# CLH report

# Proposal for Harmonised Classification and Labelling

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

# Substance Name: Flutianil

**EC Number:** not assigned

**CAS Number:** 958647-10-4

**Index Number:** not assigned

**Contact details for dossier submitter:**

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**Chemicals Regulation Directorate**

**Health and Safety Executive**

**United Kingdom**

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# Part A.

## 1 PROPOSAL FOR HARMONISED CLASSIFICATION AND LABELLING

### 1.1 Substance

**Table 1: Substance identity**

| | |
|---|---|
| Substance name: | Flutianil |
| EC number: | not assigned |
| CAS number (ISO approved flutianil with specific Z configuration): | 958647-10-4 |
| Annex VI Index number: | not assigned |
| Degree of purity: | 985 g/kg (minimum specification) [98.5% (w/w)] |
| Impurities: | Confidential information, please refer to the technical dossier. The impurities have been taken into consideration and are not thought to be of additional toxicological concern |

### 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 listed |
| Current proposal for consideration by RAC | Repr.2; H361d - Suspected of damaging the unborn child<br>Aquatic Chronic 1; H410 – Very toxic to aquatic life with long lasting effects (M = 100) |
| Resulting harmonised classification (future entry in Annex VI, CLP Regulation) | Repr.2; H361d - Suspected of damaging the unborn child<br>Aquatic Chronic 1; H410 – Very toxic to aquatic life with long lasting effects (M = 100) |

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

**Table 3: Proposed harmonised classification according to the CLP Regulation**

| CLP Annex 1 ref | Hazard class | Proposed classification | Proposed SCLs and/or M-factors[a] | Current classification | Reason for no classification[b] |
|---|---|---|---|---|---|
| 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 |

| CLP Annex 1 ref | Hazard class | Proposed classification | Proposed SCLs and/or M-factors[a] | Current classification | Reason for no classification[b] |
|---|---|---|---|---|---|
| 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 | Not classified | Not applicable | Not classified | Conclusive but not sufficient for classification |
| | 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** | **None** | - |

| CLP Annex 1 ref | Hazard class | Proposed classification | Proposed SCLs and/or M-factors[a] | Current classification | Reason for no classification[b] |
|---|---|---|---|---|---|
| 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 | Not classified | Not applicable | Not classified | Conclusive but not sufficient for classification |
| 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** | **Chronic = 100** | Not classified | - |
| 5.1. | Hazardous to the ozone layer | Not classified | Not applicable | Not classified | Conclusive but not sufficient for classification |

a Including specific concentration limits (SCLs ) and M-factors
b Data lacking, inconclusive, or conclusive but not sufficient for classification

**Labelling:** **Pictograms: GHS08, GHS09**

**Signal word: Warning**

**Hazard statements: , H361d – Suspected of damaging the unborn child**
**H410 – Very toxic to aquatic life with long lasting effects**

**Precautionary statements: Not required as PS are 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

Flutianil is a new active substance in the scope of Regulation 1107/2009. There is no existing entry in Annex VI of CLP and it has not previously been reviewed for harmonised classification and labelling. In accordance with Article 36(2) of CLP, it is now subject to the harmonised classification and labelling procedure. All hazard classes are considered in this CLH report.

At the time of submission, there are no REACH registration dossiers for flutianil.

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

Flutianil is a new active substance with fungitoxic and fungistatic action. In June 2014 the UK published the draft assessment report (DAR) under European Commission Regulation (EC) No. 1107/2009 for first approval as the Rapporteur Member State (RMS). The conclusions of the peer review of the pesticide risk assessment were published in the EFSA Journal in 2014; 12(8):3805

[1]. This CLH report presents a classification and labelling proposal based mainly on the information presented in the DAR of flutianil under EC No. 1107/2009. The relevant sections are provided as an Annex to the technical dossier.

Concern for classification with Carc 2; H351 was raised in the peer review process based on a small increase in benign cholangioma in the liver of female rats and an increase in pancreatic cell islet adenoma in male rats in a 2 year dietary study. The conclusion of this report is that there was insufficient evidence for a treatment-related carcinogenic effect in the rat or mouse and therefore it is proposed not to classify for carcinogenicity. Refer to Section 4.10.

Concern for classification with Repr 2; H361d was raised in the peer review process based on the observation of visceral hydrocephaly in a rabbit developmental toxicity. These findings occurred in one litter at the top dose, in the absence of maternal toxicity, and the foetal incidence exceeds the historical control rate. Based on the occurrence of this rare finding, classification with Repr 2; H361d – Suspected of damaging the unborn child is proposed (refer to Section 4.11).

Concern for classification with Acute Aquatic 1; H400 and Chronic Aquatic 1; H410 were raised in the peer review process based on flutianil's apparent acute effects on fish and algae at concentrations <1 mg a.s./L, with the long-term aquatic data showing toxicity <0.1 mg/L. Further investigation of the data indicates that acute toxicity is not envisaged at the limit of solubility of flutianil and so no acute aquatic classification is proposed. However, the chronic data do indicate that a classification of Chronic Aquatic 1; H410 is warranted and a chronic M-factor of 100 is also proposed (refer to Section 5.4.1).

## 2.3 Current harmonised classification and labelling

### 2.3.1 CURRENT CLASSIFICATION AND LABELLING IN ANNEX VI, TABLE 3.1 IN THE CLP REGULATION

Not currently listed on Annex VI of the CLP Regulation.

## 2.4 Current self-classification and labelling

### 2.4.1 CURRENT SELF-CLASSIFICATION AND LABELLING BASED ON THE CLP REGULATION CRITERIA

At the time of submission there are no entries in the classification and labelling inventory for flutianil. The following self-classification is proposed by the applicant:

Aquatic Acute; H400 – Very toxic to aquatic life

Aquatic Chronic: H410 – Very toxic to aquatic life with long lasting effects.

# 3 JUSTIFICATION THAT ACTION IS NEEDED AT COMMUNITY LEVEL

Flutianil is an active substance in the scope of Reg 1107/2009. As it does not have an existing entry in Annex VI of CLP it is subject to the harmonised classification and labelling process in accordance with Article 36(2) of CLP.

# Part B.

## SCIENTIFIC EVALUATION OF THE DATA

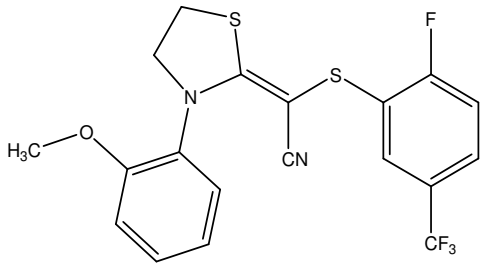
### 1 IDENTITY OF THE SUBSTANCE

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

**Table 4: Substance identity**

| | |
|---|---|
| EC number: | not assigned |
| EC name: | Not assigned |
| CAS number (ISO approved flutianil with specific Z configuration): | 958647-10-4 |
| CAS name: | Acetonitrile, 2-[[2-fluoro-5-(trifluoromethyl)phenyl]thio]-2-[3-(2-methoxyphenyl)-2-thiazolidinylidene]-,(2Z)- |
| IUPAC name: | (2Z)-{[2-fluoro-5-(trifluoromethyl)phenyl]thio}[3-(2-methoxyphenyl)-1,3-thiazolidin-2-ylidene]acetonitrile |
| CLP Annex VI Index number: | not assigned |
| Molecular formula: | $C_{19}H_{14}F_4N_2OS_2$ |
| Molecular weight range: | 426.45 |

**Structural formula:**




#### 1.2 Composition of the substance

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

| Constituent | Typical concentration | Concentration range | Remarks |
|---|---|---|---|
| Flutianil | 98.5 % | 98.5-99.9% | - |

Current Annex VI entry: Not applicable

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

| Impurity | Typical concentration | Concentration range | Remarks |
|---|---|---|---|
| - | - | - | No impurities of toxicological concern |

Current Annex VI entry: Not applicable

The manufacturer has requested that the impurity profile remains confidential, therefore this information is presented in the technical dossier only. The typical purity of flutianil is >98.5% and there are three process impurities present. These have been taken into consideration in the classification and are not considered to be of additional concern.

**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

Where available, the purity of the tested material is provided in the relevant sections. The reported studies are considered to be representative of the material as specified above.

## 1.3 Physico-chemical properties

All studies were completed to an acceptable standard and the results were considered valid in the review of the active substance. References are taken from the Draft Assessment Report Volume 3, Annex B.2: Physical and Chemical Properties – June 2013.

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

| Property (guideline[a], GLP status) | Value | Comment (e.g. measured or estimated) | Reference |
|---|---|---|---|
| State of the substance at 20°C and 101,3 kPa (US EPA D: 63—2,3,4; GLP) | White crystalline powder | Measured | [2] |
| Melting/freezing point (EC A.1, OECD 102, GLP) | 178°C - 179°C | Measured | [3] |
| Boiling point (EC A.2, OECD 103; GLP) | Reaction or decomposition started above 242ºC - 255ºC | Measured | [3] |
| Relative density (EC A.3, OECD 109, GLP) | 1.45 | Measured | [4] |
| Vapour pressure (OECD 104 [Knudsen effusion method], GLP) | $1.530 \times 10^{-7}$ Pa (20ºC)<br>$2.581 \times 10^{-7}$ Pa (25ºC) | Measured | [5] |
| Surface tension (not applicable) | Not measured | Test not required since water solubility is<1 mg/L. | - |
| Water solubility (EC A.6, OECD 105 [column elution]; GLP) | $<0.1 \times 10^{-3}$ g/L at pH 4,7 and 10 and 20°C | Measured | [6] |
| Partition coefficient n-octanol/water | The Log Pow is proposed to be 3.1 at pH 4, 7 and 10 | Measured | [7] |

| Property (guideline[a], GLP status) | Value | Comment (e.g. measured or estimated) | Reference |
|---|---|---|---|
| (EC A.8, OECD 117; GLP – HPLC method) | based upon the most critical measurement obtained using the HPLC method (92/69/EEC Method A.8 and OECD test guideline No. 117). It is noted that this figure should be considered unreliable due to the low water solubility of flutianil. It is also noted that a Rekker calculation would predict a Log $P_{ow}$ of 6.5 | | |
| Flash point (not applicable) | Not measured | Not applicable, melting point is >40°C | - |
| Flammability (EC A.10) | Flutianil does not ignite and is not classified for flammability | Measured | [8] |
| Explosive properties (EC A.14) | Flutianil is not considered explosive after a theoretical consideration of the chemical structure. No sharp exotherm was observed by differential scanning calorimtery up to 600°C. | Measured | [8] |
| Self-ignition temperature (EC A.16; GLP) | No relative self-ignition (autoflammability) was observed below 400ºC | Measured | [8] |
| Oxidising properties (EC A.17; GLP) | Flutianil does not exhibit oxidising properties | Measured | [8] |
| Dissociation constant (not applicable) | Not measured | The dissociation constant was not determined due to the low water solubility of flutianil. | - |
| Viscosity (not applicable) | Not measured | Not applicable as flutianil is a powder | - |

a Where appropriate, methods employed were guideline compliant

# 2 MANUFACTURE AND USES

## 2.1 Manufacture

Not manufactured in the EU.

## 2.2 Identified uses

Flutianil is classed as a thiazolidine fungicide exhibiting both fungitoxic and fungistatic contact action. It is a new active that is to be used in the EU and will also be imported into the EU and stored for despatching outside of the EU for the same uses.

# 3 CLASSIFICATION FOR PHYSICO-CHEMICAL PROPERTIES

## 3.1 Physico-chemical properties

In a standard study (EEC A10), flutianil did not ignite and consequently 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.

Following a theoretical consideration of the chemical structure, flutianil is not considered to be explosive. Further, no sharp exotherm was observed by differential scanning calorimetry up to 600°C.

In a standard study (EEC A17), flutianil did not exhibit oxidising properties. Consequently, it does not meet the criteria for classification as an oxidising solid.

### 3.1.1 Conclusions on classification and labelling

| **Not classified: Conclusive but not sufficient for classification** |
|---|

# 4 HUMAN HEALTH HAZARD ASSESSMENT

The human health assessment is mainly based on the information presented in Draft Assessment Report for Flutianil – DAR-Volume 3, Annex B.6: Toxicology and Metabolism – June 2013.

Note that references in the CLH report have been redacted to protect confidential information where necessary.

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

### 4.1.1 Non-human information

The following summary is based upon the pesticide Draft Assessment Report (DAR) made for review under the Regulation (EC) 1107/2009.

The toxicokinetics of flutianil have been investigated in rats after single high and low dose administration and repeat dose administration.

Absorption: Flutianil was poorly-absorbed, following the administration of a single low dose of 10 mg/kg bw; the majority of radioactivity was recovered in the faeces. Maximum plasma concentrations were attained within 2 hours to 7 hours, whilst elimination was notably protracted, with elimination half-life up to 26 hours from plasma and 68 hours from blood. Saturation kinetics are highly likely between 10 and 1000 mg/kg bw flutianil.

Experiments in bile duct cannulated rats confirmed that biliary elimination was a relatively significant route of excretion. Using figures for biliary and urinary excretion, oral absorption at the low dose (10 mg/kg bw) was estimated to be 18%, whilst a high dose of 1000 mg/kg bw yielded mean average oral absorption value of 2%. A study at 250 mg/kg bw measured an absorption of 5% (in the urine).

Distribution: Absorbed flutianil was widely distributed throughout the tissues. Generally residues were associated with the organs of metabolism, excretion and fatty tissues – highest tissue levels were in the liver. Residues at 120 hours were very low or non-detectable in the majority of tissues. The exceptions were the gastro-intestinal tract, liver, kidney, lung, fat, blood and plasma.

The evidence indicates a lack of bioaccumulation, with residues in all tissues showing declines. At 5 days post-dose, tissue (including fat) residues were <0.1% of the administered dose.

Metabolism: Unchanged flutianil accounted for nearly all (approximately 95%) of the faecal excretion, whilst the metabolism of absorbed test substance was extensive. The available data indicates numerous metabolites in urine and bile, with less than 0.5% of the administered dose being excreted as unchanged flutianil in urine or bile.

Only one metabolite was detected at greater than 5% of the applied dose. This was a mercapturate conjugate of a hydroxylated trifluoromethyl ring structure. Glutathione conjugation, mercepturate formation and sulphation are proposed as major biotransformation steps. Limited oxidative defluorination is also proposed; the data indicate that the fluorine was not widely systemically released.

Excretion: The majority (70% to 98%) of the administered dose was eliminated *via* faeces within 24 hours, whilst urinary excretion accounted for 4% to 19% and biliary excretion approximately 11% of the administered dose. In cannulated rats, in addition to a slight decrease in faecal elimination, urinary excretion was also slightly depressed when compared to intact rats which may be evidence of some enterohepatic circulation of biliary metabolites. This also indicates that a small proportion of the faecal residues may originate from biliary excretion.

### 4.1.2 Human information

No relevant information available.

### 4.1.3 Summary and discussion on toxicokinetics

The toxicokinetics of flutianil were investigated orally in single and repeat dose studies in rats. Following single and repeat dose administration, the majority of the dose was excreted in the faeces as unchanged parent compound. The absorbed dose was extensively metabolised, widely distributed in body tissues, and excreted in urine and bile. There was evidence of some enterohepatic recirculation. There was no evidence of accumulation.

## 4.2 Acute toxicity

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

**Table 9: Summary of experimental studies on acute dose toxicity**

| Method | Results | Remarks [reference] |
|---|---|---|
| Single dose, rat (Wistar) (3 females/gp – 6 animals in total)<br><br>oral: gavage<br><br>2000 mg/kg bw<br><br>14-day observation period<br><br>OECD 423 (2002 [acute toxic class]), GLP | **2000 mg/kg bw: no treatment related effects and no deaths**<br>$LD_{50}$: >2000 mg/kg bw | Well conducted, GLP-compliant study<br>Purity: 99.22%<br>[9] |
| Single dose, rat (Wistar) (5 animals/sex/gp)<br><br>dermal: occluded, 24 h | **2000 mg/kg bw: no treatment related effects and no deaths**<br>$LD_{50}$: >2000 mg/kg bw | Well conducted, GLP-compliant study<br>Purity: 99.22% |

| Method | Results | Remarks [reference] |
|---|---|---|
| 2000 mg/kg bw<br><br>14-day observation period<br><br>OECD 402 (1987), GLP | | [10] |
| Single dose, rat (Wistar)<br>(5 animals/sex/gp)<br><br>inhalation: nose only, 4 h<br><br>5.17 mg/L<br><br>14-day observation period<br><br>OECD 403 (1981), GLP | **$LC_{50}$ > 5.17 mg/L: no deaths.**<br>Clinical signs included wet fur, staining of the head, snout and dorsal region, an unkempt appearance and vocalisation. Recovery from clinical signs was complete by day 2 except for one male which had staining up to day 4, and one female in which there was vocalisation up to day 13. All animals gained body weight during the study. Histopathological findings included slight reddening of the left maxilloturbinate of the nasal cavity in one animal. | Well conducted, GLP-compliant study<br>Purity: 99.22%<br>[11] |

### 4.2.1 Non-human information

#### 4.2.1.1 Acute toxicity: oral

In an acute oral study [9] conducted on fasted female Wistar rats, no treatment related clinical signs of toxicity or effects on body weight were observed. No abnormalities were recorded at necropsy. The acute oral $LD_{50}$ for female rats was >2000 mg/kg bw.

#### 4.2.1.2 Acute toxicity: dermal

In an acute dermal study [10] conducted on Wistar rats, no treatment related clinical signs of toxicity or effects on body weight were observed. No abnormalities were recorded at necropsy. The acute oral $LD_{50}$ for female rats was >2000 mg/kg bw.

#### 4.2.1.3 Acute toxicity: inhalation

In an acute inhalation study [11], Wistar rats were exposed by inhalation route to an aerosol of flutianil for 4 hours (nose only) at a concentration of 5.17 mg/L. No abnormalities were found at necropsy except for slight reddening of the left maxilloturbinate of the nasal cavity in one animal. The 4 hour inhalation $LC_{50}$ of flutianil for males and females was ≥5.17 mg/L.

#### 4.2.1.4 Acute toxicity: other routes

No relevant information.

### 4.2.2 Human information

No relevant information available.

### 4.2.3 Comparison with criteria

$LD_{50}$ values of >2000 mg/kg bw were obtained from the acute oral and dermal toxicity studies. In an acute inhalation study a concentration of >5.17 mg/L flutianil did not result in any deaths.

It is concluded that the $LD_{50}$ / $LC_{50}$ for oral, dermal and inhalation routes, exceed the values for which classification for acute toxicity is required (i.e., > 2000 mg/kg bw *via* the oral and dermal route and > 5 mg/L *via* inhalation for dust/mist).

Flutianil is not classified for acute oral, dermal or inhalation toxicity.

#### 4.2.4 Conclusion on classification and labelling

**Not classified: Conclusive but not sufficient for classification**

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

Specific target organ toxicity (single exposure) is defined as a specific, non-lethal target organ toxicity arising from a single exposure to a substance of concern that leads to impaired function both reversible and irreversible, immediate and/or delayed and not addressed by other hazard classes.

The information gained from the acute toxicity studies in rats is provided in (Table 9). There is no indication that flutianil causes toxicity to specific organs after a single exposure. Refer to Section 4.4.3 for information on respiratory irritation.

#### 4.3.1 Comparison with criteria

Substances that have produced significant toxicity in humans or that, on the basis of evidence from studies in experimental animals, can be presumed to have the potential to produce significant toxicity in humans following single exposure are classified in STOT-SE 1 or 2. Classification is supported by evidence associating single exposure to the substance with a consistent and identifiable toxic effect.

Classification in STOT-SE 3 is reserved for transient target organ effects and is limited to substances that have narcotic effects or cause respiratory tract irritation.

There were no clinical signs of toxicity following oral and dermal exposure to flutianil. Signs following inhalation exposure to flutianil were indicative of non-specific, general toxicity. As there was no clear evidence of specific toxic effects on a target organ or tissue, no signs of respiratory tract irritation or narcotic effects, no classification for specific target organ toxicity (single exposure) is proposed.

#### 4.3.2 Conclusion on classification and labelling

**Not classified: Conclusive but not sufficient for classification**

### 4.4 Irritation

The potential of flutianil to cause skin and eye irritation has been investigated in rabbits.

**Table 10: Summary of relevant irritation studies**

| Method | Results | Remarks [reference] |
|---|---|---|
| Single dose, rabbit (Japanese White)<br>(3 females) | The primary skin irritation index obtained from the results of skin reactions at 1, 24, 48 or 72 hours was 0 in all 3 animals. | Well conducted, GLP-compliant study<br>Purity: 99.22% |

| Method | Results | Remarks [reference] |
|---|---|---|
| 4 h exposure on intact skin<br><br>3-day observation period<br><br>OECD 404 (2002), GLP | | [12] |
| Single dose, rabbit (Japanese White)<br>(3 females/gp, 6 animals in total)<br><br>Washed and unwashed eye gp<br><br>3-day observation period<br><br>OECD 405 (2002), GLP | *Unwashed eyes*: Grade 1 conjunctival redness and discharge in 3/3 animals at 1 hour only; fully resolved in all animals by 24 hours<br>*Washed eyes*: no eye irritation reactions were observed in the cornea, iris or conjunctivae of any treated animal | Well conducted, GLP-compliant study<br>Purity: 99.22%<br>[13] |

### 4.4.1 Skin irritation

#### 4.4.1.1 Non-human information

In a standard guideline compliant study [12] in rabbits, flutianil was pulverized (0.5 g) and moistened uniformly with 0.5 mL of distilled water, put on a piece of lint sheet (2.5 *x* 2.5 cm) and then applied to the clipped dorsal skin of 3 rabbits for 4 hours. Skin irritation reactions were evaluated in accordance with Draize's criteria 1, 24, 48 or 72 hours after removal of the test article. No skin irritation reactions were observed in any animal at any time after removal of the test article. There were no test article related abnormalities in clinical signs or body weight in any animal.

#### 4.4.1.2 Human information

No relevant information available.

#### 4.4.1.3 Comparison with criteria

No skin irritation reactions were observed in any animal at any time after removal of the test article (all scores were 0). Therefore, flutianil does not meet the criteria for classification as a skin irritant.

#### 4.4.1.4 Conclusion on classification and labelling

**Not classified: Conclusive but not sufficient for classification**

### 4.4.2 Eye irritation

#### 4.4.2.1 Non-human information

In a standard guideline compliant study [13] in rabbits, pulverised flutianil (0.1 g) was instilled into the conjunctival sac of the left eye of young adult female Japanese White rabbits. One group of 3 rabbits was exposed to the test article without eye washing and a second group was left for 30 seconds with the test article then the eyes were washed for 30 seconds with distilled

water. In the group without eye washing, conjunctival redness and discharge were observed in all animals and conjunctival chemosis was observed in 1/3 animals at 1 hour after application. These conjunctival changes had completely disappeared at 24 hours after application. There were no corneal or iridal changes in any animal. No other ocular changes were observed in any animal during the observation period.

#### 4.4.2.2 Human information

No relevant information available.

#### 4.4.2.3 Comparison with criteria

In the unwashed eyes, there were no corneal or iridal changes in any animal. Conjunctival redness and discharge were observed in all 3 animals and conjunctival chemosis was observed in 1/3 animals at 1 hour after application. All effects had resolved by 24 hours and therefore, flutianil does not meet the criteria for classification as an eye irritant.

#### 4.4.2.4 Conclusion on classification and labelling

**Not classified: Conclusive but not sufficient for classification**

### 4.4.3 Respiratory tract irritation

#### 4.4.3.1 Non-human information

No evidence of respiratory tract irritation was found in the acute inhalation study in rats (Table 9).

#### 4.4.3.2 Human information

No relevant information available.

#### 4.4.3.3 Summary and discussion of respiratory tract irritation

There are currently no validated animal tests that deal specifically with respiratory tract irritation, therefore this endpoint was not investigated directly. However, no signs of respiratory irritation were observed in the acute inhalation study (see Section 4.2)

#### 4.4.3.4 Comparison with criteria

There is limited evidence available. No signs of respiratory tract irritation were observed in an acute inhalation study in the rat. No repeat dose inhalation studies are available.

#### 4.4.3.5 Conclusion on classification and labelling

**Not classified: Conclusive but not sufficient for classification**

## 4.5 Corrosivity

### 4.5.1 Non-human information

Flutianil was not corrosive when tested for skin and eye irritation in the rabbit (Table 10).

### 4.5.2 Human information

No human data available.

### 4.5.3 Summary and discussion of corrosivity

No signs of corrosivity were observed in *in vivo* skin and eye irritation studies in the rabbit.

#### 4.5.3.1 Conclusion on classification and labelling

**Not classified: Conclusive but not sufficient for classification**

## 4.6 Sensitisation

### 4.6.1 Skin sensitisation

**Table 11: Summary of relevant skin sensitisation studies**

| Method | Doses | Results | Remarks [reference] |
|---|---|---|---|
| Guinea pig (Hartley)<br>10 females in the test gp, 5 in the negative control gp<br>Maximisation test<br>OECD 406 (1992), GLP | ***Induction:***<br>Intradermal: 2% (in olive oil)<br>Topical: 50% (in olive oil)<br>***Challenge*:**<br>25% (in olive oil)<br>Preliminary study; erythema at intradermal injection concentration of 2% and topical application of 50%. No skin reaction observed at 25%. | ***Test:***<br>0/10 animals were sensitised at 24 and 48 hours<br>***Negative Control:***<br>0/5 animals were sensitised at 24 and 48 hours<br>Background positive data confirmed the sensitivity of the test system<br>Non-sensitising | Well conducted, GLP-compliant study<br>Purity: 99.22%<br>[14] |

#### 4.6.1.1 Non-human information

In a guideline compliant GLP dermal sensitisation study [14], ten young adult Hartley strain female guinea pigs were tested using the Magnusson and Kligmann test. Flutianil was pulverised and suspended in an olive oil vehicle. Induction and challenge dose were based on the results of a preliminary test. The control (five animals) received olive oil for intradermal and topical induction. No reaction to the test article was seen in the test article sensitisation group. On the basis of the results, it was concluded that flutianil had no skin sensitization potential under the conditions of this study.

#### 4.6.1.2 Human information

No relevant information available.

#### 4.6.1.3 Summary and discussion of skin sensitisation

Flutianil did not induce skin sensitisation in a well conducted guinea pig maximisation study.

#### 4.6.1.4 Comparison with criteria

The criteria for classification as a skin sensitiser (≥30% of animals exhibiting a positive reaction in the guinea pig maximization test/adjuvant assay) were not met.

#### 4.6.1.5 Conclusion on classification and labelling

| **Not classified: Conclusive but not sufficient for classification** |
|---|

### 4.6.2 Respiratory sensitisation

#### 4.6.2.1 Non-human information

Not data available.

#### 4.6.2.2 Human information

No data available.

#### 4.6.2.3 Summary and discussion of respiratory sensitisation

Not applicable.

#### 4.6.2.4 Comparison with criteria

Not applicable.

#### 4.6.2.5 Conclusion on classification and labelling

| **Not classified – data lacking** |
|---|

## 4.7 Repeated dose toxicity

The short-term repeated-dose toxicity of flutianil has been investigated by the oral route in mice (28-day and 90-day), rats (28-day and 90-day) and dogs (28-day, 90-day and 1-year) and by the dermal route in rats (28-day). In addition, there are chronic toxicity studies in rats and mice. These have been described in Section 4.10.

### 4.7.1 Non-human

#### 4.7.1.1 Repeated dose toxicity: oral

**Mouse**

**Table 12: Summary of experimental studies on repeated dose toxicity after oral administration in the mouse**

| Method | Results (effects of major toxicological significance) | Remarks [reference] |
|---|---|---|
| 28d, mouse (CD1)<br>(6 animals/sex/gp)<br>(dosing: 28 Dec 2004 – 25 Jan 2005)<br><br>oral: feed<br><br>0, 100, 1000, 3000, 10000 ppm<br>equiv. to 0, 14, 138, 424, 1393 (♂) and 0, 16, 155, 497, 1601 mg/kg bw/d (♀)<br><br>OECD 407 (1995), GLP<br><br>**STOT-RE Cat 2 for rat 28-day study = 300 mg/kg bw/d[a]** | **100 ppm (14/16 mg/kg bw/d in ♂/♀), 1000 ppm (138/155 mg/kg bw/d in ♂/♀), 3000 ppm (424/497 mg/kg bw/d in ♂/♂), 10,000 ppm (1393/1601 mg/kg bw/d in ♂/♀):**<br>No adverse effects noted<br><br>NOAEL: *ca* 10000 ppm (1393/1601 mg/kg bw/d ♂/♀) | Well conducted, GLP-compliant study<br><br>Purity: 99.38%<br>[15] |
| 90d, mouse (CD1)<br>(10 animals/sex/gp)<br>(dosing: 13 June 2005 – 13 Sept 2005)<br><br>oral: feed<br><br>0, 1000, 3000, 10000 ppm<br>equiv. to 0, 138, 409, 1387 (♂) and 0, 159, 481, 1555 mg/kg bw/d (♀)<br><br>OECD 408 (1998), GLP<br><br>**STOT-RE Cat 2 for rat 90-day study = 100 mg/kg bw/d[a]** | **10000 ppm (1387/1555 mg/kg bw/d in ♂/♀):**<br>1/10 ♂: atrophy of the seminiferous tubules of the testes.<br>2/10 ♀: hepatic microgranuloma<br><br>**3000 ppm (409/481 mg/kg bw/d in ♂/♀):**<br>1/10 ♂: died after 5 days. Had 33% bw loss and atrophy of the seminiferous tubules of the testes,<br><br>**1000 ppm (138/159 mg/kg bw/d in ♂/♀):**<br>no adverse effects.<br><br>NOAEL: *ca* 1000 ppm (138 mg/kg bw/d) | Well conducted, GLP-compliant study<br><br>Purity: 99.26%<br>[16] |

*NB: The values for NOAEL and LOAEL are provided for information only: they are the values derived from the DAR for flutianil. ↓ = decrease compared to control. ↑= increase compared to control*

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### 28-day oral study in mice ([redacted] (2009) [15])

In a GLP and guideline compliant 28-day study, groups of CD1 mice (6/sex/gp) were administered flutianil on a continuous basis in the diet for a minimum of 28-days. Dose levels were 100, 1000, 3000 and 10000 ppm (equivalent to 14, 138, 424, 1393 mg/kg bw/day and 16, 155, 497, 1601 mg/kg bw/day for males and females, respectively). A concurrent control group received basal diet.

There was no evidence of treatment related toxicity in males or females up to the top dose of 10000 ppm (1393/1601 mg/kg bw/day in males/females).

### 90-day oral study in mice ([redacted] (2009) [16])

In a GLP and guideline compliant 90-day study, groups of CD1 mice (10/sex/gp) were administered flutianil on a continuous basis in the diet for a minimum of 90-days. Dose levels were 1000, 3000 and 10000 ppm (equivalent to 138, 409, 1387 mg/kg bw/day and 159, 481, 1555 mg/kg bw/day for males and females, respectively). A concurrent control group received basal diet.

There were hepatic microgranulomas in two females at the top dose. These were within the laboratory historical control range (0/10-2/10) and are therefore considered to be not treatment-related [17].

Atrophy of seminiferous tubules occurred in one male in the 10,000 ppm dose group, and also in one male in the 3000 ppm group that was found dead in week 1. This animal had a 33% bodyweight loss. The very low incidence of this finding was within the laboratory historical control range (0/10 – 1/10) [17]. Therefore, the observation of atrophy of the seminiferous tubules in this study is considered to be not treatment-related.

No other treatment-related effects were seen at any dose level in all of the parameters investigated.

In conclusion, there was no evidence of a treatment-related effect in either sex up to the highest dose tested of 10000 ppm (equivalent to 1387/1555 mg/kg bw/day in males/females).

## Rat

**Table 13: Summary of experimental studies on repeated dose toxicity after oral administration in the rat**

| Method | Results (effects of major toxicological significance) | Remarks [reference] |
|---|---|---|
| 28 d, rat WISTAR<br>(6 animals/sex/gp)<br>(dosing: 26 March 2004 – 26 April 2004)<br><br>oral: feed<br>0, 20, 200, 2000, 20000 ppm equiv. to 0, 2, 16, 159, 1555 (♂) and 0, 2, 17, 171, 1714 mg/kg bw/d (♀) (analytical conc.)<br><br>OECD 407 (1995), GLP<br><br>**STOT-RE Cat 2 for rat 28-day study = 300 mg/kg bw/d[a]** | **20000 ppm (1555/1714 mg/kg bw/d in ♂/♀):**<br>Kidney: ↑ absolute (12%) and relative (14%) wt in ♂; 5/6♂ with hyaline droplet deposition in the proximal tubular cells<br><br>**2000 ppm (159/171 mg/kg bw/d in ♂/♀):**<br>Kidney: 2/6 ♂ with hyaline droplet deposition in the proximal tubular cells<br><br>**200 ppm (16/17 mg/kf bw/d in ♂/♀):**<br>Kidney: 2/6 ♂ with hyaline droplet deposition in the proximal tubular cells<br><br>**20 ppm (2 mg/kg bw/d in ♂/♀):**<br>No observed adverse effects.<br><br>NOAEL: *ca* 20,000 ppm (1555 mg/kg bw/d) | Well conducted, GLP-compliant study<br><br>Purity: 99.38%<br>[18] |
| 90 d, rat WISTAR<br>(10 animals/sex/gp)<br>(dosing: 22 June 2004 – 24 Sept 2004)<br><br>oral: feed<br>0, 20, 200, 2000, 20000 ppm equiv. to 0, 1, 13, 122, 1271 (♂) and 0, 1, 14, 149, 1500 mg/kg bw/d (♀)<br><br>OECD 408 (1998), GLP<br><br>**STOT-RE Cat 2 for rat 90-day study = 100 mg/kg bw/d[a]** | **20000 ppm (1271/1500 mg/kg bw/d in ♂/♀):**<br>Kidney: 10/10 ♂ with hyaline droplet deposition in the proximal tubular cells;<br>Liver: ↑ relative wt (9% in ♂; 13% in ♂), with accompanying centrilobular hepatocellular hypertrophy (7/10 ♂).<br>↓ total bilirubin (14% in ♂ and 29% in ♀);<br><br>**2000 ppm (122/149 mg/kg bw/d in ♂/♀):**<br>Kidney: 10/10 ♂ with hyaline droplet deposition in the proximal tubular cells;<br><br>**200 ppm (13/14 mg/kg bw/d in ♂/♀):**<br>No observed adverse effects<br><br>NOAEL: 2000 ppm (122 mg/kg bw/d) | Well conducted, GLP-compliant study<br><br>Purity: 99.38%<br>[19] |

*NB: The values for NOAEL and LOAEL are provided for information only: they are the values derived from the DAR for flutianil. ↓ = decrease compared to control. ↑= increase compared to control.*

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**28-day oral study in rats ([redacted], (2009) [18])**

In a GLP and guideline compliant 28-day study, groups of Wistar rats (6/sex/dose) were administered flutianil on a continuous basis in the diet for a minimum of 28-days. Dose levels were 20, 200, 2000, 20000 ppm (equivalent to 2, 16, 159, 1555 mg/kg bw/day and 2, 17, 172 and 1714 mg/kg bw/day for males and females, respectively). A concurrent control group received basal diet.

The only significant findings in this study were effects in the kidney in males from 200 ppm (16 mg/kg bw/day) consistent with hyaline droplet deposition in the proximal tubular cells. Hyaline droplet formation in the kidney was also seen in the 90 day and chronic rat studies and stained positive for $\alpha_{2\mu}$-globulin, indicating that the kidney findings were due to $\alpha_{2\mu}$-globulin nephropathy, which is a condition specific to male rats and considered not relevant to humans.

In conclusion, in this 28-day study in rats there were no treatment related effects relevant to humans up to the highest dose tested of 20,000 ppm (equivalent to 1555/1714 mg/kg bw/day in males/females).

**90-day oral study in rats ([redacted], (2009) [19])**

In a GLP and guideline compliant 90-day study, groups of Wistar rats (10/sex/dose) were administered flutianil on a continuous basis in the diet for a minimum of 90-days. Dose levels were 20, 200, 2000, 20000 ppm (equivalent to 1, 13, 122, 1271 mg/kg bw/day and 1, 14, 149, 1500 mg/kg bw/day for males and females, respectively). A concurrent control group received basal diet.

Relative liver weights were statistically significantly increased in males and females in the 20,000 ppm group (9% and 13%, respectively). An increased incidence of centrilobular hepatocellular hypertrophy was restricted to 7/10 males in the high dose group.

Statistically significant decreases in total bilirubin were seen in males and females at 20000 ppm. There were no notable changes in any other blood parameters.

In the kidneys a significant increase in the severity of hyaline droplet deposition in the proximal tubular cells was seen from 2000 ppm. Immunohistochemical staining confirmed that hyaline droplets in the proximal tubular cells were positive for $\alpha_{2\mu}$-globulin. Therefore, the kidney effects were not considered relevant to humans.

In conclusion, in this 90-day study in the rat, effects relevant to humans were increases in liver weight in males and females accompanied by hypertrophy in males and decreases in total bilirubin levels in males and females at the top dose of 20000 ppm (1271/1500 mg/kg bw/day in males/females).

## Dog

**Table 14: Summary of experimental studies on repeated dose toxicity after oral administration in the dog**

| Method | Results (effects of major toxicological significance) | Remarks [reference] |
|---|---|---|
| 28 d dog (Beagle)<br>(2 animals/sex/gp 6 months of age at start of dosing)<br>(dosing 31 Aug 2004 – 27 Sept 2004)<br><br>oral: capsule<br><br>0, 10, 300, 1000 mg/kg/ bw/day | **1000 mg/kg bw/d**:<br>Testes: ↑ absolute (22%) and relative (16 %) wt; 1/2 (*vs* 0/2 in controls) with immature organ;<br>Prostate: ↑ absolute (62%) and relative (50%) wt; 1/2 (*vs* 2/2 in controls) with immature organ;<br>Uterus: ↑ (59%) relative and (61%) absolute wt.<br><br>**300 mg/kg bw/d**:<br>Prostate: ↑ absolute (54%) and relative (33%) wt; 1/2 (*vs* 2/2 in controls) with immature organ; | Well conducted, GLP-compliant study<br><br>Purity: 99.38%<br>[20] |

| Method | Results (effects of major toxicological significance) | Remarks [reference] |
|---|---|---|
| OECD 409 (1998), GLP<br><br>**STOT-RE Cat 2 for rat 28-day study = 300 mg/kg bw/d**[a] | Testes: 2/2 (*vs* 0/2 in controls) with immature organ;<br>Uterus: ↑ absolute (10%) and relative (27%) wt.<br><br>**10 mg/kg bw/d**:<br>Testes: 1/2 (*vs* 0/2 in controls) with immature organ;<br><br>NOAEL: *ca* 1000 mg/kg bw/d | |
| 90 d dog (Beagle)<br>(4 animals/sex/gp 6 months of age at start of dosing)<br>(dosing 15 June 2005 – 12 Sept 2005)<br><br>oral: capsule<br><br>0, 30, 300, 1000 mg/kg/day<br><br>OECD 409 (1998), GLP<br><br>**STOT-RE Cat 2 for rat 90-day study = 100 mg/kg bw/d**[a] | **1000 mg/kg bw/d**:<br>Prostate: 2/4 with cell infiltrate (1 mild, 1 minimal) *vs* 1/4 in controls (minimal); 3/4 (*vs* 4/4 in controls) with immature organ<br>Testes: 3/4 atrophy of seminiferous tubules (2 mild, 1 minimal) *vs* 1/4 in controls (minimal);<br>Uterus: ↑ wt (300%)<br><br>**300 mg/kg bw/d**:<br>Prostate: 2/4 with cell infiltrate (both minimal) *vs* 1/4 in controls (minimal); 2/4 (*vs* 4/4 in controls) with immature organ<br>Uterus: ↑ wt (300%)<br><br>**30 mg/kg bw/d**:<br>Testes: 3/4 atrophy of seminiferous tubules (all minimal) *vs* 1/4 in controls (minimal);<br>Prostate: 3/4 (*vs* 4/4 in controls) with immature organ<br>Uterus: ↑ wt (200%)<br><br>NOAEL: *ca* 300 mg/kg bw/d | Well conducted, GLP-compliant study<br><br>Purity: 99.38%<br>[21] |
| 52 wk dog (Beagle)<br>(4 animals/sex/gp 6 months of age at start of dosing)<br>(dosing 19 Oct 2006 – 18 Oct 2007)<br><br>oral: capsule<br><br>0, 30, 300, 1000 mg/kg/d (analytical conc.)<br><br>OECD 452 (1981), GLP<br><br>**STOT-RE Cat 2 for rat 1-yr study = 25 mg/kg bw/d**[a] | **1000 mg/kg bw/d:**<br>No adverse effects<br><br>NOAEL: *ca* 1000 mg/kg bw/d | Well conducted, GLP-compliant study<br><br>Purity: 99.22%<br>[22] |

*NB: The values for NOAEL are provided for information only: they are the values derived from the DAR for flutianil. ↓ = decrease compared to control. ↑= increase compared to control*

a STOT-RE trigger classification levels provided for information only

### 28-day oral study in dogs ([redacted], (2006) [20])

In a GLP and guideline compliant 28-day study, groups of beagle dogs (2/sex/dose) aged 6 months at the start of dosing were administered flutianil *via* oral capsules once daily, 7 days/week for a minimum of 28 days at doses of 10, 300 and 1000 mg/kg bw/day. A concurrent control group received empty capsules.

The finding of faeces containing test-article like material in the top dose suggests that a certain amount of test article remained unabsorbed at this dose and that 1000 mg/kg bw/day may have

exceeded the maximum achievable dose *via* capsule administration of the test substance. A similar finding was also seen in the 90-day and 1-year studies.

The only findings in this study were changes in testes, prostate and uterus weight from 300 mg/kg bw/day. In addition, there was an increased incidence of immature testes from 10 mg/kg bw/day and a decreased incidence of immature prostate from 300 mg/kg bw/d. However, there was no clear dose-response relationship for these histopathological changes. Furthermore, given the small number of animals per group used, the significance of these findings is un-interpretable. Therefore, no firm conclusions can be drawn from this study about the relation to treatment of these effects.

**90-day oral study in dogs ([redacted], (2009) [21])**

In a GLP and guideline compliant 90-day study, groups of beagle dogs (4/sex/dose) aged 6 months at the start of dosing were administered flutianil *via* oral capsules once daily, 7 days/week for a minimum of 90 days at doses of 30, 300 and 1000 mg/kg/day. A concurrent control group received empty capsules. Discharge of faeces containing test article-like substance occurred in the top dose.

There were histopathological findings in the testes (atrophy of seminiferous tubules) and prostate (cell infiltration) from a dose of 30 mg/kg bw/day. In addition, there was a large increase in uterus weight from the same dose level.

Whilst a relation to treatment cannot be excluded for these findings, it is noted that they were not replicated in the 52 week study at the same dose levels following a much longer period of treatment. This questions their toxicological significance.

**1-year oral study in dogs ([redacted], (2009) [22])**

In a GLP and guideline compliant 1-year study, groups of beagle dogs (4/sex/dose) aged 6 months at the start of dosing were administered 0, 30, 300 and 1000 mg/kg bw/day flutianil *via* oral capsules once daily, 7 days/week for a minimum of 52 weeks. A concurrent control group received empty capsules. Increased discharge of faeces containing test article-like substance and increased vomiting occurred at the top dose.

In contrast to the previous short-term dog studies, there were no notable changes in the reproductive organs.

In conclusion, there were no adverse findings in either sex in this 1-year dog study up to the limit dose of 1000 mg/kg bw/day.

### 4.7.1.2 Repeated dose toxicity: inhalation

No relevant information.

### 4.7.1.3 Repeated dose toxicity: dermal

**Table 15: Summary of experimental studies on repeated dose toxicity after dermal administration**

| Method | Results (effects of major toxicological significance) | Remarks [reference] |
|---|---|---|
| 28d, rat (Wistar)<br>(10 animals/sex/gp)<br>(dosing: 5 Nov 2007 – 18 Dec 2007)<br><br>dermal: occluded, 6 h/d, | **1000 mg/kg bw/d:**<br>No observed adverse effects<br><br>NOAEL: *ca* 1000 mg/kg bw/d | Well conducted, GLP-compliant study<br><br>Purity: 99.22%<br>[23] |

| Method | Results (effects of major toxicological significance) | Remarks [reference] |
|---|---|---|
| 7 d/wk<br><br>0, 1, 100, , 500, 1000 mg/kg bw/d<br><br>OECD 407 (1995), GLP<br><br>**STOT-RE Cat 2 for rat dermal 28-day study = 600 mg/kg bw/d**[a] | | |

*NB: The values for NOAEL and LOAEL are provided for information only: they are the values derived from the DAR for flutianil. ↓ = decrease compared to control. ↑= increase compared to control.*

a STOT-RE trigger classification levels provided for information only

**28-day dermal study in rats ([redacted] (2008) [23])**

In a guideline and GLP compliant 28-day dermal study, groups of Wistar rats (10/sex/gp) were administered flutianil suspended in peanut oil and applied to a shaved area of skin ranging from 10 to 15% of the total surface area of the rat for 6 hours/day, 7 days/week. Dose levels were 1, 100, 500 and 1000 mg/kg bw/day. A concurrent control group received basal diet. A 14-day treatment free period was included.

There were no treatment-related findings in this study after dermal administration of flutianil up to the maximum dose of 1000 mg/kg bw/day.

#### 4.7.1.4 Repeated dose toxicity: other routes

No relevant information.

#### 4.7.1.5 Human information

No relevant information.

#### 4.7.1.6 Other relevant information

The repeated dose toxicity of flutianil has also been investigated in guideline cancer bioassays in rats and mice (see Section 4.10).

In the rat, hyaline droplet nephropathy was observed in males from a dose of 82 mg/kg bw/day. This was associated with accumulation of $\alpha_{2\mu}$-globulin and it is therefore considered not relevant to humans. Liver effects (increased weight and decreased bilirubin) were seen in females at the high dose of 1130 mg/kg bw/day. In addition, isolated histopathological findings of the uterus (cysts, luminal dilatation, hyperplasia and polyps) were seen in females at 1130 mg/kg bw/day and a slight increase in the incidence of histopathological findings of the male reproductive organs (atrophy of testes, seminal vesicle and coagulating gland and oligospermia of epididymis) were observed at the top dose of 249 mg/kg bw/day. Given the low incidences of these isolated findings in the uterus and male reproductive organs, it is unclear whether these observations were treatment-related or incidental.

In the mouse, there were no clear treatment-related effects, including effects on the male reproductive organs up to a dietary concentration in excess of the limit dose (1084/1063 mg/kg bw/d in males/females).

#### 4.7.1.7 Summary and discussion of repeated dose toxicity

The short-term repeated-dose toxicity of flutianil has been investigated by the oral route in mice (28-day and 90-day), rats (28-day and 90-day) and dogs (28-day, 90-day and 1-year) and by the dermal route in rats (28-day). In addition, there are chronic toxicity studies in rats and mice.

In the mouse, no treatment-related effects on any organ were seen in the 28-day, 90-day chronic studies up to dietary concentrations well in excess of the limit dose. Testis atrophy was noted in single males in the 90-day study from a dose of 409 mg/kg bw/day, but the incidence was within the laboratory historical control range. Testis atrophy was also noted in the chronic toxicity study at the top dose of 1086 mg/kg bw/day, but, again, it was considered unrelated to treatment as it fell within the laboratory historical control range. These findings on the reproductive organs of mice are discussed further in the reproductive toxicity (Section 4.11).

In the rat, the kidney and liver were the main target organs of toxicity. Hyaline droplet nephropathy of the kidney was noted in males from a dose of 16 mg/kg bw/day for 28 days, 122 mg/kg bw/day for 90 days and 82 mg/kg bw/day for 2 years. These findings were associated with accumulation of $\alpha_{2\mu}$-globulin and are therefore considered not relevant to humans. Increased liver weight (usually associated with hepatocellular hypertrophy) and decreases in bilirubin were noted at the high dose of 1271/1500 mg/kg bw/day (males/females) for 90 days and at 1130 mg/kg bw/day for 2 years. In addition, in the chronic/carcinogenicity study, isolated histopathological findings of the uterus (cysts, luminal dilatation, hyperplasia and polyps) were seen in females at 1130 mg/kg bw/day and a slight increase in the incidence of histopathological findings of the male reproductive organs (atrophy of testes, seminal vesicle and coagulating gland and oligospermia of epididymis) was observed at the top dose of 249 mg/kg bw/day. These findings on the reproductive organs of rats are discussed further in the reproductive toxicity (Section 4.11). There were no treatment-related effects on any organ in the 28-day dermal study up to the limit dose.

In the dog, there were no clear treatment-related effects up to the limit dose in the 28-day, 90-day and 1-year studies. Organ weight changes of testis, prostate and uterus, and histopathological findings in testes (atrophy of seminiferous tubules) and prostate (cell infiltration) were seen from relatively low doses (10-30 mg/kg bw/day) in the 28-day and 90-day studies, but were not confirmed in the 1-year study at similar dose levels after a much longer period of treatment. Therefore, these findings were considered to be of no toxicological significance. These observations on the reproductive organs of dogs are discussed further in the reproductive toxicity (Section 4.11).

## 4.8 Specific target organ toxicity (CLP Regulation) – Repeated exposure (STOT RE)

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

The short-term repeated-dose toxicity of flutianil has been investigated by the oral route in mice (28-day and 90-day), rats (28-day and 90-day) and dogs (28-day, 90-day and 1-year) and by the dermal route in rats (28-day). In addition, there are chronic toxicity studies in rats and mice.

Classification with STOT- RE is triggered by the occurrence of *significant* (and/or *severe* for Category 1) toxic effects at doses below specified guidance values. For STOT-RE Category 2, the relevant guidance values for oral exposure are 100 mg/kg bw/day (rat 90-day study) and 300 mg/kg bw/day (rat 28-day study).

As described in Section 4.7.1.7 above, in the mouse, no treatment-related effects on any organ were seen in the 28-day, 90-day and chronic studies up to dietary concentrations well in excess of the limit dose. Therefore, in the mouse, no significant toxic effects occurred at any dose.

In the rat, the kidney and liver were the main target organs of toxicity. The kidney effects (hyaline droplet nephropathy associated with $\alpha_{2\mu}$-globulin accumulation in males) were considered not relevant to humans. The liver effects (increased liver weight, hepatocellular hypertrophy and decreases in bilirubin) were noted at the high dose of 1271/1500 mg/kg bw/day (males/females) for 90 days and at 1130 mg/kg bw/day for 2 years. Therefore, in the rat, the only significant toxic effects of relevance to humans were seen in the liver; however, these occurred at dose levels well in excess of the specified guidance values.

In addition, in the rat chronic/carcinogenicity study, isolated histopathological findings of the uterus were seen in females at 1130 mg/kg bw/day and a slight increase in the incidence of histopathological findings of the male reproductive organs was observed at the top dose of 249 mg/kg bw/day. These findings on the reproductive organs of rats are discussed further in the reproductive toxicity (Section 4.11) as they are more relevant to classification for reproductive toxicity rather than STOT-RE.

In the dog, there were no clear treatment-related effects up to the limit dose in the 28-day, 90-day and 1-year studies. Therefore, in the dog, no significant toxic effects occurred at any dose.

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

As described above in Section 4.8.1, in the mouse and dog, no significant toxic effects occurred at any dose. In the rat, the only significant toxic effects of relevance to humans were seen in the liver; however, these occurred at dose levels well in excess of the specified guidance values for classification with STOT-RE Category 2.

On this basis, classification of flutianil with STOT-RE is not warranted.

#### 4.8.3 Conclusion on classification and labelling

**Not classified: Conclusive but not sufficient for classification**

### 4.9 Germ cell mutagenicity (mutagenicity)

The genotoxic potential of flutianil has been investigated in several *in vitro* and *in vivo* studies.

#### 4.9.1 Non-human information

##### 4.9.1.1 *In vitro* data

Three standard *in vitro* tests are available as summarised in Table 16.

**Table 16: Overview of (experimental) *in vitro* genotoxicity studies**

| Method | Results | Remarks [reference] |
|---|---|---|
| Bacterial reverse mutation assay (Ames)<br>*S. typhimurium* TA98, TA100, TA1535, TA1537; | Plate incorporation: Negative ±S9<br>Precipitate at 1581 µg/plate and above.<br>Toxicity observed in all strains at 5000 µg/plate<br><br>Pre-incubation method: Negative ±S9 | Well conducted, GLP-compliant study<br><br>Purity: 99.38% |

| Method | Results | Remarks [reference] |
|---|---|---|
| *E. coli* WP2*uvr*A<br><br>Plate-incorporation assay<br>0 - 5000 µg/plate +/-S9 all strains<br>Pre-incubation assay<br>0 - 5000 µg/plate -S9 all strains<br>0 - 2500 µg/plate +S9 TA100, TA1535, WP2*uvr*A.<br>0 – 1000 µg/plate +S9 TA98, TA1537<br><br>OECD 471 (1997), GLP | Precipitate observed at 2000 µg/plate and above (all strains, -S9 ) and 1000 µg/plate and above (TA100, A1535, WP2*uvr*A, +S9) or 400 µg/plate and above (TA98, TA1537, +S9)<br>Toxicity observed at 5000 µg/plate (TA1535, TA1537, WP2*uvr*A, -S9), 1000 µg/plate (TA100, TA1535, WP2*uvr*A, +S9), 400 µg/plate (TA98, TA1537, +S9) | [24] |
| Mammalian cell gene mutation test using mouse lymphoma L5178Y $tk^{+/-}$ cells<br><br>Expt 1:<br>±S9 (3hr): 0 - 200 µg/mL<br>Expt 2:<br>±S9 (3 hr): 0 - 150 µg/mL<br><br>OECD 476 (1997), GLP | Negative without S9<br>3 hr -S9; with 85% RTG (Exp 1) or 65% (Exp 2) in the presence of precipitate<br><br>Negative with S9<br>3 hr +S9; with 65% (Expt 1) or 55% (Expt 2) RTG in the presence of precipitate | Well conducted, GLP-compliant study<br><br>Purity: 99.38%<br>[25] |
| Mammalian cell chromosome aberration assay in human peripheral blood lymphocytes<br>Expt 1:<br>3 hr + 17 hr recovery –S9:<br>0 - 235.9 µg/mL<br>3 hr + 17 hr recovery +S9:<br>0 – 294.9 µg/mL<br>Expt 2:<br>3 hr + 17 hr recovery +S9:<br>0 – 450 µg/mL<br>20 hr + 0 hr recovery –S9:<br>0 - 364.5 µg/mL<br><br>OECD 473 (1997), GLP | Negative for structural chromosome aberrations ±S9 up to and including precipitating doses.<br><br>A mitotic index was reduced to:<br>-S9: 3 hr+17hr: 75%; 20 hr+0 hr 57%<br>+S9: Expt 1: 73%; Expt 2: 65% | Well conducted, GLP-compliant study<br><br>Purity: 99.38%<br>[26] |

**Bacterial mutagenicity assay ([redacted], (2005) [24])**

In a GLP and guideline compliant Ames assay with *S. typhimurium* TA98, TA100, TA1535, and TA1537 and *E coli* WP2*uvr*A, in the presence and absence of S9, there were no increases in revertant colonies in any of the strains tested. There was a clear negative result, with and without S9 when tested up to a maximum recommended dose in accordance with current regulatory requirements for this assay type.

**Mammalian cell gene mutation assay ([redacted], (2005) [25])**

In a GLP and guideline compliant study, flutianil was assayed for its ability to induce mutation at the *tk* locus (5-trifluorothymidine resistance) in mouse lymphoma cells using a fluctuation protocol. A preliminary toxicity test was undertaken using flutianil up to 400 µg/mL.

Precipitation at the end of treatment was observed at doses of 100 µg/mL and above with and without S9.

In the main test, there was no dose-related increase in mutant frequency either in the presence or absence of S9. Toxicity, as measured by relative total growth (RTG) was reduced to 85% and 65% following a 3 hour treatment without S9, in experiments 1 and 2 respectively. In the 3 hour treatment in the presence of S9, RTG was reduced to 65% and 55% in experiments 1 and 2, respectively. The maximum dose tested in all treatment conditions was limited by precipitate observed at the end of the treatment period.

Flutianil showed no evidence of gene mutation potential in this test system using mouse lymphoma L5178Y $tk^{+/-}$ cells following 3 (+ and –S9) and 24 (-S9) hour treatments when tested in excess of its solubility limit.

**Mammalian cell chromosome aberration assay ([redacted], (2005) [26])**

In a GLP and guideline compliant mammalian chromosomal aberration assay, human lymphocyte cells were exposed to flutianil in either the presence or absence of metabolic activation.

Following a range finding trial, the doses chosen for the main test ranged from 96.6 to 235.9 µg/mL (-S9) and 188.7 to 294.9 µg/mL (+S9) for the 3 hour treatments in experiment 1. For the continuous (20 hour) treatment, concentrations ranged from 114.4 to 364.5 µg/mL (-S9), and from 174.3 to 450 µg/mL for a repeat test with the 3 hour treatment (+S9). The maximum doses selected were limited by solubility in the cell culture media

No biologically relevant increases in structural chromosomal aberrations or polyploidy were observed in any of the treatment conditions tested. Positive controls induced the appropriate response.

Flutianil did not induce chromosome aberrations in cultured human peripheral blood lymphocytes when tested in excess of its solubility limit in both the absence and presence of rat liver metabolic activation system.

### 4.9.1.2 *In vivo* data

A well conducted mouse bone marrow micronucleus assay is available, as summarised below.

**Table 17: Overview of (experimental) *in vivo* genotoxicity studies**

| Method | Results | Remarks [reference] |
|---|---|---|
| Mouse (NMRI BR (SPF) bone marrow micronucleus assay (5 male/sex/dose)<br><br>Intraperitoneal injection<br><br>0, 500, 1000, 2000 mg/kg bw<br><br>OECD 474 (1997), GLP | Negative (24 and 48 h exposure)<br><br>No evidence of bone marrow toxicity | Well conducted, GLP-compliant study<br><br>Purity: 99.1%<br>[27] |

**Bone marrow micronucleus test ([redacted], (2010) [27])**

In a GLP and guideline compliant mouse bone marrow micronucleus assay, doses were selected from a pilot toxicity study where male and female mice were dosed at 2000 mg/kg bw. As clinical signs of toxicity were limited to rough coat and hunched posture, the maximum dosed in

accordance with current regulatory guidelines was deemed to be a suitable maximum dose. In the main study doses of 0, 500, 1000 and 2000 mg/kg bw were administered.

Negative control groups were treated with vehicle only (corn oil), and positive control groups were treated with cyclophosphamide (CPA, 40 mg/kg bw) in accordance with the guideline.

There were no statistically significant increases in the frequency of micronuclei in any treatment group. Positive control treatment induced the appropriate response.

No deaths or clinical signs of toxicity were observed in the flutianil dosed groups, vehicle or positive control groups. No reduction in mean PCE/total erythrocyte ratio was observed. Although there was no direct evidence of bone marrow toxicity (e.g. from reduced PCE ratio) the use of intraperitoneal injection up to 2000 mg/kg bw was considered to be at the limits of reasonable testing.

In conclusion, flutianil was not genotoxic in this study where it is reasonable to assume that adequate target organ exposure occurred.

### 4.9.2 Non-human information

No relevant data available.

### 4.9.3 Other relevant information

No relevant data available.

### 4.9.4 Summary and discussion of mutagenicity

Flutianil has been thoroughly evaluated in a range of genotoxicity assays both *in vitro* (-/+S9) and *in vivo*.

Flutianil was non-mutagenic in a bacterial (Ames) assay for gene mutation when tested up to the maximum recommended dose (5000 µg/plate). In human peripheral cultured blood lymphocytes flutianil did not induce chromosomal aberrations when tested in excess of its solubility and in a mammalian cell gene mutation test assessing mutation at the *tk* locus in mouse lymphoma cells, flutianil was deemed not mutagenic up to precipitating doses.

The *in vivo* mouse bone marrow study was negative confirming the lack of potential to induce genotoxic damage *in vivo*. Overall, it is concluded that flutianil is not of genotoxic concern.

### 4.9.5 Comparison with criteria

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

### 4.9.6 Conclusion on classification and labelling

| **Not classified: Conclusive but not sufficient for classification** |
|---|

## 4.10 Carcinogenicity

The chronic toxicity and carcinogenic potential of flutianil have been investigated in rats and mice.

### 4.10.1 Non-human information

#### 4.10.1.1 Carcinogenicity: oral

The results of experimental studies are summarised in the following table:

**Table 18: Summary of the main treatment related findings in relevant carcinogenicity studies**

<table>
<tr><th>Method</th><th>Results (effects of major toxicological significance)</th><th>Remarks [reference]</th></tr>
<tr>
<td>Combined chronic and carcinogenicity study<br><br>Rat, Wistar<br><br>Dietary<br>♂: 0, 60, 600, 2000, 6000 ppm equiv. to equiv. to 0, 2.5, 25, 82, 249 mg/kg bw/d<br><br>♀: 0, 60, 2000, 6000, 20000 ppm approx. equiv. to 0, 3, 111, 334, 1130 mg/kg bw/d<br><br>Carcinogenicity study: 51 ♂ and ♀/gp<br><br>Chronic study: 12 ♂ and ♀/gp, except 21 ♂ and 21 ♀/gp at the top dose<br>(dosing: 17 Mar 2005 – 28 Mar 2007)<br><br>OECD 453 (1981), GLP</td>
<td>Note: Every animal in each dose group was given a gross pathological examination. However, microscopic evaluations were only made in all animals in the control and top dose groups and those animals that died early or had gross abnormalities at the end of the study.<br><br><u>Neoplastic findings</u>:<br><br>Liver:
<table>
<tr><th>Dose (ppm)</th><th>0</th><th>60</th><th>600</th><th>2000</th><th>6000</th><th>20000</th></tr>
<tr><td>♂: cholangioma (Be)</td><td>0/51</td><td>0/18</td><td>0/6</td><td>0/11</td><td>0/51</td><td>-</td></tr>
<tr><td>♀: cholangioma (Be)</td><td>0/51</td><td>0/21</td><td>-</td><td>0/12</td><td>1/17</td><td>1/51</td></tr>
<tr><td>♂: cholangiocarcinoma (Ma)</td><td>0/51</td><td>1/18</td><td>0/6</td><td>0/11</td><td>0/51</td><td>-</td></tr>
<tr><td>♀: cholangiocarcinoma (Ma)</td><td>0/51</td><td>0/21</td><td>-</td><td>0/12</td><td>0/17</td><td>0/51</td></tr>
</table>
Pancreas:
<table>
<tr><th>Dose (ppm)</th><th>0</th><th>60</th><th>600</th><th>2000</th><th>6000</th><th>20000</th></tr>
<tr><td>♂: Pancreas: islet cell adenoma (Be)</td><td>1/51</td><td>0/10</td><td>0/4</td><td>0/6</td><td>4/51</td><td>-</td></tr>
<tr><td>♀: Pancreas: islet cell adenoma (Be)</td><td>1/51</td><td>0/17</td><td>-</td><td>0/7</td><td>0/13</td><td>0/51</td></tr>
<tr><td>♂: Pancreas: islet cell carcinoma (Ma)</td><td>1/51</td><td>0/10</td><td>0/4</td><td>1/6</td><td>0/51</td><td>-</td></tr>
<tr><td>♀: Pancreas: islet cell carcinoma (Ma)</td><td>1/51</td><td>0/17#</td><td>-</td><td>0/7#</td><td>0/13#</td><td>2/51</td></tr>
</table>
<u>Non-neoplastic effects</u>:<br>20,000 ppm (in ♀ only = 1130 mg/kg bw/d):<br><u>Liver</u>: ↑ relative wt (17%- not statistically significant) at 52 wks;<br><br><u>Bile duct</u>: ↑ hyperplasia (33% vs. 8% in controls) at 52 wks;<br>↑ severity of hyperplasia at 104 wks, but same incidence as controls;<br>↓ bilirubin (43%);<br><br><u>Uterus</u>: isolated histopathological findings at 52 wks and 104 wks;<br><br>6000 ppm (249/334 mg/kg bw/d in ♂/♀):<br><u>Kidney:</u> hyaline droplet deposition in proximal tubular cells in ♂;<br><br><u>Pancreas</u>: islet cell hyperplasia in 2/51 ♂ (vs. 0/51 in controls);<br><br><u>Reproductive organs in ♂</u>: atrophy of testis seminiferous tubule (10/51 vs.7/51 in controls); oligospermia of</td>
<td>Well conducted, GLP-compliant study<br><br>Purity: 99.26%<br>[28]</td>
</tr>
</table>

<table>
<tr><th>Method</th><th>Results (effects of major toxicological significance)</th><th>Remarks [reference]</th></tr>
<tr><td></td><td>epididymis (6/51 vs. 4/51 in controls), atrophy of seminal vesicle (2/51 vs. 0/51 in controls) and atrophy of the glandular epithelial cell of the coagulating gland (2/51 vs. 0/51 in controls);<br><br>2000 ppm (82/111 mg/kg bw/d in ♂/♀):<br>Kidney: hyaline droplet deposition in proximal tubular cells in ♂ (not relevant for human risk assessment);<br><br>♂: A NOAEL of 2000 ppm equivalent to 82 mg/kg bw/d;<br>♀: A NOAEL of 6000 ppm equivalent to 334 mg/kg bw/d</td><td></td></tr>
<tr><td>78wk, mouse (CD1)<br>(52 animals/sex/gp)<br>(dosing: 15 Dec 2005 – 25 June 2007)<br><br>oral: feed<br><br>0, 1000, 3000, 10,000 ppm equiv. to 0, 106, 321, 1084 mg/kg bw/d (♂) and 0, 105, 316, 1063 mg/kg bw/d (♀)<br><br>OECD 451, GLP</td><td>Neoplastic findings:<br><br>Liver:
<table>
<tr><th>Dose (ppm)</th><th>0</th><th>1000</th><th>3000</th><th>10000</th></tr>
<tr><td>♂: Liver: hepatocellular adenoma (Be)</td><td>15/52</td><td>11/35</td><td>18/35</td><td>16/52</td></tr>
<tr><td>♀: Liver: hepatocellular adenoma (Be)</td><td>3/52</td><td>1/16</td><td>1/21</td><td>0/52</td></tr>
<tr><td>♂: Liver: hepatocellular carcinoma (Ma)</td><td>5/52</td><td>10/35</td><td>9/35</td><td>10/52</td></tr>
<tr><td>♀: Liver: hepatocellular carcinoma (Ma)</td><td>1/52</td><td>1/16</td><td>0/21</td><td>0/52</td></tr>
</table>
Non-neoplastic effects:<br>10,000 ppm (1084/1063 mg/kg bw/d in ♂/♀):<br>Testes: ↑ testes softening at gross pathology (21% vs. 5.8% in controls), ↑ testis atrophy at gross pathology (15% vs. 1.9% in controls), ↑ testis atrophy at microscopic pathology (34.6% vs. 25% in controls), ↑ interstitial cell hyperplasia (3.8% vs. 1.9% in controls);<br><br>Epididymis: ↑ oligospermia (21% vs. 11.5% in controls);<br><br>3000 ppm (321/316 mg/kg bw/d in ♂/♀):<br>Testes: ↑atrophy at microscopic pathology (30.8% vs. 25% in controls);<br><br>1000 ppm (106/105 mg/kg bw/d ♂/♀):<br>No treatment-related effects identified<br><br>♂: A NOAEL of 1000 ppm equivalent to 105 mg/kg bw/d;<br>♀: A NOAEL of 10000 ppm equivalent to 1063 mg/kg bw/d</td><td>Well conducted, GLP-compliant study<br><br>Purity: 99.26%<br>[29]</td></tr>
</table>

*NB: The values for NOAEL are provided for information only: they are the values derived from the DAR for flutianil. ↓ = decrease compared to control. ↑= increase compared to control.*
Ma = malignant, Be = benign
# Only tissues of animals showing macroscopic lesions were examined

**Combined chronic toxicity / carcinogenicity study in the rat ([redacted], (2009) [28])**

In a GLP and guideline compliant combined toxicity/carcinogenicity study, male and female Wistar rats (51/sex/dose) were administered flutianil on a continuous basis in the diet for a total of 104 weeks. Dose levels were 60, 600, 2000, 6000 ppm for males and 60, 2000, 6000, 20000 ppm for females (the maximum dose administered was lower in males due to male specific kidney toxicity). An interim necropsy took place during week 52 of the study, with selected tissues examined microscopically (12/sex/dose, with 21/sex/dose for the high dose group).

There were no treatment related effects on survival. At the end of the study body weights and body weight gains for males and females of the treated groups were comparable to the control group.

Total bilirubin levels in top dose females were consistently reduced from week 14 onwards (up to 43% reduction compared to control). This was accompanied by an increase in relative liver weight (by 17%).

The male kidney contained a number of non-neoplastic lesions in both the chronic and carcinogenic phases. At 52 weeks there was a significant increase in hyaline droplet deposition in the proximal tubular cell at 2000 and 6000 ppm. Immunohistochemical staining demonstrated that the hyaline droplets in the proximal tubular cells were positive for $\alpha_{2\mu}$-globulin. This lesion is specific to male rats and is not considered relevant to humans. Other kidney lesions that occurred at greater frequency in treated male rats included urinary casts, tubular basophilic change and calculus.

In both the chronic and carcinogenicity phases of the study an apparent dose related increase in both relative and absolute uterus weights was observed, but did not reach statistical significance due to large inter-animal variation. The increased uterus weights in both phases of the study were within the ranges of the laboratory historical controls [30]. Therefore, the increase in uterus weight in this study was considered to be unrelated to treatment.

At 52 weeks, there was a slight increase in benign endometrial stromal polyps of the uterus in the top dose females (3/21), which marginally exceeded the laboratory historical control incidence (a maximum of 2/21) [30]. At 104 weeks, several findings in the uterine horn occurred at a marginally higher rate in the top dose females - namely cysts (in one animal), luminal dilatation of endometrial gland (in 8 animals *vs*. 6 in controls) and hyperplasia of the endometrium (in 2 animals *vs*. none in controls). However, there were no treatment-related benign or malignant tumours of the uterus at any dose at 52 or 104 weeks.

In conclusion, a slight increase in the incidence of some histopathological findings of the uterus was observed in this study at the top dose of 20,000 ppm (refer to Section 4.11.4 for further discussion).

In the top dose (6000 ppm) group males, a slight increase in the incidence of histopathological findings of the reproductive organs was noted at 104 weeks; these included atrophy of the seminiferous tubule (10/51 compared to 7/51), oligospermia of epididymis (6/51 compared to 4/51), atrophy of seminal vesicle (2/51 compared to 0/51) and atrophy of the glandular epithelial cell of the coagulating gland (2/51 compared to 0/51). Given the low incidence observed, it is unclear whether these findings were treatment-related or incidental (refer to Section 4.11.4 for further discussion).

With regard to possible tumour findings, an increase in islet cell adenoma of the pancreas was observed in males in the top dose group (4/51 - 8% compared to 1/51 – 2% in controls). This finding marginally exceeded (by one animal) the laboratory historical control upper range of 3/51 (6%). However, it is noted that this incidence was well within the historical control upper range value of 44% from the RCC database [31] and of 15.8% from the publication of Caruls *et al* [32] (Table 19).

Islet cell hyperplasia was also observed in top dose males, but the incidence was low (2/51 *vs.* 0/51 in controls) and only marginally exceeded the laboratory historical control incidence rate (1/51). Hyperplasia was not seen at 52 weeks. The grading of the hyperplasia was ‘slight’ in one animal that died in week 100 and ‘moderate’ at terminal kill in the other animal. No toxic effects were noted in the islet cells or other tissues of the pancreas. These findings may be indicative of a slight treatment related tumourigenic effect of flutianil on the islet cells of the pancreas; however, considering the adenoma exceeded only marginally the laboratory historical

control range (by one animal); the hyperplasia was graded as slight/moderate; and no toxicity was noted in the pancreas; the evidence for a treatment related effect is considered equivocal.

There was one case of islet cell carcinoma in one male at 2000 ppm, but as the same tumour was also seen in one control male and none were evident at the top dose, this was not considered to have been treatment-related.

No islet cell hyperplasia or adenoma was reported in females, which raises further doubt about the relation to treatment of the findings in males.

Islet cell carcinoma of the pancreas was seen in 2/51 top dose group females (*vs.* 1/51 in controls), both of whom died before the end of the study. The laboratory historical control range for this finding was 0/51 - 1/51 (Table 19). There was some doubt about the aetiology of one of these tumours in one of the two top dose females affected. This female was killed *in extremis* during week 94 and had multiple tumours; in addition to islet cell carcinoma, the female presented with pituitary adenocarcinoma and adenocarcinoma of the uterine horn, both of which had metastasised. It is possible therefore that the pancreatic tumour was a secondary one (although this was not confirmed unequivocally in the study report). Overall, as there were no pre-neoplastic lesions or benign islet cell tumours in females; the increase above concurrent and historical controls was marginal; and it is possible that one of the tumours could have occurred as a consequence of metastasis from another tissue, the weight of evidence suggests that the female pancreatic carcinomas were not related to treatment.

Overall, the absence of a consistent toxic response to flutianil in this organ and the sex-specific nature of the response (adenomas in males) bring into question the biological plausibility of its relation to treatment.

In conclusion, there is insufficient evidence in this study for a treatment-related carcinogenic effect of flutianil in the islet cells of the pancreas.

**Table 19: Summary of main findings in the islet cells of the pancreas in the rat carcinogenicity study and historical control data (HCD) for islet cell tumours from the laboratory and public domain sources**

| Parameter | | Males | | | | | Females | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Dose level (ppm) | wk | 0 | 60 | 600 | 2000 | 6000 | 0 | 60 | 2000 | 6000 | 20000 |
| Islet cell hyperplasia (killed in extremis or found dead) | 104 | 0/8 | 0/10 | 0/4 | 0/6 | 1/9 | 0/11 | 0/17 | 0/7 | 0/13 | 0/13 |
| Islet cell hyperplasia (all animals) | 104 | 0/51 | 0/10a | 0/4a | 0/6a | 2/51 | 0/51 | 0/17a | 0/7a | 0/13a | 0/51 |
| Islet cell hyperplasia historical control incidence[b] | | Total: 5/408 (1.23%)<br>Range: 0/51 – 1/51 [2007 – 2013] | | | | | Total: 5/408 (1.23%)<br>Range: 0/51 – 2/51 [2007 – 2013] | | | | |
| Islet cell **adenoma** (ben) | *In extremis* | 0/8 | 0/10 | 0/4 | 0/6 | 0/9 | 1/11 | 0/17 | 0/7 | 0/13 | 0/13 |
| | 104 | 1/51 | 0/10[a] | 0/4[a] | 1/6[a] | 4/51 | 1/51 | 0/19[a] | 0/9[a] | 0/16[a] | 0/51 |
| Islet cell adenoma lab HCD[b] | | Total: 10/408 (2.45%)<br>Range: 0/51 – 3/51 [2007 – 2013] | | | | | Total: 8/408 (1.96%)<br>Range: 0/51 – 3/51 [2007 – 2013] | | | | |
| Islet cell adenoma RCC HCD (RccHan:WIST Hannover) [31] | | Total: 131/2442 (5.36%)<br>Range: 0 – 44.44% [1982 – 2000] | | | | | Total: 46/2404 (1.91%)<br>Range: 0 – 6.00% [1982 – 2000] | | | | |
| Islet cell adenoma HCD from Caruls *et al* (2013) [32] | | Total: 37/455<br>Range: 2-15.8% (mean: 8.1%) | | | | | Total: 5/462<br>Range: 0-2% (mean: 1.1%) | | | | |

| Parameter | | Males | | | | | Females | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Dose level (ppm) | wk | 0 | 60 | 600 | 2000 | 6000 | 0 | 60 | 2000 | 6000 | 20000 |
| Islet cell **carcinoma** (ma) | *In extremis* | 0/8 | 0/10 | 0/4 | 1/6 | 0/9 | 0/11 | 0/17 | 0/7 | 0/13 | 2/13 |
| | 104 | 1/51 | 0/10[a] | 0/4[a] | 1/6[a] | 0/51 | 1/51 | 0/17[a] | 0/7[a] | 0/13[a] | 2[c]/51 |
| Islet cell carcinoma lab HCD[b] | | Total: 6/408 (1.47%)<br>Range: 0/51 – 1/51 [2007 – 2013] | | | | | Total: 3/408 (0.74%)<br>Range: 0/51 – 1/51 [2007 – 2013] | | | | |
| Islet cell carcinoma HCD from Caruls *et al* (2013) [32] | | Total: 9/455<br>Range: 0-5.3% (mean: 2%) | | | | | Total: 3/462<br>Range: 0-1.7% (mean: 0.6%) | | | | |

Ma = malignant, Be = benign
a only animals with macroscopic lesions examined (incidence/total examined)
b laboratory historic control groups (Wistar Hannover GALAS) . Range: min.- max. [30]
c this includes animal 742 which had metastatic adenocarcinoma

Bile duct cholangioma, a benign lesion, occurred at an incidence of 2% in 1/17 and 1/51 females in the 6000 and 20,000 ppm dose groups, respectively, but not in the concurrent or historical controls (0%). However, it is noted that this incidence was within the historical control upper range value of 2% from the RCC database [31] and of 6% from public domain sources (Table 20).

In females, the incidence of bile duct hyperplasia (graded as 'slight') was slightly increased (7/21-33% *vs.* 1/12 -8% in controls) at the top dose at 52 weeks but not at 104 weeks. However, at 104 weeks, the severity of the hyperplasia was more pronounced than in controls. There were no malignant bile duct tumours in any female rat and, despite the slightly increased incidence/severity of bile duct hyperplasia in the top dose females, no toxic effects were reported in this organ in both sexes. In males, there were no benign tumours of the bile duct. Malignant chloangiocarcinoma was seen in 1/18 low dose males. However, there were no such tumours in any other dose group. In addition, there were ascites and severe hepatocellular necrosis in this animal. Therefore, this isolated carcinoma finding in a low dose group male is considered to be un-related to treatment.

Overall, the absence of a clear toxic response to flutianil in this organ and the sex-specific nature of the response (adenomas in females) bring into question the biological plausibility of its relation to treatment.

In conclusion, there is insufficient evidence in this study for a treatment-related carcinogenic effect of flutianil on the bile duct.

**Table 20: Summary of liver and bile duct effects in the rat carcinogenicity study and historical control data (HCD) for bile duct cholangioma from the laboratory and public domain sources**

| Parameter | | Males | | | | | Females | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Dose level (ppm) | wk | 0 | 60 | 600 | 2000 | 6000 | 0 | 60 | 2000 | 6000 | 20000 |
| Abs. liver weight (g) | 104 | 11.06 | 12.12 | 10.23 | 11.65 | 12.15 | 7.19 | 7.54 | 7.87 | 8.40 | 9.17 |
| Rel. liver weight (%) | 104 | 2.15 | 2.44 | 2.21 | 2.22 | 2.47 | 2.29 | 2.30 | 2.24 | 2.45 | 2.69 |
| Bile duct hyperplasia[a] | 52 | 0/12 | - | - | - | 0/12 | 1/12 | - | - | - | 7/21 |
| | *In extremis* | 1/8 | 0/10 | 1/4 | 0/6 | 0/9 | 2/11 | 5/17 | 1/7 | 2/13 | 6/13 |
| | Grade[b] | 1/0/0 | - | 1/0/0 | - | - | 1/0/1 | 4/1/0 | 0/1/0 | 1/1/0 | 4/2/0 |
| | 104 | 21/51 | 1/18[a] | 1/6[a] | 0/11[a] | 20/51 | 26/51 | 7/21[a] | 2/12[a] | 4/17[a] | 28/51 |
| | Grade[b] | 15/6/0 | 0/1/0 | 0/1/0 | - | 14/5/1 | 20/4/2 | 6/1/0 | 1/1/0 | 3/1/0 | 14/12/2 |
| Bile duct hyperplasia, HCD[c] | | Total: 164/408 (40.2%) | | | | | Total: 233/408 (57.1%) | | | | |

| Parameter | | Males | | | | | Females | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Dose level (ppm) | wk | 0 | 60 | 600 | 2000 | 6000 | 0 | 60 | 2000 | 6000 | 20000 |
| (104 weeks) | | Range: 10/51 – 29/51 [2007 – 2013] | | | | | Range: 17/51 – 40/51 [2007 – 2013] | | | | |
| **Cholangioma** (ben.) | 104 | 0/51 | 0/18[a] | 0/6[a] | 0/11[a] | 0/51 | 0/51 | 0/21[a] | 0/12[a] | 1/17[a] | 1/51 |
| **Cholangioma, lab HCD[c]** | | No incidence [2007 – 2013] | | | | | No incidence [2007 – 2013] | | | | |
| Cholangioma, RCC HCD (RccHan:WIST Hannover) [31] | | Total: 1/2619 (0.04%) Range: 0 – 1.27% [1982 – 2000] | | | | | Total: 1/2571 (0.04%) Range: 0 – 2.00% [1982 – 2000] | | | | |
| Cholangioma, HCD from Weber *et al* [33] (RccHan:WIST Hannover) | | Total: 1/3737 (0.03%) Range: not reported [1981 – 2006] | | | | | Total: 8/3686 (0.22%) Range: not reported [1981 – 2006] | | | | |
| Cholangioma, HCD from Weber *et al* (2011) [33] (Crl:WI(Han) Hannover) | | Total: 0/555 (0.0%) Range: n/a [1981 – 2006] | | | | | Total: 1/555 (0.18%) Range: not reported [1981 – 2006] | | | | |
| Cholangioma, HCD from Weber *et al* (2011) [33] (Ico:OFA-SD) | | Total: 0/330 (0.0%) Range: n/a [1981 – 2006] | | | | | Total: 5/330 (1.5%) Range: not reported [1981 – 2006] | | | | |
| Cholangioma, HCD from Eiben & Bomhard (1999) [34] (WISW SPF Cpb) | | Mean: 0.1% Range: 0-2% [1975 – 1994] | | | | | Mean: 0.6% Range: 0-6% [1975 – 1994] | | | | |
| Cholangioma, HCD from Walsh & Poteracki (1998) [35] (Wistar Han) | | 2/685 (0.29%) Range: 0-2% [1980-1990] | | | | | 2/685 (0.29%) Range: 0-0.8% [1980-1990] | | | | |
| Cholangioma, HCD from Poteracki & Walsh (1994) [36] (Crl:(WI)BR) | | Total: 0/465 (0.0%) Range: n/a [1990 – 1995] | | | | | Total: 3/465 (0.7%) Range: 0 – 2.0% [1990 – 1995] | | | | |
| **Cholangio-carcinoma** (mal.) | *In extremis* | 0/8 | 1/10 | 0/4 | 0/6 | 0/9 | 0/11 | 0/17 | 0/7 | 0/13 | 0/13 |
| | 104 | 0/51 | 1/18 | 0/6 | 0/11 | 0/51 | 0/51 | 0/21 | 0/12 | 0/17 | 0/51 |

Ma = malignant, Be = benign
a only animals with macroscopic lesions examined (incidence/total examined)
b number of lesions graded as slight/moderate/severe
c. laboratory historic control (Wistar Hannover GALAS). Range: min.- max. [40]
d finding only seen at terminal kill

In summary, flutianil was not carcinogenic in the rat up to the limit dose in females and up to a dose causing kidney toxicity in males.

**Carcinogenicity study in the mouse ([redacted], (2009) [29])**

In a GLP and guideline compliant study flutianil was administered to 52 male and 52 female CD1 mice/group for a minimum of 78 weeks. Dose levels were 1000, 3000 and 10000 ppm (equivalent to 106/105, 321/316 and 1084/1063 mg/kg/day in males/females). At termination of treatment, all surviving animals were euthanised and subjected to haematology (limited to analysis of the leukocyte population), necropsy and histopathology. Organ weights were performed on 10 animals/sex/group. Animals killed *in extremis* or found dead during the treatment period were subjected to necropsy and histopathology.

There were no treatment related effects on survival. At the end of the study body weights and body weight gains for males and females were comparable to the control group. No notable changes in any organ weight, irrespective of sex/dose were observed.

A marginal increase in hepatocellular carcinoma was seen in males in all dose groups. The increase did not reach statistical significance, but exceeded the maximum laboratory historical control rate (Table 21) by a single incidence in both the low and high dose groups.

Hepatocellular adenoma was increased in the mid dose group but showed no dose response relationship. These findings in males are considered to be incidental as there was no associated increase in pre-neoplastic findings or benign tumours, and similar findings were not seen in females.

**Table 21: Histopathology: male neoplastic liver data for the mouse carcinogenicity study**

| Parameter | Historical control incidence[a] | | Dose level (ppm) | | | |
|---|---|---|---|---|---|---|
| | | | 0 | 1000 | 3000 | 10000 |
| Hepatocellular adenoma[b] (Ben) | Total: 58/308 (18.8%) Range: 1/15 – 6/20 [2003 – 2012] | KE/ FD | 5/16 | 2/19 | 3/15 | 8/21 |
| | Total: 230/891 (25.8%) Range: 7/52 – 18/52 [2003 – 2012] | Ter | 10/36 | 9/16 | 15/20 | 8/31 |
| | | All | 15/52 | 11/35[c] | 18/35[c] | 16/52 |
| Hepatocellular carcinoma[b] (Mal.) | Total: 25/308 (8.1%) Range: 0/15 – 5/19 [2003 – 2012] | KE/ FD | 3/16 | 5/19 | 4/15 | 6/21 |
| | Total: 52/891 (5.8%) Range: 0/52 – 9/52 [2003 – 2012] | Ter | 2/36 | 5/16 | 5/20 | 4/31 |
| | | All | 5/52 | 10/35[c] | 9/35[c] | 10/52 |

KE – killed *in extremis*; FD: found dead; Ter: terminal sacrifice; All: total (KE/FD + Ter); Ben. – benign; Mal. - malignant
a laboratory historic control range (SPF ICR [Crlj:CD1(ICR)]) Total = n/total no. examined. Range min.- max [37]
b data not subjected to statistical analysis
c examined on animals that showed macroscopic lesions

Non-neoplastic findings were reported in the testes at gross necropsy. Statistically significant increases in the incidence of testis softening (11/52 *vs.* 3/52 in controls) and atrophy (8/52 *vs.* 1/52 in controls) were observed in top dose males (Table 22). These exceeded the laboratory historical control ranges.

**Table 22: Gross necropsy: findings in the testes in the mouse carcinogenicity study**

| Parameter | Historical control incidence[a] | | Dose level (ppm) | | | |
|---|---|---|---|---|---|---|
| | | | 0 | 1000 | 3000 | 10000 |
| Testis: softening | 14/308 (4.5%) Range: 0/24 – 3/16 (2003 – 2012) | KI/ FD | 1/16 | 0/19 | 1/15 | 3[b]/21 |
| | 70/891 (7.9%) Range: 1/52 – 10/52 (2003 – 2012) | Ter | 2/36 | 4/33 | 2/37 | 8*/31 |
| | | All | 3/52 | 4/52 | 3/52 | 11*/52 |
| Testis: atrophy | 14/308 (4.5%) Range: 0/24 – 3/19 (2003 – 2012) | KI/ FD | 0/16 | 0/19 | 0/15 | 1[b]/21 |
| | 27/891 (3.0%) Range: 0/52 – 4/52 (2003 – 2012) | Ter | 1/36 | 3/33 | 2/37 | 7 */31 |
| | | All | 1/52 | 3/52 | 2/52 | 8*/52 |

KI – killed *in extremis*; FD: found dead; Ter: terminal sacrifice; All: total (KE/FD + Ter)
a laboratory historic control range ( (SPF) ICR [Crlj:CD1(ICR)]) Total = n/total no. examined. Range min.- max [37]
b animal 193 displayed atrophy and softening of the testes at necropsy. Histopathological examination failed to show any abnormalities in the macroscopic lesion
* $p \leq 0.05$

At microscopy, there was an increase in atrophy of the seminiferous tubules of the testes at 3000 and 10,000 ppm, but this did not reach statistical significance and was within the range of the historical controls. Other findings included a slight increase in interstitial cell hyperplasia of the testes and oligospermia of the epididymis at the top dose, which fell within the historical control ranges.

In conclusion, whilst findings in the testes were observed at gross pathology, these were not replicated at the histopathological examination (Table 23), where findings were within the historical control range. Gross necropsy examination is relatively crude compared with histopathology and it is considered that the incidence of the histopathological findings is more reliable for the assessment of a relation to treatment. Therefore, it is concluded that there are no treatment-related effects on the male reproductive organs in this chronic study in the mouse.

**Table 23: Histopathology: findings in the testis and epididymis for the mouse carcinogenicity study**

| Parameter | Historical control incidence[a] | | Dose level (ppm) | | | |
|---|---|---|---|---|---|---|
| | | | 0 | 1000 | 3000 | 10000 |
| Non-neoplastic lesions | | | | | | |
| Testis: Atrophy of seminiferous tubules | Total: 72/308 (23.4%)<br>Range: 1/20 – 10/20 [2003 – 2012] | KE/ FD | 3/16 | 4/19 | 3/15 | 3/21 |
| | Total: 289/891 (32.4%)<br>Range: 8/52 – 31/52 [2003 – 2012] | Ter | 10/36 | 7/33 | 14/37 | 15/31 |
| | | All | 13/52 | 11/52 | 17/52 | 18/52 |
| Testis: Interstitial cell hyperplasia | Total: 1/308 (0.3%)<br>Range: 0/24 – 1/16 [2003 – 2012] | KE/ FD | 1/16 | 0/19 | 0/15 | 0/32 |
| | Total: 13/891 (1.5%)<br>Range: 0/52 – 3/52 [2003 – 2012] | Ter | 0/36 | 0/33 | 0/37 | 2/31 |
| | | All | 1/52 | 0/52 | 0/52 | 2/52 |
| Epididymis: oligospermia | Total: 41/308 (13.3%)<br>Range: 0/16 – 7/20 [2003 – 2012] | KE/ FD | 0/16 | 2/19 | 0/15 | 1/21 |
| | Total: 163/891 (18.3%)<br>Range: 3/52 – 18/52 [2003 – 2012] | Ter | 6/36 | 1/1[b] | 0/2[b] | 10/31 |
| | | All | 6/52 | 3/20 | 0/17 | 11/52 |

KE – killed *in extremis*; FD: found dead; Ter: terminal sacrifice; All: total (KE/FD + Ter); Ben - benign
a laboratory historic control range ((SPF) ICR [Crlj:CD1(ICR)]) Total = n/total no. examined. Range min.- max [37]
b examined on animals that showed macroscopic lesions

In summary, there is no evidence of a carcinogenic effect of flutianil in the mouse up to the limit dose.

#### 4.10.1.2 Carcinogenicity: inhalation

No relevant data available.

#### 4.10.1.3 Carcinogenicity: dermal

No relevant data available.

### 4.10.2 Human information

No relevant data available.

### 4.10.3 Other relevant information

Flutianil was negative in a series of *in vitro* and *in vivo* assays to detect its genotoxic potential (see Section 4.9).

### 4.10.4 Summary and discussion of carcinogenicity

The carcinogenicity of flutianil has been examined in GLP- and guideline-compliant 2 year rat and 18 month mouse studies.

In rats, an increase in islet cell adenoma of the pancreas was observed in males (but not in females) in the top dose (294 mg/kg bw/day) group (4/51 compared to 1/51 in controls). This finding marginally exceeded (by one animal) the historical control upper range of 3/51. Islet cell hyperplasia was also observed in top dose males, but the incidence was low (2/51 *vs* 0/51 in controls) and only marginally exceeded the laboratory historical control incidence rate (1/51). No toxic effects were noted in the islet cells or other tissues of the pancreas in both sexes. There was no increase in malignant tumours in males, but 2 tumours were observed in top dose (1130 mg/kg bw/day) females. However, as there were no pre-neoplastic lesions or benign Islet cell tumours in females; the increase above concurrent and historical controls was marginal; and it is possible that one of the tumours could have occurred as a consequence of metastasis from another tissue, the weight of evidence suggests that the female pancreatic carcinomas were not related to treatment. Overall, the absence of a consistent toxic response to flutianil in this organ and the sex-specific nature of the response (adenomas in males) bring into question the biological plausibility of its relation to treatment. In conclusion, there is insufficient evidence in this study for a treatment-related carcinogenic effect of flutianil in the islet cells of the pancreas.

Also in rats, bile duct cholangioma, a benign lesion, occurred in 1/17 and 1/51 females at 334 and 1130 mg/kg bw/day, respectively, but not in the concurrent or historical controls. In females, the incidence of bile duct hyperplasia (graded as 'slight') was slightly increased at the top dose at 52 weeks but not at 104 weeks. However, at 104 weeks, the severity of the hyperplasia was more pronounced than in controls. There were no malignant bile duct tumours in any female rat and, despite the slightly increased incidence/severity of bile duct hyperplasia in the top dose females, no toxic effects were reported in this organ in both sexes. In males, there were no benign tumours of the bile duct. Malignant cholangiocarcinoma was seen in 1/18 low dose males (at 2.5 mg/kg bw/day). However, there were no such tumours in any other dose group. In addition, there were ascites and severe hepatocellular necrosis in this animal. Therefore, this isolated carcinoma finding in a low dose group male is considered to be un-related to treatment. Overall, the absence of a clear toxic response to flutianil in this organ and the sex-specific nature of the response (adenomas in females) bring into question the biological plausibility of its relation to treatment. In conclusion, there is insufficient evidence in this study for a treatment-related carcinogenic effect of flutianil on the bile duct.

In summary, flutianil was not carcinogenic in the rat up to the limit dose in females and up to a dose causing kidney toxicity in males.

In the 18-month carcinogenicity study in mice, a marginal increase in hepatocellular carcinoma was seen in males, which exceeded the historical control range by a single incidence. These changes however were not considered treatment related as they were not accompanied by a dose-related response, with no associated increase in pre-neoplastic findings. No such effects were seen in females. It is concluded that there was no evidence of a carcinogenic effect in mice up to the limit dose.

### 4.10.5 Comparison with criteria

As there is insufficient evidence for a carcinogenic effect in rats and mice, and there are no other concerns about the potential carcinogenicity of flutianil, no classification is proposed.

### 4.10.6 Conclusions on classification and labelling

**Not classified: Conclusive but not sufficient for classification**

## 4.11 Toxicity for reproduction

The reproductive toxicity of flutianil has been investigated in a multi-generation and two developmental studies.

### 4.11.1 Effects on fertility

#### 4.11.1.1 Non-human information

The effects of flutianil on reproductive performance and fertility have been investigated in a guideline multi-generation study in rats.

**Table 24: Overview of multi-generation studies**

| Method | Results (effects of major toxicological significance) | Remarks [reference] |
|---|---|---|
| Range finder study:<br>rat (Wistar)<br>(8 animals/sex/gp)<br>(dosing: 14 Feb 2006 – 29 April 2006)<br><br>oral: feed<br><br>0, 20, 200, 2000, 20000 ppm equiv. 0, 1, 11, 111, 1146 mg/kg bw/day (♂) and 0, 2, 21, 227, 2271 mg/kg bw/day (♀)<br><br>no relevant guideline, GLP | **20,000 ppm:**<br>16% ↑ relative liver weight in maternal ♀;<br>↑ relative and absolute uterus weight by 30% & 27% respectively in $F_1$ 25-day weanlings (not statistically significant);<br><br>**2000 ppm:**<br>13% ↑ relative liver weight in maternal ♀;<br><br>**200 and 20 ppm**:<br>No adverse effects. | Well conducted, GLP-compliant study<br><br>Purity: 99.22%<br>[38] |
| Main study:<br>rat (Wistar)<br>(24 animals/sex/gp)<br>(dosing: 13 Jul 2006 – 29 Oct 2007)<br>oral: feed<br><br>0, 200, 2000, 20000 ppm equiv. 0, 12, 123, 1286 mg/kg bw/day ($F_0$ ♂) and 0, 19, 199, 2002 mg/kg bw/day ($F_0$ ♀)<br><br>OECD 416 (2001), GLP | **<u>Parental findings</u>:**<br><br>**20,000 ppm:**<br><u>Liver</u><br>P ♂: ↑ abs. / rel. liver weight (22% / 15%);<br>$F_1$ ♂: ↑ abs. /rel. liver weight (14%/14%);<br>P ♀: ↑ abs. /rel. liver weight (18%/15%);<br>$F_1$ ♀: ↑ abs. /rel. liver weight (10%/12%);<br>P ♂: ↑ centrilobular hepatocyte hypertrophy in 5/23 *vs.* 0 in controls.<br><br><u>Kidney</u>:<br>P ♂: ↑ proximal tubular hyaline droplet deposition in 23/23 *vs.* 17/24 controls;<br>$F_1$ ♂: ↑ proximal tubular hyaline droplet deposition in 20/21 *vs.* 18/22 controls;<br><br>**2,000 ppm:**<br><u>Kidney</u>:<br>P ♂: ↑ proximal tubular hyaline droplet deposition in 22/23 *vs.* 17/24 controls; | Well conducted, GLP-compliant study<br><br>Purity: 99.22%<br>[39] |

| Method | Results (effects of major toxicological significance) | Remarks [reference] |
|---|---|---|
| | **200 ppm:**<br>No adverse findings.<br><br>**Reproductive findings**:<br><br>**20,000 ppm**<br>$F_1$: ↓ mean number of pups delivered (10 *vs*. 11.8 in controls);<br><br>**2000 and 200 ppm:**<br>No adverse findings.<br><br>**Offspring findings**:<br><br>**20,000 ppm**<br>Uterus:<br>$F_1$ weanlings: ↑ abs. / rel. uterus weight 9% / 4% (not statistically significant);<br>$F_2$ weanlings: ↑ abs. / rel. uterus weight 10% / 11% (not statistically significant);<br><br>**2000 and 200 ppm:**<br>No adverse findings.<br><br>Parental NOAEL of 2000 ppm (123/197 mg/kg bw/d in ♂/♀)<br>Reproductive NOAEL of 2000 ppm (123/197 mg/kg bw/d in ♂/♀)<br>Offspring NOAEL of 2000 ppm (123/197 mg/kg bw/d in ♂/♀) | |

*NB: The values for NOAEL are provided for information only: they are the values derived from the DAR for flutianil. ↓ = decrease compared to control. ↑= increase compared to control.*

**Reproduction range-finder study in rats ([redacted], (2009) [38])**

In a GLP range-finding study, flutianil was administered on a continuous basis in the diet for up to 11 weeks to parental animals (8/sex/dose group) from three weeks prior to mating through to weaning of $F_1$ pups. Dose levels were 20, 200, 2000, 20,000 ppm. Male rats were administered treated diet for up to 3 weeks prior to mating; therefore the effects of flutianil on male reproduction could not be determined from this study.

Parental findings: There were no mortalities or clinical signs of toxicity. In parental females there was a significant increase in relative liver weights in the 2000 and 20,000 ppm dose groups which increased by 13% and 16%, respectively.

Reproductive performance: There were no treatment-related changes in reproductive performance, with oestrus cycles, mating, fertility and gestation indices unaffected. Duration of gestation, number of implantation sites and sex ratio of pups in treated groups were all comparable to the control group.

Offspring: There were no statistical differences in organ weights between the control and treated groups; however there was a dose-related trend for increased absolute and relative uterus weights of $F_1$ female weanlings. In the high dose group a 31% and 27% increase in absolute and relative uterus weights, respectively, were observed.

**Two-generation reproduction study in rats ([redacted], (2009) [39])**

In a GLP and guideline compliant rat two-generation study, flutianil was administered on a continuous basis in the diet from 10 weeks prior to mating in parental animals (24/sex/dose group), at 4 weeks of age from pre-mating through to weaning of $F_1$ pups. Dose levels were 20, 200, 2000, 20,000 ppm.

Parental findings: There were no mortalities and no clinical signs of toxicity. Rats in the parental generations (P and $F_1$) showed increased absolute and relative liver weights at the top dose. Parental male rats showed a higher frequency of hepatocyte centrilobular hypertrophy at 20,000 ppm.

Hyaline droplet deposition in proximal tubular cells of the kidney was observed in males from 2000 ppm. This finding was also observed in the 90-day and 52 week rat studies, where the hyaline droplets stained positive for $\alpha_{2\mu}$-globulin indicating that the findings in the kidney are due to $\alpha_{2\mu}$-globulin nephropathy, which is a condition specific to male rats and not relevant to humans.

Reproductive findings: In the $F_1$ generation, the mean number of $F_2$ pups delivered in the high dose group (10.0) was significantly lower than that in the concurrent control group (11.8) and also marginally below the laboratory historical control range for this finding (10.4 – 12.8) [40]. However, in the absence of effects on any other reproductive parameters and given this finding was only just outside the laboratory historical control range, did not occur in $F_1$ pups and was noted at a dietary concentration well in excess of the limit dose, it is unlikely to represent a significant effect on reproduction.

There was no evidence of any treatment-related changes in sperm-parameters in the P and $F_1$ males. There were no other treatment-related changes in oestrus cycles, mating, fertility or gestation indices.

Offspring: There were no treatment related clinical signs or increases in pup mortality. Terminal body weights were comparable to control animals. A slight increase in absolute and relative uterus weights occurred in the $F_1$ and $F_2$ weanlings in the top dose group, but failed to reach statistical significance. As these values were within the laboratory historical control ranges, these changes were not considered treatment related [40].

Overall, in this guideline two-generation study in the rat, with the exception of a slight decrease in the mean number of $F_2$ pups delivered, there were no significant effects on fertility and reproductive performance up to the top dose of 20,000 ppm (1286/2002 mg/kg bw/day in males/females) at which liver and kidney toxicity occurred. No offspring toxicity was observed up to the top dose.

#### 4.11.1.2 Human information

No relevant data available.

### 4.11.2 Developmental toxicity

#### 4.11.2.1 Non-human information

The developmental toxicity of flutianil has been investigated in rats and rabbits.

**Table 25: Overview of developmental toxicity studies**

| Method | Results (effects of major toxicological significance) | Remarks [reference] |
|---|---|---|
| Range finder study:<br>**Rat (Wistar)** | No maternal or developmental toxicity observed up to highest dose tested. | Well conducted, GLP-compliant study |

| Method | Results (effects of major toxicological significance) | Remarks [reference] |
|---|---|---|
| (8 animals/sex/gp)<br>(dosing: 8 Mar 2004 – 25 Mar 2004)<br><br>oral: gavage<br><br>0, 100, 333, 1000 mg/kg bw/d<br><br>no relevant guideline, GLP | | Purity: 99.38%<br>[41] |
| Main study:<br>**Rat (Wistar)**<br>(25 animals/sex/gp)<br>(dosing: 21 Jun 2004 – 6 Jul 2004)<br><br>oral: gavage<br><br>0, 100, 333, 1000 mg/kg bw/d (analytical conc.)<br><br>OECD 414 (2001), GLP | **Maternal findings:**<br>No adverse effects up to the highest dose tested (1000 mg/kg bw/day)<br><br>**Developmental findings:**<br>**1000 mg/kg bw/d:**<br>↑ asymmetric sternal centra (2/22 litters *vs*. 0/21 in control; 2/135 foetus *vs*. 0/114 in control)<br><br>**333 mg/kg bw/d:**<br>↑ asymmetric sternal centra (1/22 litters *vs*. 0/21 in control; 1/129 foetus *vs*. 0/114 in control)<br><br>NOAEL for maternal effects of 1000 mg/kg bw/d<br>NOAEL for developmental effects of 100 mg/kg bw/d | Well conducted, GLP-compliant study<br><br>Purity: 99.38%<br>[42] |
| Range finder study:<br>**rabbit (New Zealand White)**<br>(6 animals/sex/gp)<br>(dosing: 6 Jan 2007 – 28 Jan 2007)<br><br>oral: gavage<br><br>0, 100, 300, 1000 mg/kg bw/d (analytical conc.)<br><br>no relevant guideline, GLP | No maternal or developmental toxicity observed up to highest dose tested. | Well conducted, GLP-compliant study<br><br>Purity: 99.22%<br>[43] |
| Main study:<br>**rabbit** (New Zealand White)<br>(25 animals/sex/gp)<br>(dosing: 19 Feb 2007 – 15 Mar 2007)<br><br>oral: gavage<br><br>0, 100, 300, 1000 mg/kg bw/day<br><br>OECD 414 (2001), GLP | **Maternal findings:**<br>No adverse effects up to the highest dose tested.<br><br>**Developmental findings:**<br>**1000 mg/kg bw/day:**<br>3 pups in 1 litter with visceral hydrocephaly (no incidence in control)<br><br>**300 mg/kg bw/day:**<br>No adverse effects.<br><br>**100 mg/kg bw/day:**<br>1 pup with visceral hydrocephaly (this pup also had multiple malformations)<br><br>NOAEL for maternal effects of 1000 mg/kg bw/d<br>NOAEL for developmental effects of 300 mg/kg bw/d | Well conducted, GLP-compliant study<br><br>Purity: 99.22%<br>[44] |

*NB: The values for NOAEL are provided for information only: they are the values derived from the DAR for flutianil. ↓ = decrease compared to control. ↑= increase compared to control.*

**Developmental toxicity range finder in rats ([redacted], (2006) [41])**

In a GLP study conducted to select dose levels for the main developmental toxicity study in rats, flutianil was administered by oral gavage to pregnant Wistar rats (8 females/group) from gestation day (GD) 6 to 19, Dose levels were 0, 100, 333 and 1000 mg/kg bw/day.

There were no treatment related effects at any dose.

**Developmental toxicity main study in rats ([redacted], (2006) [42])**

In a GLP and guideline compliant study, flutianil was administered by oral gavage to pregnant rats (25 females/group) from GD 6 to 19 at a dose of 100, 333 and 1000 mg/kg bw/day.

No mortality was observed in the dams, with all rats surviving to the scheduled necropsy. No discernible effects on maternal bodyweight/bodyweight gain or food consumption were observed. All clinical signs were considered unrelated to treatment.

In the foetus, there was a low incidence of skeletal variations (asymmetry) of the sternal centra at 333 and 1000 mg/kg bw/day. Relation to treatment of these findings cannot be excluded (refer to Section 4.11.4 for further discussion).

In conclusion, a very low incidence of only one type of skeletal variation (asymmetry of the sternal centra) occurred in the rat from 333 mg/kg bw/day in the absence of maternal toxicity.

**Developmental toxicity range finder in rabbits ([redacted], (2007) [43])**

In a GLP range-finding study, flutianil was administered by oral gavage to time-mated pregnant New Zealand White rabbits (6 females/group) from GD 6 to 28. Dose levels were 0, 100, 300, 1000 mg/kg bw/day.

There were no treatment related effects at any dose.

**Developmental toxicity main study in rabbits ([redacted], (2007) [44])**

In a GLP and guideline compliant study, flutianil was administered by oral gavage to time-mated pregnant New Zealand White rabbits (25 females/group) from GD 6 to 28. Dose levels were 0, 100, 300, 1000 mg/kg bw/day.

No discernible effects on maternal bodyweight/bodyweight gain or food consumption were observed. No treatment-related gross pathological findings were evident in any dose group at necropsy on GD 29 in the dams.

The top dose group had a slightly increased incidence of post-implantation loss (mean 0.9 pups/dam *vs.* mean of 0.4 pups/dam in controls). This increased loss was mainly due to the increased number of late resorptions (mean of 0.5 pups/dam *vs.* mean of 0 pups/dam in controls), which was attributed to a single animal which had 11 late resorptions. Therefore, this finding is regarded as incidental.

The total number of foetuses with any malformation was the same in the top dose and control groups (4 foetuses in 2 litters at 1000 mg/kg bw/day *vs.* 4 foetuses in 4 litters in controls) (Table 26).

**Table 26: Summary of malformations in the rabbit development toxicity main study**

| Parameter | Dose level (mg/kg bw/day) | | | |
|---|---|---|---|---|
| | 0 | 100 | 300 | 1000 |
| No. of litters examined | 25 | 20 | 22 | 22[a] |

| Parameter | Dose level (mg/kg bw/day) | | | |
|---|---|---|---|---|
| | 0 | 100 | 300 | 1000 |
| No. of fetuses examined | 219 | 173 | 187 | 185 |
| External malformations (%/litter ±sd) [no. of fetuses affected/no. of litters affected) | | | | |
| Fetal oedema (localised) | (0 ±0)<br>[0/0] | (0.6 ±2.8)<br>[1/1] | (0 ±0)<br>[0/0] | (0 ±0)<br>[0/0] |
| Micropthalmia and/or anopthalmia | (0 ±0)<br>[0/0] | (0.6 ±2.8)<br>[1/1] | (0 ±0)<br>[0/0] | (0 ±0)<br>[0/0] |
| Visceral malformations (%/litter ±sd) [no. of fetuses affected/no. of litters affected) | | | | |
| Hydrocephaly | (0 ±0)<br>[0/0] | (0.6 ±2.8)<br>[1/1] | (0 ±0)<br>[0/0] | (1.5 ±7.1)<br>[3/1] |
| Interventricular septal defect | (0 ±0)<br>[0/0] | (0.6 ±2.8)<br>[1/1] | (0 ±0)<br>[0/0] | (0 ±0)<br>[0/0] |
| Skeletal malformations | | | | |
| Sternebrae fused | (0.7 ±3.3)<br>[1/1] | (0.6 ±2.8)<br>[1/1] | (0 ±0)<br>[0/0] | (0.5 ±2.1)<br>[1/1] |
| Vertebral anomaly with or without associated rib anomaly | (0.4 ±2.0)<br>[1/1] | (0.6 ±2.8)<br>[1/1] | (0 ±0)<br>[0/0] | (0 ±0)<br>[0/0] |
| Total no. of fetuses with any malformation | (1.8 ±4.5)<br>[4/4] | (0.6 ±2.8)<br>[1/1] | (1.8 ±4.8)<br>[3/3] | (2.0 ±7.2)<br>[4/2] |

Values expressed as in () = mean % per litter ± standard deviation
Values in [] = number of foetuses/litters affected
a 23 ♀ survived to necropsy but one ♀ (animal no. 48771) had no viable foetuses

In the 1000 mg/kg bw/day group, three foetuses in the same litter had visceral hydrocephalus (presenting as increased cavitation of the lateral, bilateral and third ventricles). Visceral hydrocephalus was also seen in one foetus (in one litter) at 100 mg/kg bw/day (Table 26). Relevant laboratory historical control data in time mated rabbits show a maximum incidence of 2 foetuses in a single litter for this malformation (Table 27). Therefore, although this finding was seen only in one litter at the top dose and in one litter at the low dose, the foetal incidence (3/185) at the top dose exceeded the relevant historical control range (maximum 2/189). On this basis, the occurrence of this malformation in 3 foetuses at the top dose was considered to be treatment-related. The hydrocephalus observed in the low dose group occurred in one foetus with multiple malformations (localised oedema of thorax, bilateral micropthalmia, interventricular septal defect) and was within the relevant historical control range. Therefore, this finding at the low dose was considered to have arisen naturally.

**Table 27: Summary Relevant laboratory historical control data for visceral hydrocephaly in time mated New Zealand White rabbits.**

| Study date | Total no. of fetuses (litters) examined | Visceral hydrocephaly incidence/study (no. of fetuses /no. of litters) | Study date | Total no. of fetuses (litters) examined | Visceral hydrocephaly incidence/study (no. of fetuses /no. of litters) |
|---|---|---|---|---|---|
| Dec 03 – Jan 04 | 158 (21) | 0/0 | Feb 05 – Mar 05 | 163 (19) | 0/0 |
| Mar 04 – Apr 04 | 190 (21) | 1/1 | Mar - Apr 05 | 204 (23) | 0/0 |
| Mar 04 – Apr 04 | 182 (21) | 1/1 | Jan 05 – Feb 05 | 209 (25) | 0/0 |

| Study date | Total no. of fetuses (litters) examined | Visceral hydrocephaly incidence/study (no. of fetuses /no. of litters) | Study date | Total no. of fetuses (litters) examined | Visceral hydrocephaly incidence/study (no. of fetuses /no. of litters) |
|---|---|---|---|---|---|
| Jun 04 – Jul 04 | 185(20) | 0/0 | May 05 – Jun 05 | 173 (18) | 0/0 |
| Aug 04 – Sept 04 | 166 (20) | 0/0 | Jun 05 – Jul 05 | 183 (22) | 0/0 |
| Aug 04 – Sept 04 | 177 (20) | 0/0 | Oct 05 – Nov 05 | 157 (20) | 0/0 |
| Aug 04 – Oct 04 | 197 (22) | 0/0 | Jan 06 – Feb 06 | 161 (20) | 0/0 |
| Jun 04 – Jul 04 | 177 (20) | 1/1 | Sept 05 - Oct 05 | 165 (19) | 0/0 |
| Apr 04 – May 04 | 191 (22) | 0/0 | Feb - Mar 06 | 173 (21) | 0/0 |
| Aug 04 – Sept 04 | 177 (20) | 0/0 | Nov - Dec 05 | 153 (18) | 0/0 |
| Dec 04 – Jan 05 | 182 (19) | 0/0 | Mar - Apr 06 | 189 (22) | 1/1 |
| Apr 05 – May 05 | 165 (20) | 0/0 | Apr - May 06 | 158 (22) | 0/0 |
| May 05 – Jun 05 | 147 (18) | 0/0 | May - Jun 06 | 155 (20) | 0/0 |
| Jun 05 – Jul 05 | 184 (20) | 0/0 | May - Jun 06 | 153 (22) | 0/0 |
| Feb 05 – Mar 05 | 189 (22) | 2/1 | | | |

There was no treatment-related increase in variations.

In conclusion, there was a slight increase in the foetal incidence of visceral hydrocephalus at the top dose of 1000 mg/kg bw/day in rabbits in the absence of maternal toxicity. Although this increase marginally exceeds (by 1 foetus) the historical control range and there is no difference in the total number of foetuses with any malformations between this dose group and the controls, relation to treatment cannot be excluded.

#### 4.11.2.2 Human information

No relevant data available.

### 4.11.3 Other relevant information

Minor findings in the reproductive organs were reported in mice, rats and dogs in the available guideline repeated dose toxicity studies (see Section 4.7). As these may be indicative of reproductive toxicity and are therefore more relevant to classification for reproductive toxicity, they are described here as well.

In the mouse, testis atrophy was noted in single males in a 90-day study from a dose of 409 mg/kg bw/day, but the incidence was within the laboratory historical control range. Testis atrophy was also noted in the chronic toxicity/carcinogenicity study at the top dose of 1086 mg/kg bw/day, but, again, it was considered unrelated to treatment as it fell within the laboratory historical control range.

In the rat chronic/carcinogenicity study, isolated histopathological findings of the uterus (cysts, luminal dilatation, hyperplasia and polyps) were seen in females at 1130 mg/kg bw/day and a slight increase in the incidence of histopathological findings of the male reproductive organs (atrophy of testes, seminal vesicle and coagulating gland and oligospermia of epididymis) was observed at the top dose of 249 mg/kg bw/d. Given the low incidences of these isolated findings in the uterus and male reproductive organs, it is unclear whether these observations were treatment-related or incidental.

In the dog, organ weight changes of testis, prostate and uterus, and histopathological findings in testes (atrophy of seminiferous tubules) and prostate (cell infiltration) were seen from relatively low doses (10-30 mg/kg bw/day) in the 28-day and 90-day studies, but were not confirmed in the 1-year study at similar dose levels after a much longer period of treatment. Therefore, these findings were considered to be of no toxicological significance.

### 4.11.4 Summary and discussion of reproductive toxicity

The reproductive toxicity of flutianil has been investigated in a guideline multi-generation study in the rat and in guideline developmental toxicity studies in rats and rabbits. Further relevant information is also available from guideline repeated dose toxicity studies in mice, rats and dogs (see Section 4.7).

**Reproduction performance and fertility**

In a guideline two-generation study in rats, there were no significant effects on fertility and reproductive performance up to the top dose of 20,000 ppm (1286/2002 mg/kg bw/day in males/females) at which liver and kidney toxicity occurred. The mean number of $F_2$ pups delivered in the high dose group (10.0) was significantly lower than that in the concurrent control group (11.8) and also marginally below the laboratory historical control range for this finding (10.4 – 12.8). However, in the absence of effects on any other reproductive parameters and given this finding was only just outside the laboratory historical control range, did not occur in $F_1$ pups and was noted at a dietary concentration well in excess of the limit dose, it can be concluded that there is insufficient evidence of an effect of flutianil on reproduction in this study.

Minor findings in the reproductive organs were reported in mice, rats and dogs in the available guideline repeated dose toxicity studies (see Section 4.7).

In the mouse, testis atrophy was noted in single males in a 90-day study from a dose of 409 mg/kg bw/day, but the incidence was within the laboratory historical control range. Testis atrophy was also noted in the chronic toxicity/carcinogenicity study at the top dose of 1086 mg/kg bw/day, but, again, it was considered unrelated to treatment as it fell within the laboratory historical control range.

In the dog, organ weight changes of testis, prostate and uterus, and histopathological findings in testes (atrophy of seminiferous tubules) and prostate (cell infiltration) were seen from relatively low doses (10-30 mg/kg bw/day) in the 28-day and 90-day studies, but were not confirmed in the 1-year study at similar dose levels after a much longer period of treatment. Therefore, these findings were considered to be of no toxicological significance.

In the rat chronic/carcinogenicity study, isolated histopathological findings of the uterus (cysts, luminal dilatation, hyperplasia and polyps) were seen in females at 1130 mg/kg bw/day and a slight increase in the incidence of histopathological findings of the male reproductive organs (atrophy of testes, seminal vesicle and coagulating gland and oligospermia of epididymis) was observed at the top dose of 249 mg/kg bw/day. Given the low incidences of these isolated findings in the uterus and male reproductive organs, it is unclear whether these observations were treatment-related or incidental. However, after taking into account that they were not reproduced in the rat two-generation study, in which no clear functional effects on fertility were observed, it can be concluded that these findings in the reproductive organs of rat do not represent a hazard to reproduction.

Overall, therefore, the available evidence shows that flutianil has no effects on reproductive performance and fertility.

**Development**

In a guideline developmental toxicity study in rats, a very low incidence of only one type of skeletal variation (asymmetry of the sternal centra) was noted from a dose 333 mg/kg bw/day in the absence of maternal toxicity. These findings are considered to be of minimal toxicological significance and do not represent a significant developmental hazard.

In a guideline developmental toxicity study in rabbits, there was a slight increase in the foetal incidence of visceral hydrocephalus at the top dose of 1000 mg/kg bw/day (3 foetuses in 1 litter *vs.* 0 in controls) in the absence of maternal toxicity. Although this increase marginally exceeds the historical control range (maximum of 2 foetuses in a single litter) and there is no difference in the total number of foetuses with any malformations between this dose group and the controls, relation to treatment of this malformation cannot be excluded.

Overall, there is limited evidence that flutianil is a developmental toxicant in rabbits.

### 4.11.5 Comparison with criteria

**Fertility**

Comparison with criteria for Category 1A classification: In accordance with the criteria in the CLP regulation, classification in reproductive toxicity Category 1A is reserved for substances known to be reproductive toxicants in humans. Since there is no evidence of flutianil having caused reproductive toxicity in humans, classification in Category 1A is not justified.

Comparison with criteria for Category 1B classification: In accordance with the criteria in the CLP regulation, classification in reproductive toxicity Category 1B is reserved for substances that are presumed to be reproductive toxicants in humans, and is largely based on data from animal studies where there is *clear* evidence of an adverse effect on sexual function and fertility in the absence of other toxic effects, or not as a secondary non-specific consequence of other toxic effects. There is insufficient evidence of an effect of flutianil on reproductive performance and fertility from the available multi-generation study and repeated dose toxicity studies. Therefore, classification of flutianil for fertility in Category 1B is not justified.

Comparison with criteria for Category 2 classification: In accordance with the criteria in the CLP regulation, classification in reproductive toxicity Category 2 is reserved for substances where there is *some* evidence from experimental animals of an adverse effect on sexual function and fertility but where the evidence is not sufficiently convincing to place the substance in Category 1. Any effects should be in the absence of other toxic effects, or not as a secondary non-specific consequence of other toxic effects. The available evidence shows that flutianil has no effects on reproductive performance and fertility. Therefore, classification of flutianil for fertility in Category 2 is not justified.

**Development**

Comparison with criteria for Category 1A classification: In accordance with the criteria in the CLP regulation, classification in reproductive toxicity Category 1A is reserved for substances known to be developmental toxicants in humans. Since there is no evidence of flutianil having caused developmental toxicity in humans, classification in Category 1A is not justified.

Comparison with criteria for Category 1B classification: In accordance with the criteria in the CLP regulation, classification in reproductive toxicity Category 1B is reserved for substances that are presumed to be developmental toxicants in humans, and is largely based on data from animal studies where there is *clear* evidence of an adverse effect on development in the absence of other toxic effects, or not occur as a secondary non-specific consequence of other toxic effects. Flutianil is not a developmental toxicant in rats. In rabbits, there is only limited evidence

that flutianil poses a developmental hazard. Although a slight increase in visceral hydrocephalus was seen at the top dose of 1000 mg/kg bw/day (3 foetuses in 1 litter *vs* 0 in controls) in the absence of maternal toxicity, this increase only marginally exceeded the historical control range (maximum of 2 foetuses in a single litter). In addition, there was no difference in the total number of foetuses with any malformations between this dose group and the controls, casting further doubt on its relation to treatment. Overall, therefore, classification of flutianil for developmental toxicity in Category 1B is not justified.

Comparison with criteria for Category 2 classification: In accordance with the criteria in the CLP regulation, classification in reproductive toxicity Category 2 is reserved for substances where there is *some* evidence from experimental animals of an adverse effect on development but where the evidence is not sufficiently convincing to place the substance in Category 1. Any effects should be in the absence of other toxic effects, or not occur as a secondary non-specific consequence of other toxic effects. Flutianil is not a developmental toxicant in rats. In rabbits, there is only limited evidence that flutianil poses a developmental hazard (see above). On this basis, classification of flutianil for developmental toxicity Category 2 is justified.

#### 4.11.6 Conclusions on classification and labelling

**Conclusive - Reproductive toxicant Category 2 H361d - Suspected of damaging the unborn child.**

### 4.12 Other effects

#### 4.12.1 Non-human information

##### 4.12.1.1 Neurotoxicity

No acute, sub-acute or delayed neurotoxicity studies have been conducted with flutianil, as the chemical structure of this molecule has no relationship with compounds known to induce neurotoxicity or delayed neurotoxicity. In addition, no specific clinical signs of toxicity indicative of neurological effects have been seen in the toxicity tests in rodents or dogs.

##### 4.12.1.2 Immunotoxicity

No immunotoxicity studies have been conducted with flutianil as no evidence of immunological effects have been seen in the toxicity tests in rodents or dogs

##### 4.12.1.3 Specific investigations: other studies

No relevant information available.

##### 4.12.1.4 Human information

No relevant information available.

# 5 ENVIRONMENTAL HAZARD ASSESSMENT

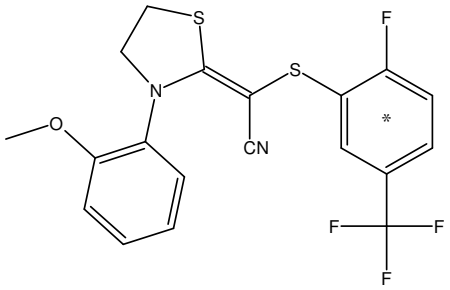
## 5.1 Degradation

The environmental fate properties assessment for flutianil is based on the Draft Assessment Report, the Addendum to the Draft Assessment Report and the EFSA Scientific Report on the peer review of flutianil [1].

Note that references in the CLH report have been redacted to protect confidential information where necessary.

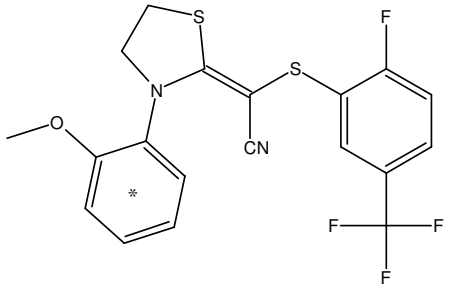
All of the studies on the fate and behaviour of flutianil in the environment were performed under GLP and according to the appropriate guidelines. They are considered to be sufficient and reliable for hazard classification purposes. Radiolabelled studies were conducted with flutianil labelled in two different positions with a specific radioactivity of 3770 MBq/g and a radiochemical purity of >99% and [$CF_3$Ph-U-$^{14}$C]-flutianil with a specific radioactivity of 3772 MBq/g and a radiochemical purity of >99%. The labelling position can be seen in the structure below.

Structure of [$CF_3$Ph-U-$^{14}$C]-Flutianil:




Site of labelling (*)

Structure of [MeOPh-U-$^{14}$C]-flutianil are shown below:




Site of labelling (*)

**Table 28: Summary on the relevant information on degradation**

| Method | Results | Remarks | Reference |
|---|---|---|---|
| Stability | | | |
| Hydrolysis<br>OECD 111 (2004), GLP | pH 4 and pH 7: Hydrolytically stable at 50°C. (>99% remaining after 7 days)<br>pH 9: Hydrolytically stable at 50°C. (97.5% remaining after 7 days) | MeOPh-U-$^{14}$C]-flutianil (>99%) | [45] |
| Aqueous photolysis<br>OECD 316 (2000) [draft guidance], GLP | $DT_{50}$: 1.1 – 1.2 days in natural water with radiation source adjusted to UK/US (25 w/$m^2$) | [MeOPh-U-$^{14}$C]-flutianil (98.95%) | [46] |

| Method | Results | Remarks | Reference |
|---|---|---|---|
| | | and [$CF_3$Ph-U-$^{14}$C]-flutianil (98.9%) | |
| Soil photolysis<br>SETAC (1995), GLP | Flutianil was degraded by photolysis from 91.8% to 69.1% AR over 45 days with the MeOPh labelled test material and from 97.4% to 68.3% AR over 45 days with the CF3Ph labelled test material. There was minimal degradation in the dark controls. | [MeOPh-U-$^{14}$C]-flutianil (98.95%) and [$CF_3$Ph-U-$^{14}$C]-flutianil (98.9%) | [47] |
| Biodegradation | | | |
| Ready biodegradability<br>OECD 301B (1992), GLP | Not readily biodegradable. | Flutianil 99.22% | [48] |
| Aerobic water/sediment<br>OECD 308 (2002), GLP | Dissipation from water $DT_{50}$ <1 day (MeOPh and $CF_3$Ph labelling positions).<br>Degradation in sediment $DT_{50}$ >1000 days (MeOPh and $CF_3$Ph labelling positions).<br>Degradation whole system $DT_{50}$ 504 and 651 day (MeOPh label), 550 and 725 days ($CF_3$Ph label).<br>Mineralization / bound residue<br>System 1(MeOPh) 2.5% / 14.8%<br>System 1($CF_3$Ph) 0.2% / 19.7%<br>System 2(MeOPh) 0.6% / 5.5%<br>System 2($CF_3$Ph) 0.1% / 6.0% | [MeOPh-U-$^{14}$C]-flutianil (98.9%) and [$CF_3$Ph-U-$^{14}$C]-flutianil (99%) | [49] |
| Aerobic soil degradation<br>OECD 307 (2002), GLP | Flutianil degraded in soil with a normalized $DT_{50}$ ranging from 261.2 to 338.4 days (n=4). Geometric mean $DT_{50}$ of 297.3 days | [MeOPh-U-$^{14}$C]-flutianil (>99%) and [$CF_3$Ph-U-$^{14}$C]-flutianil (>99%) | [50] |
| Terrestrial field dissipation<br>Council Directive 91/414/EEC of 15 July 1991, GLP | The rate of Flutianil degradation in the field varied considerably and did not follow first order kinetic at most sites. The best fit $DT_{50}$ values ranged from 0.083 to 161.1 days from 6 trial sites. The variation was considered to be due to photo degradation which was more pronounced in the Southern EU trials. | Flutianil 5% EC<br>Purity: 4.99% (w/v) | [51], [52] |

### 5.1.1 Stability

#### 5.1.1.1 Hydrolysis

In a standard OECD 111 hydrolysis study, performed reliably and to GLP [45], flutianil was shown to be hydrolytically stable at pH 5, 7 and 9 at 50°C. At pH 9 a small amount of degradation was observed after 7 days, with flutianil decreasing to 97.5% of the applied dose.

#### 5.1.1.2 Dissociation constant

Not relevant for flutianil due to low water solubility and lack of chemical groups in its structure that could be easily ionised.

#### 5.1.1.3 Aqueous photolysis

The aqueous photolysis study [46] was conducted in accordance with OECD 316 (2000) [draft guidance] and to GLP with both [MeOPh-U-$^{14}$C]-flutianil and [$CF_3$Ph-U-$^{14}$C]-flutianil using natural surface water and sterile aqueous buffer solution (0.02 M phosphate buffer, pH 7) ([redacted] (2007)). The test was run for 32 days using light source radiation of 300 - 400 nm with the intensity of 25.1 watts/$m^2$. It was considered that 1 day of continuous test lamp irradiation was approximately equivalent to the amount of sunlight radiation that would be experienced in one day in the UK/US. Despite some problems with low mass balances, which were assume to be due to the loss of volatile material, it is clear that flutianil degrades by photolysis with a $DT_{50}$ of just over 1 day. The study indicates that under suitable conditions sunlight may contribute to the dissipation of flutianil in the aqueous environment

#### 5.1.1.4 Soil photolysis

The soil photolysis of flutianil was conducted in accordance with the draft OECD guideline and to GLP [47] by treating a thin layer of dry soil with both [MeOPh-U-$^{14}$C]-flutianil and [$CF_3$Ph-U-$^{14}$C]-flutianil and irradiating with a xenon light source with wavelengths <290 nm removed. The intensity was adjusted so that light received in 24 hours was equivalent to one days natural summer sunlight at 30 to 50°N. Results from the irradiated samples were compared with dark controls. After 45 days flutianil declined from 91.8% to 69.1% of the applied radioactivity with the MeOPh labelled treatment and declined from 97.4 to 68.3% with the $CH_3$Ph labelled treatment. There was minimal degradation in the dark controls. Mineralisation reached 10.4% and 3.1% of AR in the MeOPh and the $CH_3$Ph labelled treatment respectively after 45 days. Unextracted residues increased to 7.6% and 7.4% of AR in the MeOPh and the $CH_3$Ph labelled treatment respectively. A significant photolysis product was detected from the $CH_3$Ph labelled treated soil at a maximum of 10.7% AR. In subsequent field dissipation trials photolysis was shown to be a significant route of degradation under certain climatic conditions.

### 5.1.2 Biodegradation

#### 5.1.2.1 Biodegradation estimation

The ready biodegradation of flutianil was studied according to OECD guidelines (No. 301B, $CO_2$ Evolution Test) and GLP [48]. Under the conditions of the test, 0% of the theoretical $CO_2$ from flutianil was produced at day 28. The mean biodegradation of the reference substance, sodium benzoate, was 73% on day 14 and 88% on day 28. The biodegradation of the reference substance, sodium benzoate, indicated that the test system was suitable for determining biodegradation. Also in the toxicity control the degradation of the reference substance, sodium benzoate was practically unaffected by the presence of Flutianil. Therefore flutianil cannot be classed as readily biodegradable.

#### 5.1.2.2 Simulation tests

##### 5.1.2.2.1 Water/sediment systems

Aerobic sediment/water studies [49] were conducted in accordance with OECD 308 and to GLP with two different water-sediment systems, described as ‘Site A’ and ‘Swiss Lake’ with both [MeOPh-U-$^{14}$C]-flutianil and [$CF_3$Ph-U-$^{14}$C]-flutianil. Site A is a silt loam sediment with moderate to high organic carbon (4.2%) and a water pH of 8.14. The Swiss Lake is a sandy

sediment, but with a low carbon content (0.6%) and a water pH of 5.99. Site A is considered to be a fine sediment and Swiss Lake is considered to be 'coarse' in the terms of OECD 308.

The distribution and degradation of [MeOPh-U-$^{14}$C]-flutianil (label 1) and [$CF_3$Ph-U-$^{14}$C]-flutianil (label 2) was studied in each of the systems in accordance with the OECD 308 guidance.

In the Site A system, recovery of radioactivity was 95.1% - 101.1% for samples treated with [MeOPh-U-$^{14}$C]-flutianil and 91.2% - 97.7% for samples treated with [$CF_3$Ph-U-$^{14}$C]-flutianil.

In the Swiss Lake system, recovery of radioactivity was 93.9% - 98.2% for samples treated with [MeOPh-U-$^{14}$C]-flutianil and 93.2% - 99.6% for samples treated with [$CF_3$Ph-U-$^{14}$C]-flutianil.

The radioactivity in the Site A water steadily decreased from 52.5% / 49.8% AR (label 1 / label 2) at day 0 to 7% / 6.3% AR at the end of the 100 day incubation period. The radioactivity in the Swiss lake water decreased from 51.1% / 55.1% AR at day 0 to 7.0% / 6.8% AR at study termination.

Extractable $^{14}$C residues [MeOPh-U-$^{14}$C/$CF_3$Ph-U-$^{14}$C] in the Site A sediment increased from 42.6% / 44.8% AR at day 0 to a maximum of 88.9% / 85.7% AR at day 61 / day 30 respectively and then decreased to 74.1% / 84.9% AR at study termination. Extractable $^{14}$C residues in the Swiss Lake sediment increased from 43.4% / 38.2% AR at day 0 to a maximum of 86.2% / 87.0% AR at day 100 / 61 respectively.

Non-extractable $^{14}$C residues in Site A sediments were 14.8% / 19.7% AR at day 100 and day 61 respectively. Non-extractable $^{14}$C residues in Swiss Lake sediments were 5.5% / and 6.0% / AR at day 61 and day 100 respectively.

Total volatiles for Site A were a maximum of 2.5% / 0.2% at day 100 and 61 respectively. Total volatiles for Swiss lake were a maximum of 0.6% / 0.1% AR at day 61 and day 30 respectively.

In the Site A water the flutianil concentration decreased from 51.7% / 49.3% AR at day 0 to 2.0% / 2.0% of the applied radioactivity at day 61 for [MeOPh-U-$^{14}$C / $CF_3$Ph-U-$^{14}$C] respectively. The water was not assessed for flutianil at study termination (presumably because of the low levels anticipated). In the Swiss Lake water the flutianil concentration decreased from 49.4% /53.2% AR to 4.5% / 3.8% AR at study termination for [MeOPh-U-$^{14}$C/$CF_3$Ph-U-$^{14}$C] respectively.

In the Site A sediment the concentration of flutianil increased from 41.3% /43.5% AR to 94.6% / 83.8% AR at day 30 /day 30 and then declined to 71.9% / 81.0% AR at study termination. However, unexpectedly high levels of AR were unextracted in sediment samples for Site A at day 100 [MeOPh-U-$^{14}$C]-flutianil and day 61 ($CF_3$Ph-U-$^{14}$C]-flutianil at 14.8% /19.7% AR respectively.

Similar metabolites were identified in the sediment as were found in the soil metabolism studies: OC 53276, OC 53279 and OC 56574. The formation and occurrence of the metabolites suggests they are formed in the sediment and then partition into the water

The summary of the kinetic evaluation of the flutianil degradation is presented in Table 29 below:

**Table 29: Summary of the kinetic evaluation of the degradation of flutianil**

| Water / sediment system | pH water phase | pH sed | t. °C | $DT_{50}$-$DT_{90}$ whole sys. | % $\chi^2$ | $DT_{50}$-$DT_{90}$ water | % $\chi^2$ | $DT_{50}$-$DT_{90}$ sed | Method of calculation |
|---|---|---|---|---|---|---|---|---|---|
| Site A | 8.14 | 7.3 | 20 | 504/550† - 1673/1826† | 0.516 / 0.804† | <1/<1† - 15/14† | 0.946 / 0.917† | 1000 days | SFO |
| Swiss Lake | 5.99 | 6.0 | 20 | 651/752† - 2162/2498† | 0.441 / 0.463† | <1/ <1† - 19/26† | 0.799 / 0.821† | 1000 days | SFO |
| Geometric mean/media | | | | 607 / 2015 | | 1 / 18 | | | |

† MeOPh and $CF_3$Ph label respectively
SFO = Single First Order kinetics

The physical properties of flutianil (low water solubility and high Kfoc), indicate that the $DT_{50}$ of <1 days in water phase is due to rapid partition of flutianil into sediment. Degradation appears to take place in the sediment and was similar to soil in terms of metabolites formed. The structures of the metabolites and the proposed metabolic pathway for flutianil in water-sediment systems is presented in Annex II.

#### 5.1.2.2.2 Aerobic soil metabolism

The route and rate of degradation of OK-5203 (flutianil) was studied in a single soil with the rate of degradation also investigated in a further 3 soils under aerobic conditions at 20 ± 2°C [50]. The study was undertaken according to OECD307 guideline and to GLP. The four soils were incubated in the dark for a total of 365 days. Biomass determinations were made in all four soils on the day of dosing (because acetonitrile was used as the application solvent), at the end of incubation (day 365) and all soils remained microbially active at study end. The test substance $^{14}$C-flutianil (as either [$CF_3$Ph-U-$^{14}$C]-flutianil or [MeOPh-U-$^{14}$C]-flutianil) was applied at a nominal test concentration of 1.3 µg/50g soil (≡ 0.026 mg flutianil/kg soil, equivalent to a rate of 19.5 g/ha in the field (5 cm depth, 1.5 g/cm$^3$ bulk density). This is slightly less than proposed maximum rate of pesticidal use of 25 g flutianil/ha.

In general, degradation of parent flutianil in aerobic laboratory soils was slow, with between 66% and 77% remaining after 120 days. The data indicate that flutianil is degraded under aerobic conditions in soil forming a single major metabolite at the end of the 120/365 day study – OC 56574. The data also show this metabolite is still increasing after 365 days.

**Table 30: Rate of degradation of flutianil in soil**

| Time point | Soil 1 | Soil 2 | Soil 3 | Soil 4 |
|---|---|---|---|---|
| 0 | 98.8 / 98.4 | 91.1 | 97..5 | 99.1 |
| 14 | 95.5 / 84.3 | 90.7 | 92.4 | 93.4 |
| 30 | 83.7 / 80.2 | 87.4 | 89.0 | 91.0 |
| 58 | 77.6 / 72.0 | 84.2 | 85.3 | 86.9 |
| 90 | 71.8 / 66.5 | 80.4 | 77.3 | 79.4 |
| 120 | 71 / 66.4 | 76.6 | 74.6 | 77.7 |
| 181 | 64.0 / 59.1 | - | - | - |
| 269 | 53.0 / 50.1 | - | - | - |
| 365 | 55.6 / 52.1 | - | - | - |
| Kinetic evaluation using SFO | | | | |

| Time point | Soil 1 | Soil 2 | Soil 3 | Soil 4 |
|---|---|---|---|---|
| Normalised $DT_{50}$ | 312.9 | 338.4 | 281.2 | 262.6 |

SFO = Single First Order

The rate of degradation was recalculated and normalized by the RMS in a number of ways and the final agreed end point values are presented in Table 30. The geometric mean normalised $DT_{50}$ is 297.3 days.

#### 5.1.2.2.3 Field soil dissipation

Field dissipation studies were carried out at sites in Germany, Northern and Southern France and Spain in accordance with GLP ([51], [52]). Spray applications of a nominal 5% EC formulation of flutianil were made to the soil surface on bare soil plots in June at a rate of 240 g formulation/ha. Soil sample were analysed for flutianil and the metabolites OC 53276, OC 56574 and OC 56635. The results were quite variable and flutianil exhibited very low to high persistence. The variability was at least in part considered to be due to the formation of the photolysis product OC 56635.

Field accumulation from applications in successive years was investigated at the German and Spanish sites. After 4 years of applications, parent flutianil residues in the top 10 cm soil layer were 1.6 and 1.1 times higher after the last application at the German and Spanish sites respectively than was measured after the first application. Some difficulty was experienced in deriving acceptable kinetic analysis of the results, however the following results were derived in conjunction with the RMS.

**Table 31: Field studies – Summary of best fit data for flutianil**

| Location and Trial No. | pH ($CaCl_2$) | $DT_{50}$ /$DT_{90}$ (field days) | $\chi^2$ | $DT_{50}$ (20°C, pF2) | $\chi^2$ | Method of calculation |
|---|---|---|---|---|---|---|
| Heidelberg, Germany, 2554/080/1 | 7.4 | 161.1/535.3 | 16.0 | † | † | SFO |
| Carlet, Spain, 2554/080/2 | 7.2 | 2.53/11.46 | 9.8 | † | † | DFOP |
| Heidelberg, Germany, 2554/016/1 | 7.1 | 7.1/520.5 | 16.0 | † | † | DFOP |
| Cosswiller, Northern France, 2554/016/2 | 5.2 | 2.07/10.11 | 7.2 | † | † | DFOP |
| Elne, Southern France, 2554/016/3 | 6.6 | 0.083/2.68 | 3.5 | † | † | FOMC |
| Carlet, Spain, 2554/016/4 | 7.4 | 5.63/520.5 | 10.1 | † | † | DFOP |
| Geometric mean (n=6) | | | | | | |

† Normalisation of the field data showed less robust $Chi^2$ values when the normalised data were re assessed with FOCUS kinetics - the laboratory data were used for modelling calculations

SFO = Single First Order, FOMC = First Order Multi-Compartment, DFOP = Double First-Order in Parallel kinetics

### 5.1.3 Summary and discussion of degradation

Flutianil was found to be stable to hydrolysis, however in an aqueous photolysis study degradation was quite rapid with an estimated half-life of just over 1 day. Photolysis on soil surface was quite slow in a laboratory study with approximately 69% flutianil remaining after 45 days. However in field dissipation studies it was show that photolysis could contribute

significantly to the degradation under certain conditions. Photolysis could be a significant rote of degradation in the environment.

Flutianil was not degraded in the ready biodegradability test and is therefore not considered to be readily biodegradable. In a water sediment study, flutianil rapidly dissipated from the water phase into the sediment with a half-life of <1 day. Flutianil slowly degraded in the sediment to givean overall system $DT_{50}$ of 504 to 752 days. In aerobic soil metabolism study flutianil was again slow to degrade with significant amount of parent remaining even after 365 days. The geometric mean of the estimated $DT_{50}$ values from the laboratory study was 297.3 days. Field dissipation studies with flutianil on bare soil resulted in a degradation rates ranging from 2 days to 161 days, but the data was variable and difficult to interpret. Degradation at some sites was accelerated by photolysis at some sites making evaluation difficult. It is clear that under some circumstances repeated annual application could lead to limited accumulation in the soil.

## 5.2 Environmental distribution

**Table 32: Summary of relevant information on distribution**

| Method | Results | Remarks | Reference |
|---|---|---|---|
| Adsorption/Desorption<br>OECD 106 (2000), GLP | Kfoc ranged from 20631 to 79448 from 5 soils, with an arithmetic mean of 35492. The mean 1/n value was 1.0277. | [MeOPh-U-$^{14}$C]-flutianil (98.95%) | [53] |
| Half-life in air<br>Atkinson calculation, non-GLP | Half-life in air estimated at 3.418 hours under environmental conditions with diurnal cycle of 12 hours. | Calculation | [54] |

### 5.2.1 Adsorption/Desorption

The adsorption/desorption of flutianil was determined for five soils in a GLP compliant report, conducted in accordance with the OECD 106 guideline [53]. Tests were conducted using the batch equilibrium method at 20°C. A summary of the results from the adsorption phase is shown in Table 33.

**Table 33: Adsorption/desorption of flutianil in 5 soils**

| Soil origin: | SK179618 UK | SK566696 UK | Matanuska Alaska | North Dakota, USA | Iberaki, Japan | Mean value |
|---|---|---|---|---|---|---|
| Soil type (UK) | Clay loam | Loamy sand | Silt loam | Silt loam | Loam | - |
| pH (supernatant in adsorption test) | 7.59 | 4.63 | 5.63 | 6.65 | 7.30 | - |
| Organic carbon [%] | 3.8 | 0.8 | 3.2 | 2.4 | 4.4 | |
| $K_{F(ads)}$ [mL/g] | 783.97 | 635.58 | 1093.60 | 533.23 | 923.38 | 793.95 |
| 1/n | 1.0697 | 1.0565 | 1.0434 | 0.9550 | 1.10140 | 0.9911 |
| $Kf_{oc(ads)}$ [mL/g] | 20631 | 79448 | 34175 | 22218 | 20986 | 35492 |

There was no indication of pH dependency in the adsorption to soil and the high $K_{foc}$ values indicate that flutianil is not mobile in soil.

### 5.2.2 Volatilisation

Flutianil has a vapour pressure of 2.58 x $10^{-7}$ and a Henry's Law Constant of 8.259 x $10^{-3}$ Pa.m$^3$ it is therefore considered unlikely that any significant volatilisation will occur plant surfaces, soil

or water. In addition a calculation of the potential for photo-oxidation of flutianil in the atmosphere predicted a first order half-life of 3.42 hours (Atkinson calculation) [54]. Therefore any flutianil that was volatilised would be rapidly degraded.

### 5.2.3 Distribution modelling

Not relevant to classification. Distribution would be dependent on the product and proposed uses and emissions. However, the high $K_{foc}$ values indicate that flutianil is likely to partition rapidly to sediment and soil, as demonstrated in Section 5.1.2.2.

## 5.3 Aquatic Bioaccumulation

According to CLP regulation substances with a log $K_{OW}$ >4 require further evaluation of bioaccumulation potential. Flutianil has a log $K_{OW}$ of 3.1 therefore further assessment is not required, a fish bioaccumulation study has however been conducted under pesticide regulations and is summarised in Table 34.

**Table 34: Summary on the relevant information on degradation**

| Method | Results | Remarks | Reference |
|---|---|---|---|
| Fish bioconcentration<br>OECD 305 (1996), GLP | The kinetic bioconcentration factors (BCFk) for the whole fish were 380 and 345 for 0.5 µg/L and 5.0 µg/L concentrations, respectively. | [MeOPh-U-$^{14}$C]-flutianil (98.6%) | [55] |

### 5.3.1 Aquatic bioaccumulation

#### 5.3.1.1 Bioaccumulation estimation

The substance has a log $K_{OW}$ of 3.1 which implies a moderate bioaccumulation potential. A fish bioconcentration study has been conducted and is summarised in Section 5.3.1.2.

#### 5.3.1.2 Measured bioaccumulation data

A fish bioconcentration study with radiolabelled flutianil has been conducted according to OECD 305 and GLP [55]. *Oncorhynchus mykiss* (rainbow trout) were continuously exposed to [$^{14}$C]-Flutianil, at 2 concentrations, nominally 0.5 µg/L and 5.0 µg/L for a period of 28 days under flow-through conditions. Thereafter, the remaining fish were transferred to untreated tanks containing dilution water only for a depuration period of 15 days under flow through conditions. Uptake of radioactivity within fish tissue was rapid and was similar at both the 0.5 µg/L and 5.0 µg/L concentrations. A plateau was reached during the 28 day exposure phase. The kinetic bioconcentration factors (BCFk) for the whole fish (calculated as a function of the lipid content but not including growth corrections) were 380 and 345 for the 0.5 µg/L and 5.0 µg/L concentrations, respectively.

Depuration of radioactivity from fish tissue was rapid and was similar at both the 0.5 µg/L and 5.0 µg/L concentrations (metabolism was not investigated in the study). The $DT_{50}$ for whole fish tissue was calculated to be 1.49 days and the corresponding $DT_{95}$ was 6.45 days for the 0.5 µg/L and 1.97 and 8.50 at the 5.0 µg/L concentrations. After 14 days depuration, 5.31 and 5.09% of the radioactivity present after the 28-day uptake period in the whole fish remained for both the 0.5 µg/L and 5.0 µg/L concentrations respectively.

### 5.3.2 Summary and discussion of aquatic bioaccumulation

Flutianil has a log $K_{OW}$ of 3.1 therefore further assessment according to CLP regulation is not required, however a fish bioaccumulation study has been conducted which gave a maximum kinetic bioconcentration factor of 380 (for whole fish). This fish BCF value is less than the trigger of 500 in the CLP Regulation requiring consideration of the impact of bioconcentration on its chronic classification or M-factor.

## 5.4 Aquatic toxicity

A summary of the aquatic toxicity studies conducted with flutianil is presented in Table 35. The key studies highlighted in **bold** were considered valid and reliable for the purposes of hazard classification. Note that many of the studies were conducted at concentrations in excess of the limit of water solubility of flutianil (around 0.0079 mg a.s./L at 20°C) and so endpoints are often presented as 'greater than' the concentrations which could be achieved and measured in each test.

**Table 35: Summary of relevant information on aquatic toxicity of fluitanil**

| Method | Test species | Test duration | Effect parameter | Effect (mg/L) | Reference |
|---|---|---|---|---|---|
| OECD 203 (1992), GLP | *Oncorhynchus mykiss* (rainbow trout) | 96 h (static) | $LD_{50}$ | >0.01 m* | [56] |
| | *Oncorhynchus mykiss* | 96 h (semi-static) | $LD_{50}$ | >0.9 m | [57] |
| | *Pimephales promelas* (fathead minnow) | 96 h (static) | $LD_{50}$ | >0.00472 m* | [58] |
| | *Cyprinus carpio* (carp) | 96 h (semi-static) | $LD_{50}$ | **>0.87 m** [a] | [59] |
| OECD 210 (1992), GLP | *Pimephales promelas* | Early life stage (flow-through) | NOEC | 0.008 n (survival) | [60] |
| | | | NOEC | **0.000781 m (length)** [a] | |
| OECD 202 (2004), GLP | *Daphnia magna* | 48 h (semi static) | $EC_{50}$ | >0.009 m (filtered)*<br>32.3 m (unfiltered)* | [61] |
| | | 48 h (static) | $EC_{50}$ | **>1.0 n** [a] | [62] |
| OECD 211 (1989), GLP | | 21 days | NOEC | **0.00697 m** [a] | [63] |
| OECD 201 (2006), GLP | *Pseudokirchneriella subcapitata* | 96 h | $EC_{50}$ | **>0.0127 m** [a] | [64] |
| | | | $EC_{50}$ (cell density) | >0.067 m | [65] |
| OECD 218 (2004), GLP | *Chironomus riparius (aquatic insect-midge)* | 28 days | NOEC spiked sediment | 718 mg/kg m | [66] |

* study not considered fully reliable
a endpoints used in the classification for the respective groups.
m measured concentration of the test substance
n nominal concentration of the test substance

### 5.4.1 Fish

#### 5.4.1.1 Short-term toxicity to fish

i) A semi-static 96 hour acute toxicity study was conducted with flutianil on the rainbow trout (*Oncorhynchus mykiss*) by [56].

At the start of the test, 15 *Oncorhynchus mykiss* were randomly selected from a holding At the start of the test, 15 *Oncorhynchus mykiss* were randomly selected from a holding stock and added to each test vessel in concentration order. At the end of the test, the total length and mean wet weight of ten fish selected at random from those used in the definitive test was determined. The test media in each vessel was aerated using an oil free supply of compressed air, bubbled through the test media *via* single glass tubes.

This limit test was conducted at a single nominal concentration of 100 mg/L. The dilution water used was dechlorinated mains water that had been passed through particulate and activated charcoal filters and treated with ozone for improved water clarity. On each renewal day two lots of concentrated stock media were prepared at 400 mg/L. The stock was added to individual 30 L constructed glass aquariums, made up to 20 L with water and stirred for four days in the dark, to give 2 x 100 mg/L (unfiltered) test media. The two 20 L volumes were combined and then filtered through a 1 µm and a 0.45 µm filter, to give *ca.* 38 L of filtered test media at a nominal concentration of 100 mg/L. The filtered test media was divided equally between duplicate 30 L constructed glass aquariums (*ca.* 18 L of test media in each vessel). A solvent control was prepared by adding 0.2 mL of DMF into 20 L of water in a nominal 30 L constructed glass aquarium. A water control was prepared by adding 20 L of water only into a nominal 30 L constructed glass aquarium. Final control or solvent control volumes were reduced to 18 L to ensure equivalent loading rates in all test vessels.

The results of the fish observations during the definitive test are presented in Table 36. There was no mortality or sub-lethal effects during the limit test. Therefore, the 24, 48, 72 and 96 hour $LC_{50}$ toxicity values were considered to be greater than the mean measured concentration achieved in test media (>0.01 mg/L). The corresponding NOEC was considered to be equivalent to 0.01 mg/L as flutianil. The highest concentration at which no mortality occurred was 0.01 mg/L. The validity criteria for control mortality (less than 10%) and dissolved oxygen (>60% air saturation value) were both satisfied.

**Table 36: Fish mortality during the definitive test**

| Treatment (mg/L) | % mortality | | | | |
|---|---|---|---|---|---|
| | 3 hours | 24 hours | 48 hours | 72 hours | 96 hours |
| Negative control | 0 | 0 | 0 | 0 | 0 |
| Solvent control | 0 | 0 | 3.33 | 3.33 | 3.33 |
| 0.01[a] | 0 | 0 | 0 | 0 | 0 |

a mean measured concentration

Flutianil was not toxic to *Oncorhynchus mykiss* during the study. There was no mortality or sub-lethal effects during the test. The 24, 48, 72 and 96 hour $LC_{50}$ toxicity values were considered to be greater than the mean measured concentration achieved in test media (>0.01 mg/L). The corresponding NOEC was considered to be equivalent to 0.01 mg/L as flutianil. The highest concentration at which no mortality occurred was >0.01 mg/L. This study was however not considered reliable for regulatory purposes during the approval process under Regulation 1107/2009 [1] due to excessively low recovery of the active substance in the test medium, insufficient information available on the fish used within the test and insufficient information on the appearance and behaviour of the test medium.

ii) A 96 hour semi static acute toxicity test of flutianil technical to *Pimephales promelas* was conducted [58]. The study was carried out according to OECD 203 (1992), OPPTS 850.1075 and JMAFF Method No. 2-7-1 and in compliance with GLP.

At the start of the test, 7 *Pimephales promelas* were randomly selected from a holding stock were added to each test vessel in concentration order. The fish were not fed during the test. The test media in each vessel was aerated using an oil free supply of compressed air, bubbled through the test media *via* single glass tubes.

All fish in each test vessel were observed at 3 hours and then at 24 hour intervals (24, 48, 72 and 96 hours). The number of dead fish and those fish exhibiting toxic symptoms or modified behaviour were recorded. Observations were performed before the fish were transferred to fresh test media to avoid disturbance to the fish. Observations were classified using up to five categories and were performed on each test vessel, in concentration order. Statistical analysis was not required

The mortality results are presented in Table 37. There was no mortality or sub-lethal effects during the test, therefore, the 24, 48, 72 and 96 hour $LC_{50}$ toxicity values were considered to be greater than the highest overall mean measured concentration achieved in test media (>0.00472 mg/L). The corresponding NOEC was considered to be equivalent to 0.00472 mg/L as flutianil. The lowest concentration at which 100% mortality occurred could not be determined in this study. The validity criteria of control mortality being less than 10% and maintaining a dissolved oxygen concentration above 60% of the air saturation value were satisfied.

**Table 37: Percentage (%) fish mortality**

| Treatment (mg/L) | % mortality | | | | |
|---|---|---|---|---|---|
| | 3 hours | 24 hours | 48 hours | 72 hours | 96 hours |
| Control | 0 | 0 | 0 | 0 | 0 |
| Solvent control | 0 | 0 | 3.33 | 3.33 | 3.33 |
| 0.00077 | 0 | 0 | 0 | 0 | 0 |
| 0.00103 | 0 | 0 | 0 | 0 | 0 |
| 0.00115 | 0 | 0 | 0 | 0 | 0 |
| 0.00263 | 0 | 0 | 0 | 0 | 0 |
| 0.00472 | 0 | 0 | 0 | 7.1 | 7.1 |

a mean measured concentration

Flutianil was not acutely toxic to *Pimephales promelas at the concentrations tested*. There was no mortality or sub-lethal effects during the test outside of validity criteria, therefore, the 24, 48, 72 and 96 hour $LC_{50}$ toxicity values were considered to be greater than the highest overall mean measured concentration achieved in test media (>0.00472 mg/L). The corresponding NOEC was considered to be equivalent to 0.00472 mg/L flutianil. This study was however not considered reliable for regulatory purposes during the approval process under Regulation 1107/2009 [1] due to excessively low recovery of the active substance in the test medium and insufficient information on the appearance and behaviour of the test medium.

iii) A 96 hour semi-static acute toxicity test assessing the effects of flutianil on carp (*Cyprinus carpio)* was conducted by [59]. The study was carried out according to JMAFF No. 12-Nousan-8147, Method No. 2-7-1-1 and in compliance with GLP.

At the initiation of the test 10 fish/replicate were introduced to each test vessel after measurement of pH, dissolved oxygen and temperature of all test solutions.

The test design was semi-static, with renewal of the test media at 48 hours after initiation of exposure. The fish were not fed during the test. At the end of the test, the total length

and mean wet weight of 20 fish selected at random from those used in the definitive test were determined. A preliminary toxicity test confirmed that the maximum maintainable concentration was 1.0 mg a.s./L. For the main study a 10 mL of stock solution of the test material was dissolved with the solvent (DMF: hydrogenated cast oil 1:1) in a 10 mL volumetric. A 2 mL aliquot of the stock solution was added to a test vessel which contained 20 L of dilution water and mixed to prepare the test solution. The solvent control was prepared by mixing 2 mL of the solvent with 20 L of dilution water. The test media was kept mixed throughout the test period by use of a Teflon bar.

Cumulative mortality and toxic symptoms were recorded by comparison with those of the solvent control at 24 hour intervals. The test material concentrations in all test solutions were analysed at the initiation and termination of the semi-static exposure of the test solutions. Statistical analysis was not required.

The measured concentrations of the test material in solution decreased to 78% (slightly outside the range of ±20% nominal) before the renewal of test solution at 48 hours. In the other measurements however the measured concentrations were maintained at 80 - 96% of the nominal during 48 – 96 hours. The total mean measured concentration resulted in 87% and this was used to express the endpoint. These findings suggest that fish were exposed to a constant concentration of flutianil. Test material concentrations both in the control and solvent control were less than the quantification limit (0.00357 mg a.s./L).

In this limit test with carp exposed to 0.87 mg a.s./L of flutianil technical (mean measured), no mortality nor clinical observations were observed in the test material treated group compared to the solvent control group. Therefore, the 24, 48, 72 and 96 hour $LC_{50}$ toxicity values were considered to be greater than 0.87 mg a.s/L and the 96 hour NOEC was 0.87 mg a.s/L (measured concentration).

iv) A 96 hour semi-static acute toxicity test assessing the effects of flutianil on rainbow trout (*Oncorhynchus mykiss*) was conducted by [59]. The study was carried out according to OECD 203 (1992), OPPTS 850.1075 (1996) and JMAFF Method No. 2-7-1-1 (2000) and in compliance with GLP. The 96 hour $LC_{50}$ value of flutianil technical grade to carp (*Cyprinus carpio)* was >0.87 mg/L (measured concentration) and the NOEC was 0.87 mg/L (measured concentration). Measured concentrations of flutianil in the test solutions were maintained (78 - 96% of nominal concentration) during exposure.

Three replicate groups of 10 rainbow trout were tested in each treatment group (30 fish in total per treatment group). Fish were acclimatised for 48 days and mortality during the 7 days before exposure was zero. After acclimatisation the fish were exposed to flutianil for 96 hours under semi-static conditions with test media renewed at 24 hourly intervals. The test was carried out as a limit test at nominal concentration of 1 mg a.s./L after a preliminary test demonstrated more acceptable recoveries at this concentration. A control group and a solvent control group with 100 µL solvent mixture/L (1:1 DMF:hydrogenated castor oil) were included. Observations of the appearance and behaviour of the test media were made on each day. The fish were not fed during the test. It was not stated whether the test vessels were aerated, although the dilution water was aerated prior to use. Observations of mortality and behavioural effects were made at 2, 3, 4, 6, 24, 48, 72 and 96 hours. Wet weight and total length were measured at the study termination from 20 randomly selected survivors.

Measured levels of flutianil ranged from 0.70 - 1.10 mg a.s./L (70 - 110% of nominal concentrations). These were consistent throughout the study and across replicates. As these are outside the 80 - 120% range the toxicity of flutianil is expressed in terms of the overall mean measured concentration of 0.90 mg a.s./L. There were no mortalities or toxic symptoms observed in any replicate of any treatment group at any point in the study.

The fish had a mean length of 4.6 cm (4.5 - 4.8 cm) and a mean wet weight of 0.72 g (0.64 - 0.81 g) at the end of the study. The mean fish loading rate was not provided, but was calculated to be 0.36 g/L and fish measurements were all acceptable. In the 1 mg a.s./L test group no precipitate was formed although a surface film was formed after 1 hour. The temperatures, dissolved oxygen, pH and lighting regime were within the recommended ranges throughout the study. The vessels were not aerated throughout the test but this is not considered to have adversely affected the study as the dissolved oxygen was within the recommended range. The $LC_{50}$ of flutianil to rainbow trout was >0.90 mg a.s./L (mean measured). The NOEC was 0.90 mg a.s./L (mean measured), the highest concentration tested.

#### 5.4.1.2 Long-term toxicity to fish

A fish early life stage test with *Pimephales promelas* was carried out according to OECD 210 (1992) and in compliance with GLP [60].

On receipt eggs derived from *Pimephales promelas* and acclimatised for 1 hour prior to being added to the test vessels. During this time the stages of embryonic development was assessed under a binocular microscope. The definitive test was conducted at nominal flutianil concentrations of 0.024, 0.076, 0.244, 0.781, 2.5 and 8.0 µg/L. On weekly occasions, concentrated solvent stock media was prepared at a nominal concentration of 2 mg/mL in THF. A series of stock media solutions at nominal concentrations of 160, 50, 15.62, 4.88, 1.52 and 0.48 µg/mL were prepared by serially diluting the 2 mg/mL stock solution with THF. Individual solvent stocks (including a solvent only control) were then diluted with dilution water and delivered continuously to each respective test vessel using peristaltic pumps. A dilution water control was prepared by delivering dilution water only continuously into a nominal 3 litre constructed glass aquarium.

The flow rate of each solvent stock and dilution water feed to each test vessel was measured daily. Dilution water was set at a nominal flow rate of 15 mL/min to achieve a total volume replacement rate (VRR) of 7.2/24 hour period. Solvent stocks were replenished at approximately weekly intervals. The volume of solvent stock remaining after each renewal was used to establish the accuracy of the test media delivery system. The % deviations from the nominal concentrations were calculated and adjustments to the delivery system were made as required. The test was conducted with continuous renewal of the test media (flow through).

Nominally 45 eggs were added to each of 16 glass scintillation vials half filled with treated mains water and each vial was randomly assigned a test vessel (2 vessels/concentration). The post-hatch phase started once all of the viable eggs were considered to have hatched in the control groups. Eggs hatched in each treatment were then assessed relative to control group performance. Once all viable eggs had hatched across all treatments and fish were actively feeding and mobile, the chambers were removed. At the start of the post-hatch phase (within 72 hours of >90% hatching in the control) an estimate of hatching success was determined. On Day 28 of the post-hatch phase, hatching success was further confirmed by definitive counts of all surviving fish. These counts, for each vessel, were then corrected for all larvae lost during the post-hatch phase. Discrepancies between the corrected hatched larvae counts and the number of eggs added to each chamber were considered to be due to losses during the pre-hatch phase. On Day 28 the total numbers of surviving larvae were counted and individual total fish lengths and wet weights of all remaining fish were determined. Prior to weighing the fish were blotted dry and maintained between drying paper; this guaranteed that all surface water was removed prior to weight determination. The % post-hatch survival was determined by expressing the number of surviving larvae on Day 28 post-hatch as a percentage of the hatched larvae at Day 0 (post-hatch).

Concentrations of flutianil in treated mains water were determined by GC with μECD detection. Samples of the 0.48, 1.52, 4.88, 15.62, 50 and 160 μg/mL solvent stocks (THF) were taken on Day 0 and Day 8 post-hatch. The results of these representative samples of solvent stocks were used to assess the stability of the solvent stocks over the duration of the test. Samples of test media from each of the flutianil treatments and from the control treatments were taken for analysis from freshly prepared test media prior to egg addition, Day 0 of the pre-hatch period and on Days 0, 1, 7, 14, 21, 263 and 28 during the post-hatch period. Statistical analysis of results was performed with the appropriate tests.

The results showed the measured concentrations of flutianil in the THF stock solutions were 0.544, 1.69, 5.35, 16.0, 54.4 and 168 μg/mL, respectively. These values correspond to 113, 111, 110, 103, 109 and 105% of the nominal concentrations, thereby demonstrating suitable stability in solvent stocks over the duration of the test.

During the conduct of the test, concentrations of flutianil (0.007 to 1.96 μg/L) were detected above the limit of detection in the diluent control. Extensive tests of glassware, fish tanks, water supply and cleaning procedures were all investigated but the results were inconclusive and the source of the contamination could not be determined. As there were no toxic effects associated with exposure to any of the flutianil concentrations in the test the contamination was not considered to have had an impact on the study.

No test material was detected above the limit of detection in the solvent controls during the test.

Hatching success in the control and solvent control groups were 70 and 75% respectively for pooled vessels. As no obvious trend in the pattern of hatching was observed following exposure to flutianil, NOEC and LOEC for hatching success were determined as 8.0 and >8.0 mg/L, respectively.

The % larval survival rates for each control and treatment group were 72, 56, 66, 84, 99, 84, 82 and 79%, based on pooled replicate vessels at each level, at 0 (control), 0 (solvent control), 0.024, 0.076, 0.244, 0.781, 2.5 and 8.0 μg/L, respectively. The mean post hatch survival in the diluent control group was 72% (acceptable with guideline criteria of 70%) and 56% in the solvent control group. Although the survival in the solvent control group was below the guideline requirement this was considered to be a result of vessel 3 being outside the standard deviation of the data-set. Therefore, in the interests of animal welfare vessel 3 could be considered to represent an anomaly and outlier. Post hatch survival in the second solvent control was 71% and therefore acceptable. In terms of nominal concentrations, the NOEC and LOEC for larval survival until Day 28 post hatch were 8.0 and >8.0 μg/L, respectively.

In relation to total length, a NOEC of 0.244 μg a.s./L was identified statistically in comparison to the solvent control though it was not considered to be biologically significant. There was however, a clear treatment-related effect on total length at the top two dose concentrations and so a NOEC of 0.781 μg a.s./L was identified statistically in comparison to the negative and combined controls. This key endpoint was agreed in EFSA peer review.

There was considerable variation observed in the weight data for control and treatment groups and therefore for completeness statistical comparisons of effects have been made against the control, solvent control and combined control groups. The results of statistical analysis were considered to be very conservative when interpreted biologically (e.g. standard deviation overlap and biomass) and considered to be potentially unreliable due to the variable weight data obtained. In terms of the nominal concentrations and based on biological interpretation, the NOEC and LOEC for wet weights on Day 28 post hatch under the conditions of this test were considered to equal 8.0 and ≥8.0 μg/L, respectively.

In this fish early life stage test with flutianil, the nominal NOEC based on total length was considered to be 0.781 µg a.s./L (0.000781 mg a.s./L). Reliable NOECs for all other measured parameters were considered to be 8 µg a.s./L.

### 5.4.2 Aquatic invertebrates

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

i) The 48 hour acute toxicity of flutianil to freshwater planktonic crustacean, *Daphnia magna* under semi static conditions (24 hour renewal of test media) according to OECD 202 (2004), US EPA OPPTS 850.1010 and JMAFF 2-7-2-1 and in compliance with GLP [61].

No test article was detected above the limit of determination (0.0015 mg/L) in the control or solvent control group during the test. The respective geometric mean measured concentrations for unfiltered test media were 0.0882, 1.05, 6.90 and 32.3 mg/L, corresponding to 88.2, 105, 69.0 and 32.3% of the nominal flutianil concentrations respectively. The respective geometric mean measured concentrations for filtered test media were not detected, 0.006, 0.009 and 0.002 mg/L, corresponding to not detected, 0.609, 0.0927 and 0.0017% of the nominal flutianil concentrations respectively. As the measured concentrations were outside the 80 to 120% range, the toxicity of flutianil to *Daphnia magna* is expressed in terms of the nominal (geometric) mean measured concentrations for filtered test media.

No immobility was observed in the control treatment throughout the test. The 24 and 48-hour $EC_{50}$ toxicity and NOEC values could not be calculated due to lack of graded immobility. Therefore, the time-point toxicity values are both considered to be greater than the highest measured concentrations achieved in the filtered and unfiltered test media (>0.009 and 32.3 mg/L respectively). The highest nominal concentration at which no immobility occurred could not be determined in this test. The lowest nominal concentration at which at which 100% immobility occurred could not be determined but was considered to be >100 mg/L. The validity criteria of a maximum of 10% immobility in the control treatment and achieving dissolved oxygen concentrations of >60% in the control and test vessels at the end of the test were both satisfied..

Both 24 and 48 hour $EC_{50}$ and 24 and 48 hour $EC_{50}$ NOEC values in filtered and unfiltered for flutianil to *Daphnia magna* were greater than 0.009 and 32.3 mg/L (measured concentration), respectively. Measured concentrations of flutianil in the test solution at the intended concentrations were not maintained during exposure. Consequently both the $LC_{50}$ and the NOEC values have been expressed in terms of the geometric mean. This study was however considered to have a number of limitations during the renewal of approval process under Regulation 1107/2009 [1] in particular the very low recovery of flutianil and several of the measured concentrations were below the limit of determination of the analytical method.

ii) The 48 hour acute toxicity of flutianil to the freshwater planktonic crustacean, *Daphnia magna* was investigated according to JMAFF No. 12-Nousan-8147 (2-7-2-1) and in compliance with GLP [62].

Five juvenile *Daphnia magna* (<24 hours old) were added to each replicate vessel, using a wide bore glass pipette. The animals were not fed during the test. The test design was static.

The measured concentrations of the test article in the test solution at initiation and termination of exposure were 100% and 91% of the nominal, i.e., indicating constancy during the exposure. The test substance concentrations in the control and the solvent

control were less than quantification limit (0.00357 mg/L) at initiation and termination of exposure.

Since the deviation of measured concentrations were within ± 20% from the nominal during the exposure, the definitive values, $EC_{50}$ and NOEC, are based on the nominal concentration. No immobility was observed in the control treatment throughout the test.

Both 24 and 48 hour $EC_{50}$ values for flutianil to *Daphnia magna* were greater than 1.0 mg/L, respectively and the NOEC was 1.0 mg/L (nominal),

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

A 21-day reproduction test was conducted to determine the chronic toxicity of flutianil to *Daphnia magna* according to OECD 211 (1998), OPPTS 850.1300, JMAFF 2-7-2-3 and in compliance with GLP [63].

One juvenile *Daphnia magna* (<24 hours old) was added to each replicate vessel (10 vessels/treatment concentration). The reproduction study was conducted as a semi-static test at nominal flutianil concentrations of 0, 0.191, 0.61, 1.95, 6.25 and 20 µg/L. At the start of the test and at approximately weekly intervals during the test, a concentrated solvent stock was prepared at a nominal flutianil concentration of 200 µg/mL in DMF and this was used to prepare other test media concentrations. A control treatment was prepared by the addition of only ASTM standard hard dilution water to the test vessels. An equivalent procedure to that described above was performed on each renewal occasion.

At 24 hour intervals adult *Daphnia* magna were observed for immobility, the presence or absence of eggs developing in the brood pouch and mortality.

The overall mean measured concentrations of flutianil in samples of each solvent stock were 1.83, 5.93, 19.1, 60.9 and 195 µg/mL, corresponding to 96, 97, 98, 97 and 98% of the nominal concentrations, respectively. The overall mean measured concentrations of flutianil in samples of test media were 0.0784, 0.226, 0.798, 2.47 and 6.97 µg/L, corresponding to 41, 37, 41, 40 and 35% of the nominal concentrations, respectively. Mean measured test media concentrations were <80% of the nominal concentration. The toxicity of flutianil to *Daphnia magna* during the reproduction test has therefore been expressed in terms of the geometric mean measured concentrations.

The adult mortality of *Daphnia magna* across all treatments including control groups was 0%, therefore the validity criteria for adult mortality not exceeding 20% over the duration of the test was met.

The Day 14 and Day 21 $EC_{50}$ toxicity values, based on the reduction in the numbers of juveniles produced in each treatment relative to the solvent control, could not be determined. This was due to the number of juveniles produced in all treatment at both time-points being greater than the number of juveniles produced in the solvent control group.

Therefore, in terms of measured flutianil concentrations, the Day 14 and Day 21 $EC_{50}$ toxicity values for juvenile production were both considered to be >6.97 µg/L as flutianil, the highest measured concentration  There were no dead juveniles recorded during the test. No effect on either dry weight or carapace actual lengths following treatment with flutianil.

Based on mean measured concentrations, the NOEC and LOEC for *Daphnia magna* carapace lengths and dry weights, determined on Day 21, were 6.97 µg/L flutianil/L.

### 5.4.3 Algae and aquatic plants

i) The effect on growth of freshwater green alga, *Pseudokirchneriella subcapitata* when exposed to flutianil was assessed over a 96 hour exposure period carried out according to JMAFF 2-7-7, OECD Guideline No. 201 (2006), the Official Journal of the European Communities L383A (EC, 1992), OPPTS 850.5400 (1996), and in compliance with GLP [65].

Growth media (100 mL) inoculated with green alga, *Pseudokirchneriella subcapitata* in exponential growth (1 x $10^4$ cells/mL) were added to each test vessel (6/treatment). Following a preliminary solubility test, the highest attainable concentration of flutianil was 320 µg/L, with the aid of DMF / hydrogenated castor oil (1:1 w/w). Cultures were exposed for a total of 96 hours. Therefore the definitive test was conducted as a limit test at this nominal concentration.

For the definitive test, a stock solution was prepared in the solvent mix and diluted in test medium to provide the test media concentration. The solvent control was prepared by mixing the solvent mixture (0.01% v/v) in the test medium. The negative control was composed of only test medium.

Cell densities were determined at 24 hour intervals. At 72 and 96 hours, microscopic observations were conducted to detect any morphological effects. Measurement of pH and temperature in each test solution were recorded at 0, 72 and 96 hours. Intensity of illumination (photosynthetically-active solution) at five points in the test area was measured at 0, 72 and 96 hours.

At treatment initiation the % of nominal concentration was 103%, this decreased over time with the measure concentration resulting in 35 and 27 µg/L (11 and 8.4% of nominal, respectively) at 72 and 96 hours, respectively. Mean measured concentrations in each test solution at 0-72 and 0-96 hours were calculated to be 85 µg/L (27% of nominal) and 67 µg/L (21% of nominal), respectively.

No effect on cell density, biomass or grow rates were observed for *Pseudokirchneriella subcapitata* following exposure to flutianil.

No effect on cell densities, growth rate or biomass were observed when compared to both the concurrent solvent and negative control were observed, therefore the $EC_{50}$ and NOEC were estimated to be greater than or equal to the nominal test concentration (320 µg/L) .

Based on mean measured concentrations the $EC_{50}$ and NOEC for growth rate inhibition (0 - 72 hours) were determined to be >85 and 85 µg/L, respectively (the same values were also applicable based on area under the growth curve - biomass).

Based on mean measured concentrations the $EC_{50}$ and NOEC values for cell density (96 hours) were determined to be >67 and 67 µg/L, respectively.

ii) The effect on growth of freshwater green alga, *Pseudokirchneriella subcapitata* when exposed to flutianil was assessed over a 96 hour exposure period carried out according to OECD 201 (2006), US EPA OPPTS 850.5400, and JMAFF Notification No. 12-Nousan-8147, Method No. 2-7-7 and in compliance with GLP [64].

At the respective nominal flutianil concentrations of 3.13, 6.25, 12.5, 25, 50 and 100 mg/L the geometric mean measured concentrations were <LOD, <LOD, 0.00281, 0.00263, 0.00309 and 0.01269 mg/L, corresponding to 0, 0, 0.023, 0.011, 0.0062 and 0.013% of the nominal concentrations, respectively.

As all mean measured concentrations of flutianil were outside 80 to 120% of the nominal concentrations, the toxicity of flutianil to *Pseudokirchneriella subcapitata* has been expressed in terms of the geometric mean measured concentrations.

The limit of determination for the analytical method was 0.00125 mg/L.

There was no significant growth inhibition relative to the control treatment during the definitive test, the 0 - 96-hour growth rate, biomass and yield $EC_{50}$ values could not be calculated and are therefore considered to be >100 mg/L based on nominal concentrations and >0.0127 mg/L based on geometric mean measured concentrations, which was considered to represent the practical limit of water solubility in this test using added solvent. Endpoints for 0 - 72 hours were not reported.

The corresponding NOEC for the area under the growth curve and the specific growth rate were both considered to be ≥100 mg/L based on nominal concentrations and ≥0.0127 mg/L based on the geometric mean measured concentrations.

Flutianil was considered not to be toxic to *Pseudokirchneriella subcapitata*, the 96 hour biomass and growth rate $EC_{50}$ values could not be calculated and are therefore assumed to be >0.0127 mg/L respectively, the corresponding NOEC was 0.0127 mg/L. Based on geometric mean measured concentrations this value was considered to represent the practical limit of solubility under the conditions of this test and was the highest concentration tested.

### 5.4.4 Other aquatic organisms (including sediment)

A study was carried out to assess the toxicity of flutianil to the sediment-dwelling phase of the non-biting midge *Chironomus riparius*, using a static sediment spiked test system over a 28 day period [66].

Flutianil did not have an adverse effect on the emergence success or sex ratio of emerged midges at the maximum tested concentration. There were no effects on the development rate of *Chironomus riparius* under the conditions of this test and the $EC_{50}$ and NOEC were considered to be >718 mg/kg and 718 mg/kg, respectively. However, as this test was based on concentrations in the sediment, which have not been related to classification criteria and concentrations in the water phase, this study has not been reported in detail.

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

Both acute and chronic aquatic toxicity tests have been conducted for fish, aquatic invertebrates and algae. Whilst these were all affected by the low limit of solubility of flutianil, adequate reliable acute and chronic endpoints are available for each trophic group.

The 96 hour acute $LC_{50}$ endpoint for fish is derived from the reliable *Cyprinus carpio* study, with a measured $LC_{50}$ value of >0.87 mg/L. It could be argued to use the equally reliable but slightly higher $LC_{50}$ for rainbow trout of >0.9 mg/L, however the more precautionary endpoint is chosen. In relation to chronic toxicity to fish, the flow-through 28 day early life stage study with *Pimephales promelas* resulted in a nominal NOEC of 0.000781 mg/L based on total length.

The acute nominal 48 hour $EC_{50}$ for aquatic invertebrates (Daphnia magna) is >1.0 mg/L, with a chronic 21 day measured NOEC of 0.00697 mg/L.

The 96 hour growth rate $EC_{50}$ and the corresponding NOEC for algae (*Pseudokirchneriella subcapitata)* were a measured >0.0127 mg/L and 0.0127 mg/L, respectively, the highest dose tested.

### 5.6 Conclusions on classification and labelling for environmental hazards (Section 5.1 – 5.4)

Flutianil was found to be stable to hydrolysis. In an aqueous photolysis study degradation was quite rapid with an estimated half-life of just over 1 day, however this is of uncertain relevance for classification purposes. Flutianil was not degraded in the ready biodegradability test and is therefore not considered to be readily biodegradable. In a water sediment study, flutianil rapidly dissipated from the water phase into the sediment with a water phase half-life of <1 day. Flutianil slowly degraded in the sediment to give an overall whole system $DT_{50}$ of 504 to 752 days. On this basis it is concluded the substance should be considered to be not readily or rapidly degradable. The BCF value of flutianil is 380 (whole fish), this is lower than the trigger value of 500 which is used to indicate a potential for bioaccumulation under CLP. Therefore, flutianil is not considered bioaccumulative.

The ecotoxicity test results indicate that the substance may exhibit acute aquatic toxicity to fish and algae at concentrations <1 mg/L, however, the data are affected by the low limit of solubility of flutianil and the respective $EC_{50}$ values for fish and algae are greater than the highest concentrations tested. *Daphnia* showed an acute $EC_{50}$ of >1.0 mg/L, though results are also based on the highest concentration tested. These results indicate that acute aquatic toxicity is not envisaged at the limit of solubility for flutianil and therefore, in accordance with CLP guidance, it is proposed that flutianil need not be classified with regard to acute effects.

The long-term aquatic data shows toxicity at concentrations <0.1 mg/L. The results indicate that fish are the most chronically sensitive taxa with a NOEC of 0.000781 mg a.s./L for *Pimephales promelas*. Based on this result and in accordance with the chronic ecotoxicity criteria in the CLP Regulation, flutianil requires an aquatic hazard classification of Chronic category 1. The associated hazard statement is H410: Very toxic to aquatic life with long lasting effects.

In accordance with Article 10 of CLP, when a substance is classified as Chronic category 1 a multiplying factor (M-factor) has to be assigned. For flutianil, a chronic M-factor of '100' is set based on the test substance being not rapidly biodegradable and the fish NOEC of 0.000781 mg/L being between 0.0001 and 0.001 mg/L.

In summary, the proposed environmental hazard classification of flutianil based on CLP criteria is:

**Aquatic Chronic category 1; H410 - Very toxic to aquatic life with long lasting effects**

**Chronic M-factor = 100**

## 6. OTHER INFORMATION

This substance has been reviewed by the United Kingdom Competent Authority under Council Directive EC No. 1107/2009. The studies evaluated in this dossier were largely taken from the draft assessment report produced under this review programme, although additional information in the form of revised historical control ranges from the testing laboratories and relevant public domain data have been obtained by the Notifier to support the mammalian toxicity observations after the assessment report had been published.

**REFERENCES**


| | | a |
|---|---|---|
| 1 | EFSA (European Food Safety Authority), 2014. Conclusion on the peer review of the pesticide risk assessment of the active substance flutianil. *EFSA Journal* 2014;12(8):3805, 89 pp. doi:10.2903/j.efsa.2014.3805 | N |
| 2 | [redacted] (2004). Determination of appearance of OK-5203. [redacted]. Unpublished report no.: 395459. [redacted] report no.: [redacted]-0015-FR | Y |
| 3 | [redacted]. (2004). Determination of the melting and boiling temperature of OK-5203 by differential scanning calorimetry. [redacted] Unpublished report no.: 395415. [redacted] report no.: [redacted]-0011-FR | Y |
| 4 | [redacted]. (2004). Determination of the density of OK-5203. [redacted]. Unpublished report no.: 395426. [redacted] report no.: [redacted]-0012-FR | Y |
| 5 | [redacted] (2010). OK-5203: Evaluation of vapour pressure. [redacted]. Unpublished report no.: 8231838. [redacted] report no.: [redacted]-399-FR | Y |
| 6 | [redacted] (2004). Determination of the water solubility of OK-5203 at 3 pH values. [redacted]. Unpublished report no.: 395505. [redacted] report no.: [redacted]-0020-FR | Y |
| 7 | [redacted]. (2004). Determination of the partition coefficient (n-octanol/water) of OK-5203 at 3 pH values. [redacted]. Unpublished report no.: 395516. [redacted] report no.: [redacted]-0021-FR (Final report) | Y |
| 8 | [redacted]. (2007). OK-5203 technical: evaluation of physico-chemical properties. [redacted]. Unpublished report no.: 2554/058-D2149. [redacted] report no.: [redacted]-0183-FR | Y |
| 9 | [redacted]. (2006). Acute oral toxicity study of flutianil technical in rats. [redacted]. Unpublished report no.: B-5886. [redacted] report no.: [redacted]-0139-FR | Y |
| 10 | [redacted]. (2006). Acute dermal toxicity study of OK-5203 technical in rats. [redacted]. Unpublished report no.: B-5887. [redacted] report no.: [redacted]-0140-FR | Y |
| 11 | [redacted]. (2007). Flutianil technical: Acute inhalation toxicity study in the rat + Amendment 1. [redacted]. Unpublished report no.: 2554/061. [redacted] report no.: [redacted]-0185-FR (Final report); [redacted]-0185-RA1 (Amendment to final report 1) | Y |


a Data Protection Claimed (Y/N) according to Regulation (EC) 1107/2009

| | | a |
|---|---|---|
| 12 | [redacted]. (2006). An skin irritation study of OK-5203 technical in rabbits. [redacted]. Unpublished report no.: I-2680. [redacted] report no.: [redacted]-0143-FR | Y |
| 13 | [redacted]. (2006). An eye irritation study of OK-5203 technical in rabbits. [redacted]. Unpublished report no.: I-2679. [redacted] report no.: [redacted]-0146-FR | Y |
| 14 | [redacted]. (2006). A skin sensitisation study of flutianil technical in guinea pigs (Maximisation test). [redacted]. Unpublished report no.: I-2681. [redacted] report no.: [redacted]-0144-FR | Y |
| 15 | [redacted]. (2009). Flutianil technical: 28-day repeated dose oral toxicity study in mice. [redacted]. Unpublished report no.: [redacted] 04-0155. [redacted] report no.: [redacted] -0064-FR | Y |
| 16 | [redacted]. (2009). Flutianil technical: 90-Day repeated dose oral toxicity study in mice. [redacted]. Unpublished report no.: [redacted] 04-0156. [redacted] report no.: [redacted]-0076-FR | Y |
| 17 | [redacted]. (2014). OK-5203: Historical control data in [redacted] (mouse 90-day data and mouse carcinogenicity data). [redacted]. Unpublished report no.: 14-C023 | Y |
| 18 | [redacted]. (2009). Flutianil technical: Repeated dose 28-day oral toxicity study in rats. [redacted]. Unpublished report no.: [redacted] 04-0121. [redacted] report no.: [redacted]-0010-FR | Y |
| 19 | [redacted]. (2009). Flutianil technical: 90-day repeated dose oral toxicity study in rats. [redacted]. Unpublished report no.: [redacted] 04-0022. [redacted] report no.: [redacted]-0077-FR | Y |
| 20 | Nagashima, Y. (2006). A 28-day repeated dose oral toxicity study on Flutianil technical in beagle dogs. [redacted]. Unpublished report no.: B-5370. [redacted] report no.: [redacted]-0033(EN)-FR | Y |
| 21 | [redacted]. (2009). A 90-day repeated dose oral toxicity study of OK-5203 technical in beagle dogs. [redacted]. Unpublished report no.: B-5468. [redacted] report no.: [redacted]-0087-FR | Y |
| 22 | [redacted]. (2009). A 52-week oral toxicity study of OK-5203 technical in beagle dogs. [redacted]. Unpublished report no.: B-5944. [redacted] report no.: [redacted]-0156-FR | Y |
| 23 | [redacted]. (2008). OK-5203 technical: 28-day dermal administration toxicity study in the rat with a 14-day treatment-free period. [redacted]. Unpublished report no.: 2554/076. [redacted] report no.: [redacted]-0198-FR. | Y |

a Data Protection Claimed (Y/N) according to Regulation (EC) 1107/2009

| | | a |
|---|---|---|
| 24 | [redacted]. (2005). Flutianil technical: Reverse mutation in four histidine-requiring strains of *Salmonella typhimurium* and one tryptophan requiring strain of *Escherichia coli*. [redacted]. Unpublished report no.: 275/121-D6171. [redacted] report no.: [redacted]-0023-FR. | Y |
| 25 | [redacted]. (2005). OK-5203 technical: Mutation at the thymidine kinase (*tk*) locus of mouse lymphoma L5178Y cells (MLA) using the Microtitre® fluctuation technique. [redacted]. Unpublished report no.: 275/123. [redacted] report no.: [redacted]-0025-FR | Y |
| 26 | [redacted] (2005). OK-5203 Technical: Induction of chromosome aberrations in cultured human peripheral blood lymphocytes. [redacted], Unpublished report no.: 275/122. [redacted] report no.: [redacted]-0024-FR. | Y |
| 27 | [redacted]. (2010). Micronucleus test in bone marrow cells of the mouse with flutianil technical. [redacted]. Unpublished report no.: 494419. [redacted] report no.: [redacted]-0424-FP | Y |
| 28 | [redacted]. (2009) OK-5203 Technical: Combined chronic toxicity and carcinogenicity study in rats. [redacted]. Unpublished report no.: [redacted] 04-0154 [redacted] report no.: [redacted]-0041-FR; [redacted]-0041-Annex 1, [redacted]-0041-Annex 2, [redacted]-0041-Annex 3, [redacted]-0041-Annex 4, [redacted]-0041-Annex 5 | Y |
| 29 | [redacted]. (2009). OK-5203 technical: Carcinogenicity study in mice. [redacted]. Unpublished report no.: [redacted] 04-0157. [redacted] report no.: [redacted]-0115-FR | Y |
| 30 | [redacted]. (2014). OK-5203: Historical control data in [redacted] (rat chronic/carcinogenicity and reproduction data). [redacted]. Unpublished report no.: 14-C021 | Y |
| 31 | RCC Ltd. Historical control data on non-neoplastic findings in Wistar (planned sacrifices prior to 103 weeks). Compiled from 2-year bioassays performed at RCC Ltd, Itingen, Switzerland | N |
| 32 | Carlus, M., Elies, L., Fouque, M-C., Maliver, P. & Schorsch, F. (2013). Historical control data of neoplastic lesions in the Wistar Hannover rat among eight 2-year carcinogenicity studies. *Exper. Toxicol. Pathol.* **65**, pp 243-253 | N |
| 33 | Weber, K., Hardisty, J. Razinger, T., Mann, P., Martel, K., Frische, E., Blumbach, K., Hillen,, S., Song, S., Anzai, T. & Chevalier, H-J. (2011). Differences in rat models used in routine toxicity studies. *Int. J. Toxicol.* **30**, pp 162-173 | N |
| 34 | Eiben, R. & Bomhard, E.M. (1999). Trends in mortality, body weight and tumor incidences of Wistar rats over 20 years. *Exp. Toxic. Pathol.* **51**, pp 523-536 | N |
| 35 | Walsh, K.M. & Poteracki, J. (1994). Spontaneous neoplasms in control Wistar rats. *Fund. Appl. Toxicol.* **22**, pp 65-72 | N |


a Data Protection Claimed (Y/N) according to Regulation (EC) 1107/2009

| | | a |
|---|---|---|
| 36 | Poteracki, J. & Walsh, K.M. (1998). Spontaneous neoplasms in control Wistar rats: A comparison of reviews. *Toxicol. Sci.* **45**, pp 1-8 | N |
| 37 | [redacted]. (2014). OK-5203: Historical control data in [redacted] (mouse 90-day data and mouse carcinogenicity data). [redacted]. Unpublished report no.: 14-C023 | Y |
| 38 | [redacted]. (2009). Flutianil technical: A reproduction toxicity study in rats. Dose range-finding study, [redacted]. Unpublished report no.: [redacted] 05-0157. [redacted] report no.: [redacted]-0118-FR | Y |
| 39 | [redacted]. (2009) OK-5203 Technical: A reproduction toxicity study in rats (and Annex: Historical control data on the number of implantations sites in Wistar Hannover rats from [redacted] reproduction toxicity studies). [redacted]. Unpublished report no.: [redacted] 05-0158. [redacted] report no.: [redacted]-0149-FR | Y |
| 40 | [redacted]. (2014). OK-5203: Historical control data in [redacted] (rat chronic/carcinogenicity and reproduction data). [redacted]. Unpublished report no.: 14-C021 | Y |
| 41 | [redacted]. (2006). Oral (gavage) dosage-range developmental toxicity study of OK 5203 Technical in Wistar Hannover GALAS rats. [redacted]. Unpublished report no.: 315-008P. [redacted] report no.: [redacted]-0026-FR | Y |
| 42 | York, R.G. (2006). Oral (gavage) developmental toxicity study of OK-5203 technical in Wistar Hannover GALAS rats. [redacted]. Unpublished report no.: 315-008. [redacted] report no.: [redacted]-0008-FR | Y |
| 43 | [redacted]. (2007). A dose range-finding prenatal developmental toxicity study of OK-5203 technical grade in rabbits. [redacted]. Unpublished report no.: [redacted]-292004. [redacted] report no.: [redacted]-0167-FR | Y |
| 44 | [redacted]. (2007). A prenatal developmental toxicity study of flutianil technical grade in rabbits. [redacted]. Unpublished report no.: [redacted] -292005. [redacted] report no.: [redacted]-0159-FR | Y |
| 45 | [redacted]. (2007). [$^{14}$C]-OK-5203: Hydrolytic Stability (+ Amendment 1). [redacted]. Unpublished report no.: 2554/029-D2149. [redacted] report no.: [redacted]-0119-FR (Final report); [redacted]-0119-RA1 (Amended final report 1) | Y |
| 46 | [redacted]. (2007). [$^{14}$C]-OK-5203: Photodegradation and quantum yield in sterile, aqueous solution (+ Amendment 1 & 2). [redacted]. Unpublished report no.: 2554/030-D2149. [redacted] report no.: [redacted]-0120-FR (Final report); [redacted]-0120-RA1 (Amended final report 1); [redacted]-0120-RA2 (Amended final report 2) | Y |

a Data Protection Claimed (Y/N) according to Regulation (EC) 1107/2009

| | | a |
|---|---|---|
| 47 | [redacted]. (2008). [$^{14}$C]-OK-5203: Photodegradation on a Soil Surface (+ Amendment 1). [redacted]. Unpublished report no.: 2554/006-D2149. [redacted] report no.: [redacted]-0101-FR (Final report); [redacted]-0101-RA1 (Amended final report 1) | Y |
| 48 | [redacted]. (2007). OK-5203: Assessment of Ready Biodegradability by Measurement of $CO_2$ Evolution. [redacted]. Unpublished report no.: 2554/050-D2149. [redacted] report no.: [redacted]-0174-FR | Y |
| 49 | [redacted]. (2008). [$^{14}$C]-OK-5203: Degradation and Retention in Water-Sediment Systems. [redacted]. Unpublished report no.: 2554/029-D2149. [redacted] report no.: [redacted]-0129-FR | Y |
| 50 | [redacted]. (2008). [$^{14}$C]-OK-5203: Aerobic Soil Metabolism and Degradation (+ Amendments 1, 2, 3 & 4). [redacted]. Unpublished report no.: 2554/002-D2149. [redacted] report no.: [redacted]-0102-FR (Final report); [redacted]-0102-RA1 (Amended final report 1); [redacted]-0102-RA2 (Amended final report 2); [redacted]-0102-RA3 (Amended final report 3); [redacted]-0102-RA4 (Amended final report 4) | Y |
| 51 | [redacted]. (2010). OK-5203: The dissipation of residues in soil in Northern and Southern Europe. [redacted]. Unpublished report no.: 2554/080-D2149. [redacted] report no.: [redacted]-0220-FR | Y |
| 52 | [redacted]. (2011). OK-5203: The dissipation and accumulation of residues in soil in Northern and Southern Europe. [redacted]. Unpublished report no.: 2554/016-D2149. [redacted] report no.: [redacted]-0142-FR | Y |
| 53 | [redacted]. (2007) [14C]-OK-5203: Adsorption/desorption in soil. [redacted]. Unpublished report No.: 2554/014-D2149 [redacted] report no.: [redacted]-0130-FR (Final report); [redacted]-0130-RA1 (Amended final report 1) | |
| 54 | [redacted]. (2010). The estimation of the half-life of flutianil in the atmosphere. [redacted]. Unpublished report no.: [redacted]/03/02-AOP. [redacted] report no.: [redacted]-0444-FR | Y |
| 55 | [redacted]. (2010) [$^{14}$C]-OK-5203: Fish bioconcentration study. [redacted]. Unpublished report no.: 2554/044. [redacted] report no.: [redacted]-0181-FR | Y |
| 56 | [redacted]. (2010). OK-5203: Acute toxicity to *Oncorhynchus mykiss*. [redacted]. Unpublished report no.: 2554/042. [redacted] report no.: [redacted]-0171-FR | Y |
| 57 | [redacted] (2007). Acute toxicity test of OK-5203 technical to rainbow trout (*Oncorhynchus mykiss*). [redacted]. Unpublished report no.: 0604EFA-3. [redacted] report no.: [redacted]-0237-FR | Y |

a Data Protection Claimed (Y/N) according to Regulation (EC) 1107/2009

a

58 [redacted]. (2010). OK-5203: Acute toxicity to *Pimephales promelas*. [redacted]. Unpublished report no.: 2554/054. [redacted] report no.: [redacted]-0178-FR Y

59 [redacted] (2006). Acute toxicity test of OK-5203 technical grade to carp (*Cypomus carpio*) (+ English translation). [redacted]. Unpublished report no.: 0604EFA-2. [redacted] report no.: [redacted]-0186(EN)-FR Y

60 [redacted]. (2010). OK 5203: Fish early stage testing *Pimephales promelas*. [redacted]. Unpublished report no.: 2554/041. [redacted] report no.: [redacted]-0275-FR. Y

61 [redacted] (2010). OK-5203: Acute toxicity to *Daphnia magna*. [redacted]. Unpublished report no.: 2554/039. [redacted] report no.: [redacted]-169-FR Y

62 [redacted]. (2006). Acute immobilisation test of OK-5203 technical grade to *Daphnia magna* (+ English translation). [redacted]. Unpublished report no.: 0604EDI. [redacted] report no.: [redacted]-0187(EN)-FR Y

63 [redacted] (2007). OK-5203: Chronic effects in *Daphnia magna*. [redacted]. Unpublished report no.: 2554/040. [redacted] report no.: [redacted]-0200-FR. Y

64 [redacted] (2010). OK-5203: Inhibition of growth to the alga, *Pseudokirchneriella subcapitata*. [redacted]. Unpublished report no.: 2554/045. [redacted] o report no.: [redacted]-0201-FR Y

65 [redacted] (2007). Growth inhibition test of OK-5203 technical with freshwater green alga, *Pseudokirchneriella subcapitata*. [redacted]. Unpublished report no.: 0604EAI. [redacted] report no.: [redacted]-0233-FR. Y

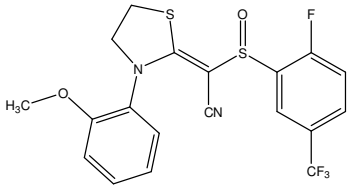
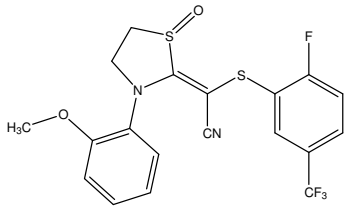
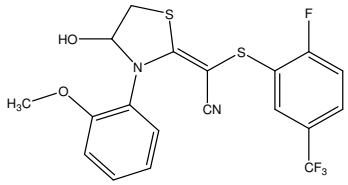
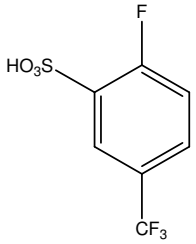
66 [redacted] (2010). OK-5203: Sediment-water *Chironomus riparius* toxicity test using spiked sediment [redacted]. Unpublished report no.: 2554/038. [redacted] report no.: [redacted]-0274-FR Y

# ANNEX I – ABBREVIATIONS

| | |
|---|---|
| ♀ | Female |
| ♂ | Male |
| Abs | Absolute |
| AR | Application Rate |
| BCFk | kinetic bioconcentration factors |
| Ben. | Benign |
| bw | bodyweight |
| *ca* | *Circa* (approximately) |
| CD | Caesarean Derived |
| CLH | Harmonised classification and labelling |
| CLP | Classification Labelling and Packaging |
| CMC | Carboxymethyl Cellulose |
| $CO_2$ | Carbon dioxide |
| CPA | Cyclophosphamide |
| Crl | Charles River Laboratories |
| Crlj | Charles River Laboratories Japan |
| d | Day |
| DMF | Dimethyl Formamide |
| DT | Degradation rate |
| EC | Effect Concentration |
| equiv | *equivalent* |
| *et al* | *et alii* (and others) |
| EU | European Union |
| FD | Found Dead |
| GALAS | Global Alliance for Laboratory Animal Standardization |
| GD | Gestation Day |
| GLP | Good Laboratory Practice |
| gp | Group |
| Han | Hannover |
| hr | hour |
| HSE | Health Safety Executive |
| ICR | Institute for Cancer Research |
| *i.e.* | *id est* (that is) |
| kg | kilograms |
| KI | Killed *In extremis* |
| L | Litre |
| LC | Lethal Concentration |

| | |
|---|---|
| LD | Lethal Dose |
| LOD | Limit Of Detection |
| LOEC | Lowest Observed Effect Concentration |
| LOQ | Limit Of Quantification |
| Mal. | Malignant |
| Max. | Maximum |
| mg | miligrams |
| Min. | Minimum |
| mL | millilitres |
| no. | number |
| NOAEC | No Adverse Effect Concentration |
| NOAEL | No Adverse Effect Level |
| NOEC | No Observable Effect Concentration |
| OECD | Organisation for Economic Co-operation and Development |
| P | Parental |
| p | probability |
| pH | Negative log of the hydrogen ion |
| ppm | parts per million |
| Rel | Relative |
| RTG | Relative Total Growth |
| -S9 | Absence of rat liver enzyme homogenate |
| +S9 | Presence of rat liver enzyme homogenate obtained following centrifugation at 9000*g* |
| sd | standard deviation |
| SD | Sprague Dawley |
| SPF | Specific Pathogen Free |
| STOT SE | Specific Target Organ Toxicity Single Exposure |
| Ter | Terminal sacrifice |
| *tk* | thymidine kinase |
| wk | week |
| µg | microgram |
| *vs.* | versus |
| WIST / WI | Wistar |

## ANNEX II -
## METABOLITE STUCTURES AND METABOLIC PATHWAY

| ID No. | Chemical Abstracts Name | Structure | Where found |
|---|---|---|---|
| OC 53276 | (Z)-2-(2-fluoro-5-(trifluoromethyl)phenylsulfinyl)-2-(3-(2-methoxyphenyl) thiazolidin-2-ylidene) acetonitrile |  | Soil, Water, Sediment |
| OC 56574 | (Z)-2-(2-fluoro-5-(trifluoromethyl)phenylsulfinyl)-2-(3-(2-methoxyphenyl) 1-oxo-thiazolidin-2-ylidene) acetonitrile |  | Soil, Water, Sediment |
| OC 53279 | (Z)-2-(2-fluoro-5-(trifluoromethyl)phenylthio)-2-(4-hydroxy-3-(2-methoxyphenyl)thiazolidin-2-ylidene)acetonitrile |  | Soil, Water, Sediment |
| OC 56635 | 2-fluoro-5-(trifluoromethyl) benzenesulfonic acid |  | Soil (photolysis), Water (aqueous photolysis) |

**Proposed metabolite pathway for flutianil in water-sediments systems**