isomer ratio of cc:ct in soils sampled on days 0, 37 and 120 of the study.

The $DT_{50}$ in the anaerobic soil (mean of both radiolabels from day 30 onwards) was estimated to be 779 days. Mineralisation ($^{14}CO_2$ formation) was between 0.6 and 3.8% of the applied radioactivity after 120 days. Metabolites formed are considered further below.

**Table 28: Identification of radioactive components from aerobic/anaerobic soil after treatment with [$^{14}$C]-ipconazole at the rate of 0.288 mg/kg.**

| Identity | % Applied radioactivity (days after application) | | | | | |
|---|---|---|---|---|---|---|
| | 0 | 30 | 37 | 60 | 91 | 120 |
| [$^{14}$C TRIAZOLE] IPCONAZOLE | | | | | | |
| Ipconazole | 94.5 | 87.1 | 87.2 | 83.3 | 82.0 | 79.7 |
| Polar metabolites | <0.1 | 1.8 | 1.3 | 1.0 | 1.3 | 1.3 |
| KNF-317-M-1 (see text) | 0.5 | 0.7 | 0.6 | 0.8 | 0.8 | 0.6 |
| Unknown metabolite | 0.2 | 1.6 | 1.1 | 1.5 | 1.4 | 1.5 |
| KNF-317-M-11 (see text) | 0.7 | 0.6 | 0.6 | 0.5 | 0.9 | 1.1 |
| Others | 0.9 | 1.7 | 1.8 | 2.4 | 2.1 | 4.0 |
| [$^{14}$C BENZYL METHYLENE] IPCONAZOLE | | | | | | |
| Ipconazole | 94.8 | 86.1 | 84.8 | 83.9 | 81.6 | 80.1 |
| Polar metabolites | <0.1 | <0.1 | 0.1 | 0.3 | <0.1 | 0.2 |
| KNF-317-M-1 (see text) | 0.6 | 0.6 | 0.7 | 0.6 | 0.7 | 0.6 |
| Unknown metabolite | 0.2 | 1.4 | 1.4 | 1.4 | 1.3 | 1.6 |
| KNF-317-M-11 (see text) | 0.7 | 0.5 | 0.7 | 0.5 | 0.6 | 0.6 |
| Others | 2.1 | 1.8 | 2.4 | 1.4 | 2.6 | 2.9 |

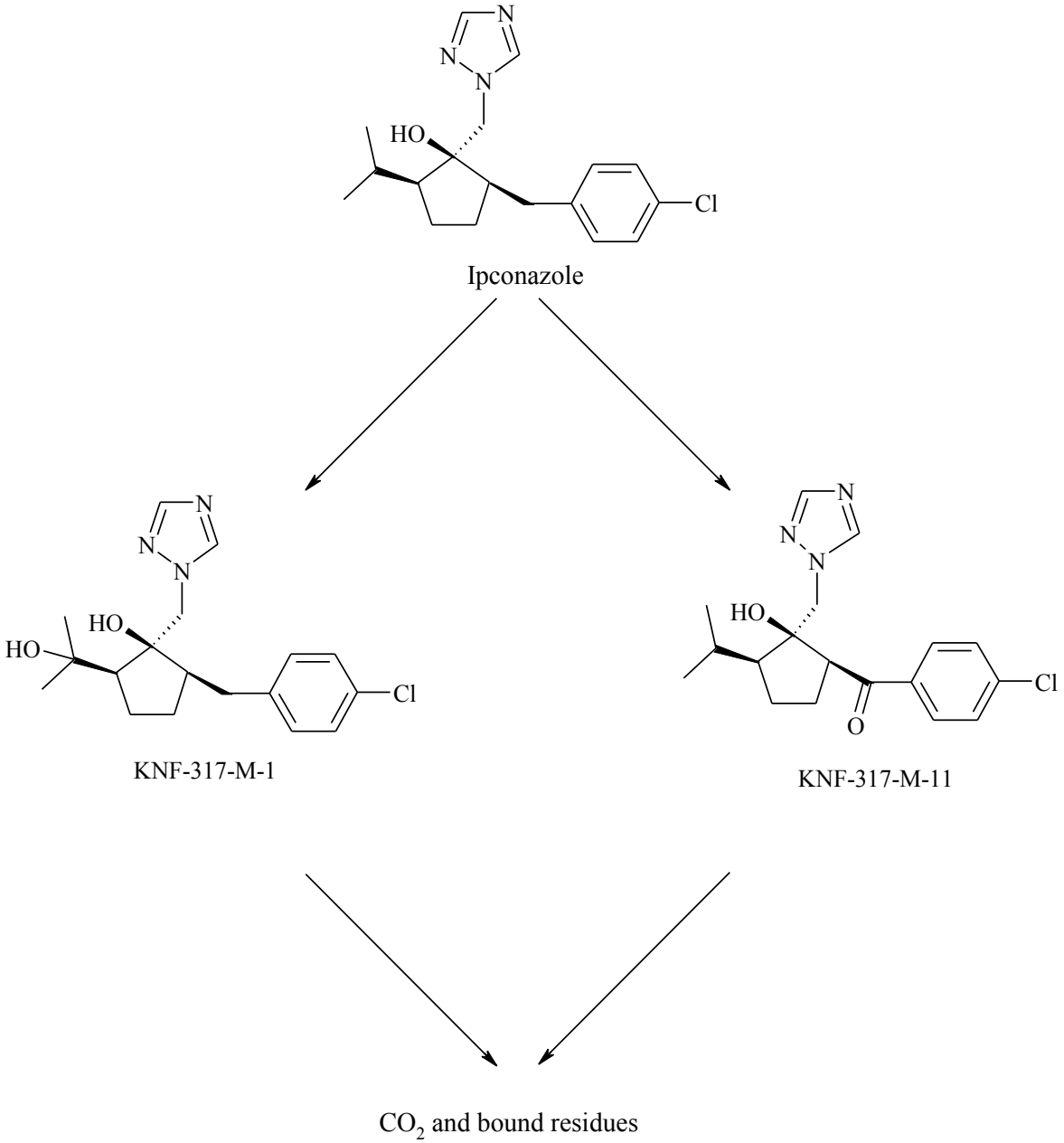
Notes: Others: Radioactivity not associated with specific components.

## Route of degradation in soil

The route and rate of degradation of ipconazole was investigated in a single soil with two appropriate radiolabelling positions (triazole and benzyl methylene positions). In addition, a rate of degradation study with a single radiolabelling position (triazole position) provided further appropriate route of degradation information in an additional three soils. Aerobic degradation led to the formation of a number of minor metabolites and there was no difference in metabolites between the two different radiolabelling positions. The amounts of metabolites formed were generally low, below 5% of the applied radioactivity. The proposed route of degradation in aerobic soil is summarised in Figure 8.

A study investigating route of degradation of ipconazole under anaerobic conditions on a single soil was also conducted using both radiolabelled positions. There were apparently fewer metabolites observed in this study, but KNF-317-M-1 (max 0.8% AR) and KNF-317-M-11 (max 1.1% AR at end of study) were observed. This indicates that the route of degradation under anaerobic conditions is likely to be very similar to aerobic conditions, but occurring at a slower rate.

**Figure 8: Proposed pathway for degradation of ipconazole in aerobic soil**




### 5.1.3 Summary and discussion of degradation

Ipconazole is not readily biodegradable. Laboratory simulation studies have shown that ipconazole has a relatively long biodegradation half-life ($DT_{50}$ >>100 days) in whole water-sediment systems and in soil. No aqueous photolysis study is available but this is considered a relatively minor route of degradation given a lack of significant absorption at >290 nm. Photolysis on soil surfaces has been shown to occur but the $DT_{50}$ is around 241 days of summer sunlight at 40°N. Ipconazole is hydrolytically stable at pH 5, 7 and 9.

Based on the available data, ipconazole is considered to degrade only slowly in the environment and so should be considered to be 'not rapidly degradable' for the purpose of environmental hazard classification.

## 5.2 Environmental distribution

### 5.2.1 Adsorption/Desorption

The $K_{oc}$ for ipconazole has been determined in two studies, covering six different soil types (Hatzenbeler and Long, 2001b[75]; Wanner, 2006) [76]. The $K_{oc}$ determined was in the range 1,724 to 3,214 ml $g^{-1}$, with mean value of 2,431 ml $g^{-1}$.

### 5.2.2 Volatilisation

The Henry's law constant for ipconazole has been calculated to be $3\times10^{-5}$ Pa $m^3$ $mol^{-1}$ (Comb, 2007[4]).

### 5.2.3 Distribution modelling

Based on the Henry's law constant of $3\times10^{-5}$ Pa $m^3$ $mol^{-1}$, ipconazole is expected to have a low potential for volatilisation from aquatic systems. The mean $K_{oc}$ for the substance has been determined to be 2,431 ml $g^{-1}$ and so partitioning from the water phase to the sediment phase is expected to occur (as demonstrated in the simulation studies at 5.1.2.3).

## 5.3 Aquatic Bioaccumulation

The available data on aquatic bioaccumulation are summarised in Table 29.

**Table 29: Summary of relevant information on aquatic bioaccumulation**

| Method | Results | Remarks | Reference |
|---|---|---|---|
| Log $K_{ow}$ (OECD 117, HPLC method) | 4.65 ipconazole cc<br>4.44 ipconazole ct | GLP Study<br>Temperature is not given<br>Substance is surface active | Riggs, 2001<br>[65] |
| Log $K_{ow}$ (EEC A8 (Shake flask)) | 4.49 at 20°C ipconazole cc<br>4.28 at 20°C ipconazole ct | GLP Study<br>(Reliable without restriction) | Comb, 2012a and 2012b [12] [13] |
| Bioconcentration in fish (OECD 305) | Steady state BCF 225-283 l/kg in *Lepomis macrochirus* | GLP Study<br>(Reliable without restriction) | Kureha Corporation (2006)<br>[66] |

Note: a) The surface tension of ipconazole (98.1% purity) has been determined as 56.5 mN $m^{-1}$ at 20°C for a 90% saturated aqueous solution using the OECD 115 methodology (GLP study) (Comb, 2005).

### 5.3.1 Aquatic bioaccumulation

#### 5.3.1.1 Bioaccumulation estimation

Ipconazole has a log $K_{ow}$ of 4.28-4.65 (see Section 1.3) which suggests that the substance has some potential to bioconcentrate in aquatic organisms.

#### 5.3.1.2 Measured bioaccumulation data

### 5.3.2 Summary and discussion of aquatic bioaccumulation

The bioconcentration factor for ipconazole has been measured in an OECD 305 guideline study using bluegill sunfish (*Lepomis macrochirus*) carried out according to GLP (2006[66]).

The substance tested was [$^{14}$C-triazole]ipconazole mixed with non-radiolabelled ipconazole with a purity of 98.4% (cc 91.7% and ct 6.7%). The test was carried out using a flow-through system and the study consisted of a 28 day exposure period followed by an 11 day depuration period. The temperature during the test was in the range 22.0-23.7°C and the mean pH of the water was 7.3.

Two exposure concentrations were used, nominally 1.3 and 13 µg/l. The mean measured concentration (± standard deviation) in the two exposure groups during the exposure period was 1.43±0.08 µg/l and 13.9±0.6 µg/l respectively based on radioactivity measurements. [$^{14}$C-triazole]ipconazole accounted for >98% of the radioactivity present in solution.

Four fish from each treatment were sampled on days 1, 3, 7 14, 21 and 28 of the exposure period and days 1, 3, 7 and 11 of the depuration period. The fish were separated into fillet (edible portion),

skeleton and viscera prior to analysis for total $^{14}C$. The fish lipid contents were determined on two pooled samples (each of three fish) collected on the first and last day of exposure and on the last day of depuration. The mean measured concentrations in the fish found during the study are summarised in Table 30.

For the 1.3 µg/l (nominal) treatment group, uptake of the radioactivity was rapid and the concentrations in fish reached steady-state between days 7 and 28 of the uptake period. The mean concentrations in whole fish were in the range 0.285-0.365 mg/kg at steady state.

In the 13 µg/l (nominal) group, initial uptake of radioactivity in fish was again rapid. The concentrations of radioactivity in non-edible portions and whole-fish increased rapidly to day 1 of exposure and were generally consistent and only increased slightly throughout the remaining exposure period. Concentrations in edible portions of fish increased rapidly during the first 24 hours and remained constant to day 7 before increasing again at day 14. Steady-state concentrations were therefore achieved between days 14 and 28 at this exposure concentration. The mean concentrations in whole fish were in the range 3.56-4.15 mg/kg at steady-state.

**Table 30: Mean concentrations in edible tissues, non-edible tissues and whole fish during exposure to [$^{14}C$ triazole] ipconazole**

| Time (days) | Mean[a] concentrations (± standard deviation) of radioactivity (µg equivalents/g) | | | | | |
|---|---|---|---|---|---|---|
| | Nominal test concentration1.3 µg/l | | | Nominal test concentration 13 µg/l | | |
| | Edible | Non-edible | Whole fish | Edible | Non-edible | Whole fish |
| Exposure day 0 | nd | nd | nd | nd | nd | nd |
| Exposure day 1 | 0.109±0.020 | 0.520±0.045 | 0.390±0.023 | 1.52±0.29 | 3.89±0.16 | 3.27±0.36 |
| Exposure day 3[b] | 0.093±0.016 | 0.361±0.031 | 0.279±0.033 | 1.53±0.33 | 3.79±0.76 | 3.12±0.47 |
| Exposure day 7 | 0.116±0.028 | 0.348±0.075 | 0.285±0.058 | 1.53±0.36 | 3.80±0.24 | 3.21±0.19 |
| Exposure day 14 | 0.141±0.063 | 0.403±0.052 | 0.331±0.021 | 2.24±0.55 | 4.06±0.88 | 3.56±0.72 |
| Exposure day 21 | 0.138±0.074 | 0.364±0.063 | 0.306±0.037 | 2.43±0.53 | 4.37±0.64 | 3.88±0.62 |
| Exposure day 28 | 0.147±0.038 | 0.445±0.114 | 0.365±0.073 | 2.43±0.21 | 4.73±0.54 | 4.15±0.48 |
| Depuration day 1 | 0.023±0.002 | 0.063±0.005 | 0.051±0.004 | 0.417±0.146 | 0.828±0.083 | 0.721±0.092 |
| Depuration day 3 | 0.012±0.003 | 0.023±0.002 | 0.020±0.001 | 0.125±0.053 | 0.247±0.109 | 0.214±0.093 |
| Depuration day 7 | 0.006±0.001 | 0.015±0.001 | 0.012±0.001 | 0.054±0.008 | 0.119±0.023 | 0.100±0.019 |
| Depuration day 11 | 0.003±0.002 | 0.010±0.001 | 0.007±0.001 | 0.015±0.017 | 0.073±0.016 | 0.054±0.014 |

Notes: a) mean of four fish.

b) mean of three fish (results from fourth fish were considered anomalous and not included in the mean calculation.

nd: not detected.

The mean lipid content in the fish was found to increase from 2.79% tissue weight at day 0 of the exposure phase, to 4.19-5.53% at the end of the exposure phase (exposure day 28) and 3.98-6.07% by day 11 of the depuration phase.

The mean steady state bioconcentration factors (for whole fish) were determined to be 225 l/kg for the 1.3 µg/l treatment group and 283 l/kg for the 13 µg/l treatment group. These values were not normalised to a standard lipid content of 5%. However, as noted above, the lipid contents of the fish

at the end of the exposure phase and the end of the depuration phase were close to 5% and so lipid normalisation would not significantly affect the results.

Depuration of the radioactivity from the fish was rapid. By day 1 of depuration the whole fish concentration of radioactivity had declined by 86% (in the 1.3 µg/l treatment group) and 82.6% (in the 13 µg/l treatment group) compared with the day 28 of uptake value and >95% of the radioactivity had been eliminated by day 7 of depuration. The time for 50% depuration ($DT_{50}$) was estimated to be around 0.29-0.37 days for whole fish.

The authors of the study calculated kinetic bioconcentration factors of 246 l/kg for the 1.3 µg/l treatment group and 274 l/kg for the 13 µg/l treatment group. However it should be noted that the rapid uptake seen in the study means that the uptake kinetics are effectively estimated from only a limited number of data points. The kinetic bioconcentration factors were not lipid normalised or growth-corrected. However, as noted above, lipid normalisation would be unlikely to affect the results significantly and, as very rapid overall depuration was seen in the test, growth dilution would be unlikely to significantly contribute to the depuration (and hence kinetic bioconcentration factor) seen in this test.

## 5.4 Aquatic toxicity

The available aquatic toxicity data for ipconazole are summarised in Table 31.

**Table 31: Summary of relevant information on aquatic toxicity**

| Method | Results | Remarks | Reference |
|---|---|---|---|
| Acute toxicity to fish (OECD 203) | 96h-$LC_{50}$ = 1.5 mg/l mm (*Oncorhynchus mykiss*) | GLP Study | Kureha Corporation (2001a) [67] |
| Acute toxicity to fish (OECD 203) | 96h-$LC_{50}$ = **1.3 mg/l mm** (*Lepomis macrochirus*) | GLP Study | Kureha Corporation (2001b) [68] |
| Long-term toxicity to fish (OECD 210) | 28d-NOEC = **0.00044 mg/l mm** (*Pimphales promelas*) | GLP Study | Kureha Corporation (2007) [69] |
| Acute toxicity to *Daphnia magna* (OECD 202) | 48h-$EC_{50}$ = 1.7 mg/l mm | GLP Study | Palmer et al., 2001c [70] |
| Long-term toxicity to *Daphnia magna* (OECD 211) | 21d-NOEC = 0.0109 mg/l mm | GLP Study | Flatman, 2007 [71] |
| Toxicity to algae (OECD 201) | 96h-$E_rC_{50}$ >2.2 mg/l n (*Pseudokirchneriella subcapitata*)<br>96h-$NOEC_r$ = 0.22 mg/l n (*Pseudokirchneriella subcapitata*) | GLP Study | Flatman, 2006a [72] |
| Long-term toxicity to *Chironomus riparius* (OECD 219) | 28d-NOEC = 3.52 mg/l im[a] | GLP Study<br>Test substance initially added to the water phase of a water-sediment system. | Flatman, 2006b [73] |

Notes: mm = endpoint based on mean measured test concentrations.
n = endpoint based on nominal test concentrations.
a) concentration relates to the initial measured concentration added to the water phase.
Values in **bold** indicate the most sensitive endpoints used for acute and chronic classifications

### 5.4.1 Fish

#### 5.4.1.1 Short-term toxicity to fish

Two studies are available on the short-term toxicity of ipconazole to fish.

Study 1:
The first study was an OECD 203 guideline study with rainbow trout (*Oncorhynchus mykiss*) carried out according to GLP [67]. The test substance used had a purity of 99.2%. Stock solutions of the test substance were dissolved in acetone and acetone (0.1 ml/l) was subsequently present in the test solutions. In the test, two replicate groups of 10 fish each were exposed to nominal concentrations of ipconazole of 0.31, 0.63, 1.3, 2.5 or 5.0 mg/l for 96 hours using a flow-through

system (12 volume additions every 24 hours). The test included duplicate control groups (dilution water alone) and solvent control groups (dilution water containing acetone at 0.1 ml/l).

During the test, the temperature ranged between 10.7°C and 12.2°C and the pH ranged from 8.0 to 8.3. The dissolved oxygen concentration was at least 76% of the air saturation value. The concentration of test substance present in exposure tanks was verified by HPLC analysis on samples taken on day 0, 2 and 4 of the test. The water samples were centrifuged prior to analysis. Non-centrifuged samples were also analysed from the 0.31, 1.3 and 5.0 mg/l treatments on days 0, 2 and 4 of the test. The measured concentrations are summarised in Table 32.

**Table 32: Measured concentration of ipconazole in water during the test with *Oncorhynchus mykiss***

| Nominal test concentration (mg/l) | Measured concentration (mg/l) | | | Mean measured concentration (mg/l)[b] | Mean measured as % of nominal[b] |
|---|---|---|---|---|---|
| | 0 days | 2 days | 4 days | | |
| Centrifuged samples | | | | | |
| Control | <LOQ[a] | <LOQ[a] | <LOQ[a] | - | - |
| Solvent control | <LOQ[a] | <LOQ[a] | <LOQ[a] | - | - |
| 0.31 | 0.122 | 0.127 | 0.138 | 0.13 | 42 |
| 0.63 | 0.356 | 0.307 | 0.356 | 0.34 | 54 |
| 1.3 | 0.606 | 0.828 | 0.835 | 0.76 | 58 |
| 2.5 | 1.39 | 1.39 | 1.32 | 1.4 | 56 |
| 5.0 | 2.24 | 2.12 | 1.92 | 2.1 | 42 |
| Non-centrifuged samples | | | | | |
| 0.31 | 0.276 | 0.279 | 0.276 | 0.28 | 90 |
| 1.3 | 0.838 | 1.096 | 1.118 | 1.0 | 77 |
| 5.0 | 2.42 | 2.39 | 2.23 | 2.3 | 46 |

Notes: a) Limit of quantification (LOQ) was 0.1 mg/l for ipconazole cc and 0.01 mg/l for ipconazole ct.
b) Results were the sum of the ipconazole cc and ct stereoisomers.

As can be seen above, the concentrations measured in the centrifuged samples were lower than both the nominal concentrations and also the non-centrifuged samples. The authors of the study noted that the limit of solubility of the substance in the test medium may have been exceeded and therefore based the results on the mean measured in the centrifuged samples.

A dose-related increase in mortality was evident in the fish after 96 hours exposure. Mortality in the control and solvent control groups was 0%, and no mortality was seen in the treatment groups at mean measured concentrations of 0.13, 0.34 and 0.76 mg/l (signs of sublethal effects were evident at 0.76 mg/l). Mortality was 30% at a mean measured concentration of 1.4 mg/l and 100% at a mean measured concentration of 2.1 mg/l. The 96h-$LC_{50}$ based on the mean measured concentrations was calculated to be 1.5 mg/l (95% confidence interval: 0.76 mg/l to 2.1 mg/l).

Study 2:
The second study was an OECD 203 guideline study with bluegill sunfish (*Lepomis macrochirus*) carried out according to GLP [68]. The test substance used had a purity of 99.2%. The method used, and the nominal test concentrations used, was essentially the same as that used in the Palmer et al. (2001a) study above with rainbow trout.

During the test, the temperature ranged from 21.6 to 21.8°C and the pH ranged from 7.9 to 8.1. The dissolved oxygen concentration was at least 76% of the air saturation value. The mean measured concentrations determined in both the centrifuged and non-centrifuged (only collected from the 0.31 mg/l treatment group on days 0, 2, and 4) are summarised in Table 33.

**Table 33: Measured concentration of ipconazole in water during the test with *Lepomis macrochirus***

| Nominal test concentration (mg/l) | Measured concentration (mg/l) | | | Mean measured concentration (mg/l)[b] | Mean measured as % of nominal[b] |
|---|---|---|---|---|---|
| | 0 days | 2 days | 4 days | | |
| Centrifuged samples | | | | | |
| Control | <LOQ[a] | <LOQ[a] | <LOQ[a] | - | - |
| Solvent control | <LOQ[a] | <LOQ[a] | <LOQ[a] | - | - |
| 0.31 | 0.130 | 0.140 | 0.161 | 0.14 | 45 |
| 0.63 | 0.326 | 0.329 | 0.324 | 0.33 | 52 |
| 1.3 | 0.754 | 0.704 | 0.738 | 0.73 | 56 |
| 2.5 | 1.64 | 1.74 | 2.02 | 1.8 | 72 |
| 5.0 | 2.59 | 2.15 | - | 2.4 | 48 |
| Non-centrifuged samples | | | | | |
| 0.31 | 0.280 | 0.273 | 0.285 | 0.28 | 90 |

Notes: a) Limit of quantification (LOQ) was 0.15 mg/l for ipconazole cc and 0.015 mg/l for ipconazole ct
b) Results were the sum of the ipconazole cc and ct stereoisomers.

The measured concentrations in the centrifuged samples were again lower than the nominal concentrations, and also the non-centrifuged samples and so the $LC_{50}$ was determined using mean measured concentrations from the centrifuged samples.

No mortality or visual evidence of sublethal effects was seen in the control, solvent control, 0.14 mg/l, 0.33 mg/l or 0.73 mg/l treatment groups. Mortality in the 1.8 mg/l treatment group was 90% after 96 hours and 100% mortality was seen in the 2.4 mg/l treatment group. The 96h-$LC_{50}$ based on the mean measured concentration was calculated to be 1.3 mg/l (95% confidence interval: 0.73-1.8 mg/l).

#### 5.4.1.2 Long-term toxicity to fish

The long-term toxicity of ipconazole to fish has been studied in an OECD 210 guideline study (fish early-life stage) with fathead minnow (*Pimephales promelas*) carried out according to GLP [69]. The substance used in the test had a purity of 98.1%, consisting of 91.2% cc and 6.9% ct. Stock solutions of the test substance were prepared in tetrahydrofuran (THF).

In the test, newly-fertilised eggs (less than 48 hours old) were exposed to ipconazole under flow-through conditions at nominal test concentrations of 0.077, 0.19, 0.48, 1.2 or 3.0 µg/l. Controls and solvent controls were also maintained in duplicate. The test system was a flow-through system (flow rate 140 ml/minute) and the developing embryos were exposed for a period of 4 days pre-hatch and 28 days post-hatch (32 days total). From day 0 to 8 post-hatch the fry were fed twice daily with newly hatched brine shrimp (*Artemia* sp.) nauplii and from day 10 the fry were fed 2-3 times per day with rinsed 48-hour-old *Artemia* nauplii cultures. Excess food and detritus were removed from the exposure tanks daily.

During the test the temperature was in the range 23.7°C to 25.9°C and the pH was between 7.6 and 8.6. The dissolved oxygen concentration was in the range 7.9 to 10.0 mg $O_2$/l. The concentration of test substance in the exposure tanks was analytically verified. The mean measured concentrations are summarised in Table 34.

**Table 34: Measured concentration of ipconazole in water during the test with *Pimephales promelas***

| Nominal test concentration (µg/l) | Mean measured concentration (µg/l) | Mean measured as % of nominal[a] |
|---|---|---|
| Control | ND | - |
| Solvent control | ND | - |
| 0.077 | 0.075 | 97 |
| 0.19 | 0.18 | 95 |
| 0.48 | 0.44 | 92 |
| 1.2 | 1.1 | 92 |
| 3.0 | 2.9 | 97 |

Notes: ND: not detected.

a) Calculated from rounded data

As can be seen the mean measured concentrations were close to the nominal concentrations. The toxicity was expressed in terms of the mean measured concentrations.

The main biological responses are summarised in Table 35.

**Table 35: Total numbers of hatched and surviving fry, hatching success and percentage survival**

| Mean measured conc. (µg/l) | Total no. eggs (A) | Total no. hatched fry[a] (B) | Total no. live fry[b] | Total no. dead hatched larvae[c] | Total no. dead eggs[c] | Total no. remaining viable eggs | Total no. surviving fry at day 28 (C) | Total hatch (%)[d] | Post-hatch survival (%)[e] | Overall survival (%)[f] |
|---|---|---|---|---|---|---|---|---|---|---|
| Control | 60 | 52 | 58 | 0 | 0 | 6 | 48 | 87 | 92 | 80 |
| Solvent control | 60 | 59 | 59 | 0 | 0 | 0 | 53 | 98 | 90 | 88 |
| 0.075 | 60 | 59 | 59 | 0 | 0 | 0 | 48 | 98 | 81 | 80 |
| 0.18 | 60 | 57 | 57 | 0 | 0 | 0 | 48 | 95 | 84 | 80 |
| 0.44 | 61 | 61 | 61 | 0 | 0 | 0 | 41 | 100 | 67 | 67 |
| 1.1 | 60 | 59 | 59 | 0 | 0 | 0 | 47 | 98 | 80 | 78 |
| 2.9 | 60 | 58 | 58 | 0 | 0 | 0 | 13 | 97 | 22 | 22 |

Notes: a) Back-calculated from numbers of fry surviving to Day 28 by adding the sum of observed larval mortalities throughout exposure period.

b) Number of live larvae on Day 0 post hatch, including remaining viable eggs.

c) Numbers of dead larvae and dead eggs on Day 0 post hatch.

d) Total hatch calculated from the total number of hatched larvae as a percentage of the total number of eggs (B/A).

e) Based on total number of successfully hatched eggs (C/B).

f) Based on total number of eggs at initiation (C/A).

Hatching success

Hatching commenced on the first day after initiation of the study, with 87 % of control eggs having hatched successfully. By day 4 (pre-hatch), hatching was complete for all egg chambers. Hatching success ranged from 87-102% across all the levels. Embryo development was unaffected by the presence of ipconazole (Table 34). The day that >90% control hatching had been achieved was designated as day 0 post-hatch.

Post-hatch survival

On day 28 post-hatch, mortality was statistically significant at concentrations of 0.44 and 2.9 µg/l (33 and 78%, respectively), but not at the interim level of 1.1 µg/l where mortality was only 20% and therefore within the guideline acceptance criterion of 30%. The remaining fish from each vessel were counted and the percent post-hatch survival calculated by comparison with the number of hatched larvae in each exposure vessel. The 28-day $LC_{50}$ was determined to be 2.48 µg/l and the mean measured NOEC for mortality was determined to be 1.1 µg/l.

Sub-lethal observations

No clear sub-lethal effects were noted in the appearance and behaviour of fry in either the controls or exposure levels.

Weight and length

The weight and length of the surviving fry were determined on day 28 post-hatch. The data are summarised in Table 36. There were statistically significant effects on both weight and length in both the 1.1 and 2.9 µg/l treatment groups. The $EC_{50}$ for weight and length were determined to be 1.63 µg/l and >2.9 µg/l, respectively. The mean measured NOEC for weight and length were determined to be 0.44 µg/l.

**Table 36: Mean length and weight for surviving fry at day 28 post-hatch**

| Mean measured concentration (µg/l) | Mean fry length (mm) | Mean fry dry weight (mg) |
|---|---|---|
| Control | 15.6 | 8.1 |
| Solvent control | 16.8 | 8.0 |
| 0.075 | 16.6 | 9.4 |
| 0.18 | 15.5 | 7.0 |
| 0.44 | 16.1 | 7.0 |
| 1.1 | 14.9* | 4.3* |
| 2.9 | 11.3** | 1.5** |

Notes: * Statistically significant compared to the solvent control at p<0.05.
** Statistically significant compared to the solvent control at p<0.001.

The control and solvent control groups met the validity criteria of ≥66% hatching success with post hatch survival of ≥70%.

Overall the lowest mean measured chronic NOEC from this study was 0.44 µg/l (0.00044 mg/l) based on fish weight and length.

### 5.4.2 Aquatic invertebrates

#### 5.4.2.1 Short-term toxicity to aquatic invertebrates

The acute toxicity of ipconazole has been determined in an OECD 202 guideline study carried out according to GLP using *Daphnia magna* (Palmer et al., 2001c[70]). The test substance used had a purity of 99.2%.

Stock solutions of the test substance were dissolved in dimethylformamide (DMF) and DMF (0.1 ml/l) was subsequently present in the test solutions. In the test, two replicate groups of 10 animals each were exposed to nominal concentrations of ipconazole of 0.31, 0.63, 1.3, 2.5 or 5.0 mg/l for 48 hours using a flow-through system (28 volume additions over 24 hours). The test included duplicate control groups (dilution water alone) and solvent control groups (dilution water containing DMF at 0.1 ml/l).

During the test, the temperature ranged between 19.8°C and 19.9°C and the pH ranged from 8.1 to 8.3. The dissolved oxygen concentration was at least 91% of the air saturation value. The concentration of test substance present in exposure tanks was verified by HPLC analysis on samples taken on day 0 and 2 of the test. The water samples were centrifuged prior to analysis. Non-

centrifuged samples were also analysed from the 0.31, 1.3 and 5.0 mg/l treatment on days 0 and 2 of the test. The measured concentrations are summarised in Table 37.

**Table 37: Measured concentration of ipconazole in water during the test with *Daphnia magna***

| Nominal test concentration (mg/l) | Measured concentration (mg/l) | | Mean measured concentration (mg/l)[b] | Mean measured as % of nominal[b] |
|---|---|---|---|---|
| | 0 days | 2 days | | |
| Centrifuged samples | | | | |
| Control | <LOQ[a] | <LOQ[a] | - | - |
| Solvent control | <LOQ[a] | <LOQ[a] | - | - |
| 0.31 | 0.133 | 0.126 | 0.13 | 42 |
| 0.63 | 0.352 | 0.351 | 0.35 | 56 |
| 1.3 | 0.787 | 0.729 | 0.76 | 58 |
| 2.5 | 1.63 | 1.55 | 1.6 | 64 |
| 5.0 | 2.93 | 3.74 | 3.3 | 66 |
| Non-centrifuged samples | | | | |
| 0.31 | 0.290 | 0.283 | 0.29 | 94 |
| 1.3 | 1.06 | 1.00 | 1.0 | 77 |
| 5.0 | 3.30 | 4.15 | 3.7 | 74 |

Notes: a) Limit of quantification (LOQ) was 0.1 mg/l for ipconazole cc and 0.01 mg/l for ipconazole ct.
b) Results were the sum of the ipconazole cc and ct stereoisomers.

The mean measured concentrations in the centrifuged samples were lower than both the nominal concentrations and the concentrations measured in non-centrifuged samples. The toxicity was therefore expressed in terms of mean measured concentrations from the centrifuged samples.

No immobilised *Daphnia* were seen in the control, solvent control or 0.13 mg/l treatment groups after 48 hours exposure. A dose-related increase in mortality (immobilised *Daphnia*) was evident from concentrations of 0.35 mg/l (5% immobilisation) to 3.3 mg/l (80% immobilisation). The 48h-$EC_{50}$ based on the mean measured exposure concentrations in the centrifuged samples was 1.7 mg/l (95% confidence interval: 1.2-2.6 mg/l).

#### 5.4.2.2 Long-term toxicity to aquatic invertebrates

The long-term toxicity of ipconazole to *Daphnia magna* has been studied in an OECD 211 guideline reproduction study carried out according to GLP (Flatman, 2007[71]). The substance tested had a purity of 98.1% and consisted of 91.2% cc and 6.9% ct. Stock solutions of the test substance were prepared in tetrahydrofuran (THF).

The test system used was a semi-static test system whereby groups of 10 first instar *Daphnia* were exposed to ipconazole for 21 days. In the first test, the mean measured exposure concentrations used were 0.050, 0.112, 0.215, 0.434, 0.84 and 1.7 mg/l. However in this test 100% mortality was seen at the highest two concentrations and effects on reproduction were seen at all exposure concentrations. As a result a second test was conducted at lower mean measured concentrations of 0.0014, 0.0038, 0.0109, 0.0329 and 0.0969 mg/l. Controls and solvent control groups were also

included. Owing to the very shallow dose response seen the author of the study considered that it was necessary to combine the data from both tests in order to determine the full range of endpoints in the study.

During the tests the temperature ranged from 19°C to 22°C and the pH was in the range 7.3 to 8.1. The *Daphnia* were fed suspensions of *Chlorella vulgaris* on a daily basis. The test concentrations were verified by HPLC analysis of samples taken from freshly prepared test media on days 0, 7, 9 and 14 and on expired test media on days 2, 9 and 12 in the first test. In the second test analysis was carried out on samples from freshly prepared test media on days 0, 10, 14 and 19 and on expired test media on days 3, 12, 17 and 21. The measured concentrations are summarised in Table 38.

**Table 38: Measured concentrations of ipconazole during a 21-day chronic *Daphnia magna* study**

| Test | Nominal concentration (mg/l) | Mean measured concentration (mg/l) | Mean measured concentration as % nominal |
|---|---|---|---|
| 1 | Solvent control | ND | - |
| | 0.053 | 0.050 | 94 |
| | 0.106 | 0.112 | 106 |
| | 0.213 | 0.215 | 101 |
| | 0.425 | 0.434 | 102 |
| | 0.85 | 0.84 | 99 |
| | 1.70 | 1.7 | 100 |
| 2 | Solvent control | ND | - |
| | 0.0013 | 0.0014* | 106 |
| | 0.0039 | 0.0038 | 98 |
| | 0.0117 | 0.0109 | 93 |
| | 0.0353 | 0.0329 | 93 |
| | 0.106 | 0.0969 | 91 |

Notes: * Calculated based on solvent stock recoveries.
ND: Not detected.

The measured concentrations were close to nominal concentrations. The toxicity endpoints were expressed in terms of mean measured concentrations.

The biological responses found in the study are summarised in Table 39.

**Table 39: Chronic toxicity of ipconazole to *Daphnia magna* in a 21-day study**

| Test | Mean measured concentration (mg/l) | % Immobilisation after 21 days | Cumulative number of young per female after 21 days | Cumulative number of dead young after 21 days compared to solvent control | Mean length per surviving female (mm) |
|---|---|---|---|---|---|
| 1 | Control | 0 | 86 | 10.6 | a |
| | Solvent control | 10 | 96 | 0.0 | a |
| | 0.050 | 0 | 56 | 41.9 | a |
| | 0.112 | 20 | 34 | 64.2 | a |
| | 0.215 | 30 | 71 | 25.8 | a |
| | 0.434 | 40 | 34 | 64.2 | a |
| | 0.84 | 100 | - | - | a |
| 2 | Control | 5 | 122 | -16.8 | 4.53 |
| | Solvent control | 5 | 104 | 0.0 | 4.45 |
| | 0.0014 | 10 | 95 | 7.9 | 4.44 |
| | 0.0038 | 0 | 73 | 29.8 | 4.37 |
| | 0.0109 | 0 | 103 | 1.6 | 4.40 |
| | 0.0329 | 10 | 100 | 4.7 | 4.25 |
| | 0.0969 | 10 | 106 | -1.5 | 4.28 |

Notes: a) Lengths were not determined in the first test.

Fecundity

In the first test, a statistically significant ($p$<0.001) reduction in neonate production occurred at all test concentrations with the exception of the 0.215 mg/l group. This anomaly was thought to be a result of the very flat dose response across the test range. In the second test, the only test level at which a statistically significant reduction in neonate production occurred compared to the solvent control was the second lowest concentration, 0.0038 mg/l. The three higher test concentrations showed no significant reduction in fecundity and it was therefore concluded that the reduction at 0.0038 mg/l was an anomaly and not treatment related.

In the second test, there was a statistically significant difference in the mean number of neonates per adult between the control and the solvent control groups (122 and 104, respectively). The mean numbers of neonates produced per adult were very similar in the control and solvent control during the range finding test (20 and 25, respectively) and in the first test (86 and 96, respectively). It was therefore considered that the difference in the second test was unlikely to be attributable to the solvent. The numbers of neonates produced in the two main tests were in excess of the validity criteria of 60 neonates per surviving adult, confirming the health and acceptability of the test animals. As a result of the variation in reproduction observed between the two control groups, all statistical evaluations were made between the test groups and the solvent control group.

Based on the combined data set from both tests, the 21-day NOEC for fecundity was determined to be 0.215 mg/l.

Time to first brood

In the first test, time to first brood was significantly delayed at all treatment levels but in the second test a significant effect only occurred in the highest treatment level, 0.0969 mg/l. Based on the combined data set from both tests, the NOEC for time to first brood was determined to be 0.0329 mg/l.

Parental growth

The lengths of adults surviving to day 21 were determined in the second test only. A significant treatment related effect on parental body length was identified at the two highest test concentrations of 0.0329 and 0.0969 mg/l.

The 21-day NOEC for parental growth was therefore determined to be 0.0109 mg/l. This was also the overall NOEC and was based on mean measured test concentrations.

The study was conducted in accordance with OECD test guideline 211 and to GLP. Although the test was carried out in two parts and for some endpoints the data from both parts of the test were combined, the most sensitive endpoint was parental growth and the NOEC for this was determined using data from the second test only and so the result is considered to be reliable.

### 5.4.3 Algae and aquatic plants

The toxicity of ipconazole to *Pseudokirchneriella subcapitata* (formerly known as *Selenastrum capricornutum*) has been studied in an OECD 201 guideline study carried out according to GLP (Flatman, 2006a[72]). The substance used in the test had a purity of 98.1% and consisted of 91.2% of cc and 6.9% ct,

Stock solutions of the test substance were prepared in tetrahydrofuran (THF) and algal cultures were exposed in triplicate to nominal concentrations of 0.046, 0.10, 0.22, 0.46, 1.0 or 2.2 mg/l, together with a control group and solvent control group. The cultures were incubated under continuous illumination with continuous agitation. Cell densities were determined after 0, 24, 48, 72 and 96 hours incubation.

During the test, the temperature ranged between 23°C and 24°C and the pH in the control samples was between 7.7 and 8.9. The concentrations of the test substance in the nutrient medium were verified by analysis using an HPLC method at the start of the test and after 96 hours exposure. The measured concentrations are summarised in Table 40.

As the measured concentrations at test initiation and at 96 hours were generally within 80% of the nominal concentration (or just outside 80% of nominal) the authors calculated the toxicity endpoints based on the nominal concentrations.

**Table 40: Measured concentrations of ipconazole during the algal toxicity study *Pseudokirchneriella subcapitata***

| Time | Nominal concentration (mg/l) | Measured concentration (mg/l) | Measured concentration as % nominal |
|---|---|---|---|
| 0 hours (fresh solution) | Control | ND | - |
| | 0.046 | 0.0485 | 105 |
| | 0.10 | 0.105 | 105 |
| | 0.22 | 0.240 | 109 |
| | 0.46 | 0.464 | 101 |
| | 1.0 | 1.05 | 105 |
| | 2.2 | 2.37 | 108 |
| 96 hours (expired solution) | Control | ND | - |
| | 0.046 | 0.0382 | 83.1 |
| | 0.10 | 0.0856 | 85.6 |
| | 0.22 | 0.174 | 79.1 |
| | 0.22 (no algae) | 0.257 | 117 |
| | 0.46 | 0.364 | 79.1 |
| | 1.0 | 0.907 | 90.7 |
| | 2.2 | 2.242 | 102 |

Notes: ND: Not detected. Limit of detection was 0.01 mg/l ipconazole (0.00912 mg/l for ipconazole cc and 0.00069 mg/l ipconazole ct).

The effect of exposure on biomass and growth rate is summarised in Table 41.

The growth of the untreated control was significantly less than that observed in the solvent control and the growth in the lower ipconazole treatments was comparable to that of the solvent control. The study author therefore concluded that the solvent used acted as a mild growth stimulant. In evaluating the data, comparisons for the impact of ipconazole on the growth of the algae were therefore made between the treatment groups and the solvent control. This is considered to be acceptable. Cell concentrations in non-solvent control cultures increased by a factor of 208 within 96 hours. Cell concentrations in solvent control cultures increased by a factor of 322 within 96 hours

**Table 41: Inhibition of algae following exposure to ipconazole**

| Parameter | Nominal concentration (mg/l) | % Inhibition compared with solvent control | Statistical significance compared with solvent control (*p*) |
|---|---|---|---|
| Area under curve to 96 hours (biomass) | Control | 42.9 | <0.001***[T] |
| | Solvent control | 0.0 | - |
| | 0.046 | -14.6 | 0.127[D] |
| | 0.10 | 15.3 | 0.103[D] |
| | 0.22 | -10.3 | 0.428[D] |
| | 0.46 | 26.6 | 0.002**[D] |
| | 1.0 | 66.4 | <0.001***[D] |
| | 2.2 | 87.4 | <0.001***[D] |
| Growth rate | Control | 7.5 | <0.001***[T] |
| | Solvent control | 0.0 | - |
| | 0.046 | -3.3 | >0.999[W] |
| | 0.10 | 3.7 | 0.396[W] |
| | 0.22 | -0.1 | 0.396[W] |
| | 0.46 | 8.1 | <0.001***[W] |
| | 1.0 | 24.1 | <0.001***[W] |
| | 2.2 | 47.8 | <0.001***[W] |

Notes: *P* values for the comparison with solvent control using Williams' test (W), Dunnett's test (D) and the *t*-test (T)
* $p<0.05$
** $p<0.01$
*** $p<0.001$

The 96-hour $E_bC_{50}$ of ipconazole to *Pseudokirchneriella subcapitata* was determined to be 0.62 mg/l and the 96-hour $E_rC_{50}$ was determined to be >2.2 mg/l. The 96h-NOEC was determined to be 0.22 mg/l based on both biomass and growth rate. The $E_rC_{50}$ and $NOE_rC$ endpoints at 72 hours are not available, however the data indicate that effects at 96 hours were similar or slightly greater than at 72 hours and so the 72-h $ErC_{50}$ is also expected to be >2.2 mg/l and the 72h-$NOE_rC$ unchanged at 0.22 mg/l. The results are based on the nominal concentration (the measured concentration was generally within 80% of the nominal value during the test).

### 5.4.4 Other aquatic organisms (including sediment)

The long-term toxicity of ipconazole to sediment-dwelling invertebrates has been studied in an OECD guideline 219 study carried out to GLP using *Chironomus riparius* [73]. The test was carried out using [$^{14}$C-benzyl methylene]ipconazole mixed with non-radiolabelled ipconazole with a purity of 98.1% and consisted of 91.2% cc and 6.9% ct.

The test was carried out using artificial sediment consisting of 5% dry weight sphagnum peat, 20% dry weight kaolin clay and 75% dry weight industrial sand (acid-washed fine sand). The pH of the sediment was adjusted to pH 6.6. The test was carried out using glass beakers (8 cm diameter) containing a 2 cm deep layer of sediment with 8 cm of overlying water (400 ml volume). The water-sediment system was allowed to settle and acclimate to the test conditions for one week prior to the addition of the larvae.

Stock solutions of the test substance were prepared by suspending radiolabelled test substance, diluted with non-radiolabelled ipconazole, in DMF (dimethylformamide). The required volume of each dose solution (or DMF for the solvent control group) was dispensed into the overlying water in the test vessels. Dispersion of the test substance was facilitated by airflow through the system for approximately 5 minutes prior to sampling at time zero.

The test was carried out under static conditions (no renewal of test medium) for 28 days. Four groups, each of 20 first instar *C. riparius* were exposed to nominal ipconazole concentrations of 0.219, 0.438, 0.875, 1.75 and 3.50 mg/l (the concentrations relate to the initial concentration in the water phase). Control groups (four replicate groups of 20) and solvent control groups (eight replicate groups of 20; each containing DMF at 0.1 ml/l) were also established. Four additional test vessels containing ipconazole (two at 0.219 mg/l and two at 3.5 mg/l) were prepared for use as 'destructive' samples for the analysis of water, sediment and pore water. The larvae were fed daily at a rate of 0.5 mg/larvae from days 1 to 9 and 1.0 mg/larvae from day 10 to study termination. The test vessels were aerated throughout the test. The temperature during the test was 20°C±2°C.

A sub-sample (5 ml) of the overlying water from each of the treated test vessels (including those intended for destructive sampling) and all control/solvent control vessels was removed following test substance application and after 1, 3, 7, 14 and 28 days incubation and triplicate aliquots were subjected to radioassay by liquid scintillation counting (LSC). The concentration of radioactivity in pore water was measured for destructive samples at days 0 and 7; for all remaining samples, concentration in pore water was measured at Day 28. Following removal of the pore water, sediment from the destructive samples (day 7) and all other samples (day 28) were extracted with acetonitrile; aliquots of the acetonitrile extract were taken for radioassay by LSC. The sediments were then allowed to air-dry for up to 10 days at room temperature before taking aliquots for combustion/radioassay. Control and solvent control sediments at day 28, and destructive samples at day 0, were air-dried for 5 to 7 days then ground and combusted directly without extraction.

The measured concentrations of the test substance in the overlying water at day 0 of the test were 0.205, 0.411, 0.791, 1.62 and 3.33 mg/l, for the nominal 0.219, 0.438, 0.875, 1.75 and 3.50 mg/l treatment groups respectively. The concentration of radiolabel in the overlying water decreased from between 90.5-94.6% of the applied radioactivity on day 0 to 17.0-19.7% of the applied radioactivity on Day 7, and finally to between 8.3 and 9.9% of the applied radioactivity on day 28 (see Table 42). The concentration of radiolabel in the sediment-phase increased from between 7.36-7.46% of the applied radioactivity on day 0 to 59.4-70.1% of the applied radioactivity on day 7, and finally to 60.2-76.6% of the applied radioactivity on day 28. Radioactivity in pore water accounted for <0.1% of the applied radioactivity throughout the study. A maximum of 1.4% AR was recovered from test vessel rinses. In all fractions, total radioactivity on day 28 accounted for 69.6-84.9% of the applied radioactivity.

**Table 42: Summary of measured concentrations of total radioactivity in the overlying water**

| Nominal treatment group (mg/l) | Initial measured concentration added to water (mg/l) | Mean measured concentration as a % of applied radioactivity | | | | | |
|---|---|---|---|---|---|---|---|
| | | Day 0 | Day 1 | Day 3 | Day 7 | Day 14 | Day 28 |
| Solvent control | Solvent control | ND | ND | ND | ND | ND | ND |
| Control | Control | ND | ND | ND | ND | ND | ND |
| 0.219 | 0.219 | 93.6 | 55.7 | 34.0 | 17.8 | 11.9 | 8.4 |
| 0.438 | 0.436 | 94.1 | 49.5 | 30.3 | 17.0 | 12.1 | 8.3 |
| 0.875 | 0.874 | 90.5 | 50.4 | 33.0 | 19.1 | 14.0 | 9.9 |
| 1.75 | 1.76 | 92.2 | 49.8 | 32.3 | 18.6 | 12.3 | 8.3 |
| 3.50 | 3.52 | 94.6 | 55.3 | 34.5 | 19.7 | 14.3 | 9.4 |

Notes: ND: None detected, <0.001 mg/l

The concentration data showed that ipconazole partitioned from the water phase to the sediment phase during the study.

Chironomid growth and development were monitored daily. From day 13, when the first midges emerged, adults were sexed and removed from the vessels. The 28-day percentage emergence success was calculated for each treatment. The main findings are summarised in Table 43.

**Table 43: Summary of the toxicity of ipconazole to *Chironomus riparius***

| Nominal treatment group (mg/l) | Initial measured concentration added to water (mg/l) | Mean development rate at day 28 | Mean % emergence at day 28 |
|---|---|---|---|
| Solvent control | Solvent control | 0.0655 | 95.5 |
| Control | Control | 0.0682 | 93.4 |
| 0.219 | 0.219 | 0.0635 | 92.3 |
| 0.438 | 0.436 | 0.0649 | 94.3 |
| 0.875 | 0.874 | 0.0597 | 90.6 |
| 1.75 | 1.76 | 0.0613 | 91.9 |
| 3.50 | 3.52 | 0.0600 | 83.6 |

The mean emergence from the control and solvent control groups were 93.4 and 95.5% respectively. The mean emergence from the treatment groups was in the range 83.6 and 94.3% and was not statistically significantly different from the solvent control group at any treatment group. Similarly the development rate was not significantly reduced ($p<0.05$) compared with the solvent control group and there was no significant difference ($p>0.05$) in the sex ratio of the emerged midges from each treatment group compared to the solvent control group.

As no significant treatment related effects on emergence or delayed development was seen in this study the NOEC was determined to be 3.52 mg/l (the highest dose tested) and the $EC_{50}$ was >3.52 mg/l. The results are based on the initial measured applied radioactivity in the water column.

The study is considered to be reliable. However, the results are difficult to use in relation to aquatic classification as the substance partitioned significantly from water to sediment during the course of the study.

### 5.5 Comparison with criteria for environmental hazards (sections 5.1 – 5.4)

Ipconazole is hydrolytically stable, not readily biodegradable and has a half-life in whole water-sediment systems >>16 days. Therefore the substance is considered to be 'not rapidly degradable' for the purposes of hazard classification.

Despite a log $K_{ow}$ of 4.28-4.65, the substance has a low potential for bioaccumulation as the steady-state BCF has been experimentally determined in fish to be 225-283 l/kg. This is less than the CLP cut-off value of 500 l/kg.

The toxicity of ipconazole to aquatic species is summarised in Table 31 and the key acute and chronic classification endpoints are included below in Table 44.

**Table 44:** Key acute and chronic aquatic toxicity endpoints for ipconazole

| **Species** | **Acute Toxicity L/$EC_{50}$ (mg/l)** | **Chronic Toxicity NOEC (mg/l)** |
|---|---|---|
| Fish | 96h-$LC_{50}$: 1.3 mg/l (*Lepomis macrochirus*) | 28d-NOEC: 0.00044 mg/l (*Pimephales promelas*) |
| *Daphnia magna* | 48 h-$EC_{50}$: 1.7 mg/l | 21d-NOEC: 0.0109 mg/l |
| Algae | 96h-$E_rC_{50}$: >2.2 mg/l (*Pseudokirchneriella subcapitata)* | 96h-$NOE_rC$: 0.22 mg/l (*Pseudokirchneriella subcapitata*) |

The lowest reliable acute/short-term L(E)$C_{50}$ is 1.3 mg/l for fish (mean measured), which is above the CLP Aquatic Acute Toxicity criterion of ≤1 mg/l. Therefore it is proposed that the substance is not classified for aquatic acute toxicity.

Chronic/long-term toxicity data are available for fish, *Daphnia magna* and algae. Chronic NOECs <0.1 mg/l were obtained with both fish and *Daphnia magna*. The lowest chronic NOEC is the 28-day mean measured value of 0.00044 mg/l for *Pimephales promelas* from a reliable fish early-life stage study. At the time of writing, no longer-term fish study, e.g. investigating potential endocrine disruption, is available. These data indicate that the substance should be classified as Chronic Category 1 (H410). It is further proposed that the appropriate Chronic M-factor is 100 since ipconazole is not rapidly degradable and its lowest NOEC is between 0.0001 and 0.001 mg/l.

**Note**: The available information on environmental degradants of ipconazole is considered briefly in Annex IV. These were mainly assessed in the DAR in relation to their formation, persistence and risks to soil and none were identified in the EFSA Conclusion (2013) [74] as being 'major' (i.e. formed at ≥10% Applied Radioactivity) in water or sediment. It would, in any case, appear from the limited ecotoxicity data available on 1*H*-1,2,4-triazole and the self-classifications of 4-chlorobenzoic acid and 4-chlorobenzaldehyde, that these predominantly soil degradants are less aquatically toxic than the parent substance. Therefore, degradants are not considered to affect the proposed environmental classification of ipconazole.

**5.6 Conclusions on classification and labelling for environmental hazards (sections 5.1 – 5.4)**

| |
|---|
| **Aquatic Acute Category: Not classified (conclusive but not sufficient for classification)** |
| **Aquatic Chronic Category 1: H410: Very toxic to aquatic life with long lasting effects** |
| **Chronic M-factor = 100** |

# 6 OTHER INFORMATION

No other relevant information.

# 7 REFERENCES

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# 8 ANNEXES

## 8.1 Annex I: Mean body weights of rat fetuses with and without external malformations (developmental toxicity studies)

Table 1 Study no. 89-0062 KNF-317; Teratogenicity Study in Rats (Preliminary Study)
Mean body weights of fetuses with or without external malformations

1) Information on all fetuses

| | | B.W. (mg) | |
|---|---|---|---|
| Category | n | Mean | S.D. |
| Without microphthalmia | 371 | 3226 | 522 |
| With microphthalmia | 10 | 1851 | 461 |
| Without kinky/short tail | 373 | 3222 | 524 |
| With kinky/short tail | 8 | 1705 | 367 |
| Without microphthalmia/kinky and/or short tail | 366 | 3245 | 498 |
| With microphthalmia/kinky and/or short tail | 15 | 1847 | 420 |

2) Information in each treatment group

| | Dose level (mg/kg/day) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | 0 | | | 1 | | | 10 | | |
| | | B.W. (mg) | | | B.W. (mg) | | | B.W. (mg) | |
| Category | n | Mean | S.D. | n | Mean | S.D. | n | Mean | S.D. |
| Without microphthalmia | 94 | 3369 | 313 | 87 | 3526 | 352 | 79 | 3360 | 410 |
| With microphthalmia | 0 | – | – | 1 | 1411 | – | 0 | – | – |
| Without kinky/short tail | 94 | 3369 | 313 | 88 | 3502 | 416 | 79 | 3360 | 410 |
| With kinky/short tail | 0 | – | – | 0 | – | – | 0 | – | – |
| Without microphthalmia/kinky and/or short tail | 94 | 3369 | 313 | 87 | 3526 | 352 | 79 | 3360 | 410 |
| With microphthalmia/kinky and/or short tail | 0 | – | – | 1 | 1411 | – | 0 | – | – |

| | Dose level (mg/kg/day) | | | | | |
|---|---|---|---|---|---|---|
| | 50 | | | 100 | | |
| | | B.W. (mg) | | | B.W. (mg) | |
| Category | n | Mean | S.D. | n | Mean | S.D. |
| Without microphthalmia | 84 | 2928 | 466 | 27 | 2296 | 554 |
| With microphthalmia | 2 | 2173 | 569 | 7 | 1822 | 443 |
| Without kinky/short tail | 85 | 2918 | 476 | 27 | 2347 | 517 |
| With kinky/short tail | 1 | 2260 | – | 7 | 1626 | 314 |
| Without microphthalmia/kinky and/or short tail | 83 | 2936 | 463 | 23 | 2393 | 530 |
| With microphthalmia/kinky and/or short tail | 3 | 2202 | 406 | 11 | 1790 | 392 |

3) Information in each litter with external malformations

| | Litter no. and dose level (mg/kg/day) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | 1038 (1) | | | 1012 (50) | | | 1015 (100) | | |
| | | B.W. (mg) | | | B.W. (mg) | | | B.W. (mg) | |
| Category | n | Mean | S.D. | n | Mean | S.D. | n | Mean | S.D. |
| Without microphthalmia | 16 | 3344 | 159 | 8 | 2724 | 453 | 5 | 1832 | 473 |
| With microphthalmia | 1 | 1411 | – | 2 | 2173 | 569 | 3 | 1563 | 345 |
| Without kinky/short tail | 17 | 3230 | 494 | 9 | 2653 | 513 | 4 | 1837 | 443 |
| With kinky/short tail | 0 | – | – | 1 | 2260 | – | 4 | 1625 | 443 |
| Without microphthalmia/kinky and/or short tail | 16 | 3344 | 159 | 7 | 2790 | 446 | 2 | 1916 | 745 |
| With microphthalmia/kinky and/or short tail | 1 | 1411 | – | 3 | 2202 | 406 | 6 | 1669 | 353 |

| | Litter no. and dose level (mg/kg/day) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | 1016 (100) | | | 1017 (100) | | | 1019 (100) | | |
| | | B.W. (mg) | | | B.W. (mg) | | | B.W. (mg) | |
| Category | n | Mean | S.D. | n | Mean | S.D. | n | Mean | S.D. |
| Without microphthalmia | 3 | 1920 | 299 | 0 | – | – | 3 | 2146 | 347 |
| With microphthalmia | 2 | 1634 | 50 | 1 | 2404 | – | 1 | 2394 | – |
| Without kinky/short tail | 2 | 2073 | 197 | 1 | 2404 | – | 4 | 2208 | 310 |
| With kinky/short tail | 3 | 1627 | 37 | 0 | – | – | 0 | – | – |
| Without microphthalmia/kinky and/or short tail | 2 | 2073 | 197 | 0 | – | – | 3 | 2146 | 347 |
| With microphthalmia/kinky and/or short tail | 3 | 1627 | 37 | 1 | 2404 | – | 1 | 2394 | – |

Table 2 Study no. 89-0063 KNF-317; Teratogenicity Study in Rats
Mean body weights of fetuses with or without external malformations

1) Information on all fetuses

| | | B.W. (mg) | |
|---|---|---|---|
| Category | n | Mean | S.D. |
| Without microphthalmia | 1367 | 3410 | 339 |
| With microphthalmia | 2 | 1999 | 74 |
| Without kinky/short tail | 1369 | 3408 | 343 |
| With kinky/short tail | 0 | – | – |

2) Information in each treatment group

| | Dose level (mg/kg/day) | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 0 | | | 3 | | | 10 | | | 30 | | |
| | | B.W. (mg) | | | B.W. (mg) | | | B.W. (mg) | | | B.W. (mg) | |
| Category | n | Mean | S.D. | n | Mean | S.D. | n | Mean | S.D. | n | Mean | S.D. |
| Without microphthalmia | 324 | 3496 | 296 | 352 | 3434 | 375 | 339 | 3445 | 324 | 352 | 3272 | 312 |
| With microphthalmia | 0 | – | – | 0 | – | – | 0 | – | – | 2 | 1999 | 74 |
| Without kinky/short tail | 324 | 3496 | 296 | 352 | 3434 | 375 | 339 | 3445 | 324 | 354 | 3265 | 325 |
| With kinky/short tail | 0 | – | – | 0 | – | – | 0 | – | – | 0 | – | – |

3) Information in each litter with external malformations

| | Litter no. and dose level (mg/kg/day) | | | | | |
|---|---|---|---|---|---|---|
| | 77 (30) | | | 80 (30) | | |
| | | B.W. (mg) | | | B.W. (mg) | |
| Category | n | Mean | S.D. | n | Mean | S.D. |
| Without microphthalmia | 17 | 3305 | 170 | 14 | 3334 | 324 |
| With microphthalmia | 1 | 2051 | – | 1 | 1947 | – |
| Without kinky/short tail | 18 | 3235 | 338 | 15 | 3241 | 475 |
| With kinky/short tail | 0 | – | – | 0 | – | – |

## 8.2 Annex II: Developmental toxicity studies in rats: individual fetal data on sex, body weights and external malformations

Appendix 1 Study no. 89-0062 KNF-317; Teratogenicity Study in Rats (Preliminary Study)
Fetal sex, body weights and external malformations – Individual data

50 mg/kg/day

| Dose level (mg/kg/day) | Animal no. | Total no. of live fetuses | Sex/ (m/f) | B. W./ (mg) | Findings |
|---|---|---|---|---|---|
| 50 | 1008 | 12 | m | 3216 | N |
| 50 | 1008 | 12 | m | 3204 | N |
| 50 | 1008 | 12 | m | 2636 | N |
| 50 | 1008 | 12 | m | 3179 | N |
| 50 | 1008 | 12 | m | 3436 | N |
| 50 | 1008 | 12 | m | 3131 | N |
| 50 | 1008 | 12 | f | 2464 | N |
| 50 | 1008 | 12 | f | 3279 | N |
| 50 | 1008 | 12 | f | 2804 | N |
| 50 | 1008 | 12 | f | 3291 | N |
| 50 | 1008 | 12 | f | 2826 | N |
| 50 | 1008 | 12 | f | 2865 | N |
| 50 | 1009 | 11 | m | 2386 | N |
| 50 | 1009 | 11 | m | 2974 | N |
| 50 | 1009 | 11 | m | 1843 | N |
| 50 | 1009 | 11 | m | 2328 | N |
| 50 | 1009 | 11 | m | 2472 | N |
| 50 | 1009 | 11 | f | 2610 | N |
| 50 | 1009 | 11 | f | 3194 | N |
| 50 | 1009 | 11 | f | 2801 | N |
| 50 | 1009 | 11 | f | 2480 | N |
| 50 | 1009 | 11 | f | 2642 | N |
| 50 | 1009 | 11 | f | 2521 | N |
| 50 | 1010 | 14 | m | 1951 | N |
| 50 | 1010 | 14 | m | 3495 | N |
| 50 | 1010 | 14 | m | 2915 | N |
| 50 | 1010 | 14 | m | 2406 | N |
| 50 | 1010 | 14 | m | 2258 | N |
| 50 | 1010 | 14 | m | 2706 | N |
| 50 | 1010 | 14 | m | 2221 | N |
| 50 | 1010 | 14 | f | 3014 | N |
| 50 | 1010 | 14 | f | 1989 | N |
| 50 | 1010 | 14 | f | 2825 | N |
| 50 | 1010 | 14 | f | 2325 | N |
| 50 | 1010 | 14 | f | 3303 | N |
| 50 | 1010 | 14 | f | 2302 | N |
| 50 | 1010 | 14 | f | 3036 | N |
| 50 | 1011 | 11 | m | 3270 | N |
| 50 | 1011 | 11 | m | 3575 | N |
| 50 | 1011 | 11 | m | 3398 | N |
| 50 | 1011 | 11 | m | 3751 | N |
| 50 | 1011 | 11 | f | 3444 | N |
| 50 | 1011 | 11 | f | 3455 | N |
| 50 | 1011 | 11 | f | 3217 | N |
| 50 | 1011 | 11 | f | 3424 | N |
| 50 | 1011 | 11 | f | 2736 | N |
| 50 | 1011 | 11 | f | 3203 | N |
| 50 | 1011 | 11 | f | 3576 | N |

Appendix 1 Study no. 89-0062 KNF-317; Teratogenicity Study in Rats (Preliminary Study)
Fetal sex, body weights and external malformations – Individual data

50 mg/kg/day –continued

| Dose level (mg/kg/day) | Animal no. | Total no. of live fetuses | Sex/ (m/f) | B. W./ (mg) | Findings |
|---|---|---|---|---|---|
| 50 | 1012 | 10 | f | 2575 | Microphthalmia |
| 50 | 1012 | 10 | f | 1770 | Microphthalmia |
| 50 | 1012 | 10 | m | 2260 | Kinky tail |
| 50 | 1012 | 10 | f | 2883 | N |
| 50 | 1012 | 10 | f | 3246 | N |
| 50 | 1012 | 10 | f | 2512 | N |
| 50 | 1012 | 10 | m | 2348 | N |
| 50 | 1012 | 10 | m | 3076 | N |
| 50 | 1012 | 10 | m | 3286 | N |
| 50 | 1012 | 10 | m | 2177 | N |
| 50 | 1013 | 14 | m | 3110 | N |
| 50 | 1013 | 14 | m | 3199 | N |
| 50 | 1013 | 14 | m | 2081 | N |
| 50 | 1013 | 14 | m | 2128 | N |
| 50 | 1013 | 14 | m | 2900 | N |
| 50 | 1013 | 14 | m | 2872 | N |
| 50 | 1013 | 14 | m | 3252 | N |
| 50 | 1013 | 14 | m | 2513 | N |
| 50 | 1013 | 14 | f | 2978 | N |
| 50 | 1013 | 14 | f | 2468 | N |
| 50 | 1013 | 14 | f | 2625 | N |
| 50 | 1013 | 14 | f | 3101 | N |
| 50 | 1013 | 14 | f | 2707 | N |
| 50 | 1013 | 14 | f | 2802 | N |
| 50 | 1014 | 14 | m | 3603 | N |
| 50 | 1014 | 14 | m | 3416 | N |
| 50 | 1014 | 14 | m | 3330 | N |
| 50 | 1014 | 14 | m | 3591 | N |
| 50 | 1014 | 14 | m | 3535 | N |
| 50 | 1014 | 14 | f | 3132 | N |
| 50 | 1014 | 14 | f | 3234 | N |
| 50 | 1014 | 14 | f | 3175 | N |
| 50 | 1014 | 14 | f | 3351 | N |
| 50 | 1014 | 14 | f | 3387 | N |
| 50 | 1014 | 14 | f | 2885 | N |
| 50 | 1014 | 14 | f | 3474 | N |
| 50 | 1014 | 14 | f | 3538 | N |
| 50 | 1014 | 14 | f | 3364 | N |

Appendix 1 Study no. 89-0062 KNF-317; Teratogenicity Study in Rats (Preliminary Study)
Fetal sex, body weights and external malformations – Individual data

100 mg/kg/day

| Dose level (mg/kg/day) | Animal no. | Total no. of live fetuses | Sex/ (m/f) | B. W./ (mg) | Findings |
|---|---|---|---|---|---|
| 100 | 1015 | 8 | f | 1825 | Microphthalmia |
| 100 | 1015 | 8 | f | 1691 | Microphthalmia |
| 100 | 1015 | 8 | m | 1172 | Microphthalmia, Kinky tail, Micrognathia, Meningoencephalocele |
| 100 | 1015 | 8 | f | 2211 | Kinky tail |
| 100 | 1015 | 8 | m | 1433 | Kinky tail |
| 100 | 1015 | 8 | f | 1683 | Short tail |
| 100 | 1015 | 8 | m | 1389 | N |
| 100 | 1015 | 8 | f | 2443 | N |
| 100 | 1016 | 5 | f | 1598 | Microphthalmia, Kinky tail |
| 100 | 1016 | 5 | m | 1669 | Microphthalmia, Kinky tail, Short tail |
| 100 | 1016 | 5 | m | 1615 | Kinky tail, Omphalocele |
| 100 | 1016 | 5 | m | 2212 | N |
| 100 | 1016 | 5 | f | 1934 | N |
| 100 | 1017 | 1 | f | 2404 | Microphthalmia |
| 100 | 1018 | 0 | | | |
| 100 | 1019 | 4 | f | 1750 | N |
| 100 | 1019 | 4 | m | 2400 | N |
| 100 | 1019 | 4 | m | 2394 | Microphthalmia |
| 100 | 1019 | 4 | f | 2287 | N |
| 100 | 1020 | 3 | m | 2718 | N |
| 100 | 1020 | 3 | m | 1862 | N |
| 100 | 1020 | 3 | m | 1424 | N |
| 100 | 1021 | 13 | m | 2778 | N |
| 100 | 1021 | 13 | m | 2522 | N |
| 100 | 1021 | 13 | m | 2563 | N |
| 100 | 1021 | 13 | m | 2507 | N |
| 100 | 1021 | 13 | m | 2629 | N |
| 100 | 1021 | 13 | m | 2414 | N |
| 100 | 1021 | 13 | f | 2424 | N |
| 100 | 1021 | 13 | f | 1679 | N |
| 100 | 1021 | 13 | f | 3552 | N |
| 100 | 1021 | 13 | f | 2756 | N |
| 100 | 1021 | 13 | f | 3071 | N |
| 100 | 1021 | 13 | f | 2761 | N |
| 100 | 1021 | 13 | f | 2968 | N |

Appendix 2 Study No. 89-0063 KNF-317; Teratogenicity Study in Rats
Fetal sex, body weights and external malformations – Individual data

10 mg/kg/day

| Dose level (mg/kg/day) | Animal no. | Total no. of live fetuses | Sex/ (m/f) | B. W./ (mg) | Findings |
|---|---|---|---|---|---|
| 10 | 49 | 15 | f | 3216 | N |
| 10 | 49 | 15 | f | 3268 | N |
| 10 | 49 | 15 | f | 3083 | N |
| 10 | 49 | 15 | f | 3391 | N |
| 10 | 49 | 15 | m | 3603 | N |
| 10 | 49 | 15 | m | 3696 | N |
| 10 | 49 | 15 | m | 3605 | N |
| 10 | 49 | 15 | m | 3457 | N |
| 10 | 49 | 15 | f | 3308 | N |
| 10 | 49 | 15 | f | 3396 | N |
| 10 | 49 | 15 | f | 3499 | N |
| 10 | 49 | 15 | m | 3615 | N |
| 10 | 49 | 15 | f | 3381 | N |
| 10 | 49 | 15 | f | 3446 | N |
| 10 | 49 | 15 | m | 3789 | N |
| 10 | 50 | 14 | m | 3276 | N |
| 10 | 50 | 14 | m | 3028 | N |
| 10 | 50 | 14 | m | 3111 | N |
| 10 | 50 | 14 | m | 3606 | N |
| 10 | 50 | 14 | m | 3294 | N |
| 10 | 50 | 14 | m | 3179 | N |
| 10 | 50 | 14 | f | 2959 | N |
| 10 | 50 | 14 | f | 2925 | N |
| 10 | 50 | 14 | m | 3354 | N |
| 10 | 50 | 14 | f | 3007 | N |
| 10 | 50 | 14 | m | 3194 | N |
| 10 | 50 | 14 | f | 3147 | N |
| 10 | 50 | 14 | f | 3332 | N |
| 10 | 50 | 14 | f | 3408 | N |
| 10 | 51 | 17 | f | 3334 | N |
| 10 | 51 | 17 | m | 3769 | N |
| 10 | 51 | 17 | f | 3787 | N |
| 10 | 51 | 17 | m | 3677 | N |
| 10 | 51 | 17 | m | 3745 | N |
| 10 | 51 | 17 | m | 3921 | N |
| 10 | 51 | 17 | f | 3721 | N |
| 10 | 51 | 17 | m | 3597 | N |
| 10 | 51 | 17 | f | 3321 | N |
| 10 | 51 | 17 | f | 3376 | N |
| 10 | 51 | 17 | m | 3123 | N |
| 10 | 51 | 17 | f | 3544 | N |
| 10 | 51 | 17 | m | 3721 | N |
| 10 | 51 | 17 | m | 3785 | N |
| 10 | 51 | 17 | m | 3753 | N |
| 10 | 51 | 17 | m | 3824 | N |
| 10 | 51 | 17 | m | 3505 | N |

Appendix 2 Study No. 89-0063 KNF-317; Teratogenicity Study in Rats
Fetal sex, body weights and external malformations - Individual data

10 mg/kg/day -continued

| Dose level (mg/kg/day) | Animal no. | Total no. of live fetuses | Sex/ (m/f) | B. W./ (mg) | Findings |
|---|---|---|---|---|---|
| 10 | 52 | 15 | f | 2481 | N |
| 10 | 52 | 15 | m | 3149 | N |
| 10 | 52 | 15 | f | 3191 | N |
| 10 | 52 | 15 | m | 3053 | N |
| 10 | 52 | 15 | f | 3085 | N |
| 10 | 52 | 15 | m | 3264 | N |
| 10 | 52 | 15 | m | 3190 | N |
| 10 | 52 | 15 | f | 2829 | N |
| 10 | 52 | 15 | m | 3479 | N |
| 10 | 52 | 15 | m | 3604 | N |
| 10 | 52 | 15 | m | 2983 | N |
| 10 | 52 | 15 | f | 3056 | N |
| 10 | 52 | 15 | f | 2113 | N |
| 10 | 52 | 15 | f | 2933 | N |
| 10 | 52 | 15 | m | 3562 | N |
| 10 | 53 | 16 | m | 3442 | N |
| 10 | 53 | 16 | f | 3626 | N |
| 10 | 53 | 16 | m | 3494 | N |
| 10 | 53 | 16 | f | 3401 | N |
| 10 | 53 | 16 | m | 3969 | N |
| 10 | 53 | 16 | f | 3540 | N |
| 10 | 53 | 16 | f | 3774 | N |
| 10 | 53 | 16 | f | 3558 | N |
| 10 | 53 | 16 | f | 2789 | N |
| 10 | 53 | 16 | m | 3855 | N |
| 10 | 53 | 16 | m | 3744 | N |
| 10 | 53 | 16 | m | 3703 | N |
| 10 | 53 | 16 | m | 4010 | N |
| 10 | 53 | 16 | f | 3892 | N |
| 10 | 53 | 16 | m | 3615 | N |
| 10 | 53 | 16 | m | 3942 | N |
| 10 | 54 | 15 | m | 3661 | N |
| 10 | 54 | 15 | m | 3434 | N |
| 10 | 54 | 15 | f | 3855 | N |
| 10 | 54 | 15 | f | 3778 | N |
| 10 | 54 | 15 | f | 3152 | N |
| 10 | 54 | 15 | m | 3865 | N |
| 10 | 54 | 15 | m | 3952 | N |
| 10 | 54 | 15 | m | 3612 | N |
| 10 | 54 | 15 | m | 3759 | N |
| 10 | 54 | 15 | m | 3425 | N |
| 10 | 54 | 15 | f | 3822 | N |
| 10 | 54 | 15 | f | 3524 | N |
| 10 | 54 | 15 | f | 3810 | N |
| 10 | 54 | 15 | m | 4036 | N |
| 10 | 54 | 15 | m | 4233 | N |

Appendix 2 Study No. 89-0063 KNF-317; Teratogenicity Study in Rats
Fetal sex, body weights and external malformations - Individual data

10 mg/kg/day -continued

| Dose level (mg/kg/day) | Animal no. | Total no. of live fetuses | Sex/ (m/f) | B. W./ (mg) | Findings |
|---|---|---|---|---|---|
| 10 | 55 | 10 | f | 2953 | N |
| 10 | 55 | 10 | m | 3413 | N |
| 10 | 55 | 10 | f | 2998 | N |
| 10 | 55 | 10 | m | 3428 | N |
| 10 | 55 | 10 | f | 3391 | N |
| 10 | 55 | 10 | m | 3331 | N |
| 10 | 55 | 10 | m | 3367 | N |
| 10 | 55 | 10 | f | 3098 | N |
| 10 | 55 | 10 | m | 3368 | N |
| 10 | 55 | 10 | m | 3888 | N |
| 10 | 56 | 11 | m | 3822 | N |
| 10 | 56 | 11 | m | 4020 | N |
| 10 | 56 | 11 | f | 4035 | N |
| 10 | 56 | 11 | f | 3885 | N |
| 10 | 56 | 11 | f | 3886 | N |
| 10 | 56 | 11 | f | 3485 | N |
| 10 | 56 | 11 | m | 3919 | N |
| 10 | 56 | 11 | f | 3692 | N |
| 10 | 56 | 11 | m | 4054 | N |
| 10 | 56 | 11 | m | 4005 | N |
| 10 | 56 | 11 | f | 3608 | N |
| 10 | 57 | 16 | m | 3200 | N |
| 10 | 57 | 16 | f | 3572 | N |
| 10 | 57 | 16 | m | 3710 | N |
| 10 | 57 | 16 | f | 3655 | N |
| 10 | 57 | 16 | f | 3676 | N |
| 10 | 57 | 16 | m | 3997 | N |
| 10 | 57 | 16 | f | 3392 | N |
| 10 | 57 | 16 | m | 3744 | N |
| 10 | 57 | 16 | m | 3546 | N |
| 10 | 57 | 16 | f | 3682 | N |
| 10 | 57 | 16 | m | 3424 | N |
| 10 | 57 | 16 | m | 3509 | N |
| 10 | 57 | 16 | m | 3980 | N |
| 10 | 57 | 16 | m | 3681 | N |
| 10 | 57 | 16 | f | 3616 | N |
| 10 | 57 | 16 | f | 3657 | N |

Appendix 2 Study No. 89-0063 KNF-317; Teratogenicity Study in Rats
Fetal sex, body weights and external malformations – Individual data

10 mg/kg/day –continued

| Dose level (mg/kg/day) | Animal no. | Total no. of live fetuses | Sex/ (m/f) | B. W./ (mg) | Findings |
|---|---|---|---|---|---|
| 10 | 58 | 16 | m | 3259 | N |
| 10 | 58 | 16 | f | 3076 | N |
| 10 | 58 | 16 | f | 3301 | N |
| 10 | 58 | 16 | f | 3731 | N |
| 10 | 58 | 16 | m | 3965 | N |
| 10 | 58 | 16 | m | 3998 | N |
| 10 | 58 | 16 | m | 3980 | N |
| 10 | 58 | 16 | f | 3895 | N |
| 10 | 58 | 16 | f | 3709 | N |
| 10 | 58 | 16 | f | 3458 | N |
| 10 | 58 | 16 | m | 3952 | N |
| 10 | 58 | 16 | f | 3769 | N |
| 10 | 58 | 16 | m | 3720 | N |
| 10 | 58 | 16 | m | 4037 | N |
| 10 | 58 | 16 | f | 4160 | N |
| 10 | 58 | 16 | f | 3843 | N |
| 10 | 59 | 14 | m | 3739 | N |
| 10 | 59 | 14 | m | 3592 | N |
| 10 | 59 | 14 | m | 3425 | N |
| 10 | 59 | 14 | f | 3257 | N |
| 10 | 59 | 14 | m | 4027 | N |
| 10 | 59 | 14 | f | 3661 | N |
| 10 | 59 | 14 | m | 3753 | N |
| 10 | 59 | 14 | f | 3025 | N |
| 10 | 59 | 14 | m | 3832 | N |
| 10 | 59 | 14 | m | 3843 | N |
| 10 | 59 | 14 | f | 3269 | N |
| 10 | 59 | 14 | f | 3262 | N |
| 10 | 59 | 14 | f | 3471 | N |
| 10 | 59 | 14 | f | 3729 | N |
| 10 | 60 | 15 | f | 3265 | N |
| 10 | 60 | 15 | m | 3567 | N |
| 10 | 60 | 15 | f | 3563 | N |
| 10 | 60 | 15 | m | 3745 | N |
| 10 | 60 | 15 | f | 3555 | N |
| 10 | 60 | 15 | f | 3558 | N |
| 10 | 60 | 15 | m | 3570 | N |
| 10 | 60 | 15 | f | 3638 | N |
| 10 | 60 | 15 | m | 3672 | N |
| 10 | 60 | 15 | f | 3634 | N |
| 10 | 60 | 15 | f | 3299 | N |
| 10 | 60 | 15 | m | 3637 | N |
| 10 | 60 | 15 | m | 3916 | N |
| 10 | 60 | 15 | m | 3890 | N |
| 10 | 60 | 15 | m | 4102 | N |

Appendix 2 Study No. 89-0063 KNF-317; Teratogenicity Study in Rats
Fetal sex, body weights and external malformations - Individual data

10 mg/kg/day -continued

| Dose level (mg/kg/day) | Animal no. | Total no. of live fetuses | Sex/ (m/f) | B. W./ (mg) | Findings |
|---|---|---|---|---|---|
| 10 | 61 | 16 | f | 3051 | N |
| 10 | 61 | 16 | f | 2973 | N |
| 10 | 61 | 16 | m | 3420 | N |
| 10 | 61 | 16 | m | 3300 | N |
| 10 | 61 | 16 | m | 2953 | N |
| 10 | 61 | 16 | f | 2905 | N |
| 10 | 61 | 16 | m | 2427 | N |
| 10 | 61 | 16 | m | 3267 | N |
| 10 | 61 | 16 | m | 3482 | N |
| 10 | 61 | 16 | m | 3193 | N |
| 10 | 61 | 16 | f | 3286 | N |
| 10 | 61 | 16 | f | 3130 | N |
| 10 | 61 | 16 | m | 3189 | N |
| 10 | 61 | 16 | f | 3111 | N |
| 10 | 61 | 16 | m | 2931 | N |
| 10 | 61 | 16 | m | 3290 | N |
| 10 | 62 | 15 | m | 3282 | N |
| 10 | 62 | 15 | m | 4000 | N |
| 10 | 62 | 15 | m | 3696 | N |
| 10 | 62 | 15 | m | 3439 | N |
| 10 | 62 | 15 | m | 3527 | N |
| 10 | 62 | 15 | m | 3374 | N |
| 10 | 62 | 15 | m | 3557 | N |
| 10 | 62 | 15 | m | 3692 | N |
| 10 | 62 | 15 | f | 3169 | N |
| 10 | 62 | 15 | m | 3637 | N |
| 10 | 62 | 15 | m | 3458 | N |
| 10 | 62 | 15 | m | 3510 | N |
| 10 | 62 | 15 | f | 3243 | N |
| 10 | 62 | 15 | m | 3781 | N |
| 10 | 62 | 15 | f | 3339 | N |
| 10 | 63 | 15 | m | 3162 | N |
| 10 | 63 | 15 | m | 2979 | N |
| 10 | 63 | 15 | m | 2858 | N |
| 10 | 63 | 15 | f | 3008 | N |
| 10 | 63 | 15 | f | 3092 | N |
| 10 | 63 | 15 | m | 3046 | N |
| 10 | 63 | 15 | m | 3131 | N |
| 10 | 63 | 15 | f | 2971 | N |
| 10 | 63 | 15 | f | 2835 | N |
| 10 | 63 | 15 | m | 2939 | N |
| 10 | 63 | 15 | m | 3309 | N |
| 10 | 63 | 15 | f | 2820 | N |
| 10 | 63 | 15 | m | 3012 | N |
| 10 | 63 | 15 | m | 3229 | N |
| 10 | 63 | 15 | f | 2796 | N |

Appendix 2 Study No. 89-0063 KNF-317; Teratogenicity Study in Rats
Fetal sex, body weights and external malformations - Individual data

10 mg/kg/day -continued

| Dose level (mg/kg/day) | Animal no. | Total no. of live fetuses | Sex/ (m/f) | B. W./ (mg) | Findings |
|---|---|---|---|---|---|
| 10 | 64 | 14 | f | 3014 | N |
| 10 | 64 | 14 | m | 3840 | N |
| 10 | 64 | 14 | m | 3148 | N |
| 10 | 64 | 14 | m | 3411 | N |
| 10 | 64 | 14 | m | 3500 | N |
| 10 | 64 | 14 | f | 3089 | N |
| 10 | 64 | 14 | m | 3096 | N |
| 10 | 64 | 14 | m | 3005 | N |
| 10 | 64 | 14 | f | 3192 | N |
| 10 | 64 | 14 | f | 2861 | N |
| 10 | 64 | 14 | m | 2938 | N |
| 10 | 64 | 14 | f | 3438 | N |
| 10 | 64 | 14 | f | 3264 | N |
| 10 | 64 | 14 | f | 3384 | N |
| 10 | 65 | 15 | f | 3499 | N |
| 10 | 65 | 15 | m | 3809 | N |
| 10 | 65 | 15 | m | 3855 | N |
| 10 | 65 | 15 | m | 3686 | N |
| 10 | 65 | 15 | m | 3799 | N |
| 10 | 65 | 15 | f | 3789 | N |
| 10 | 65 | 15 | f | 3688 | N |
| 10 | 65 | 15 | f | 3806 | N |
| 10 | 65 | 15 | f | 3628 | N |
| 10 | 65 | 15 | m | 4081 | N |
| 10 | 65 | 15 | m | 3782 | N |
| 10 | 65 | 15 | m | 3892 | N |
| 10 | 65 | 15 | m | 3841 | N |
| 10 | 65 | 15 | f | 3754 | N |
| 10 | 65 | 15 | f | 3699 | N |
| 10 | 66 | 16 | f | 3182 | N |
| 10 | 66 | 16 | f | 3332 | N |
| 10 | 66 | 16 | f | 3021 | N |
| 10 | 66 | 16 | f | 3366 | N |
| 10 | 66 | 16 | f | 3484 | N |
| 10 | 66 | 16 | f | 3253 | N |
| 10 | 66 | 16 | f | 3534 | N |
| 10 | 66 | 16 | m | 3433 | N |
| 10 | 66 | 16 | m | 3621 | N |
| 10 | 66 | 16 | m | 3573 | N |
| 10 | 66 | 16 | m | 3284 | N |
| 10 | 66 | 16 | m | 3331 | N |
| 10 | 66 | 16 | f | 3295 | N |
| 10 | 66 | 16 | m | 3484 | N |
| 10 | 66 | 16 | f | 3228 | N |
| 10 | 66 | 16 | f | 3729 | N |
| 10 | 67 | 3 | m | 3556 | N |
| 10 | 67 | 3 | m | 3650 | N |
| 10 | 67 | 3 | f | 3489 | N |

Appendix 2 Study No. 89-0063 KNF-317; Teratogenicity Study in Rats
Fetal sex, body weights and external malformations – Individual data

10 mg/kg/day –continued

| Dose level (mg/kg/day) | Animal no. | Total no. of live fetuses | Sex/ (m/f) | B. W./ (mg) | Findings |
|---|---|---|---|---|---|
| 10 | 68 | 17 | f | 3111 | N |
| 10 | 68 | 17 | m | 3604 | N |
| 10 | 68 | 17 | f | 3075 | N |
| 10 | 68 | 17 | f | 3387 | N |
| 10 | 68 | 17 | m | 3814 | N |
| 10 | 68 | 17 | f | 3620 | N |
| 10 | 68 | 17 | m | 3309 | N |
| 10 | 68 | 17 | f | 2839 | N |
| 10 | 68 | 17 | m | 3378 | N |
| 10 | 68 | 17 | f | 2966 | N |
| 10 | 68 | 17 | f | 3160 | N |
| 10 | 68 | 17 | f | 3154 | N |
| 10 | 68 | 17 | m | 3717 | N |
| 10 | 68 | 17 | f | 3498 | N |
| 10 | 68 | 17 | f | 3238 | N |
| 10 | 68 | 17 | m | 3397 | N |
| 10 | 68 | 17 | f | 2965 | N |
| 10 | 69 | 6 | f | 3304 | N |
| 10 | 69 | 6 | m | 3420 | N |
| 10 | 69 | 6 | f | 3294 | N |
| 10 | 69 | 6 | m | 3540 | N |
| 10 | 69 | 6 | f | 3505 | N |
| 10 | 69 | 6 | m | 3245 | N |
| 10 | 70 | 17 | m | 3265 | N |
| 10 | 70 | 17 | m | 3356 | N |
| 10 | 70 | 17 | m | 3326 | N |
| 10 | 70 | 17 | f | 3257 | N |
| 10 | 70 | 17 | f | 3136 | N |
| 10 | 70 | 17 | m | 3339 | N |
| 10 | 70 | 17 | m | 3281 | N |
| 10 | 70 | 17 | m | 3578 | N |
| 10 | 70 | 17 | f | 3276 | N |
| 10 | 70 | 17 | m | 3281 | N |
| 10 | 70 | 17 | m | 3391 | N |
| 10 | 70 | 17 | m | 3524 | N |
| 10 | 70 | 17 | m | 3353 | N |
| 10 | 70 | 17 | f | 3500 | N |
| 10 | 70 | 17 | f | 3544 | N |
| 10 | 70 | 17 | f | 3531 | N |
| 10 | 70 | 17 | m | 3551 | N |

Appendix 2 Study No. 89-0063 KNF-317; Teratogenicity Study in Rats
Fetal sex, body weights and external malformations – Individual data

10 mg/kg/day –continued

| Dose level (mg/kg/day) | Animal no. | Total no. of live fetuses | Sex/ (m/f) | B. W./ (mg) | Findings |
|---|---|---|---|---|---|
| 10 | 71 | 17 | f | 3115 | N |
| 10 | 71 | 17 | f | 3100 | N |
| 10 | 71 | 17 | m | 3115 | N |
| 10 | 71 | 17 | f | 3123 | N |
| 10 | 71 | 17 | f | 3214 | N |
| 10 | 71 | 17 | f | 3089 | N |
| 10 | 71 | 17 | f | 3089 | N |
| 10 | 71 | 17 | m | 3481 | N |
| 10 | 71 | 17 | f | 3354 | N |
| 10 | 71 | 17 | m | 3535 | N |
| 10 | 71 | 17 | f | 3355 | N |
| 10 | 71 | 17 | m | 3328 | N |
| 10 | 71 | 17 | m | 3524 | N |
| 10 | 71 | 17 | f | 3494 | N |
| 10 | 71 | 17 | m | 3241 | N |
| 10 | 71 | 17 | f | 3050 | Vestigial tail, Anal atresia |
| 10 | 71 | 17 | f | 3317 | N |
| 10 | 72 | 14 | f | 3005 | N |
| 10 | 72 | 14 | m | 3538 | N |
| 10 | 72 | 14 | m | 3839 | N |
| 10 | 72 | 14 | f | 3360 | N |
| 10 | 72 | 14 | m | 3538 | N |
| 10 | 72 | 14 | m | 3578 | N |
| 10 | 72 | 14 | m | 3356 | N |
| 10 | 72 | 14 | f | 2991 | N |
| 10 | 72 | 14 | f | 3247 | N |
| 10 | 72 | 14 | m | 3351 | N |
| 10 | 72 | 14 | m | 3661 | N |
| 10 | 72 | 14 | m | 3465 | N |
| 10 | 72 | 14 | f | 3379 | N |
| 10 | 72 | 14 | f | 3256 | N |

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Fetal sex, body weights and external malformations – Individual data

30 mg/kg/day

| Dose level (mg/kg/day) | Animal no. | Total no. of live fetuses | Sex/ (m/f) | B. W./ (mg) | Findings |
|---|---|---|---|---|---|
| 30 | 73 | 15 | f | 2745 | N |
| 30 | 73 | 15 | f | 3325 | N |
| 30 | 73 | 15 | f | 3155 | N |
| 30 | 73 | 15 | m | 3451 | N |
| 30 | 73 | 15 | m | 3395 | N |
| 30 | 73 | 15 | m | 3405 | N |
| 30 | 73 | 15 | m | 3440 | N |
| 30 | 73 | 15 | f | 2906 | N |
| 30 | 73 | 15 | m | 3450 | N |
| 30 | 73 | 15 | m | 3481 | N |
| 30 | 73 | 15 | m | 3233 | N |
| 30 | 73 | 15 | m | 3514 | N |
| 30 | 73 | 15 | m | 3180 | N |
| 30 | 73 | 15 | f | 3289 | N |
| 30 | 73 | 15 | m | 3538 | N |
| 30 | 74 | 14 | f | 3042 | N |
| 30 | 74 | 14 | m | 3295 | N |
| 30 | 74 | 14 | f | 3226 | N |
| 30 | 74 | 14 | f | 3362 | N |
| 30 | 74 | 14 | m | 3289 | N |
| 30 | 74 | 14 | m | 3311 | N |
| 30 | 74 | 14 | m | 2976 | N |
| 30 | 74 | 14 | f | 3634 | N |
| 30 | 74 | 14 | m | 3339 | N |
| 30 | 74 | 14 | f | 3258 | N |
| 30 | 74 | 14 | m | 3524 | N |
| 30 | 74 | 14 | m | 2778 | N |
| 30 | 74 | 14 | m | 3203 | N |
| 30 | 74 | 14 | f | 3343 | N |
| 30 | 75 | 15 | f | 3659 | N |
| 30 | 75 | 15 | m | 3849 | N |
| 30 | 75 | 15 | m | 3486 | N |
| 30 | 75 | 15 | m | 4009 | N |
| 30 | 75 | 15 | f | 3290 | N |
| 30 | 75 | 15 | f | 3825 | N |
| 30 | 75 | 15 | m | 3949 | N |
| 30 | 75 | 15 | f | 2712 | N |
| 30 | 75 | 15 | f | 3943 | N |
| 30 | 75 | 15 | m | 3824 | N |
| 30 | 75 | 15 | m | 3928 | N |
| 30 | 75 | 15 | f | 3733 | N |
| 30 | 75 | 15 | f | 2700 | N |
| 30 | 75 | 15 | m | 2706 | N |
| 30 | 75 | 15 | f | 3905 | N |

Appendix 2 Study No. 89-0063 KNF-317; Teratogenicity Study in Rats
Fetal sex, body weights and external malformations – Individual data

30 mg/kg/day –continued

| Dose level (mg/kg/day) | Animal no. | Total no. of live fetuses | Sex/ (m/f) | B. W./ (mg) | Findings |
|---|---|---|---|---|---|
| 30 | 76 | 13 | f | 3385 | N |
| 30 | 76 | 13 | f | 2943 | N |
| 30 | 76 | 13 | f | 3306 | N |
| 30 | 76 | 13 | m | 3478 | N |
| 30 | 76 | 13 | f | 3251 | N |
| 30 | 76 | 13 | m | 3714 | N |
| 30 | 76 | 13 | m | 2325 | N |
| 30 | 76 | 13 | m | 3543 | N |
| 30 | 76 | 13 | m | 3569 | N |
| 30 | 76 | 13 | f | 3177 | N |
| 30 | 76 | 13 | m | 3280 | N |
| 30 | 76 | 13 | m | 2311 | N |
| 30 | 76 | 13 | m | 3557 | N |
| 30 | 77 | 18 | m | 3441 | N |
| 30 | 77 | 18 | f | 3408 | N |
| 30 | 77 | 18 | f | 2994 | N |
| 30 | 77 | 18 | m | 2051 | Microphthalmia |
| 30 | 77 | 18 | f | 2987 | N |
| 30 | 77 | 18 | m | 3255 | N |
| 30 | 77 | 18 | m | 3392 | N |
| 30 | 77 | 18 | m | 3525 | N |
| 30 | 77 | 18 | m | 3298 | N |
| 30 | 77 | 18 | f | 3344 | N |
| 30 | 77 | 18 | f | 3316 | N |
| 30 | 77 | 18 | f | 3026 | N |
| 30 | 77 | 18 | f | 3384 | N |
| 30 | 77 | 18 | m | 3269 | N |
| 30 | 77 | 18 | f | 3174 | N |
| 30 | 77 | 18 | f | 3471 | N |
| 30 | 77 | 18 | m | 3470 | N |
| 30 | 77 | 18 | m | 3431 | N |
| 30 | 78 | 17 | f | 3023 | N |
| 30 | 78 | 17 | f | 3057 | N |
| 30 | 78 | 17 | m | 3259 | N |
| 30 | 78 | 17 | m | 3383 | N |
| 30 | 78 | 17 | f | 2859 | N |
| 30 | 78 | 17 | f | 3230 | N |
| 30 | 78 | 17 | f | 3065 | N |
| 30 | 78 | 17 | m | 3366 | N |
| 30 | 78 | 17 | m | 3075 | N |
| 30 | 78 | 17 | f | 3174 | N |
| 30 | 78 | 17 | m | 3328 | N |
| 30 | 78 | 17 | f | 3129 | N |
| 30 | 78 | 17 | f | 3396 | N |
| 30 | 78 | 17 | f | 3007 | N |
| 30 | 78 | 17 | f | 3087 | N |
| 30 | 78 | 17 | m | 3440 | N |
| 30 | 78 | 17 | f | 3162 | N |

Appendix 2 Study No. 89-0063 KNF-317; Teratogenicity Study in Rats
Fetal sex, body weights and external malformations - Individual data

30 mg/kg/day -continued

| Dose level (mg/kg/day) | Animal no. | Total no. of live fetuses | Sex/ (m/f) | B. W./ (mg) | Findings |
|---|---|---|---|---|---|
| 30 | 79 | 16 | m | 3615 | N |
| 30 | 79 | 16 | f | 3150 | N |
| 30 | 79 | 16 | m | 3208 | N |
| 30 | 79 | 16 | f | 3197 | N |
| 30 | 79 | 16 | f | 3333 | N |
| 30 | 79 | 16 | f | 3401 | N |
| 30 | 79 | 16 | f | 3787 | N |
| 30 | 79 | 16 | f | 3198 | N |
| 30 | 79 | 16 | f | 3155 | N |
| 30 | 79 | 16 | m | 3567 | N |
| 30 | 79 | 16 | f | 3149 | N |
| 30 | 79 | 16 | f | 3361 | N |
| 30 | 79 | 16 | m | 3221 | N |
| 30 | 79 | 16 | m | 3256 | N |
| 30 | 79 | 16 | m | 3744 | N |
| 30 | 79 | 16 | f | 3287 | N |
| 30 | 80 | 15 | m | 3302 | N |
| 30 | 80 | 15 | f | 3397 | N |
| 30 | 80 | 15 | m | 2871 | N |
| 30 | 80 | 15 | f | 3241 | N |
| 30 | 80 | 15 | f | 3604 | N |
| 30 | 80 | 15 | m | 3677 | N |
| 30 | 80 | 15 | f | 3282 | N |
| 30 | 80 | 15 | m | 2602 | N |
| 30 | 80 | 15 | f | 3355 | N |
| 30 | 80 | 15 | m | 3690 | N |
| 30 | 80 | 15 | m | 3267 | N |
| 30 | 80 | 15 | m | 1947 | Microphthalmia, Cleft lip, Cleft palate |
| 30 | 80 | 15 | m | 3678 | N |
| 30 | 80 | 15 | m | 3629 | N |
| 30 | 80 | 15 | f | 3075 | N |
| 30 | 81 | 14 | m | 3286 | N |
| 30 | 81 | 14 | f | 3452 | N |
| 30 | 81 | 14 | m | 2996 | N |
| 30 | 81 | 14 | f | 2917 | N |
| 30 | 81 | 14 | m | 3330 | N |
| 30 | 81 | 14 | f | 2875 | N |
| 30 | 81 | 14 | m | 3088 | N |
| 30 | 81 | 14 | m | 3489 | N |
| 30 | 81 | 14 | m | 3154 | N |
| 30 | 81 | 14 | m | 3078 | N |
| 30 | 81 | 14 | m | 3149 | N |
| 30 | 81 | 14 | m | 3173 | N |
| 30 | 81 | 14 | m | 2832 | N |
| 30 | 81 | 14 | f | 3224 | N |

Appendix 2 Study No. 89-0063 KNF-317; Teratogenicity Study in Rats
Fetal sex, body weights and external malformations - Individual data

30 mg/kg/day -continued

| Dose level (mg/kg/day) | Animal no. | Total no. of live fetuses | Sex/ (m/f) | B. W./ (mg) | Findings |
|---|---|---|---|---|---|
| 30 | 82 | 15 | m | 2342 | N |
| 30 | 82 | 15 | m | 2838 | N |
| 30 | 82 | 15 | f | 2987 | N |
| 30 | 82 | 15 | f | 2228 | N |
| 30 | 82 | 15 | m | 3187 | N |
| 30 | 82 | 15 | m | 2975 | N |
| 30 | 82 | 15 | f | 2674 | N |
| 30 | 82 | 15 | m | 2611 | N |
| 30 | 82 | 15 | f | 2972 | N |
| 30 | 82 | 15 | f | 2723 | N |
| 30 | 82 | 15 | f | 2902 | N |
| 30 | 82 | 15 | f | 2173 | N |
| 30 | 82 | 15 | f | 2944 | N |
| 30 | 82 | 15 | m | 2442 | N |
| 30 | 82 | 15 | f | 2905 | N |
| 30 | 83 | 14 | m | 3470 | N |
| 30 | 83 | 14 | f | 3413 | N |
| 30 | 83 | 14 | f | 3483 | N |
| 30 | 83 | 14 | f | 3217 | N |
| 30 | 83 | 14 | m | 3409 | N |
| 30 | 83 | 14 | f | 3274 | N |
| 30 | 83 | 14 | m | 3932 | N |
| 30 | 83 | 14 | m | 3685 | N |
| 30 | 83 | 14 | m | 3736 | N |
| 30 | 83 | 14 | m | 3333 | N |
| 30 | 83 | 14 | m | 3552 | N |
| 30 | 83 | 14 | f | 3139 | N |
| 30 | 83 | 14 | f | 3327 | N |
| 30 | 83 | 14 | f | 3434 | N |
| 30 | 84 | 15 | m | 2942 | N |
| 30 | 84 | 15 | f | 2695 | N |
| 30 | 84 | 15 | f | 3071 | N |
| 30 | 84 | 15 | f | 3333 | N |
| 30 | 84 | 15 | f | 3495 | N |
| 30 | 84 | 15 | m | 3660 | N |
| 30 | 84 | 15 | f | 2920 | N |
| 30 | 84 | 15 | f | 3248 | N |
| 30 | 84 | 15 | f | 2993 | N |
| 30 | 84 | 15 | f | 2945 | N |
| 30 | 84 | 15 | m | 3779 | N |
| 30 | 84 | 15 | f | 3193 | N |
| 30 | 84 | 15 | f | 3118 | N |
| 30 | 84 | 15 | m | 3115 | N |
| 30 | 84 | 15 | m | 3389 | N |
| 30 | 85 | 0 | | | Non-pregnant |

Appendix 2 Study No. 89-0063 KNF-317; Teratogenicity Study in Rats
Fetal sex, body weights and external malformations – Individual data

30 mg/kg/day –continued

| Dose level (mg/kg/day) | Animal no. | Total no. of live fetuses | Sex/ (m/f) | B. W./ (mg) | Findings |
|---|---|---|---|---|---|
| 30 | 86 | 15 | m | 2606 | N |
| 30 | 86 | 15 | f | 3257 | N |
| 30 | 86 | 15 | m | 3416 | N |
| 30 | 86 | 15 | f | 3089 | N |
| 30 | 86 | 15 | f | 3476 | N |
| 30 | 86 | 15 | f | 3269 | N |
| 30 | 86 | 15 | f | 3259 | N |
| 30 | 86 | 15 | m | 2994 | N |
| 30 | 86 | 15 | f | 2847 | N |
| 30 | 86 | 15 | f | 2827 | N |
| 30 | 86 | 15 | f | 3355 | N |
| 30 | 86 | 15 | f | 3195 | N |
| 30 | 86 | 15 | m | 3083 | N |
| 30 | 86 | 15 | m | 3234 | N |
| 30 | 86 | 15 | f | 2986 | N |
| 30 | 87 | 15 | f | 3362 | N |
| 30 | 87 | 15 | m | 3344 | N |
| 30 | 87 | 15 | f | 3498 | N |
| 30 | 87 | 15 | m | 3729 | N |
| 30 | 87 | 15 | f | 3276 | N |
| 30 | 87 | 15 | f | 3200 | N |
| 30 | 87 | 15 | f | 3461 | N |
| 30 | 87 | 15 | f | 3586 | N |
| 30 | 87 | 15 | m | 3456 | N |
| 30 | 87 | 15 | m | 3717 | N |
| 30 | 87 | 15 | f | 3547 | N |
| 30 | 87 | 15 | m | 3665 | N |
| 30 | 87 | 15 | m | 3329 | N |
| 30 | 87 | 15 | m | 3341 | N |
| 30 | 87 | 15 | f | 3353 | N |
| 30 | 88 | 15 | f | 2763 | N |
| 30 | 88 | 15 | m | 3405 | N |
| 30 | 88 | 15 | m | 3529 | N |
| 30 | 88 | 15 | m | 3261 | N |
| 30 | 88 | 15 | m | 3621 | N |
| 30 | 88 | 15 | m | 3663 | N |
| 30 | 88 | 15 | f | 3173 | N |
| 30 | 88 | 15 | f | 3144 | N |
| 30 | 88 | 15 | m | 3234 | N |
| 30 | 88 | 15 | m | 3303 | N |
| 30 | 88 | 15 | f | 2546 | N |
| 30 | 88 | 15 | m | 3318 | N |
| 30 | 88 | 15 | f | 3362 | N |
| 30 | 88 | 15 | f | 3209 | N |
| 30 | 88 | 15 | f | 3281 | N |

Appendix 2 Study No. 89-0063 KNF-317; Teratogenicity Study in Rats
Fetal sex, body weights and external malformations – Individual data

30 mg/kg/day -continued

| Dose level (mg/kg/day) | Animal no. | Total no. of live fetuses | Sex/ (m/f) | B. W./ (mg) | Findings |
|---|---|---|---|---|---|
| 30 | 89 | 19 | m | 3296 | N |
| 30 | 89 | 19 | m | 3460 | N |
| 30 | 89 | 19 | f | 3191 | N |
| 30 | 89 | 19 | f | 3099 | N |
| 30 | 89 | 19 | f | 3206 | N |
| 30 | 89 | 19 | m | 2975 | N |
| 30 | 89 | 19 | m | 3254 | N |
| 30 | 89 | 19 | f | 2685 | N |
| 30 | 89 | 19 | f | 3279 | N |
| 30 | 89 | 19 | f | 2882 | N |
| 30 | 89 | 19 | m | 3421 | N |
| 30 | 89 | 19 | f | 3394 | N |
| 30 | 89 | 19 | f | 3434 | N |
| 30 | 89 | 19 | f | 3151 | N |
| 30 | 89 | 19 | m | 3552 | N |
| 30 | 89 | 19 | m | 3537 | N |
| 30 | 89 | 19 | m | 3471 | N |
| 30 | 89 | 19 | f | 3295 | N |
| 30 | 89 | 19 | m | 3373 | N |
| 30 | 90 | 15 | f | 2910 | N |
| 30 | 90 | 15 | f | 2842 | N |
| 30 | 90 | 15 | f | 2810 | N |
| 30 | 90 | 15 | m | 3213 | N |
| 30 | 90 | 15 | m | 3077 | N |
| 30 | 90 | 15 | m | 3740 | N |
| 30 | 90 | 15 | m | 3173 | N |
| 30 | 90 | 15 | f | 3401 | N |
| 30 | 90 | 15 | f | 2743 | N |
| 30 | 90 | 15 | f | 3077 | N |
| 30 | 90 | 15 | f | 3299 | N |
| 30 | 90 | 15 | f | 3269 | N |
| 30 | 90 | 15 | m | 3429 | N |
| 30 | 90 | 15 | f | 3177 | N |
| 30 | 90 | 15 | m | 3697 | N |
| 30 | 91 | 15 | m | 3399 | N |
| 30 | 91 | 15 | m | 3864 | N |
| 30 | 91 | 15 | f | 3320 | N |
| 30 | 91 | 15 | f | 3285 | N |
| 30 | 91 | 15 | m | 3802 | N |
| 30 | 91 | 15 | f | 3090 | N |
| 30 | 91 | 15 | f | 3476 | N |
| 30 | 91 | 15 | f | 3264 | N |
| 30 | 91 | 15 | f | 3631 | N |
| 30 | 91 | 15 | m | 3714 | N |
| 30 | 91 | 15 | m | 3712 | N |
| 30 | 91 | 15 | f | 3561 | N |
| 30 | 91 | 15 | f | 3585 | N |
| 30 | 91 | 15 | f | 3499 | N |
| 30 | 91 | 15 | f | 3324 | N |

Appendix 2 Study No. 89-0063 KNF-317; Teratogenicity Study in Rats
Fetal sex, body weights and external malformations – Individual data

30 mg/kg/day –continued

| Dose level (mg/kg/day) | Animal no. | Total no. of live fetuses | Sex/ (m/f) | B. W./ (mg) | Findings |
|---|---|---|---|---|---|
| 30 | 92 | 15 | f | 2955 | N |
| 30 | 92 | 15 | f | 2901 | N |
| 30 | 92 | 15 | f | 3212 | N |
| 30 | 92 | 15 | f | 3017 | N |
| 30 | 92 | 15 | f | 3086 | N |
| 30 | 92 | 15 | m | 3172 | N |
| 30 | 92 | 15 | m | 3509 | N |
| 30 | 92 | 15 | f | 3236 | N |
| 30 | 92 | 15 | m | 3315 | N |
| 30 | 92 | 15 | f | 3057 | N |
| 30 | 92 | 15 | f | 2236 | N |
| 30 | 92 | 15 | m | 2932 | N |
| 30 | 92 | 15 | f | 3213 | N |
| 30 | 92 | 15 | f | 3188 | N |
| 30 | 92 | 15 | m | 3051 | N |
| 30 | 93 | 15 | m | 3038 | N |
| 30 | 93 | 15 | m | 3365 | N |
| 30 | 93 | 15 | m | 3222 | N |
| 30 | 93 | 15 | f | 3341 | N |
| 30 | 93 | 15 | f | 2919 | N |
| 30 | 93 | 15 | m | 3235 | N |
| 30 | 93 | 15 | f | 3451 | N |
| 30 | 93 | 15 | f | 3494 | N |
| 30 | 93 | 15 | f | 3101 | N |
| 30 | 93 | 15 | f | 3113 | N |
| 30 | 93 | 15 | f | 3216 | N |
| 30 | 93 | 15 | f | 3587 | N |
| 30 | 93 | 15 | f | 3287 | N |
| 30 | 93 | 15 | m | 3132 | N |
| 30 | 93 | 15 | m | 3568 | N |
| 30 | 94 | 19 | m | 2915 | N |
| 30 | 94 | 19 | m | 3356 | N |
| 30 | 94 | 19 | m | 3201 | N |
| 30 | 94 | 19 | f | 3072 | N |
| 30 | 94 | 19 | m | 3107 | N |
| 30 | 94 | 19 | f | 3341 | N |
| 30 | 94 | 19 | m | 3614 | N |
| 30 | 94 | 19 | f | 3263 | N |
| 30 | 94 | 19 | f | 3380 | N |
| 30 | 94 | 19 | f | 3253 | N |
| 30 | 94 | 19 | f | 2963 | N |
| 30 | 94 | 19 | f | 3169 | N |
| 30 | 94 | 19 | f | 3260 | N |
| 30 | 94 | 19 | m | 3214 | N |
| 30 | 94 | 19 | f | 3098 | N |
| 30 | 94 | 19 | m | 3371 | N |
| 30 | 94 | 19 | f | 3256 | N |
| 30 | 94 | 19 | f | 3302 | N |
| 30 | 94 | 19 | m | 2297 | N |

Appendix 2 Study No. 89-0063 KNF-317; Teratogenicity Study in Rats
Fetal sex, body weights and external malformations – Individual data

30 mg/kg/day -continued

| Dose level (mg/kg/day) | Animal no. | Total no. of live fetuses | Sex/ (m/f) | B. W./ (mg) | Findings |
|---|---|---|---|---|---|
| 30 | 95 | 17 | f | 3116 | N |
| 30 | 95 | 17 | m | 3575 | N |
| 30 | 95 | 17 | m | 3738 | N |
| 30 | 95 | 17 | m | 3881 | N |
| 30 | 95 | 17 | f | 3427 | N |
| 30 | 95 | 17 | m | 3416 | N |
| 30 | 95 | 17 | m | 3684 | N |
| 30 | 95 | 17 | f | 3655 | N |
| 30 | 95 | 17 | m | 3640 | N |
| 30 | 95 | 17 | m | 3852 | N |
| 30 | 95 | 17 | f | 3409 | N |
| 30 | 95 | 17 | f | 3612 | N |
| 30 | 95 | 17 | m | 3671 | N |
| 30 | 95 | 17 | f | 3292 | N |
| 30 | 95 | 17 | m | 3603 | N |
| 30 | 95 | 17 | f | 3594 | N |
| 30 | 95 | 17 | f | 3694 | N |
| 30 | 96 | 13 | f | 2833 | N |
| 30 | 96 | 13 | m | 3245 | N |
| 30 | 96 | 13 | f | 3460 | N |
| 30 | 96 | 13 | f | 3561 | N |
| 30 | 96 | 13 | m | 3053 | N |
| 30 | 96 | 13 | m | 3476 | N |
| 30 | 96 | 13 | f | 3329 | N |
| 30 | 96 | 13 | f | 3261 | N |
| 30 | 96 | 13 | f | 3306 | N |
| 30 | 96 | 13 | m | 3620 | N |
| 30 | 96 | 13 | m | 3558 | N |
| 30 | 96 | 13 | f | 3084 | N |
| 30 | 96 | 13 | f | 3427 | N |

## 8.3 Annex III: Benchmark dose analysis of litter effects in preliminary rat and rabbit developmental toxicity studies: external malformations

### RAT: Benchmark dose analysis for indiv_litter_prelimdt.txt

This report was generated on `2015-06-11` using PROAST version `50.9` and `R version 3.1.1 (2014-07-10)`.

### Dose

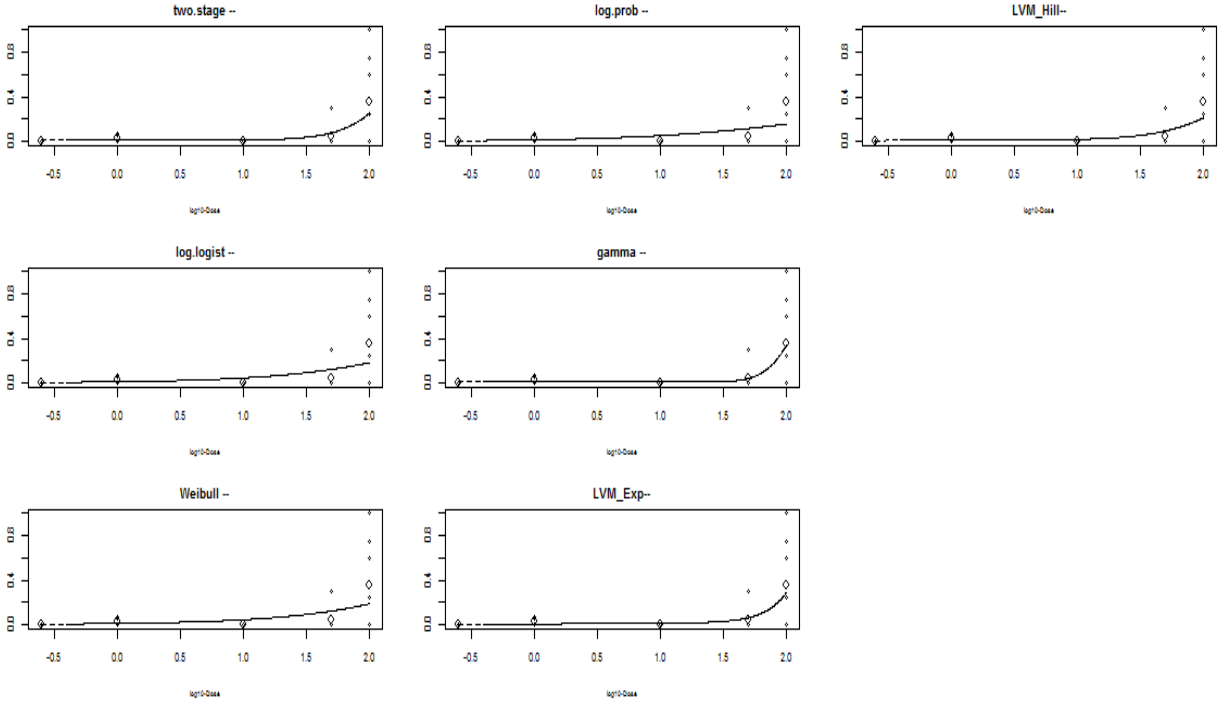
The dose variable was `'Dose'`.

### Response

The response variable was `'malformed'`, which has data type `6` (`quantal + litter effects`).

### Model results

Figure 1 shows a plot of dose vs. response for the exponential and Hill models respectively.




### Exponential model

The chosen exponential model was `LVM_Exp: m2-`, predicting a benchmark dose of `64.4074`. It had log-likelihood `-39.9109166`.
Here are the results from the exponential model:

```
##
## version: 50.9
##  model    B 12
##  log-lik   -39.91
##  alfa   0.3055
##  a-   0.6244
##  CED-   64.4074
##  dtype   6
##  b:  -0.01982
##  ces.ans   3
##  CES   0.1
##  conv   1
```

```
##  scaling on x: 1
##  selected   all
```

Table 2 shows the log-likelihood and number of parameters of the exponential models.

| model | converged | loglik | npar |
|---|---|---|---|
| full | 1 | -37.86 | 6 |
| m1- | 1 | -46.04 | 2 |
| m2- | 1 | -39.91 | 3 |
| m3- | 1 | -39.54 | 4 |
| m4- | 1 | -39.91 | 4 |
| m5- | 0 | -39.48 | 5 |
| m2- | 1 | -39.91 | 3 |

## Hill model

The chosen Hill model was `LVM_Hill: m2-`, predicting a benchmark dose of `55.5298`. It had log-likelihood `-40.8341984`.

Here are the results from the Hill model:

```
##
## version: 50.9
##  model   B 22
##  log-lik   -40.83
##  alfa   0.2285
##  a-   0.612
##  CED-   55.5298
##  dtype   6
##  b:  22.05
##  ces.ans   3
##  CES   0.1
##  conv   1
##  scaling on x: 1
##  selected   all
```

Table 3 shows the log-likelihood and number of parameters of the Hill models.

| model | converged | loglik | npar |
|---|---|---|---|
| full | | -37.86 | 6 |
| m1- | 1 | -46.04 | 2 |
| m2- | 1 | -40.83 | 3 |
| m3- | 1 | -39.49 | 4 |
| m4- | 1 | -40.83 | 4 |
| m5- | 1 | -39.48 | 5 |
| m2- | 1 | -40.83 | 3 |

## Benchmark dose by covariate

The benchmark dose predicted intervals by covariate are summarised in Table 4

Table 5. Number of pups with malformations in each litter

| Dose | dam | grpsize | malformed |
|---|---|---|---|
| 0 | 1001 | 10 | 0 |
| 0 | 1002 | 13 | 0 |
| 0 | 1003 | 16 | 0 |
| 0 | 1004 | 14 | 0 |
| 0 | 1005 | 14 | 0 |
| 0 | 1006 | 13 | 0 |
| 0 | 1007 | 14 | 0 |
| 1 | 1036 | 5 | 0 |
| 1 | 1037 | 17 | 0 |
| 1 | 1038 | 17 | 1 |
| 1 | 1039 | 17 | 0 |
| 1 | 1040 | 18 | 0 |
| 1 | 1041 | 14 | 1 |
| 10 | 1042 | 14 | 0 |
| 10 | 1043 | 17 | 0 |
| 10 | 1044 | 14 | 0 |
| 10 | 1045 | 6 | 0 |
| 10 | 1046 | 13 | 0 |
| 10 | 1047 | 15 | 0 |
| 50 | 1008 | 12 | 0 |
| 50 | 1009 | 11 | 0 |
| 50 | 1010 | 14 | 0 |
| 50 | 1011 | 11 | 0 |
| 50 | 1012 | 10 | 3 |
| 50 | 1013 | 14 | 0 |
| 50 | 1014 | 14 | 0 |
| 100 | 1015 | 8 | 6 |
| 100 | 1016 | 5 | 3 |
| 100 | 1017 | 1 | 1 |
| 100 | 1019 | 4 | 1 |
| 100 | 1020 | 3 | 0 |
| 100 | 1021 | 13 | 0 |

Value of parameter alpha = 0.4222
With p value = 0.000398

## RABBIT: Benchmark dose analysis for Indiv _litter_rabbit_prelim.txt

This report was generated on `2015-06-25` using PROAST version `50.9` and `R version 3.1.1 (2014-07-10)`.

## Dose

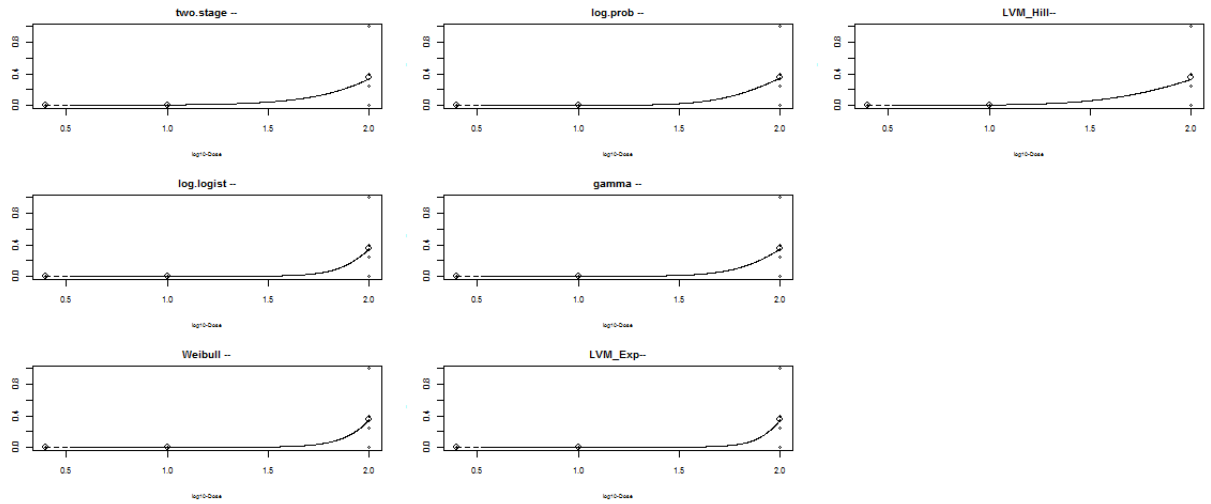
The dose variable was `'Dose'`.

## Response

The response variable was `'malf_foet'`, which has data type `6` (`quantal + litter effects`).

## Model results

Figure 1 shows a plot of dose vs. response for the exponential and Hill models respectively.




## Exponential model

The chosen exponential model was `LVM_Exp: m2-`, predicting a benchmark dose of `75.8658`. It had log-likelihood `-12.6657178`.
Here are the results from the exponential model:

```
##
## version: 50.9
##  model    B 12
##  log-lik    -12.67
##  alfa    0.7089
##  a-    2.9716
##  CED-    75.8658
##  dtype    6
##  b:   -0.03701
##  ces.ans    3
##  CES    0.1
##  conv    0
##  scaling on x: 1
##  selected    all
```

Table 2 shows the log-likelihood and number of parameters of the exponential models.

| model | converged | loglik | npar |
|---|---|---|---|
| full | 1 | -12.69 | 4 |
| m1- | 1 | -17.18 | 2 |
| m2- | 1 | -12.63 | 3 |
| m2- | 0 | -12.67 | 3 |

## Hill model

The chosen Hill model was `LVM_Hill: m2-`, predicting a benchmark dose of `42.6963`. It had log-likelihood `-12.7496115`.
Here are the results from the Hill model:

```
##
## version: 50.9
##  model    B 22
##  log-lik    -12.75
##  alfa    0.7302
##  a-    10
##  CED-    42.6963
##  dtype    6
##  b:   0.7797
```

```
##  ces.ans   3
##  CES   0.1
##  conv   1
##  scaling on x: 1
##  selected   all
```

Table 3 shows the log-likelihood and number of parameters of the Hill models.

| model | converged | loglik | npar |
|---|---|---|---|
| full | | -12.69 | 4 |
| m1- | 1 | -17.18 | 2 |
| m2- | 1 | -12.78 | 3 |
| m2- | 1 | -12.75 | 3 |

## Benchmark dose by covariate

The benchmark dose predicted intervals by covariate are summarised in Table 4.

subgroup diffBMR: 0.1 extra risk
constraint: no
P-value GoF: 0.05
including litter effects
Value of parameter alfa: 0.715377931491429
with P-value: 0.185457372767165

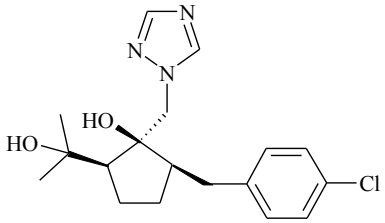
## 8.4 Annex IV – Ecotoxicity of degradation products

Ipconazole has been shown to partition rapidly and degrade slowly in water-sediment and soil systems. A number of degradation products of ipconazole have been identified (UK, 2011; EFSA, 2013), predominantly in soil. These main degradants are summarised below:

a) Abbreviation in the Draft Assessment Report (2011) and EFSA conclusion (2013) [73]: KNF-317-M-1

Name: (1RS,2SR,5RS)-2-(4-chlorobenzyl)-5-(1-hydroxy-1-methylethyl)-1-(1*H*-1,2,4-triazol-1-ylmethyl)cyclopentanol

Structure:



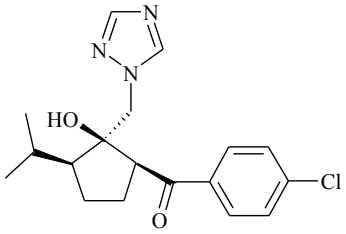

Formed in water-sediment and soil biodegradation studies.

No information is readily available for the environmental classification of this substance.

b) Abbreviation in the Draft Assessment Report (2011) and EFSA conclusion (2013) [73]: KNF-317-M-11

Name: (1RS,2SR,5SR)-2-(4-chlorobenzoyl)-5-isopropyl-1-(1*H*-1,2,4-triazol-1-ylmethyl)cyclopentanol

Structure:



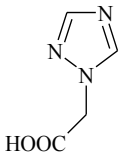

Formed in soil biodegradation studies.

No information is readily available for the environmental classification of this substance.

c) Abbreviation in the Draft Assessment Report (2011) and EFSA conclusion (2013) [73]: KNF-317-M-21

Name: 1*H*-1,2,4-triazole-1-acetic acid

Structure:



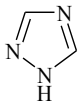
Formed in soil photolysis studies.

No information is readily available for the environmental classification of this substance.

d) Abbreviation in the Draft Assessment Report (2011) and EFSA conclusion (2013) [73]: KNF-317-M-22

Name: 1*H*-1,2,4-triazole

Structure:



Formed in soil photolysis and degradation studies.

The EFSA conclusion (2013) [73] reports ecotoxicity data for this substance and this is summarised in Table 45.

**Table 45: Summary of persistence and toxicity of 1*H*-1,2,4-triazole to aquatic species**

| Species | Acute Toxicity L/$EC_{50}$ (mg/l) | Chronic Toxicity NOEC (mg/l) |
|---|---|---|
| Fish | 96h-$LC_{50}$: 498 mg/l | 28d-NOEC: 3.2 mg/l |
| *Daphnia magna* | 48h-$EC_{50}$: >100 mg/l | No data |
| Algae | 72h-$E_rC_{50}$: 22.5 mg/l | 72h-NOEC: 4.6 mg/l (unclear if biomass, rate or both) |

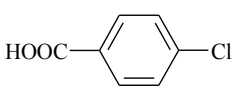
The EFSA conclusion (2013) [73] indicates that 1*H*-1,2,4-triazole has a low log $K_{ow}$ value of -0.76 and so is unlikely to bioaccumulate.

Based on these data, the 1*H*-1,2,4-triazole would not be classified for environmental effects. This is consistent with the proposed classification and self-classifications for this substance in the ECHA C&L Inventory at the time of submission.

e) Abbreviation in the Draft Assessment Report (2011) and EFSA conclusion (2013) [73]: KNF-317-M-23

Name: 4-Chlorobenzoic acid

Structure:

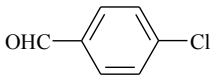


Formed in soil photolysis studies.

The self-classifications for this substance in the C&L Inventory at the time of submission indicate that the substance would not be classified for environmental effects.

f) Name: 4-Chlorobenzaldehyde

Structure:

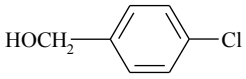



Formed in soil photolysis studies.

The majority of self-classifications for this substance in the C&L Inventory at the time of submission indicate that the substance would be classified for environmental effects as Aquatic Chronic 2, H411 toxic to aquatic life with long-lasting effects.

g) Name: 4-Chlorobenzyl alcohol

Structure:




Formed in soil photolysis studies.

No information is readily available for the environmental classification of this substance.