# CLH report

## Proposal for Harmonised Classification and Labelling

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

## Substance Name:

## Fludioxonil (ISO); 4-(2,2-difluoro-1,3-benzodioxol-4-yl)-1H-pyrrole-3-carbonitrile

**EC Number: No entry**

**CAS Number: 131341-86-1**

**Index Number: 608-RST-VW-Y**

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# Part A.



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# Part A.

## 1 PROPOSAL FOR HARMONISED CLASSIFICATION AND LABELLING

### 1.1 Substance

**Table 1: Substance identity**

| | |
|---|---|
| **Substance name:** | Fludioxonil (ISO); 4-(2,2-difluoro-1,3-benzodioxol-4-yl)-1*H*-pyrrole-3-carbonitrile |
| **EC number:** | No entry |
| **CAS number:** | 131341-86-1 |
| **Annex VI Index number:** | 608-RST-VW-Y |
| **Degree of purity:** | ≥ 95% w/w |
| **Impurities:** | No impurities present at >1% and none of the impurities at lower levels are considered relevant for the classification of the substance. Information on impurities can be found in the confidential part of IUCLID. |

### 1.2 Harmonised classification and labelling proposal

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

| | **CLP Regulation** | **Directive 67/548/EEC (Dangerous Substances Directive; DSD)** |
|---|---|---|
| **Current entry in Annex VI, CLP Regulation** | Not included | Not included |
| **Current proposal for consideration by RAC** | Aquatic Acute 1, H400, M = 1<br>Aquatic Chronic 1, H410, M = 1 | |
| **Resulting harmonised classification (future entry in Annex VI, CLP Regulation)** | Aquatic Acute 1, H400, M = 1<br>Aquatic Chronic 1, H410, M = 1 | |

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

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

| CLP Annex I ref | Hazard class | Proposed classification | Proposed SCLs and/or M-factors | Current classification[1)] | Reason for no classification[2)] |
|---|---|---|---|---|---|
| **2.1.** | Explosives | None | - | None | Conclusive but not sufficient for classification |
| **2.2.** | Flammable gases | None | - | None | Conclusive but not sufficient for classification |
| **2.3.** | Flammable aerosols | None | - | None | Conclusive but not sufficient for classification |
| **2.4.** | Oxidising gases | None | - | None | Conclusive but not sufficient for classification |
| **2.5.** | Gases under pressure | None | - | None | Conclusive but not sufficient for classification |
| **2.6.** | Flammable liquids | None | - | None | Conclusive but not sufficient for classification |
| **2.7.** | Flammable solids | None | - | None | Conclusive but not sufficient for classification |
| **2.8.** | Self-reactive substances and mixtures | None | - | None | Conclusive but not sufficient for classification |
| **2.9.** | Pyrophoric liquids | None | - | None | Conclusive but not sufficient for classification |
| **2.10.** | Pyrophoric solids | None | - | None | Conclusive but not sufficient for classification |
| **2.11.** | Self-heating substances and mixtures | None | - | None | Conclusive but not sufficient for classification |
| **2.12.** | Substances and mixtures which in contact with water emit flammable gases | None | - | None | Conclusive but not sufficient for classification |
| **2.13.** | Oxidising liquids | None | - | None | Conclusive but not sufficient for classification |
| **2.14.** | Oxidising solids | None | - | None | Conclusive but not sufficient for classification |
| **2.15.** | Organic peroxides | None | - | None | Conclusive but not sufficient for |

| CLP Annex I ref | Hazard class | Proposed classification | Proposed SCLs and/or M-factors | Current classification[1)] | Reason for no classification[2)] |
|---|---|---|---|---|---|
| | | | | | classification |
| **2.16.** | Substance and mixtures corrosive to metals | None | - | None | Conclusive but not sufficient for classification |
| **3.1.** | Acute toxicity - oral | None | - | None | Data conclusive but not sufficient for classification |
| | Acute toxicity - dermal | None | - | None | Data conclusive but not sufficient for classification |
| | Acute toxicity - inhalation | None | - | None | Data conclusive but not sufficient for classification |
| **3.2.** | Skin corrosion / irritation | None | - | None | Data conclusive but not sufficient for classification |
| **3.3.** | Serious eye damage / eye irritation | None | - | None | Data conclusive but not sufficient for classification |
| **3.4.** | Respiratory sensitisation | None | - | None | Data lacking |
| **3.4.** | Skin sensitisation | None | - | None | Data conclusive but not sufficient for classification |
| **3.5.** | Germ cell mutagenicity | None | - | None | Data conclusive but not sufficient for classification |
| **3.6.** | Carcinogenicity | None | - | None | Data conclusive but not sufficient for classification |
| **3.7.** | Reproductive toxicity | None | - | None | Data conclusive but not sufficient for classification |
| **3.8.** | Specific target organ toxicity –single exposure | None | - | None | Data conclusive but not sufficient for classification |
| **3.9.** | Specific target organ toxicity – repeated exposure | None | - | None | Data conclusive but not sufficient for classification |
| **3.10.** | Aspiration hazard | None | - | None | Data conclusive but not sufficient for classification |
| **4.1.** | Hazardous to the aquatic environment | Aquatic Acute 1 H400<br>Aquatic Chronic 1 H410 | Acute M factor: 1<br>Chronic M factor: 1 | None | - |
| **5.1.** | Hazardous to the ozone layer | None | - | None | Data lacking |

[1)] *Including specific concentration limits (SCLs) and M-factors*

[2)] *Data lacking, inconclusive, or conclusive but not sufficient for classification*

| | | |
|---|---|---|
| **Labelling:** | Signal word: | WARNING |
| | Pictogram code: | GHS09 |
| | Hazard statements: | H410 Very toxic to aquatic life with long lasting effects |

**Proposed notes assigned to an entry:**

None required according to Annex VI Section 1.1.3 of the CLP Regulation

# 2 BACKGROUND TO THE CLH PROPOSAL

## 2.1 History of the previous classification and labelling

Fludioxonil is not listed on Annex I of Directive 67/548/EEC or in Annex VI of the CLP Regulation. Fludioxonil is already listed on Annex I of Directive 91/414/EEC; here no classification for physical chemical properties nor human health effects was proposed by the RMS (Denmark) or EFSA as part of this evaluation. Based on the results of aquatic toxicity testing and the conclusion that fludioxonil is not readily biodegradable, an environmental classification is required.

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

Physicochemical hazards

Fludioxonil was examined for explosive properties following the procedures specified in test method EEC A.14 and was found to be not explosive. Fludioxonil does not require classification as an explosive material. The flammability of fludioxonil was determined following the procedures specified in test method EEC A.10 and it is considered not highly flammable. Fludioxonil does not require classification as highly flammable. Fludioxonil is not flammable, explosive or oxidising. The molecular structure of fludioxonil does not include any functional groups that indicate a hazard for self-reaction, and there have not been any instances of self-reaction during manufacture or extensive use of the active substance. The material is regarded as thermally stable and does not require classification as a self-reactive substance. Fludioxonil does not possess self-heating properties and it shall not be considered for classification as a pyrophoric solid. Fludioxonil is not flammable, explosive or oxidising. The molecular structure of fludioxonil does not include any functional groups that indicate a hazard for self-heating, and there have not been any instances of self-heating during manufacture or extensive use of the active substance. The material was examined for self-ignition using method EEC A.16 and was found not to have an auto-ignition temperature, indicating it is not an auto-flammable material. Fludioxonil does not require classification as a self-heating substance. Fludioxonil is used in aqueous formulations and does not emit flammable gases in contact with water. Fludioxonil does not require classification as a substance which in contact with water emits flammable gases. Fludioxonil was examined for oxidising properties following the procedures specified in test method EEC A.17 and was found to be not oxidising. Fludioxonil does not require classification as an oxidising solid. The organic peroxide bond grouping is not present in the chemical structure. Fludioxonil does not therefore require classification as an organic peroxide. Fludioxonil is not flammable, explosive or oxidising; and no incidences of damage to metals have occurred during manufacture and use. Fludioxonil is a stable organic molecule with no functional groups that infer strongly acidic or basic properties, and it is considered not to be corrosive to metals. Fludioxonil does not require classification as corrosive to metals.

Human health hazards

Fludioxonil is not classified for acute toxicity on the basis of experimental data for acute oral toxicity (acute oral $LD_{50}$ >5000 mg/kg bw), for acute inhalation toxicity (acute inhalation $LC_{50}$ >2.636 mg/L; stated to be the maximum technically achievable concentration) and for acute dermal toxicity (acute dermal $LD_{50}$ >2000 mg/kg bw). In the absence of any effects (clinical signs or pathology) considered to constitute significant or severe effects in the acute oral, dermal or inhalation toxicity studies, classification of fludioxonil for STOT SE (Category 1 or Category 2) is not required. In the absence of any human data or evidence from the acute inhalation toxicity of significant respiratory irritation, classification of fludioxonil in STOT SE Category 3 is not warranted.

Studies of skin and eye irritation performed in the rabbit with fludioxonil showed minimal effects; classification for skin irritation, eye irritation, skin corrosion or eye damage is therefore not required.

A study of skin sensitisation performed with fludioxonil (Maximisation method) reports an absence of sensitisation reactions; a similar negative response is reported for a Maximisation study performed with a fludioxonil-containing product. No classification is therefore required for skin sensitisation. No data are available for respiratory sensitisation; however fludioxonil is not structurally related to substances known to cause respiratory sensitisation. No classification is therefore proposed for respiratory sensitisation.

Studies of repeated dose toxicity with fludioxonil do not report any significant health effects of relevance to STOT RE classification. Findings indicate the liver, kidney and red blood cell as targets of toxicity; however adverse effects are seen only at relatively high dose levels (above those considered to be relevant for STOT RE classification), with adaptive findings or effects not considered to be of toxicological significance seen at lower dose levels. A NOAEL of 64 mg/kg bw/d is determined for the 90-day oral toxicity study in the rat, based on mild effects in the liver and kidney at the LOAEL of 428 mg/kg bw/d. In the absence of any evidence of 'significant toxicity' at low or generally moderate dose levels from repeated dose toxicity studies, fludioxonil does not require classification as STOT RE.

Negative results are reported for gene mutation *in vitro* in an Ames test and a study of mammalian cell mutation. Fludioxonil did show a clastogenic potential *in vitro* and an equivocal result in 1 of the 5 chromosome aberration tests *in vivo*. However four other *in vivo* chromosome aberration tests were negative and the test with equivocal results were replaced by a newer and better performed study. Fludioxonil, did not show any DNA damaging potential in rat hepatocytes or a genotoxic potential in germ cells of mouse in *in vivo* tests. On the basis of the available data, classification for mutagenicity is not required.

Carcinogenicity studies performed with fludioxonil in the rat and mouse do not provide any evidence of carcinogenicity. A non-significantly increased incidence of hepatocellular tumours seen in the rat at an intermediate dietary concentration was without a dose-response relationship and was within the historical control range; this finding is therefore not considered to be related to treatment with fludioxonil. Significantly increased incidences of lymphoma seen in female mice were within the laboratory's historical control range, and are not observed at the higher tested doses and are therefore not considered to be related to treatment with fludioxonil. No classification for carcinogenicity is therefore proposed for fludioxonil.

A multi-generation reproductive toxicity study performed with fludioxonil in the rat shows no effects on sexual function or fertility. Repeated dose toxicity studies with fludioxonil do not identify any effects on the male or female reproductive tract. The multi-generation study does not indicate any effect of fludioxonil on lactation, or on offspring via lactation. Studies of developmental toxicity performed with fludioxonil in the rat and rabbit do not show any effects on the developing foetus. In the absence of any effects on sexual function or fertility, on developmental toxicity, on or via lactation, no classification for reproductive toxicity is proposed for fludioxonil. There are no additional effects of fludioxonil triggering classification for effects on human health according to the criteria of Regulation (EC) No 1272/2008.

Environmental hazards

The acute toxicity of fludioxonil to fish was investigated in laboratory tests with two freshwater fish species (*Oncorhynchus mykiss*, *Lepomis macrochirus*) and one marine fish species (*Cyprinodon variegatus*); $LC_{50}$ values were found range from 0.23-1.2 mg/L. The lowest $LC_{50}$ of 0.23 mg/L is reported for *Oncorhynchus mykiss*. The acute toxicity of fludioxonil to aquatic invertebrates was investigated in two laboratory tests with one freshwater species (*Daphnia magna*). The $EC_{50}$ values for the fresh water *Daphnia magna* were found to range from 0.40-0.90 mg/L. The effects of fludioxonil on green algae were determined on two algal species *(Desmodesmus subspicatus*, *Pseudokirchneriella subcapitata*). The lowest $ErC_{50}$ value was found to be 0.21 mg/L after 48 hours of exposure. Results for algae are all based on 48-hour exposure and geomean measured concentrations (0-72 h and 0-120 h) due to excessive pH variation at 72 and 120 hours of exposure. The most sensitive organism in the short term toxicity tests is green algae (*P. subcapitata*).

A series of flow-through chronic fish studies are available. Earlier studies are not considered to be reliable due to flocculation of the test material and consequent uncertainty over the achieved test concentrations. The lowest reliable endpoint is a NOEC of 0.04 mg/L from an OECD 215 study with *Oncorhynchus mykiss*. In a fathead minnow (*Pimephales promelas*) early life stage test, mean length and weight and survival at 28-days post-hatch were the most sensitive biological parameters. Based on these observations, the overall chronic NOEC for fish was determined to be 0.039 mg/L. Three 21-day chronic toxicity studies are available with *Daphnia magna*. In one study, the most sensitive biological parameters indicating effects of fludioxonil were the number of offspring per female and the mean body length. The overall NOEC of this study was established to be 0.019 mg/L. In a second study, the fraction of dead young and the time for appearance of first brood were the most sensitive biological parameters. The overall NOEC of this test was determined to be 0.005 mg/L. Although it complied with the guideline requirement current at the time it was performed, the second study failed to meet the reproductive performance criteria in terms of number of live juveniles/adult for studies of long-term toxicity to *D. magna* according to the current state-of-the-art embodied in OECD Guideline 211 (1998 *et seq*.). Adult mortalities were recorded in the latter days of the study, with numbers of immobilised parent animals unrelated to dose. Taken together, these findings suggest that sub-optimal test conditions limited reproductive output and survival. The presence of a solvent system comprising a mixture of acetone and an unidentified alkylphenol-based surfactant reduced juvenile production still further. The NOEC provided by this study is considered to have been influenced by a combination of stress factors in addition to the presence of fludioxonil and is therefore considered to be invalid. The third and most recent study, performed according to OECD 211, provided a reliable overall NOEC of 0.035 mg/L (mean measured), based on effects on reproduction, mortality and growth.

The lowest reliable short term $L/EC_{50}$ endpoints for the three main trophic groups of aquatic organisms (fish, invertebrates and algae) are all < 1 mg/L. Classification in Acute Category 1

therefore applies to fludioxonil. As the lowest L/$EC_{50}$ is 0.21 mg/L (algae, calculated from growth rate) an acute M-factor of 1 is applied. Fludioxonil is not rapidly degradable and adequate chronic toxicity endpoints are available. The lowest reliable chronic NOEC endpoints for fish and invertebrates are both < 0.1 mg/L. Therefore, according to the CLP criteria, classification as Chronic Category 1 also applies to fludioxonil. As the lowest reliable NOEC value is 0.019 mg/L *(Daphnia magna*) a chronic M-factor of 1 is applied.

Based on these findings, fludioxonil should be classified in the following categories:

Aquatic environmental hazard acute category 1, H400.
Aquatic environmental hazard chronic category 1, H410.

For labelling purposes, H400 is subsumed by H410 and the environmental hazard classification shall be indicated by H410 alone.

The following Multiplying Factors apply to fludioxonil when it is present as a component of a mixture:

Acute toxicity M factor: 1
Chronic toxicity M factor: 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

There is currently no classification and labelling for fludioxonil in Annex VI, Table 3.1 in the CLP Regulation.

### 2.3.2 Current classification and labelling in Annex VI, Table 3.2 in the CLP Regulation

There is currently no classification and labelling for fludioxonil in Annex VI, Table 3.2 in the CLP Regulation.

## 2.4 Current self-classification and labelling

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

**Table 4 Current CLP self-classification**

| **Current self-classification according to Regulation (EC) No. 1272/2008** | |
|---|---|
| **Code:** | Aquatic Acute 1, H400, Aquatic Chronic 1, H410 |
| **Hazard statement:** | H410 Very toxic to aquatic life with long lasting effects |
| **Symbol:** |  |

| | |
|---|---|
| **Hazard Category:** | Category 1 |
| **Signal Word** | Warning |
| **Precautionary Statements:** | -- |
| **Prevention** | P273 Avoid release to the environment. |
| **Response** | P391 Collect spillage |
| **Storage** | Not applicable. |
| **Disposal** | P501 Dispose of contents/container in accordance with local regulations. |

Self-classification declared in the CLP inventory for fludioxonil includes:

H400/H410 with labelling as either H410 or H400/H410
H400 with labelling as H400
H410 with labelling as H410

# 3 JUSTIFICATION THAT ACTION IS NEEDED AT COMMUNITY LEVEL

Not applicable for biocides.

# PART B.

## SCIENTIFIC EVALUATION OF THE DATA

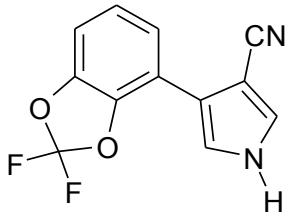
# 1 IDENTITY OF THE SUBSTANCE

## 1.1 Name and other identifiers of the substance

**Table 5: Substance identity**

| | |
|---|---|
| **EC number:** | no entry |
| **EC name:** | no entry |
| **CAS number (EC inventory):** | 131341-86-1 |
| **CAS number:** | 131341-86-1 |
| **CAS name:** | 4-(2,2-difluoro-1,3-benzodioxol-4-yl)-1*H*-Pyrrole-3-carbonitrile |
| **IUPAC name:** | 4-(2,2-difluoro-1,3-benzodioxol-4-y1)-1H-pyrrole-3-carbonitrile |
| **CLP Annex VI Index number:** | 608-RST-VW-Y |
| **Molecular formula:** | $C_{12}H_6F_2N_2O_2$ |
| **Molecular weight range:** | 248.2 g/mol |

**Structural formula:**




## 1.2 Composition of the substance

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

| Constituent | Typical concentration | Concentration range | Remarks |
|---|---|---|---|
| Fludioxonil; 1*H*-Pyrrole-3-carbonitrile, 4-(2,2-difluoro-1,3-benzodioxol-4-yl)- ; (4-(2,2-difluoro-1,3-benzodioxol-4-yl)-1H-pyrrole-3-carbonitrile) | ≥ 95% (w/w) | - | - |

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

| Impurity | Typical concentration | Concentration range | Remarks |
|---|---|---|---|
| No impurities present at >1% and none of the impurities at lower levels are considered relevant for the classification of the substance (information on impurities are provided in | - | - | - |

| | | | |
|---|---|---|---|
| IUCLID) | | | |

**Table 8: Additives (non-confidential information)**

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

### 1.2.1 Composition of test material

The composition of batches of the test material used is given in the biocide IUCLID file.

## 1.3 Physicochemical properties

**Table 9: Summary of physicochemical properties**

| Property | Value | Reference | Comment (e.g. measured or estimated) |
|---|---|---|---|
| State of the substance at 20°C and 101.3 kPa | Powder<br><br>Fine powder | Rodler, M., 1992 (IUCLID 3.1-01)<br><br>Das, R., 1998 (IUCLID 3.1-02) | Visual assessment (96.8%, technical grade of active ingredient)<br>Visual assessment (99.9%, pure active ingredient) |
| Melting/freezing point | 199.8˚C | Rodler, M., 1992 (IUCLID 3.2-01) | Measured |
| Boiling point | Not determined since thermal decomposition starts at about 306°C | Das, R., 2000 (IUCLID 3.4-01) | NA |
| Relative density | Bulk density = 1.54 x $10^3$ kg/$m^3$ corresponding to a relative density of 1.54 | Füldner, H., 1992 (IUCLID 3.5-01) | Measured |
| Vapour pressure | Temperature: 25ºC<br>3.9 x $10^{-7}$ Pa (extrapolated)<br>Vapour pressure curve:<br>$^{10}$log P [Pa] = 16.8495 - 6936.15 x 1/T [K] | Rordorf, B., 1992 (IUCLID 3.7.1-01) | Measured |
| Henry's law constant | Measured/calculated:<br>5.4 x $10^{-5}$ Pa m³/mol | Burkhard, N., 1994 (IUCLID 3.7.2-01) | Measured/calculated |
| Surface tension | 47.7 - 48.5 mN/m<br>(plate method applied to filtrates of 10 g/L suspension, concentration 1.8 mg/L, 100% saturated solution)<br>Temperature: 20ºC<br>The surface tension is lower than 60 mN/m indicating that fludioxonil is regarded as a surface active substance. | Ryser, M., 1992 (IUCLID 3.8-01) | Measured |

| Property | Value | Reference | Comment (e.g. measured or estimated) |
|---|---|---|---|
| Water solubility | 1.8 mg/L<br>pH: Fludioxonil has no dissociation within the range pH 2 to pH 12, that means the pH has no effect on the water solubility of the compound in the pH range 5 to 9<br>The solubility is low and therefore effects of temperature and pH are not expected to be significant. | Rodler, M., 1992 (IUCLID 3.9-01) | Measured |
| Partition coefficient n-octanol/water | log Pow = 4.12 ± (0.016)<br>pH: Fludioxonil has no dissociation within the range pH 2 to pH 12, that means the pH has no effect to partition coefficient of the compound in the pH range 5 to 9 | Rodler, M., 1992 (IUCLID 3.10-01) | Measured |
| Flash point | Not applicable because the physical state is powder / fine powder (i.e. solid) with a melting point at 199.8˚C | NA | NA |
| Flammability | Fludioxonil is not considered highly flammable | Jackson, W.A., 2004 (IUCLID 4.2-02) | Measured |
| Explosive properties | Not explosive | Schürch, H., 1992 (IUCLID 4.1-01) | Measured |
| Self-ignition temperature | Not auto-flammable.<br>No auto-ignition temperature | Schürch, H., 1992 (IUCLID 4.17.1-02) | Measured |
| Oxidising properties | Not oxidising | Schürch, H., 1992 (IUCLID 4.4-01) | Measured |
| Granulometry | Median: 46.6 µm<br>% undersize d(10): 17.8 µm<br>% undersize d(90): 90.1 µm | Das, R., 2009 (IUCLID 3.14-01) | Measured |
| Stability in organic solvents and identity of relevant degradation products | Not applicable because the active substance as manufactured does not include an organic solvent and is not formulated in organic solution in the biocidal product. | NA | NA |
| Dissociation constant | The estimated dissociation constants of fludioxonil in water were found to be:<br>$pK_{a1} < 0$ (basic)<br>$pK_{a2}$ ~14.1 (acidic) | Jäkel, K., 1992 (IUCLID 3.13-01) | Measured |
| Viscosity | Not applicable because the active substance is a solid. | NA | NA |

# 2 MANUFACTURE AND USES

## 2.1 Manufacture

Not relevant for hazard classification.

## 2.2 Identified uses

| | |
|---|---|
| **Product type** | Fungicide for material preservation in PT7, PT9 and PT10 (biocidal product types) |
| **Intended use pattern(s)** | PT7 Film preservatives<br>For PT7 uses, fludioxonil is formulated as the preservative product Sporgard WB which is added to paints, silicon coatings, mineral sealants and grouts. Other preservative products containing fludioxonil may be used in silicon sealants and grout.<br><br>PT9 Fibre, leather, rubber and polymerised material preservatives<br>For PT9 uses, fludioxonil is formulated as the preservative product Sporgard WB which is added to paper which is used for the production of wall linings.<br><br>PT10 Masonry preservatives<br>For PT10 uses, fludioxonil is formulated as the preservative product Sporgard WB which is added to building materials such as gypsum boards. |
| **Users** | Fludioxonil in combination with other fungicides are added during material production. The end-use treated items may be used by professional workers and by the general public (non-professional), depending on the individual item. |

# 3 CLASSIFICATION FOR PHYSICAL HAZARDS

**Table 10: Summary table for relevant physicochemical studies**

| Method | Results | Remarks | Reference |
|---|---|---|---|
| EEC A.14 | Not explosive | - | Schürch, H., 1992 (IUCLID 4.1-01) |
| EEC A.10 (Flammability of solids) | Fludioxonil is not considered highly flammable | - | Jackson, W.A., 2004 (IUCLID 4.2-02) |
| EEC A.17 | Not oxidising | - | Schürch, H., 1992 (IUCLID 4.4-01) |
| EEC A.16 (Auto-ignition) | Not auto-flammable.<br>No auto-ignition temperature | - | Schürch, H., 1992 (IUCLID 4.17.1-02) |

## 3.1 Physical hazards

### 3.1.1 Summary and discussion of physical hazards

Experimental data have been generated for explosive properties, flammability, oxidising properties, and relative self-ignition temperature. All other relevant physicochemical parameters have been assessed by considering the chemical nature of fludioxonil and the results of relevant safety tests. It

could be concluded that fludioxonil is not highly flammable, explosive, oxidising or auto-flammable, and should not be classified on the basis of its physicochemical properties.

### 3.1.2 Comparison with criteria

Explosivity

Fludioxonil was examined for explosive properties following the procedures specified in test method EEC A.14 and was found to be not explosive. Fludioxonil does not require classification as an explosive material.

Flammable solids

The flammability of fludioxonil was determined following the procedures specified in test method EEC A.10 and it is considered not highly flammable. Fludioxonil does not require classification as highly flammable.

Self-reactive substances and mixtures

Fludioxonil is not flammable, explosive or oxidising. The molecular structure of fludioxonil does not include any functional groups that indicate a hazard for self-reaction, and there have not been any instances of self-reaction during manufacture or extensive use of the active substance. The material is regarded as thermally stable and does not require classification as a self-reactive substance.

Pyrophoric solids

Fludioxonil does not possess self-heating properties and it shall not be considered for classification as a pyrophoric solid.

Self-heating substances and mixtures

Fludioxonil is not flammable, explosive or oxidising. The molecular structure of fludioxonil does not include any functional groups that indicate a hazard for self-heating, and there have not been any instances of self-heating during manufacture or extensive use of the active substance. The material was examined for self-ignition using method EEC A.16 and was found not to have an auto-ignition temperature, indicating it is not an auto-flammable material. Fludioxonil does not require classification as a self-heating substance.

Substances and mixtures which in contact with water emit flammable gases

Fludioxonil is used in aqueous formulations and does not emit flammable gases in contact with water. Fludioxonil does not require classification as a substance which in contact with water emits flammable gases.

Oxidising solids

Fludioxonil was examined for oxidising properties following the procedures specified in test method EEC A.17 and was found to be not oxidising. Fludioxonil does not require classification as an oxidising solid.

Organic peroxides

The organic peroxide bond grouping is not present in the chemical structure. Fludioxonil does therefore not require classification as an organic peroxide.

Substance and mixtures corrosive to metals

The substance is not flammable, explosive or oxidising; and no incidences of damage to metals have occurred during manufacture and use. Fludioxonil is a stable organic molecule with no functional groups that infer strongly acidic or basic properties, and it is considered not to be corrosive to metals. Fludioxonil does not require classification as corrosive to metals.

### 3.1.3 Conclusions on classification and labelling

No classification is proposed for fludioxonil in relation to its physicochemical properties, based on the available data and information.

# 4 HUMAN HEALTH HAZARD ASSESSMENT

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

### 4.1.1 Non-human information

The absorption, distribution and metabolism of fludioxonil were investigated in the rat using radiolabelled test material in four studies (Bissing, 1990: Thanei, 1992: Muller, 1995 and Thanei, 1994). Fludioxonil was rapidly absorbed following oral administration to the rat; maximum blood levels were attained within 30 minutes. Figures for the excretion of radioactivity in bile duct cannulated rats indicate that at least 77.5% of the administered dose was absorbed from the gastrointestinal tract into the systemic circulation within 48 hours. The actual amount of excreted radioactivity is likely to be greater than this due to the loss of one bile sample from one animal. It is also notable that the total amount of radioactivity recovered from excreta (urine, faeces and bile) in this study is relatively low (91.8% of the administered radioactivity), indicating a level of residual radioactivity in the carcass and/or gastrointestinal tract of approximately 8%. The incomplete excretion of radioactivity by bile duct-cannulated rats from 0-48 hours following dosing is consistent with the pattern seen for other groups, in which excretion during the first 24 hours accounted for approximately 75-90% of the administered dose and excretion within 7 days accounted for 94-97% of the administered dose. It is also known that excretion may be slower in bile duct-cannulated rats. It is therefore concluded that the total level of radioactivity absorbed by the bile duct cannulated rats is likely to exceed 80% of the administered dose.

Fludioxonil was excreted in urine (13-20%) and to a greater extent (78-83%) in faeces; the majority of the faecal radioactivity (68% of the administered dose) was found to be of biliary origin and there is evidence for a degree of enterohepatic recirculation. Residues were low in all tissues, but were comparatively high in the liver and kidney, reflecting biliary and urinary excretion; there is no evidence for accumulation. Tissue residues accounted for 0.06% of the low dose and 0.17% of the high dose after 7 days. Absorbed fludioxonil was completely metabolised, with 20 metabolites detected in urine. Unchanged fludioxonil was the only identified component in faeces (Bissig, 1990) and the unchanged parent compound was not found in urine. The metabolite pattern was complex but appeared to be independent of sex, dose level or pre-treatment.

Following a single low or high oral dose of fludioxonil to rats, absorption was rapid, as was the clearance of radioactivity from blood. AUC values indicate a slightly lower level of absorption in

both sexes at the high dose level and also indicate slightly lower systemic exposure in females at both dose levels. Tissue concentrations of radioactivity declined rapidly: no accumulation or retention of fludioxonil or its metabolites is predicted.

Metabolism involved oxidation of the pyrrole ring, hydroxylation of the phenyl ring and conjugation with glucuronic acid and sulphate. The major metabolites were identified as the glucuronide and sulphate conjugates of the hydroxylated metabolite SYN 51877. Urine from animals exposed to high dietary doses (1000 and 3000 ppm) for a prolonged period showed a blue colouration. This colouration was due to a metabolite of fludioxonil, which was identified as a dimer formed by metabolic oxidation of the pyrrole moiety, followed by autoxidative dimerisation. The coloured dimer accounted for about 1-2% of the intake of 3000 ppm fludioxonil in male rats (Thanei, 1994). The formation of this metabolite accounts for the coloration of excreta and various tissues in the standard toxicity studies.

### 4.1.2 Human information

No human data are available.

### 4.1.3 Summary and discussion on toxicokinetics

Approximately 80% of a high dose was absorbed from the gastrointestinal tract based on the data obtained for the bile duct-cannulated animals. The absorbed material was rapidly excreted, with 13-20% detected in the urine and 78-83% in the faeces within 24 hours. Excretion was mainly via the bile (68%), with evidence for a degree of enterohepatic recirculation. Considering the level of residual carcass radioactivity in cannulated rats and based on the characterisation of metabolites, the total systemic availability of fludioxonil is considered to be in excess of 80% at the high dose level and is likely to be almost quantitative at low dose levels. At $T_{max}$ (0.5 hours), tissue concentrations were below 0.05 ppm equivalents except in liver, kidneys, lungs and plasma and declined rapidly. Residues were low in all tissues, but were comparatively high in the liver and kidney, reflecting ongoing biliary and urinary excretion. As a consequence of the rapid elimination from tissues, no accumulation or retention of the test substance and/or its metabolites is expected. Tissue distribution appeared to be largely independent of sex and dose. Excretion rate and route was independent of the sex and dose level. The metabolite pattern was complex but appeared to be independent of sex, dose level, and pre-treatment. Unchanged fludioxonil was detected in faeces as the only identified component but was not found in the urine.

## 4.2 Acute toxicity

**Table 11: Summary table of relevant acute toxicity studies**

| Method | Results | Remarks | Reference |
|---|---|---|---|
| **Oral:** OECD 401 (1987), GLP 1<br>Rat, Sprague Dawley SD, five/sex<br>Test material: fludioxonil (purity 95.4%)<br>Limit test: 5000 mg/kg bw | $LD_{50}$ >5000 mg/kg bw | LD50 value does not trigger classification | Glaza, S.M, 1991a (IUCLID 8.7.1-01) |
| **Inhalation:** OECD 403 (1981), GLP 1<br>Rat Tif:RAIf (SPf<br>Test material: fludioxonil (purity97.5%)<br>Dust/solid aerosol; MMAD 2.2-2.4 µm, 1.6 µm | $LC_{50}$ >2.636 mg/L (4 h, nose only) | LC50 value does not trigger classification | Hartmann, H.R., 1989 (IUCLID 8.7.2-01) |

| Method | Results | Remarks | Reference |
|---|---|---|---|
| GSD<br>2.636 mg/ $m^3$: nose-only | | | |
| **Dermal:** OECD 402 (1987), GLP 1<br>Rat Tif:RAI f (SPF) five/sex<br>Test material: fludioxonil (purity 97.5%).<br>Vehicle: distilled water containing 0.5% carboxymethylcellulose and 0.1% polysorbate 80<br>Limit test: 2000 mg/kg bw, >10% of body surface; intact skin. | $LD_{50}$<br>>2000 mg/kg bw | $LD_{50}$ value does not trigger classification | Hartmann, H.R., 1988<br>(IUCLID 8.7.3-01) |

### 4.2.1 Non-human information

#### 4.2.1.1 Acute toxicity: oral

In the acute oral toxicity study (Glaza, 1991a), clinical signs were limited to soft stool in half of the animals on the day of dosing; all animals appeared normal on the following day. No deaths occurred during the observation period following administration of a limit dose of 5000 mg/kg bw. Bodyweight gain was unaffected by treatment. No effects of treatment were observed in any test animal at necropsy. The acute oral $LD_{50}$ of fludioxonil in the rat was therefore found to be >5000 mg/kg bw under the conditions of this study.

#### 4.2.1.2 Acute toxicity: inhalation

In the acute inhalation toxicity study (Hartmann, 1989), no deaths occurred. Signs of toxicity in exposed rats included piloerection, hunched posture and dyspnoea. All treated animals appeared normal by Day 5. Reduced weight gain was apparent in exposed males. The acute inhalation $LC_{50}$ of fludioxonil in the rat was found to be >2.636 mg/L under the conditions of this study; the tested concentration is stated to have been the maximum technically achievable.

#### 4.2.1.3 Acute toxicity: dermal

No deaths occurred in the acute dermal toxicity study at the limit dose of 2000 mg/kg bw/d (Hartmann, 1988). Signs of toxicity (including piloerection, hunched posture, ventral recumbency and dyspnoea) were observed in all animals and persisted for up to six days. Weight loss was observed in two males and one female during week 2, however all animals gained weight over the study period. Gross necropsy did not reveal any treatment-related findings. The acute dermal $LD_{50}$ of fludioxonil in the rat was therefore found to be >2000 mg/kg bw under the conditions of this study.

#### 4.2.1.4 Acute toxicity: other routes

No data are available.

### 4.2.2 Human information

No human data are available.

### 4.2.3 Summary and discussion of acute toxicity

Fludioxonil was found to be of low acute toxicity via the oral, dermal and inhalation routes. The acute oral $LD_{50}$ was >5000 mg/kg bw. The acute inhalation $LC_{50}$ of fludioxonil was found to exceed the maximum achievable concentration of 2.636 mg/L. The acute dermal $LD_{50}$ of fludioxonil was found to be >2000 mg/kg bw.

### 4.2.4 Comparison with criteria

Classification for acute oral toxicity under Regulation (EC) No 1272/20008 (Section 3.1) is required for substances with an acute oral $LD_{50}$ value (or estimated $LD_{50}$ value) of ≤2000 mg/kg bw. Fludioxonil is reported to have an acute oral $LD_{50}$ of >5000 mg/kg bw; therefore classification is not required for acute oral toxicity.

Classification for acute dermal toxicity under Regulation (EC) No 1272/20008 (Section 3.1) is required for substances with an acute dermal $LD_{50}$ value of ≤2000 mg/kg bw. Fludioxonil is reported to have an acute dermal $LD_{50}$ of >2000 mg/kg bw; therefore classification is not required for acute dermal toxicity.

Classification for acute inhalation toxicity under Regulation (EC) No 1272/20008 (Section 3.1) is required for substances (dusts and mists) with an acute inhalation $LD_{50}$ value of ≤5 mg/L. Fludioxonil is reported to have an acute inhalation $LC_{50}$ of >2.636 mg/L. This concentration is reported to be the maximum technically achievable; therefore classification is not required for acute inhalation toxicity.

### 4.2.5 Conclusions on classification and labelling

Based on the available data, no classification is required for acute oral, dermal or inhalation toxicity according to Regulation (EC) No 1272/2008.

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

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

Classification with STOT SE is appropriate for substances showing clear evidence of toxicity to a specific organ following a single exposure, especially where this is seen in the absence of lethality. Specific target organ toxicity (single exposure) is defined as specific, non-lethal target organ toxicity arising from a single exposure (Section 3.8.1.1 of Annex I of the CLP Regulation). All significant health effects that can impair function, both reversible and irreversible, immediate and/or delayed, are included in this category. Relevant data for fludioxonil are limited to the acute oral, dermal and inhalation toxicity studies discussed in Section 4.2, above. No acute neurotoxicity studies are available.

### 4.3.2 Comparison with criteria

Classification in STOT SE Category 1 is required for substances that have produced significant toxicity in humans or which, on the basis of studies in experimental animals, can be presumed to have the potential to produce significant toxicity in humans following a single exposure. Substances are classified in Category 1 on the basis of reliable and good quality evidence from

human cases, or observations from animal studies in which significant and/or severe effects of relevance to human health are seen at generally low exposure levels.

Exposure levels relevant to classification in Category 1 are defined (Annex I: 3.8.2.1.9.3 of the CLP Regulation) as ≤300 mg/kg bw (oral route, rat); ≤1000 mg/kg bw (dermal route, rat) and ≤1 mg/L (inhalation route, rat, dust). In the absence of human data and in the absence of any effects (clinical signs or pathology) considered to constitute significant or severe effects in the acute oral, dermal or inhalation toxicity studies, classification of fludioxonil in Category 1 for STOT SE is not required.

Classification in STOT SE Category 2 is required for substances showing significant toxic effects of relevance to humans, in studies in experimental animals and at generally moderate exposure levels.

In the absence of any effects (clinical signs or pathology) considered to constitute significant or severe effects in the acute oral, dermal or inhalation toxicity studies, classification of fludioxonil in Category 2 for STOT SE is not required.

### 4.3.3 Conclusions on classification and labelling

Fludioxonil does not require classification for STOT SE (Category 1 or 2) according to Regulation (EC) No 1272/2008., based on the available data.

## 4.4 Irritation

### 4.4.1 Skin irritation

**Table 12: Summary table of relevant skin-irritation studies**

| Method | Results | Remarks | Reference |
|---|---|---|---|
| OECD 404 (1992), GLP, 1<br>Rabbit NZW M(3)<br>Fludioxonil (97.5% purity)<br>No vehicle (gauze patch moistened with distilled water)<br>0.5 g fludioxonil/animal<br>4 hour exposure (7 days post exposure period) | Erythema: 0.22<br>Oedema: 0.00<br><br>Very slight erythema in two rabbits at one hour and persisted to 48 hours in one animal. | Dermal reactions do not trigger classification | Schneider,M.,1988a<br>(IUCLID 8.1-01) |
| OECD 404 (1992), GLP, 1<br>Rabbit NZW 3/sex<br>Fludioxonil (95.4% purity),<br>No vehicle (0.9% saline used for moistening the test substance)<br>0.5 g fludioxonil/animal<br>4 hour exposure (7 days post exposure period) | Erythema: 0.00<br>Oedema: 0.00<br><br>No local dermal reactions were observed in any animal at any time point | Dermal reactions do not trigger classification | Glaza,.M.,1991b<br>(IUCLID 8.1-02) |

#### 4.4.1.1 Non-human information

Fludioxonil was found to be a non-irritant to the skin in one study and a minimal skin irritant (reactions of low severity and readily reversible) in a further study.

#### 4.4.1.2 Human information

No human data are available.

#### 4.4.1.3 Summary and discussion of skin irritation

Two studies of skin irritation in the rabbit (OECD 404) are available for fludioxonil. In one study (Schneider, 1988a), findings were limited to slight erythema (mean score of 0.22 for 24-72 hours) in 2 of 3 male New Zealand white rabbits. In a second study, no signs of local irritation were observed at any time point in any of the 3 male or 3 female New Zealand white rabbits, following a 4-hour semi-occluded application of undiluted fludioxonil.

#### 4.4.1.4 Comparison with criteria

Skin irritation is defined as the production of reversible damage to the skin following the application of a test substance for up to 4 hours (Annex I: 3.2.1.1). Classification of a substance for skin irritation (Category 2) is required on the basis of an animal study showing a mean (24-72 hour) value of between 2.3-4.0 for erythema/eschar or for oedema in at least 2 of 3 tested or, if reactions are delayed, from three consecutive days after the onset of skin reactions. Classification is also required for inflammation that persists to the end of the observation period (normally 14 days) in at least 2 animals, particularly taking into account findings such as alopecia, hyperkeratosis, hyperplasia, and scaling. Classification may also be required in some cases where there is pronounced variability of response among animals, with very definite positive effects related to exposure in a single animal but less than the criteria listed above.

One of the skin irritation studies in rabbits with fludioxonil (Schneider, 1988a) mean (24-72 hour) scores of 0.22 for erythema/eschar and 0.00 for oedema were obtained. A second study (Glaza, 1991b) reports mean (24-72 hour) scores of 0.00 for erythema/eschar and for oedema. Fludioxonil is therefore shown to be a non-irritant or minimal irritant and does not require classification as a skin irritant based on comparison with the criteria detailed in Regulation (EC) No 1272/2008.

#### 4.4.1.5 Conclusions on classification and labelling

Fludioxonil is not classified for skin irritation according to Regulation (EC) No 1272/2008 on the basis of the data available.

### 4.4.2 Eye irritation

**Table 13: Summary table of relevant eye irritation studies**

| Method | Results | Remarks | Reference |
|---|---|---|---|
| OECD 405, GLP 1<br>Rabbit, NZW, f (3)<br>Fludioxonil (97.5%)<br>0.1 ml (31 mg) of fludioxonil<br>72 hours observations period after instillation | Erythema: 0.22<br>Chemosis: 0.00<br>Iris: 0.00<br>Cornea: 0.00 | Reactions do not trigger classification | Schneider, M, 1988b (IUCLID 8.2-01) |

#### 4.4.2.1 Non-human information

One eye irritation study in the rabbit is available (Schneider, 1988b). Fludioxonil was found to be a mild eye irritant under the conditions of this study; findings were limited to conjunctival erythema (mean score of 0.22 for 24-72 hours for all three animals; individual scores for 2/3 animals were 0.3 for erythema) and were reversible within 48 hours.

#### 4.4.2.2 Human information

No human data are available.

#### 4.4.2.3 Summary and discussion of eye irritation

In a rabbit study, slight redness (mean score of 0.22 for the 24-72 hours readings) of the conjunctival sac was seen in 2 of 3 female New Zealand white rabbits while no effects were observed on cornea or iris.

#### 4.4.2.4 Comparison with criteria

Serious eye damage (Category 1) is defined as the production of tissue damage in the eye, or serious physical decay of vision, following application of a substance to the anterior surface of the eye, which is not fully reversible within 21 days of application (Annex I: 3.3.1.1).

Eye irritation (Category 2) is defined as the production of changes in the eye following the application of test substance to the anterior surface of the eye, which are fully reversible within 21 days of application (Annex I: 3.3.1.1).

Classification in Category 1 is required for substances producing (in at least in one animal) effects on the cornea, iris or conjunctivae that are not expected to reverse or have not fully reversed within the observation period (normally 21 days). Classification is also required where (in at least 2 of 3 animals) mean (24-72 hour) scores of ≥3 for corneal opacity or >1 for iritis are attained.

Classification in Category 2 is required for substances producing (in at least 2 of 3 animals) mean (24-72 hour) scores of ≥1 for corneal opacity, ≥1 for iritis, ≥2 for conjunctival erythema and/or ≥2 for chemosis.

In the single study available (Schneider, 1988b), findings were limited to conjunctival erythema (mean 24-72 hour score of 0.22). Fludioxonil does therefore not require classification for serious eye damage (Category 1) or for eye irritation (Category 2) according to Regulation (EC) No 1272/2008.

#### 4.4.2.5 Conclusions on classification and labelling

Fludioxonil is not classified for eye irritation according to Regulation (EC) No 1272/2008 on the basis of the available data.

### 4.4.3 Respiratory tract irritation

#### 4.4.3.1 Non-human information

#### 4.4.3.2 Human information

No human data are available.

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

No significant signs of respiratory tract irritation were observed in the acute inhalation study in rats (Hartmann, 1989). No longer-term inhalation toxicity studies are available.

#### 4.4.3.4 Comparison with criteria

Classification in STOT SE Category 3 is required for substances showing evidence of transient effects following inhalation exposure, specifically respiratory irritation. In the absence of any human data or evidence from the acute inhalation toxicity of significant respiratory irritation, classification of fludioxonil in STOT SE Category 3 is not required.

#### 4.4.3.5 Conclusions on classification and labelling

Fludioxonil does not require classification for STOT SE Category 3 according to Regulation (EC) No 1272/2008., based on the available data.

### 4.5 Corrosivity

**Table 14: Summary table of relevant corrosivity studies**

| Method | Results (average score (24, 48 & 72 h) | Remarks | Reference |
|---|---|---|---|
| OECD 404 (1992), GLP, 1<br>Rabbit NZW M(3)<br>Fludioxonil (97.5% purity)<br>No vehicle (gauze patch moistened with distilled water)<br>0.5 g fludioxonil/animal<br>4 hour exposure (7 days post exposure period) | Erythema: 0.22<br>Oedema: 0.00<br><br>Very slight erythema in two rabbits at one hour and persisted to 48 hours in one animal. | Dermal reactions do not trigger classification | Schneider,M.,1988a<br>(IUCLID 8.1-01) |
| OECD 404 (1992), GLP, 1<br>Rabbit NZW 3/sex<br>Fludioxonil (95.4% purity),<br>No vehicle (0.9% saline used for moistening the test substance)<br>0.5 g fludioxonil/animal<br>4 hour exposure (7 days post exposure period) | Erythema: 0.00<br>Oedema: 0.00<br><br>No local dermal reactions were observed in any animal at any time point | Dermal reactions do not trigger classification | Glaza,.M.,1991b<br>(IUCLID 8.1-02) |

### 4.5.1 Non-human information

Fludioxonil was found not to be corrosive to the skin in two studies performed in the rabbit.

### 4.5.2 Human information

No human data are available.

### 4.5.3 Summary and discussion of corrosivity

No evidence of corrosivity and only minimal skin irritation was seen in two studies performed in the rabbit.

### 4.5.4 Comparison with criteria

Skin corrosion is described (Annex I: 3.2.1.1 of the CLP Regulation) as the production of irreversible damage to the skin; i.e. visible necrosis through the epidermis and into the dermis, following the application of a test substance for up to 4 hours. Corrosive reactions are typified by ulcers, bleeding, bloody scabs, and, by the end of observation at 14 days, by discolouration due to blanching of the skin, complete areas of alopecia, and scars.

Fludioxonil was investigated for skin irritation and corrosion in two studies performed in the rabbit (Schneider, 1988a; Glaza, 1991b). No evidence of skin corrosion was seen in either of these studies.

### 4.5.5 Conclusions on classification and labelling

Fludioxonil does not require classification for skin corrosion according to Regulation (EC) No 1272/2008, based on the available data.

## 4.6 Sensitisation

### 4.6.1 Skin sensitisation

**Table 15: Summary table of relevant skin sensitisation studies**

| Method | Results | Remarks | Reference |
|---|---|---|---|
| Skin sensitisation Maximization test (OECD 406, 1992), GLP, 1<br>Guinea pig (Tif:DHP) 10/sex , 5/sex (control)<br>Fludioxonil (97.5%), Vaseline<br>1% fludioxonil for intradermal induction<br>30% fludioxonil for topical induction<br>10% fludioxonil for topical challenge<br>Intradermal and topical exposure<br>48 hr for induction<br>24 hr for challenge | 0/20 animals in the test group showed any dermal reactions following challenge. | No evidence of sensitisation | Schneider, M., 1988c (IUCLID 8.3-01) |
| Skin sensitization Maximization test (OECD 406, 1999) GLP, 1<br>Celest 025 FS containing 26 g Fludioxonil/l, | 0/20 animals in the test group showed any dermal reactions | No evidence of sensitisation | Cantoreggi, S., 1999 (IUCLID 8.3-02) |

| Method | Results | Remarks | Reference |
|---|---|---|---|
| Physiological saline<br>5% for intradermal induction<br>80% for topical induction<br>5% for topical challenge<br>Intradermal and topical exposure<br>48 hr for induction<br>24 hr for challenge | following challenge. | | |

#### 4.6.1.1 Non-human information

The potential of fludioxonil to induce delayed contact hypersensitivity was investigated in a Maximisation test using ten test animals/sex and five control animals/sex. Test substance concentrations of 1% and 30% were used for intradermal and topical induction exposures, respectively. Local dermal irritation at the application site was induced using sodium lauryl sulphate prior to topical induction. Test and control animals were challenged using a concentration of 10% test substance. The challenge concentrations were considered to be too low (A concentration of 30% fludioxonil did not induce mild-to moderate skin irritation and 10% can therefore not be said to be the highest non-irritant dose) and partially invalidate the study. No dermal reactions were observed in test or control animals following the challenge exposure; therefore no evidence of sensitisation was seen under the conditions of this study. Further confirmatory data are needed to draw the final conclusion regarding skin sensitisation.

In a Maximisation test performed with the product Celest 025 FS (containing 26 g fludioxonil/L), delayed contact hypersensitivity was investigated using 10 test animals/sex and 5 control animals/sex. At pre-test, irritation was produced after intradermal induction with concentrations from 0.5-5% in physiological saline and after topical application of concentrations from 10-100% (vehicle physiological saline) in both male and female guinea pigs. Concentrations selected for the maximisation test were 5% and 80% (in physiological saline) for intradermal and topical induction, respectively; 5% was selected for topical challenge. It was not possible to document irritation by the test material during topical induction due to the colour of the test material. There were no positive skin reactions following topical challenge with test material or physiological saline either at the 24-hour or the 48-hour readings in either of the test group or the control group animals. The product Celest 025 FS was not considered to be a skin sensitiser under the conditions of the performed study.

#### 4.6.1.2 Human information

No human data are available.

#### 4.6.1.3 Summary and discussion of skin sensitisation

A Maximisation study performed with fludioxonil (Schneider, 1988c) and a Maximisation study performed with a product containing fludioxonil (Cantoreggi, 1999) are available. Neither study shows any evidence of sensitisation, however the challenge concentration of 10% in the first study (Schneider, M., 1988c) was regarded to be too low. The study is considered acceptable, but the OECD guideline was not fulfilled referring to the levels of doses selected. This might partially invalidate the study. However, a second study (Cantoreggi, S., 1999) is available with a formulation containing only one active substance, showing negative results.

Taking into account all the available information, no sensitising potential is expected of fludioxonil.

#### 4.6.1.4 Comparison with criteria

A skin sensitiser is defined as a substance that will lead to an allergic response following skin contact (Annex 1: 3.4.1.2 of the CLP Regulation). Skin sensitisers are allocated into Category 1A (strong sensitisers) or Category 1B (other sensitisers), based on a weight of evidence from reliable and good quality evidence from human cases or epidemiological studies and/or observations from appropriate studies in experimental animals.

Substances are classified as Category 1 skin sensitisers where data are not sufficient for sub-categorisation, if there is evidence in humans that the substance can lead to sensitisation by skin contact in a substantial number of persons, or if there are positive results from an appropriate animal test.

Substances are classified as Category 1A skin sensitisers where there is evidence of a high frequency of occurrence in humans and/or a high potency in animals. For the Maximisation study, substances are allocated to Category 1A where a response of ≥30% is seen at intradermal induction concentrations of ≤0.1%; or where a response of ≥60% is seen at intradermal induction concentrations of >0.1% to ≤1%.

Substances are classified as Category 1B skin sensitisers where there is evidence of a low to moderate frequency of occurrence in humans and/or a low to moderate potency in animals. For the Maximisation study, substances are allocated to Category 1B where a response of ≥30% to <60% is seen at intradermal induction concentrations of >0.1% to ≤1%; or where a response of ≥30% is seen at intradermal induction concentrations of >1%.

Since no positive reactions were seen in either Maximisation study performed with fludioxonil and there is no human data, fludioxonil is not classified as a skin sensitiser.

#### 4.6.1.5 Conclusions on classification and labelling

Taking into account all evidences fludioxonil does not require classification for skin sensitisation according to Regulation (EC) No 1272/2008, based on the available data.

### 4.6.2 Respiratory sensitisation

**Table 16: Summary table of relevant respiratory sensitisation studies**

| Method | Results | Remarks | Reference |
|---|---|---|---|
| No data are available | | | |

#### 4.6.2.1 Non-human information

No non-human data are available. There are no formally recognised and validated animal tests for respiratory sensitisation.

#### 4.6.2.2 Human information

No human data are available.

#### 4.6.2.3 Summary and discussion of respiratory sensitisation

No data are available on the potential of fludioxonil to cause respiratory sensitisation. Fludioxonil is not structurally related to substances known to cause respiratory sensitisation.

#### 4.6.2.4 Comparison with criteria

A respiratory sensitiser is described as a substance that will lead to hypersensitivity of the airways following inhalation of the substance (Annex I: 3.4.1.1 of the CLP Regulation). Respiratory sensitisers are allocated into Category 1A (strong sensitisers) or Category 1B (other sensitisers), based on a weight of evidence from reliable and good quality evidence from human cases or epidemiological studies and/or observations from appropriate studies in experimental animals. Substances are classified as Category 1 respiratory sensitisers where data are not sufficient for sub-categorisation, if there is evidence in humans that the substance can lead to specific respiratory hypersensitivity, or if there are positive results from an appropriate animal test. Substances are classified as Category 1A respiratory sensitisers where there is evidence of a high frequency of occurrence in humans, or a probability of occurrence of a high sensitisation rate in humans based on animal or other tests. Substances are classified as Category 1B respiratory sensitisers where there is evidence of a low to moderate frequency of occurrence in humans, or a probability of occurrence of a low to moderate sensitisation rate in humans based on animal or other tests. In the absence of relevant human or non-human data, fludioxonil is not classified as a respiratory sensitiser.

#### 4.6.2.5 Conclusions on classification and labelling

In the absence of any data, fludioxonil does not require classification for respiratory sensitisation according to Regulation (EC) No 1272/2008.

### 4.7 Repeated dose toxicity

**Table 17: Summary table of relevant repeated dose toxicity studies**

| Method | Results | Remarks | Reference |
|---|---|---|---|
| **Short-term oral toxicity:**<br>OECD 407<br>GLP<br>Rat Tif:RAIf (SPF) 10/sex<br>Fludioxonil (97.5%)<br>0, 10, 100, 1000 mg/kg<br>Oral 28-day | NOAEL: 100 mg/kg bw/d (M/F)<br>LOAEL: 1000 mg/kg bw/d (M/F)<br>Critical effects:<br>Effects on bodyweight, clinical chemistry increased liver weights and hepatocyte hypertrophy; increased kidney weight and associated pathological changes including blood in urine. | None | Fankhauser, H., 1990 (IUCLID 8.9.1.1-01) |
| **Short-term oral toxicity:**<br>OECD 407 (1981)<br>GLP<br>Sprague-Dawley Crl:CD (SD) Rat 6/sex<br>Fludioxonil<br>0, 1000, 5000, 10000, 20000 ppm<br>Oral 20-21 day | NOAEL: 124/698 kg bw/d (M/F) (M/F)<br>LOAEL: 624/1407 kg bw/d (M/F)<br>Tubular nephrosis (from 5000 ppm in males) and increased absolute and relative kidney weights, increased relative liver weights in the two highest dose groups. | The administration period was 20 days (not 28 as specified in the current version of OECD 407) | Courcy di Rosa, J. 1988<br>(DAR, 2006) |
| **Short-term dermal toxicity:** | NOAEL: 1000/200 mg/kg bw/d | None | Schneider, M., 1990 |

| Method | Results | Remarks | Reference |
|---|---|---|---|
| OECD 410 (1981)<br>GLP<br>Rat<br>Tif:RAIf (SPF) 5/sex<br>Fludioxonil (97.5%)<br>Dermal 28-day | (M/F)<br>LOAEL: -/1000 mg/kg bw/d<br>(M/F)<br>Enlarged macrophages in the thymic cortex and lymphophagocytosis – females only | | (IUCLID 8.9.1.3-01) |
| **Sub-chronic oral toxicity:**<br>OECD 408 (1981)<br>GLP<br>Rat Sprague Dawley 10/sex<br>Fludioxonil (96%)<br>0, 10, 100, 1000, 7000, 20000 ppm<br>Oral 90-day | NOAEL: 64/70 mg/kg bw/d (M/F)<br>LOAEL: 428/462 mg/kg bw/d<br>(M/F)<br>Effects in the kidney and liver at the two highest dose groups in both sexes (increased relative weights, chronic nephropathy, centrilobular hepatocyte hypertrophy) | None | Chang, J.C.F.1990<br>(IUCLID 8.9.1.1-02) |
| **Sub-chronic oral toxicity:**<br>OECD 408 (1981)<br>GLP<br>CD-1 Mice 10/sex<br>Fludioxonil (96%)<br>0, 10, 100, 1000, 3000, 7000 ppm<br>Oral 90-day | NOAEL: 445/559 mg/kg bw/d<br>(M/F)<br>LOAEL: 1052/1307 mg/kg bw/d<br>(M/F)<br>Increased relative liver weight in and histopathological findings in the liver and kidneys (centrilobular hepatocyte hypertrophy and nephropathy) at 7000 ppm. | None | Chang, J.C.F. & Morrissey, R.L., 1990<br>(DAR, 2006) |
| **Sub-chronic oral toxicity:**<br>OECD 409 (1981)<br>GLP<br>Dog, Beagle, 4-6/sex<br>Fludioxonil (97.5%)<br>0, 200, 2000, 15000/10000 ppm<br>Oral 13 weeks (28-day recovery) | NOAEL: 60/58.5 mg/kg bw/d<br>(M/F)<br>LOAEL: 299/351 mg/kg bw/d<br>(M/F)<br>Decreased body weight in high-dose animals, increased liver weight and histopathological changes in high-dose animals, bile duct proliferation, signs of mild anaemia in high-dose females. | None | Moysan, F., 1990<br>(IUCLID 8.9.1.1-03) |
| **Sub-chronic oral toxicity:**<br>OECD 452<br>GLP<br>Dog Beagle 4/sex<br>Fludioxonil (97.5%)<br>0, 100, 1000 or 8000 ppm<br>Oral 13 weeks (28-day recovery)<br>12 months | NOAEL: 33/33 mg/kg bw/d (M/F)<br>LOAEL: 298/331 mg/kg bw/d<br>(M/F)<br>Weight loss (m), reduced weight gain, increased relative liver weight (m/f), increased cholesterol (m), gross and histopathology (m/f) at the high dose | None | Vallet, L. 1992, 1994<br>(IUCLID 8.9.1.1-03) |

| Method | Results | Remarks | Reference |
|---|---|---|---|
| **Long-term oral toxicity:**<br>OECD 453<br>GLP<br>Rat Sprague Dawley 50 sex/group<br>Fludioxonil (95.4%)<br>0, 10, 30, 100, 1000 or 3000 ppm<br>24 months | NOAEL chronic: 37/44 mg/kg bw/d (M/F)<br>LOAEL chronic: 113/141 mg/kg bw/d (M/F)<br>High dose:<br>Reduced weight gain and body weight<br>Signs of slight anaemia (females)<br>Liver: degenerative changes;<br>kidney: nephropathy, cysts (males)<br>- | Survival less 50% after 2 years,<br>7 weeks old rats at study start (not 6 weeks or younger as recommended) | Chang, J.C. & Richter, A.G. (1993c).<br>(IUCLID 8.9.1.1-07) |
| **Long-term oral toxicity:**<br>OECD 451<br>GLP<br>Mice Crl:CD®-1 (ICR) BR.<br>10 /sex/group (12 month, interim sacrifice)<br>50 sex/group (18 months)<br>Fludioxonil (95.4%)<br>0, 3, 10, 30 100, 1000, 3000, 5000 or 7000 ppm<br>18 months | NOAEL Chronic:<br>112 / 133 mg/kg bw/d (M/F)<br>LOAEL Chronic:<br>360 / 417 mg/kg bw/d<br>Survival markedly reduced at 7000 ppm, body weight and body weight gain decreased from 5000 ppm, signs of anaemia at 7000 ppm, increased liver weight from 3000 ppm, bile duct hyperplasia at 7000 ppm (males), nephropathy from 5000 ppm. | MTD exceeded at 7000 ppm | Chang, J.C.F. & Wyand, S.D., (1993a, 1993b).<br>(IUCLID 8.9.1.1-05)<br>(IUCLID 8.9.1.1-06) |

### 4.7.1 Non-human information

#### 4.7.1.1 Repeated dose toxicity: oral

The repeated dose toxicity of fludioxonil has been investigated in a number of studies in the rat, mouse and dog.

In a 28-day rat study (Fankhauser, 1990), no animals died and the only treatment-related clinical observation was blue discoloration of the tail at the top dose level of 1000 mg/kg bw/d, associated with the excretion of the coloured metabolite SYN 518582. Weight gain was minimally reduced in high dose males but did not reach statistical significance. Cumulative body weight gain was 40% of control for high dose females and resulted in a statistically significant reduction of 12% in terminal bodyweight compared to controls. In high dose males the final body weight was 9% lower. Bodyweights in the other dose groups were unaffected. Food consumption was reduced throughout the study in 1000 mg/kg bw/d females and was marginally reduced in males during Weeks 1 and 2. Overall mean food consumption was 79% of controls in high dose females and 96% of control in high dose males. Haematological parameters were unaffected by treatment. Clinical chemistry revealed statistically significantly reduced glucose concentrations in in both sexes at the highest dose level and at 100 mg/kg bw/d in females only. Plasma cholesterol was statistically significantly increased in both sexes at the high dose and in females at 100 mg/kg bw/d (values were within the historical control range HCD data are verified. Please refer to the position paper regarding origin of the HCD data provided Syngenta 2015.07.20). Bilirubin levels were increased (with statistical significance) in females at the highest dose level. Urinalysis revealed the presence of ketones in all dose groups of males (with no clear dose-response relationship) and in females at 100 and 1000 mg/kg bw/d. Historical control data reveals a generally high incidence of ketonuria; study incidences are stated to be within the historical control range. Haematuria was detected in two high

dose males at the highest dose level. Treatment-related increases in absolute and/or relative liver and kidney weight were observed in both sexes at the high dose level. Similar findings in females at 100 mg/kg bw/d were not associated with any histopathology or clinical chemistry correlates and are therefore not considered adverse. Histopathology revealed increased incidences of hepatocyte hypertrophy in both sexes at the top dose level and renal tubular casts in males at 1000 mg/kg bw/d. The study therefore indicates that fludioxonil produces hepatotoxicity (increased liver weights and hepatocyte hypertrophy) and nephrotoxicity (increased relative kidney weight and pathological changes including blood in urine) at the highest dose level of 1000 mg/kg bw/d. **The NOAEL for this study is considered to be 100 mg/kg bw/d.** An increase in relative kidney weight in females at 100 mg/kg bw/d is not considered to be adverse as the increase is small and no clear dose-response relationship was seen. An increase in liver weight in females at 100 mg/kg bw/d is not considered to be adverse in the absence of pathological or clinical chemistry correlates.

In a 20-day rat study (Courcy di Rosa, 1988), no deaths occurred and clinical signs were limited to abnormal coloured (black) faeces in males and females from the 5000, 10000 and 20000 ppm groups. The body weight was decreased about 12 % (not statistically significant) in high-dose males after 3 weeks whereas the body weights of all other treated groups and at all other time points were similar to control group body weights.

Clinical chemistry investigations revealed slight decreases in the values of plasma, sodium and chloride in the 5000, 10000 and 20000 ppm groups (both sexes) due to individual changes in urinary specific gravity and a slight decrease in urinary volume in males from the 10000 ppm and 20000 ppm groups. These changes were considered to be potentially related to tubular nephrosis.

The relative liver weights were statistically significantly increased in both sexes from 10000 ppm. The relative and absolute kidney weights were statistically significantly increased in high-dose males but in females only at 10000 ppm.

Other organ weight changes were not dose-related and were not associated with histopathological changes and were therefore not considered to be treatment related. The microscopic observations, as summarised in the report, included tubular nephrosis in the kidneys in 1/6 males from the 5000 ppm dose group (slight), in 4/6 males and 1/6 females from the 10000 ppm dose group (minimal to marked), and 6/6 males and 3/6 females from the 20000 ppm dose group (minimal to severe); both incidences and severity of the lesions occurred in a dose related manner. Incidence, severity and morphological characters of microscopical changes in other organs examined did not suggest a treatment relationship. The statistically significantly decreased heart weights and spleen weights, and the increased relative liver weights were not associated with macroscopical or histopathological findings.

Based on these the results, the NOAEL for this study is considered to be 124 mg/kg bw/day (1000 ppm) for males; the NOAEL for females is 5000 ppm (698 mg/kg bw/day). Higher dietary levels resulted in a dose-related nephrotoxicity (tubular nephrosis – minimal to severe) and occurred in male rats from 5000 ppm and in female rats from 10000 ppm. A NOAEL of 1000 ppm is set for males although only 1/6 males at 5000 ppm showed nephrosis characterised as being slight because other studies have identified the kidney as a target organ and because occurrence of nephrosis within 20 days is not a normal finding in this strain of rats.

In a 90-day rat study (Chang, 1990), two deaths occurred in treated males; one rat at 20000 ppm was found dead on Day 36 and one rat at 7000 ppm was sacrificed moribund on Day 50 (due to pituitary adenoma). Deaths are not considered to be related to treatment. Clinical signs were limited to blue staining of the tail, abdomen, feet and perineum from Day 6-14 in the two highest

dose groups. Blue staining is associated with the urinary excretion of a coloured metabolite and is not considered to be of toxicological significance. Body weights and weight gains were statistically significantly decreased in both sexes at 20000 ppm (from Week 1) and in females at 7000 ppm (from Week 4). The relative decrements for weight gain were 3%, 14% and 41% (males); 6%, 33%, and 61% (females) at dietary concentrations of 1000 ppm, 7000 ppm and 20000 ppm, respectively. No effects were observed at the two lowest concentrations. Food consumption was statistically significantly decreased in males at 20000 ppm and in females at ≥7000 ppm throughout the study. Significantly reduced feed efficiency was observed early in the study in both sexes at the highest dose level. Haematology revealed treatment-related effects on erythrocyte parameters in females at ≥7000 ppm; similar effects were not apparent in males. Changes in clinical chemistry parameters indicative of liver and kidney toxicity were observed in both sexes at 20000 ppm; changes suggestive of liver toxicity (increased cholesterol, increased bilirubin and 5'nucleotidase) were seen at 7000 ppm. Discoloration of urine was noted at ≥1000 ppm, consistent with the excretion of the coloured metabolite. Urine volumes were lower in females at ≥7000 ppm; significant amounts of bilirubin were detected in urine samples from both sexes at these dose levels. Statistically significant differences were observed for a number of organ weights in the two highest dose groups with increased relative kidney and liver weights being the most prominent. Organ weight changes are consistent with the microscopically identified changes in the liver (centrilobular hepatocyte hypertrophy) and kidneys (chronic nephropathy with a prominent active inflammatory component) at the two highest dose levels. An increased incidence (not statistically significant) of slight centrilobular hepatocyte hypertrophy was also seen in males at 1000 ppm. In the absence of any clinical chemistry histopathological correlates, the finding is not considered to be of toxicological significance. In conclusion, therefore, 90 day oral exposure of Sprague-Dawley rats to fludioxonil resulted in clinical signs of toxicity, reduced body weight and weight gain, haematological effects (in females only), changes in clinical chemistry, organ weight changes, gross and histopathological changes consistent with kidney and liver damage in males and females in the two highest dose groups. **A NOAEL of 1000 ppm (corresponding to 64 and 70 mg/kg bw/d in males and females, respectively) is derived** for this study based on the histopathological changes observed in the liver and kidneys at dietary concentrations of ≥7000 ppm (428 mg/kg bw/d).

In a 90-day mouse study (Chang & Morrissey, 1990), there were no deaths, and no effects of treatment on food consumption or feed efficiency up to and including the highest dose of 7000 ppm. There were no effects on body weight or body weight gain in males, however body weight and weight gain was significantly reduced in females at 2000 ppm during Weeks 4, 5, 9, 11 and 12. Discoloured urine (blue, green or brown) and blue stains on the pelvis were noted in male mice from 1-2 weeks after treatment at 1000 ppm and above, and the frequencies of the observations increased with dose, the findings were not accompanied by histopathological changes and these effects were not observed in females. The staining was thought to be a result of a sex difference in the metabolism of the test substance, and is not considered to be adverse. There were no effects on measured haematology parameters, however clinical chemistry investigations revealed significantly elevated 5'nucleotidase in both sexes at 7000 ppm.

Increased gamma-glutamyl transferase were observed in males of the two lowest dose groups (values within the historical control range and without dose relation), increased total bilirubin in 1000 and 3000 ppm females (within historical control values and without dose relation), and decreased potassium in high-dose females (within historical values) and therefore not considered to be treatment related..

The relative liver weights were statistically significantly increased in high-dose males (no corresponding effects on body weight or body weight gain) and from 3000 ppm in females. The

relative kidney weights were slightly increased at most dose levels but without achieving statistical significance changes from the control group except from the high dose groups. In the high dose group both absolute and relative liver weight was statistically significant in females. Furthermore, the weight of the adrenals in relation to brain weight was statistically significantly increased in the 100 ppm dose group females, this was not observed in any other dose groups.

Treatment-related findings in males at necropsy included discoloured aglandular mucosa in the stomach and kidneys with depressed focal discolouration in males fed 7000 ppm, discoloured urine in the urinary bladder (1000 ppm and above) and discoloured cecum (7000 ppm). Treatment-related findings in females included discolouration in the mucosa of the gallbladder, cecum and stomach (aglandular portion) at 7000 ppm. Only one female at 7000 ppm exhibited focal discolouration of the kidneys.

The histopathological changes included nepropathy with significantly increased incidence in high-dose animals and centrilobular hepatocyte hypertrophy, which was recorded with significantly increased incidence in high-dose animals and with a non-significantly increase in females at 3000 ppm. The increase in serum 5' nucleotidase indicates a cholestasis, which might result from the liver cell hyperthrohy (much higher increase in females than in males). The target organs were the kidneys and the liver in both sexes of the CD-1 mice.

Under the conditions of this OECD TG 408 study dietary administration of 0, 10, 100, 1000, 3000 or 7000 ppm Fludioxonil to groups of male and female Swiss mice of the CD-1 strain for 90-days resulted in reduced body weight and body weight gain in high-dose females, and changes in clinical biochemistry, organ weights, gross necropsy and histopathology consistent with kidney and liver damage. The NOAEL is considered to be 3000 ppm (445/559 mg/kg bw/day) for both males and females based on increased relative liver weight and histopathological findings in the liver and kidney at 7000 ppm.

In a 13-week dog study incorporating a 28-day recovery period (Moysan, 1990) groups of Beagle dogs were initially given 0, 200, 2000 and 15000 ppm fludioxonil in the diet. The highest dietary concentration was reduced to 10000 ppm on Day 18 due to marked weight loss by both sexes during the first three weeks of treatment with 15000 ppm; body weights subsequently stabilised. No deaths occurred. Significantly decreased body weight was noted in high dose males from Week 4; in high dose females, bodyweights were 90% and 87% of controls at Weeks 8 and 14 respectively. Food consumption was decreased (by approximately 50%) in both sexes during the 17 days of feeding with the 15000 ppm diet. Diarrhoea was observed with increased frequency (number of observations and number of animals affected) in both sexes at 2000 and 10000 ppm. The relevance of this effect was discussed at an expert meeting when the substance was evaluated for PPP use; the meeting agreed that effects were treatment-related but not adverse. This conclusion was based on the fact that incidences of diarrhoea were episodic and transient, and that same effect was not seen in a 1-year dog study (performed in the same laboratory and using dogs from the same source) at dietary concentrations of 1000-8000 ppm (the same laboratory with dogs from the same source. Ophthalmoscopy did not reveal any effects of treatment. Changes in haematological parameters (reduced red blood cell count, haemoglobin concentration and PCV) indicative of mild anaemia were observed in high dose females. Statistically significant changes in fibrinogen concentration and platelet count at the high dose level are not considered to be clearly related to treatment as the individual values are within the historical control range. It is noted that the age of animals for historical control data (6-9 months) is less than that of the animals in this study (11 months old at study initiation). A relation to treatment cannot therefore be excluded completely. Treatment-related clinical chemistry effects were limited to a significant increase in

cholesterol concentration in high dose females at Weeks 4, 8 and 13; which was reversible during the recovery period. A trend for increased cholesterol concentration was also observed in individual high dose males at Weeks 8 and 13. Urinalysis did not reveal any effect of treatment. Increased absolute and relative liver weights were observed in both sexes at the highest dose level; organ weights were not analysed statistically. Gross necropsy did not reveal any findings of toxicological significance; effects were limited to staining of the gastrointestinal tract at the high dose level, consistent with the excretion of a coloured metabolite. Histopathological findings were limited to an increased incidence in the severity of bile duct hyperplasia in both sexes at the high dose level; a finding considered adaptive in nature. In conclusion, the administration of 0, 200, 2000 or 15000/10000 ppm fludioxonil caused episodically and transient diarrhoea and blue-coloured faeces in the mid-dose group and in all high-dose animals, decreased body weight in high dose males, signs of mild anaemia in high dose females, increased absolute and relative liver weights in high-dose animals. The increased liver weights corresponded to an increased severity of bile duct proliferation in high-dose animals. The NOAEL for this study is considered to be 2000 ppm, equivalent to 60 and 59 mg/kg bw/d in males and females respectively.

In a 12-month dog study (Vallet, 1992; 1994), Beagle dogs were administered fludioxonil at dietary concentrations of 0, 100, 1000 and 8000 ppm. No deaths occurred; signs of toxicity were limited to blue faeces in both sexes at the high dose, consistent with the excretion of a coloured metabolite. In males at the highest dose level of 8000 ppm, mean overall body weight loss of 0.4 kg was recorded; weight gain in low- and mid-dose males exceeded that of the controls. Weight gain by in low-dose females was similar to that of the controls, whereas females at 1000 ppm gained 43% less weight and high-dose females gained 51% less weight. Differences are not statistically significant). No corresponding significant effects were seen on food consumption. All haematological values were minimally changed in relation to controls and are within the normal range of the historical control data for the performing laboratory (1989-1992). Clinical chemistry showed a statistically significant increase in total cholesterol in males at 8000 ppm; all other clinical chemistry and urinalysis parameters were within the historical control range. Statistically significant increases in absolute (+14%) and relative (+36%) liver weight in females at 8000 ppm were observed; a significant increase in relative (+28%) liver weight was also seen in males. Findings correlate with gross observations of liver enlargement in 2 of 4 high-dose females. Histopathology revealed a single incidence of biliary epithelial cell proliferation in females at 8000 ppm. In conclusion, the NOAEL is considered to be 1000 ppm corresponding to 33.1 mg/kg bw/d in males and 35.5 mg/kg bw/d in females due to weight loss (males), reduced weight gain, increased total serum cholesterol (males), increased relative liver weight, gross and histopathology (females ) and at the highest dietary concentration of 8000 ppm.

In a two-year oral combined chronic and carcinogenicity study in Sprague Dawley rats (Chang & Richter, 1993c), there was no treatment-related effect on survival. Clinical signs were limited to a slightly increased frequency of diarrhoea in high dose males. Dark faeces, blue urine and blue staining on various areas of the body seen in both sexes at dose levels of ≥1000 ppm was not considered not to be of toxicological significance and are attributed to the presence of the blue-coloured metabolite. At the highest dose level, reduced weight gain resulted in bodyweights of 5% and 11% lower than controls at 12 months in males and females, respectively; bodyweights were 5% and 8% lower at termination. Reductions in cumulative weight gain were 10% and 16% for males and females respectively at 12 months and 11% for both sexes over the whole study. No treatment-related effects were apparent on food consumption or conversion efficiency. Water consumption was unaffected by treatment. Haematology revealed changes in red blood cell parameters at the high dose level in females. Slight anaemia was indicted at the 12 month time point as indicated by statistically significant decreases in red blood cell counts, haemoglobin,

haematocrit, and mean corpuscular haemoglobin concentration. Similar findings were not observed at later sacrifices during the treatment period or at the 4-month recovery following 13 months of dosing. Clinical chemistry parameters were unaffected by treatment: occasional statistically significant effects are not considered to be treatment-related in the absence of any relationship to dose level or duration of treatment. Urinalysis revealed discoloration in the high-dose group due to a coloured metabolite excreted in the urine and is not considered to be an adverse effect. A slightly higher incidence of increased levels of urobilinogen was seen at dose levels of ≥1000 ppm, findings attained statistical significance at 3000 ppm. Organ weights was largely unaffected by treatment. Increases in the absolute weights of a small number of organs were seen in females, but are considered to be secondary to variations in bodyweight; similar effects were not seen on relative organ weights and no relationship to dose level or duration of treatment was apparent. Gross necropsy reported enlarged livers in high dose males, kidneys with cysts(s) in males at ≥1000 ppm and focal or general renal discolouration in both sexes in the two highest dose groups. The discolouration is due to the renal excretion of a coloured metabolite, and is not considered adverse. Histopathology reported a lower incidence of kidneys with cysts in males at 1000 and 3000 ppm; the macroscopic finding of an increased incidence of renal cysts is therefore not considered to be of toxicological significance. Non-neoplastic histopathological findings were observed in the livers (degeneration, atrophy, inflammation, and necrosis) of high-dose animals and in the kidneys (cysts and progressive nephropathy) of high-dose males. In conclusion, this combined chronic toxicity/carcinogenicity study in Sprague Dawley rats administered dietary concentrations of 0, 10, 30, 100, 1000, or 3000 ppm fludioxonil technical for up to 2 years revealed effects in both sexes at the highest dose level including reduced body weight and weight gain, signs of mild anaemia in females, gross necropsy and histopathological findings in the liver (in both sexes) and the kidneys (males only). The NOAEL for chronic toxicity is considered to be 1000 ppm, corresponding to 37 and 44 mg/kg bw/d in male and female rats.

Two 18-month carcinogenicity studies were performed in the mouse, as it became apparent after 6 months that the highest dietary concentration of 3000 ppm in the initial study would not meet the criteria for an MTD. Therefore the second study was initiated with dose levels of up 7000 ppm. In the first study, performed using dietary concentrations of 0, 10, 100, 1000 and 3000 ppm fludioxonil, mortality was unaffected by treatment. Survival rates at 18 months were 74-86% and 71-92% in males and females respectively. Treatment-related clinical signs were limited to blue urine, dark faeces and blue staining of various areas of the body. These findings are attributable to the excretion of the blue metabolite SYN 51852 and are not considered to be of toxicological significance. Group mean bodyweights and weight gains were unaffected by treatment. Females in the high dose group had significantly increased absolute and relative liver weights after 18 months; relative liver weight was also significantly elevated after 12 months. Significant increases in relative liver weight at the interim sacrifice were also seen in 10 ppm and 1000 ppm females, but are not considered to be related to treatment in the absence of a dose-response relationship and the absence of similar findings at terminal sacrifice. A slight (but not significant) increase in relative liver weight (112% of control) was also seen for 3000 ppm males. Enlarged spleen was noted at slightly increased incidence in both sexes at 3000 ppm; there was no effect on spleen weight and no histopathological correlates were observed. High dose females had a slightly increased incidence of enlarged thymus, liver and lymph nodes; however there was no effect on thymus weight and no histopathological correlates were observed in the thymus. There were generally high incidences of chronic inflammation of the lung (34-43/60 in males; 42-51/60 in females) and the glandular mucosa of the stomach (34-48/60 in males; 27-37/60 in females) in all control and treated groups and in both sexes. Other changes recorded in this study are common findings in this strain of mouse. In the second mouse study, performed at dietary concentrations of 0, 3, 30, 5000 and

7000 ppm, survival to termination was markedly reduced in high dose animals (27% and 22% in males and females) and was associated with clinical signs including dyspnoea, hypothermia, pallor, hypoactivity, hunched posture and tremors. Increased mortality in this group became apparent from approximately one year and was largely attributable to nephropathy. Additional clinical signs associated with the excretion of the blue metabolite SYN 51852 were seen at dietary concentrations of≥5000 ppm and are not considered to be adverse. Significantly reduced weight gain by both sexes was apparent at ≥5000 ppm, while bodyweights of 7000 ppm males were significantly lower than controls from Week 4. Bodyweights of females at 5000 and 7000 ppm were significantly lower from Week 21. Food consumption was unaffected by treatment at any dose level, however slightly reduced food utilisation efficiency was seen in males at ≥5000 ppm. Haematology revealed changes in red blood cell parameters (reduced haemoglobin concentrations and haematocrit) consistent with anaemia in both sexes at 7000 ppm at 12 and 18 months; findings were associated with increased reticulocyte counts. In addition, increased lymphocyte counts, reduced red blood cell counts, mean corpuscular haemoglobin and the relative counts of segmented neutrophils were observed in 7000 ppm females at 12 and/or 18 months. Mean absolute and relative liver weights were increased in both sexes at ≥5000 ppm; findings were associated with gross observations of discoloration and (in males) with discoloured foci. An increased incidence of hepatic cysts was also observed in males at 7000 ppm. Histopathology revealed increased incidences of hepatocellular necrosis and bile duct hyperplasia in 7000 ppm males. Significantly lower absolute kidney weights were seen in males at 7000 ppm, however values in females were elevated. Weight changes in the kidney were associated with general discoloration and cysts at 7000 ppm; discoloured foci and a rough pitted surface were additionally observed at ≥5000 ppm. A dose-related increase in the incidence and severity of nephropathy was observed in both sexes at 5000 and 7000 ppm; findings were characterised by glomerular atrophy, hyaline change, tubular dilatation and protein cast formation, an irregular capsular outline due to fibrosis and thickening of the tubular basement membrane. A marked increase in the incidence of focal tubular calcification and a slightly increased severity of chronic inflammation was also apparent at ≥5000 ppm.

#### 4.7.1.2 Repeated dose toxicity: inhalation

No data are available.

#### 4.7.1.3 Repeated dose toxicity: dermal

In a 28-day dermal rat study (Schneider, 1990), no treatment-related effects were observed on mortality, clinical signs (local or systemic), bodyweights, food consumption, haematology or organ weights. Clinical chemistry showed a significantly higher creatinine concentration in males at 1000 mg/kg bw/d and in females at 200 and 1000 mg/kg bw/d (without a dose-response relationship). Values are within the historical control range (data taken from 28 -day dermal studies performed in the same test facility in the same strain of rats 1988-1992) and are therefore not considered to be toxicologically relevant. Minor (but statistically significant) increases in plasma globulin and total protein levels were seen in females at 200 and 1000 mg/kg bw/d; however the group mean values and the individual values are well within the historical control range and do not form a dose-response relationship; variations are therefore not considered to be treatment-related or toxicologically relevant. This was also the conclusion from the expert meeting when the substance was evaluated for the PPP use of fludioxonil. The only histopathological change considered to be treatment-related was enlarged cortical macrophages, often revealing lymphophagocytosis, in the thymus of females at 1000 mg/kg bw/d. While no relevant thymus effects were observed in other

studies with fludioxonil in rats (or any other species), the finding (observed all females) is considered to be adverse and a NOAEL of 200 mg/kg bw/d is therefore supported for this study.

#### 4.7.1.4 Repeated dose toxicity: other routes

No data are available.

#### 4.7.1.5 Human information

No human data are available.

#### 4.7.1.6 Other relevant information

No data are available

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

Repeated dose oral toxicity studies performed with fludioxonil in the rat, mouse and dog indicates the liver, kidney and red blood cell as the targets of toxicity, as evidenced by organ weight changes, gross and histopathology, changes in haematology, clinical chemistry and/or urinalysis parameters. Target organ toxicity is associated with reduced weight gain and/or food consumption.

The 28-day rat study (Fankhauser, 1990) reports a NOAEL of 100 mg/kg bw/d based on effects (reduced bodyweight gain, clinical chemistry, increased liver weights and hepatocyte hypertrophy, increased kidney weight and associated changes including haematuria) seen at the limit dose of 1000 mg/kg bw/d.

The 20-day rat study (Courcy di Rosa, 1988) reports a NOAEL of 124 mg/kg bw/d based on kidney effects (tubular nephrosis, increased liver and kidney weights) seen from 624 mg/kg bw/d.

The 90-day rat study (Chang, 1990) reports a NOAEL of 64/70 mg/kg bw/d based on effects in the liver and kidney (increased relative weights, chronic nephropathy, centrilobular hepatocyte hypertrophy) seen at 428/462 mg/kg bw/d and higher.

The 90-day mice study (Chang & Morissey, 1990) reports a NOAEL of 445/559 mg/kg bw/d based on effects in increased relative liver weight and histopathological findings in the liver and kidney at 1052/1307 mg/kg bw/d.

The 90-day dog study (Moysan, 1990) report a NOAEL of 60/59 mg/kg bw/d based on effects (reduced weight gain, increased liver weight and associated histopathology, bile duct proliferation and signs of mild anaemia) seen at dose levels of 299/351 mg/kg bw/d and higher.

A 12-month dog study (Vallet, 1992; 1994) reports a NOAEL of 33/33 mg/kg bw/d based on effects (weight loss or reduced weight gain, increased relative liver weight, increased cholesterol, gross and histopathology of the liver) at dose levels of 298/331 mg/kg bw/d and higher.

In the rat combined chronic toxicity/carcinogenicity study (Chang & Richter, 1993c), the NOAEL for chronic toxicity is determined as 37/44 mg/kg bw/d, based on reduced weight gain, slight anaemia, liver and kidney histopathology at the next highest dose level of 113/141 mg/kg bw/d.

In the mouse carcinogenicity studies (Chang & Wyand, 1993a,b), an overall NOAEL for chronic toxicity of 112/133 mg/kg bw/d is determined, based on effects (reduced weight gain, increased liver weight, liver and kidney pathology) seen at dose levels of 360/417 mg/kg bw/d and higher. In the kidneys nephropathy were observed in both sexes from 5000 ppm (590 mg/kg bw day for males and 715 mg/kg bw day for females). A marked reduction in survival, associated with nephropathy, was seen in this study at the highest dietary concentration of 7000 ppm (exceeded the maximum tolerated dose MTD).

The repeated dose dermal toxicity study (Schneider, 1990) reports a NOAEL of 200 mg/kg bw/d based on effects seen at the limit dose of 1000 mg/kg bw/d.

## 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

Specific target organ toxicity (repeated exposure) is defined in the CLP Regulation (Annex I, 3.9.1.1) as specific, target organ toxicity arising from repeated exposure to a substance. All significant health effects that can impair function, both reversible and irreversible, immediate and/or delayed are included in this definition. The adverse health effects relevant for STOT RE classification include consistent and identifiable toxic effects in humans, or, in experimental animals, toxicologically significant changes which have affected the function or morphology of a tissue/organ, or have produced serious changes to the biochemistry or haematology of the organism and these changes are relevant for human health. With respect to animal data, Annex 1, Section 3.9.2.5 of the CLP Regulation notes that the standard animal studies in rats or mice that provide this information are 28-day, 90-day or lifetime studies that include haematological, clinical chemistry and detailed macroscopic and microscopic examination to enable the toxic effects on target tissues/organs to be identified. Data from repeat dose studies performed in other species may also be used, if available and other long-term exposure studies such as carcinogenicity, neurotoxicity or reproductive toxicity may also provide evidence of specific target organ toxicity that could be used in the assessment of STOT RE classification.

Studies of repeated dose toxicity with fludioxonil do not report any significant health effects of relevance to STOT RE classification. Findings indicate the liver, kidney and red blood cell as targets of toxicity. Adverse effects are seen only at generally high dose levels, with adaptive findings or effects not considered to be of toxicological significance seen at lower dose levels.

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

Substances are classified in STOT RE Category 1 based on evidence of significant toxicity in humans or where there is evidence from studies in experimental animals that they can be presumed to have the potential to produce significant toxicity in humans following repeated exposure. For classification in Category 1, either reliable good quality human data (evidence from human cases or epidemiological studies) or animal data (observations from appropriate studies in experimental animals in which significant and/or severe toxic effects, of relevance to human health, were observed at generally low exposure concentrations) is required. Annex I, Section 3.9.2.9.6 of the CLP Regulation provides a ‘guidance value’ of ≤10 mg/kg bw/d from a 90-day rat study to assist in

Category 1 classification. For a 28 day study the guidance value of ≤30 mg/kg bw/d to assist in Category 1 classification.

Substances are classified in STOT RE Category 2 based on evidence from studies in experimental animals that they can be presumed to have the potential to be harmful to human health following repeated exposure. For classification in Category 2, animal data (observations from appropriate studies in experimental animals in which significant toxic effects, of relevance to human health, were observed at generally moderate exposure concentrations) is required. Annex I, Section 3.9.2.9.7 of the CLP Regulation provides a 'guidance value' of 10-100 mg/kg bw/d from a 90-day rat study to assist in Category 2 classification. For a 28 day study the guidance value of ≤300 mg/kg bw/d to assist in Category 2 classification.

The 28-day rat study with fludioxonil (Fankhauser, 1990) reports a NOAEL of 100 mg/kg bw/d based on effects on bodyweight, clinical chemistry, increased liver weight and hepatocyte hypertrophy, increased kidney weight with associated histopathology and urinalysis findings at the highest dose level of 1000 mg/kg bw/d. Effects seen in this study do not constitute 'significant toxicity' and are not seen at dose levels relevant to STOT RE classification. Other repeated dose toxicity studies (90 day rat study, 90 day mice study, 90 day dog study, 1 year dog studies and life time studies) with fludioxonil similarly do not identify effects which constitute 'significant or severe toxicity' and are not seen at dose levels relevant to STOT RE classification. A NOAEL of 64 mg/kg bw/d is determined for the 90-day oral toxicity study in the rat, based on mild effects in the liver and kidney at the LOAEL of 428 mg/kg bw/d. The liver or kidney effects observed do not warrant a classification with STOT RE as there was no indication of an impairment of organ function or any organ dysfunction at dose levels relevant to STOT RE classification. In addition, no severe suffering of animals or a reduction of the life span of rats was observed. The lower survival in the 2 year rat study became apparent towards the end of the study and is considered to be secondary to obesity, an effect common in this strain of rat and not related to treatment. In mice a marked reduction in survival, associated with nephropathy, was seen in this study at the highest dietary concentration of 7000 ppm (exceeded the maximum tolerated dose MTD).

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

In the absence of any evidence of 'significant or severe toxicity' at low or generally moderate dose levels from repeated dose toxicity studies, fludioxonil does not require classification as STOT RE.

## 4.9 Germ cell mutagenicity (Mutagenicity)

**Table 18: Summary table of relevant *in vitro* and *in vivo* mutagenicity studies**

| Method | Results | Remarks | Reference |
|---|---|---|---|
| ***In vitro*:**<br>Bacterial reverse mutation assay (OECD 471)<br>GLP<br>Fludioxonil (97.5%)<br>(20-5000 µg/plate)<br>*S. typhimurium* TA 98, TA 100, TA 1535, TA 1537; *E. coli* WP2*uvr*A | Negative both with and without metabolic activation. | None | Ogorek, B., 1989 (IUCLID 8.5.1-01) |
| ***In vitro*:** | Positive both with and without metabolic | Only 100 metaphases (instead | Strasser, F.F., 1989 |

| | | | |
|---|---|---|---|
| *In vitro* mammalian cytogenetic test (OECD 473)<br>GLP<br>Fludioxonil (97.5%)<br>(3-350 µg/ml)<br>Chinese hamster ovary (CHO) cells | activation. | of the 200 specified in the current OECD (1997 OECD guideline) were analysed) | (IUCLID 8.5.2-01) |
| ***In vitro*:**<br>*In vitro* mammalian cell gene mutation test (OECD 476)<br>GLP<br>Fludioxonil (97.5%)<br>(0.5-60 µg/ml),<br>Chinese hamster lung fibroblasts V79 | Negative both with and without metabolic activation. | None | Dollenmeier, P. 1989<br>(IUCLID 8.5.3-01) |
| ***In vitro*:**<br>Unscheduled DNA synthesis in mammalian cells *in vitro* (OECD 482, 1987)<br>GLP<br>Fludioxonil (97.5%)<br>(4.1 – 5000 µg/mL)<br>Primary rat (male) hepatocytes | Positive | Conducted to a now deleted guideline | Hertner, T. 1989<br>(DAR, 2006) |
| ***In vivo*:**<br>Micronucleus test (OECD 474) GLP<br>Fludioxonil (97.5%)<br>(1250, 2500, 5000 mg/kg bw)<br>chromosome aberrations (structural leading to micronuclei)<br>Mouse bone marrow | Negative | From OECD TG 474, 1997: only 1000 PCEs were evaluated. No sign of cytotoxicity in the bone marrow at the highest dose level tested. | Hertner, T. 1990<br>(IUCLID 8.6-01) |
| ***In vivo*:**<br>Micronucleus test<br>GLP<br>Fludioxonil (97.5%)<br>(1250, 2500, 5000 mg/kg bw)<br>chromosome aberrations (structural leading to micronuclei)<br>Rat hepatocytes | Equivocal (Significant increase, no dose-response, almost within historical data, few animals) | Non-guideline | Meyer, A. 1991<br>(DAR, 2006) |
| ***In vivo*:**<br>Micronucleus test<br>GLP<br>Fludioxonil (96.4%)<br>(50, 125, 1250 mg/kg bw)<br>chromosome aberrations (structural leading to micronuclei)<br>Rat hepatocytes | Negative | Non-guideline | Ogorek, B. 1999<br>(DAR, 2006) |
| ***In vivo*:**<br>Chromosome aberrations in rat bone marrow cells<br>GLP<br>Fludioxonil (97.5%)<br>(1250, 2500, 5000 mg/kg bw) | Negative | Similar to OECD 475 | Myhr, B.C. 1999<br>(DAR, 2006) |

| | | | |
|---|---|---|---|
| Rat bone marrow | | | |
| ***In vivo*:**<br>Chromosome aberration test (OECD 475)<br>GLP<br>Fludioxonil (96.4%)<br>1250, 2500, 5000 mg/kg bw<br>Chinese hamster bone marrow | Negative | No information on cytotoxicity as the mitotic index was not determined | Hertner, T. 1993a (IUCLID 8.6-02) |
| ***In vivo*:**<br>UDS assay (OECD 486)<br>GLP<br>Fludioxonil (96.4%)<br>2500, 5000 mg/kg bw<br>Rat hepatocytes | Negative | None | Hertner, T. 1993b (IUCLID 8.6-03) |
| ***In vivo*:**<br>Mouse dominant lethal test (OECD 478, 1984)<br>GLP<br>Fludioxonil (96.4%)<br>0, 1250, 2500, 5000 mg/kg bw | Negative | Limit dose higher than recommended in 2015 version of guideline | Hertner, T. 1992 (DAR, 2006) |

### 4.9.1 Non-human information

#### 4.9.1.1 *In vitro* data

Fludioxonil was tested at concentrations from 20-5000 µg/plate in Ames test using *S. typhimurium* TA98, TA100, TA1535, TA1537 and E. coli WP2*uvr*A (OECD 471). In the mutagenicity tests, bacterial growth was occasionally inhibited at the higher concentrations. There was no indication of mutagenic activity in any of the bacterial strains employed in any of the tests. The array of positive control chemicals used in this assay induced significant increases in mutation frequencies and confirmed the sensitivity of the assay. No evidence for a mutagenic effect of fludioxonil was seen either in the absence or presence of a metabolic activation system under the experimental conditions used.

In an *in vitro* mammalian cytogenetic test (OECD 473), fludioxonil was tested at concentrations from 2.73-43.75 µg/mL without metabolic activation and 5.47-350 µg/mL with metabolic activation in in Chinese hamster ovary cells. Marked cytotoxicity was seen at concentrations of ≥43.75 µg/ml. Both with and without metabolic activation, fludioxonil and/or its metabolites induced specific chromosome aberrations. The effect showed a concentration-response relationship. Without metabolic activation, effects were seen at the highest concentration tested. With metabolic activation, there was a statistically significant trend at the three highest concentrations. Fludioxonil also increased the number of polyploid cells, which may indicate a potential to inhibit mitotic processes and to induce numerical chromosomal aberrations. It is therefore concluded that under the experimental conditions used in this study, fludioxonil showed clastogenic potential *in vitro*.

In an *in vitro* mammalian cell gene mutation test (OECD 476), there was no evidence of mutagenicity, either in the absence or presence of metabolic activation. Slightly increased mutation frequencies seen in one experiment in the absence of metabolic activation were not reproducible and no relationship to exposure concentration was apparent. A slight increase in mutation

frequency (mutant factor 3.4) at the lowest tested concentration (1.0 µg/mL) without metabolic activation was observed in the confirmatory experiment. However, one of the acceptance criteria for a positive result is that the mutant frequency in a treated culture exceeds that of the negative control by a mutant factor of 3 and that the absolute number of clones in the treated and untreated culture differs by more than 20 clones per $10^6$ cells. The difference between the mutation frequency of the sample and the mean mutation frequency of solvent controls was less than 20 per $10^6$ cells. Furthermore, no increase in mutation frequency was observed at any other concentration of fludioxonil with or without metabolic activation in any of the experiments. The test had a mutation frequency sensitivity limit of 4 $x10^{-6}$. In all the experiments, at many concentrations of test substance as well as in negative controls, the mutation frequency was below this sensitivity limit. The mutant frequency of all these samples was set to 4 $x10^{-6}$ and the mutant factor therefore 1. Appropriate positive control compounds confirmed the sensitivity of the assay. In conclusion, fludioxonil was not mutagenic under the conditions of this study either in the absence or presence of metabolic activation.

In an *in vitro* autoradiography DNA repair test (UDS assay) on rat hepatocytes (OECD 482), fludioxonil was tested at concentrations of 4.1-5000 µg/mL in two independent experiments (following a preliminary cytotoxicity test). Fludioxonil excerted a DNA damaging effect, as there was a dose-related increase in the gross number of silver grains per nucleus. It can therefore be concluded that under the experimental conditions used in the experiment Fludioxonil showed DNA damaging potential.

Positive control tests confirmed the validity of the assay.

A general remark: According to the new data requirements for pesticides and given the low recognized sensitivity of the *in vivo* UDS test to follow-up positive results for *in vitro* gene mutation negative results in the *in vivo* UDS test is no longer recommended to overrule positive results in either of the *in vitro* gene mutation tests. As mentioned previously OECD 482 (*in vitro* UDS test) has been deleted since April 2014.

#### 4.9.1.2 *In vivo* data

In the *in vivo* micronucleus test (OECD 474) there was no significant increase in the number of micronucleated PCE found in animals treated with fludioxonil. There were no signs of cytotoxicity as the PCE/NCE ratio varied from 0.8 to 1.2 in all investigated animals. There was no sign of cytotoxicity in the bone marrow at the highest dose level tested confirming bone marrow exposure. However the ADME studies showed that fludioxonil administered orally is rapidly and widely distributed in blood and various organs and tissues including bone. After 0.25 hr in the F1 and F3 dose groups 0.41% and 0.58 % of the applied dose was found in bone tissue in male and females respectively. An initial Cmax was apparent at 15 minutes after oral administration of a low dose of 0.5 mg/kg bw, with a second smaller peak seen at 12 hours showing rapid distribution and within the time frame of the micronucleus study. Given the presence of fludioxonil in the blood and detection in bone together with a high level of oral absorption, adequate exposure of the target tissue (bone marrow) is predicted. The positive control chemical used in the test induced significant increases in the number of micronucleated PCE and therefore confirmed the adequacy of the experimental conditions for detecting the induction of micronuclei. In conclusion, fludioxonil did not show any clastogenic potential *in vivo* under the conditions of this study.

In an *in vivo* micronucleus test conducted with rat hepatocytes doses tested were from 1250-5000 mg/kg bw. The result of the test was equivocal as a slight but significant positive result was obtained in the experiment where fludioxonil was administered after treatment with a mitogenic stimulus. The increase was not dose-dependent as the increase was only observed at the lowest and intermediate dose levels. However, the results were except from one animal within the set of historical data. In the light of the equivocal result and the relatively small treatment groups (3 animal/group), the second experiment should have been repeated. Positive controls in both experiments showed significant increases. However a newer study (Ogorek, B 1999) is submitted which was negative and described below.

Table shows effects of fludioxonil on micronucleated rat hepatocytes when administered after a mitogenic stimulus (Meyer, A. 1991)

| Treatment | Concentration (mg/kg bw) | Percent of micronucleated hepatocytes | | | Statistical significance |
|---|---|---|---|---|---|
| | | Animal 1 | Animal 2 | Animal 3 | |
| Negative control (CMC 0.5%) | | 0.2 | 0 | 0 | |
| Fludioxonil | 1250 | 0.9 | 0.5 | 0.5 | *** |
| | 2500 | 0.6 | 0.1 | 0.2 | * |
| | 5000 | 0.5 | 0 | 0.02 | |
| Positive control: cyclophosphamide | 20 | 4.6 | 3.6 | 2.6 | *** |
| Historical data, 9 animals: Negative control (CMC 0.5%) | | 0.1, 0.2, 0.3, 0.3, 0.4, 0.6, 0, 0, 0.2 | | | |

* P<0.05, the treated group versus the negative control group; *** P<0.001

In a second *in vivo* micronucleus test (Ogorek, B. 1999) conducted with rat hepatocytes, there were no significant increases in the number of micronucleated hepatocytes at doses up to and including 1250 mg/kg bw. Individual animals in the fludioxonil treated and positive control groups showed marked increases in the frequency of hepatocytes in apoptosis, however the groups means showed not statistically significant difference when compared to the negative control; this effect was thought to be due the synergistic effects of treatment with fludioxonil or the positive control and the

hepatocyte necrogenic agent used in the assay (4-acetylaminofluorene). The positive control substance induced a statistically significant increase in the frequency of micronuclei, confirming the validity of the assay. In conclusion, fludioxonil was not clastogenic or aneugenic in rat hepatocytes *in vivo*, under the conditions of the study.

In an *in vivo* chromosome aberration (aneuploidy) assay conducted in rats (Myhr, B.C. 1999) fludioxonil did not cause an statistically significant dose-dependent increase in the number of bone marrow cells with numerical aberrations at doses up to and including 5000 mg/kg bw. The positive control substance caused a statistically significant increase in the number of aberrant cells.

Table shows effects of fludioxonil on the number of cells with numerical aberrations (Myhr, B.C. 1999)

| Treatment | Concentration (mg/kg bw) | Mean percent aberrant cells (5 animals/group) | |
|---|---|---|---|
| | | Males | Females |
| Negative control (CMC 0.5%) | | 2.8 | 2.6 |
| Fludioxonil | 1250 | 4.2 | 5.8 |
| | 2500 | 3.6 | 4.2 |
| | 5000 | 2.6 | 4.2 |
| Positive control: Vinblastine Sulfate | 1 | 41.0 | 44.3** |

** P<0.01

In conclusion, fludioxonil was not aneugenic *in vivo*, under the conditions of the study.

In an *in vivo* chromosome aberration test (OECD 475) there was no statistically significant increase in the number of metaphases containing specific chromosome aberrations in animals treated with fludioxonil compared to controls. Furthermore, there was no significant difference in the incidence of polyploid metaphases in the animals treated with fludioxonil compared to the negative control. There are no measurements of cytotoxicity in the bone marrow. The dose levels used in the study were high and there is sufficient information from ADME studies to show an adequate level of exposure of the target tissue (bone marrow) under the conditions of this study. The positive control compound induced significant increases in the proportion of aberrations, confirming the sensitivity of the assay. In conclusion, fludioxonil did not show any clastogenic potential or spindle toxicity *in vivo* under the conditions of the study.

In an *in vivo*/*in vitro* unscheduled DNA synthesis (UDS) assay in in rat hepatocytes (OECD 486), there were no differences in either gross or net nuclear grains in the hepatocytes from fludioxonil-treated animals, compared to the negative controls. The percentage distribution of the nuclear grain counts revealed no differences either. Positive control compounds demonstrated the sensitivity of

the assay to detect UDS and replicative DNA synthesis. In conclusion, there was no evidence of the induction of UDS in hepatocytes by treatment with fludioxonil under the condition of this study.

In an *in vivo* mouse dominant lethal test (OECD 478), there was no evidence for cytotoxic effects on the pre-implantation stages; the post-implantation mortality of embryos was slightly increased compared to the negative control group. However, the effect was not statistical significant, there was no clear dose-response and the values are within the range of historical negative control data for embryonic deaths reported from two other studies (4.6-11.5% and 2.4-11.1%).

Summary table – Effects of fludioxonil on post-implantation mortality in mouse (Hertner, T., 1992)

| Treatment | Concentration (mg/kg bw) | Percent embryonic deaths per mating period | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | I | II | III | IV | V | VI | VII | VIII |
| Negative control (CMC 0.5%) | | 6.2 | 8.3 | 7.9 | 8.7 | 7.8 | 6.6 | 5.1 | 7.3 |
| Fludioxonil | 1250 | 11.5 | 4.7 | 9.3 | 7.2 | 4.6 | 6.6 | 7.0 | 10.5 |
| | 2500 | 7.0 | 5.8 | 7.8 | 6.1 | 5.7 | 6.1 | 7.8 | 10.2 |
| | 5000 | 9.0 | 7.6 | 9.0 | 11.8 | 7.7 | 5.2 | 8.3 | 11.4 |
| Positive control: cyclophosphamide | 133 | 34.6 * | 38.7 * | 24.5 * | 5.8 | 8.0 | 11.9 * | 9.3 | 8.0 |

* $p<0.05$

The positive control caused a strong increase in post-implantation losses thereby confirming the validity of the assay. In conclusion, there was no evidence that fludioxonil increased post-implantation loss and is therefore not considered to be genotoxic in germ cells of mouse *(in vivo)* under the conditions of this assay.

### 4.9.2 Human information

No human data are available.

### 4.9.3 Other relevant information

No additional relevant information is available.

### 4.9.4 Summary and discussion of mutagenicity

The mutagenicity of fludioxonil has been investigated in an appropriate battery of studies *in vitro* and *in vivo*, covering gene mutation and chromosomal damage endpoints.

***In vitro:***

Fludioxonil did not show mutagenic potential *in vitro* studies on different strains of bacteria cells or mammalian cells (hamster). The substance showed a clastogenic potential in Chinese Hamster ovary cells where specific numerical chromosome aberrations were induced both with and without metabolic activation. The effect showed a concentration dependent tendency. Fludioxonil also increased the number of polyploid cells which may indicate a potential to inhibit mitotic processes and to induce numerical chromosomal aberrations. Fludioxonil also showed DNA damaging potential in rat UDS hepatocytes.

***In vivo:***

Of the 5 *in vivo* chromosome abberation tests made with fludioxonil four of these were negative. The substance did not show a clastogenic potential in the bone marrow of the Chinese hamster, in the bone marrow of the mouse and in 2 of the 3 tests in the rat (bone marrow and hepatocytes). However, in the two studies in rats, a slight increase in micronucleated cells or cells with numerical aberrations was seen, but the increase was either not significant or within historical data and the tests therefore considered negative. The third test in rat hepatocytes was considered equivocal as the increase in micronucleated cells was significant but almost within historical data and with only few animals treated.

Fludioxonil, did not show a DNA damaging potential in rat hepatocytes or a genotoxic potential in germ cells of mouse.

On the basis of the submitted tests the overall weight of evidence indicates that the substance is not genotoxic.

Fludioxonil did show a clastogenic potential *in vitro* and an equivocal result in 1 of the 5 chromosome aberration tests *in vivo.* However this test was not well performed and a newer test was submitted which was negative. In the present *in vivo* tests, several endpoints and different species as well as target organs have been investigated.

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### 4.9.5 Comparison with criteria

Annex I Section 3.5.1.1 of the CLP regulation defines mutation as a permanent change in the amount or structure of the genetic material in a cell. The term mutation applies both to heritable genetic changes that may be manifested at the phenotypic level and to the underlying DNA modifications. The term ‘mutagenic’ and ‘mutagen’ are used for agents giving rise to an increased occurrence of mutations in populations of cells and/or organisms. This hazard class is primarily concerned with substances that may cause mutations in the germ cells of humans that can be transmitted to the progeny. However, the results from mutagenicity or genotoxicity tests *in vitro* and in mammalian somatic and germ cells *in vivo* are also considered in classifying substances within this hazard class.

Classification for mutagenicity in Category 1 is appropriate for substances known to induce heritable mutations (Category 1A) or for substances regarded as if they induce heritable mutations in the germ cells of humans (Category 1B).

Classification in Category 1A is based on positive evidence from human epidemiological studies.

Classification in Category 1B is based on positive result(s) from *in vivo* heritable germ cell mutagenicity tests in mammals; or positive result(s) from *in vivo* somatic cell mutagenicity tests in mammals, in combination with evidence that the substance has potential to cause mutations to germ cells; or positive results from tests showing mutagenic effects in the germ cells of humans, without demonstration of transmission to progeny.

Classification for mutagenicity in Category 2 is appropriate for substances which cause concern for humans owing to the possibility that they may induce heritable mutations in the germ cells of humans. Classification in Category 2 is based on positive evidence obtained from somatic cell mutagenicity tests in mammals and/or in some cases from somatic cell mutagenicity tests in mammals and supporting data from *in vitro* experiments.

### 4.9.6 Conclusions on classification and labelling

Negative results (somatic cells) are reported for gene mutation *in vitro* in an Ames test and a study of mammalian cell mutation. There is no evidence that fludioxonil causes DNA damage in a UDS study *in vivo*. Fludioxonil did show clastogenic potential in a study *in vitro*; however no clastogenic activity is seen in studies *in vivo*. On the basis of the available data (mammalian somatic cells and cultured bacterial cells), classification for germ cell mutagenicity is not required according to Regulation (EC) No 1272/2008.

## 4.10 Carcinogenicity

Evaluated under long-term repeated dose toxicity (combined chronic and carcinogenicity studies).

**Table 19: Summary table of relevant carcinogenicity studies**

| Method | Results | Remarks | Reference |
|---|---|---|---|
| OECD 453<br>GLP<br>Rat Sprague Dawley 50 sex/group<br>Fludioxonil (95.4%)<br>0, 10, 30, 100, 1000 or 3000 ppm<br>24 months[1] | NOAEL carcinogenicity: 113/141 mg/kg bw/d (M/F)<br>LOAEL carcinogenicity: -/- | Survival less 50% after 2 years,<br>7 weeks old rats at study start (not 6 weeks or younger as recommended) | Chang, J.C. & Richter, A.G. (1993c).<br>(IUCLID 8.9.1.1-07) |
| OECD 451<br>GLP<br>Mice Crl:CD®-1 (ICR) BR.<br>10 /sex/group (12 month, interim sacrifice)<br>50 sex/group (18 months)<br>Fludioxonil (95.4%)<br>0, 3, 10, 30 100, 1000, 3000, 5000 or 7000 ppm<br>18 month[2] | NOAEL Carcinogenicity: 851 / 1008 (M/F)<br>LOAEL Carcinogenicity:- / - | MTD exceeded at 7000 ppm | Chang, J.C.F. & Wyand, S.D., (1993a, 1993b).<br>(IUCLID 8.9.1.1-05)<br>(IUCLID 8.9.1.1-06) |

[1] *Mean achieved fludioxonil intakes were calculated to be 0.37 and 0.44; 1.1 and 1.3; 3.7 and 4.4; 37 and 44; 113 and 141 mg/kg bw/d for males and females at dose levels of 10, 30, 100, 1000 and 3000 ppm respectively.*

[2] *Mean achieved daily fludioxonil intakes were 0.41, 1.4, 4.1, 13.5, 133, 417, 715 and 1008 mg/kg bw day for females and 0.33, 1.1, 3.3, 11.3, 112, 360, 590 and 851 mg/kg bw day for males at dose levels of 3, 10, 30 100, 1000, 3000, 5000, or 7000 ppm, respectively*

### 4.10.1 Non-human information

#### 4.10.1.1 Carcinogenicity: oral

The carcinogenicity of fludioxonil has been investigated in studies in the rat (Chang & Richter, 1993c) and in the mouse (Chang & Wyand, 1993a, b).

In the rat study, higher incidences of liver adenoma, carcinoma and combined adenoma/carcinoma were observed in the low dose (10 ppm) group and the high dose group in males. However the incidences did not exhibit a monotonic dose-response relationship. The incidence of hepatocellular tumours (adenoma, carcinoma and combined) were slightly increased in females at the top dose level 3000 ppm; however incidences do not attain statistical significance and are well within the laboratory's historical control range. The increased incidences in the liver adenoma and combined adenoma and carcinoma in high-dose females were not statistically significant after Bonferroni adjustment of the tail probabilities. The carcinoma incidence in the high dose females was not analysed statistically since the single occurrence in this group was insufficient for evaluation. Neoplasms were observed as solitary nodules (there was no tumour multiplicity) and were not associated with other evidence of a proliferative hepatocellular response such as increases in foci of cellular alteration. In the absence of a dose-relationship, coupled with the fact that the incidences fall within the historical control range, the hepatocellular tumours seen in females at 3000 ppm are not considered to be related to treatment. Treatment with fludioxonil had no influence on the total incidence of neoplastic lesions or on the number of tumour-bearing animals. As the historical control data were presented in the original study report (seven studies conducted at Ciba Ceigy Environmental Health Center (EHC)), the dossier submitter assumes that it was conducted within the same time period and the testing facilities were the same. However the time interval needs to be confirmed by the original data owner of the study which is the obligation of the applicant to provide. Please refer to the position paper regarding origin of the HCD data provided Syngenta 2015.07.20 in section 8 Annexes.

Incidence (% of animal examined) of hepatocellular tumours in Sprague Dawley rats

| | Males | | | | | | | Females | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **group** | **1** | **2** | **3** | **4** | **5** | **6** | | **1** | **2** | **3** | **4** | **5** | **6** | |
| **ppm** | **0** | **10** | **30** | **100** | **1000** | **3000** | **Hist** | **0** | **10** | **30** | **100** | **1000** | **3000** | **Hist** |
| Carcinoma | 0 | 5.0 | 1.7 | 3.3 | 3.3 | 2.9 | 0-5 | 0 | 0 | 0 | 0 | 0 | 1.4 | 0-1.7 |
| Adenoma | 1.4 | 5.0 | 1.7 | 0 | 1.7 | 2.9 | 0-13.3 | 0 | 3.3 | 0 | 0 | 0 | 5.7 | 0-10 |
| Adenoma + carcinoma | 1.4 | 10.0 | 3.3 | 3.3 | 5.0 | 5.7 | 1.4 -15 | 0 | 3.3 | 0 | 0 | 0 | 7.1 | 0-10 |

Hist = historical control range

Incidence (numerical) of hepatocellular tumours in Sprague Dawley rats

| Sex | Males | | | | | | Females | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Dose group ppm | 0 | 10 | 30 | 100 | 1000 | 3000 | 0 | 10 | 30 | 100 | 1000 | 3000 |

Incidence (numerical) of hepatocellular tumours in Sprague Dawley rats

| Sex | Males | | | | | | Females | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Dose group ppm | 0 | 10 | 30 | 100 | 1000 | 3000 | 0 | 10 | 30 | 100 | 1000 | 3000 |
| Animals examined | 70 | 60 | 60 | 60 | 60 | 70 | 70 | 60 | 60 | 60 | 60 | 70 |
| Adenoma | 1 | 3 | 1 | 0 | 1 | 2 | 0 | 2 | 0 | 0 | 0 | 4 |
| Carcinoma | 0 | 3 | 1 | 2 | 2 | 2 | 0 | 0 | 0 | 0 | 0 | 1 |
| Adenoma + carcinoma | 1 | 6 | 2 | 2 | 3 | 4 | 0 | 2 | 0 | 0 | 0 | 5 |

Labelling indices for liver sections from 12-month interim sacrifice and 13-month recovery sacrifice stained with Proliferating Cell Nuclear Antigen (PCNA) methodology indicated no treatment-related effects on cell proliferation. Marginally increased labelling indices for female rats administered ≥ 1000 ppm were considered incidental due to a lack of statistical significance and dose-response relationship.

Based on the above considerations, the hepatocellular tumours observed in this rat study are not considered to be related to treatment with fludioxonil. Fludioxonil was therefore not carcinogenic in male or female rats at dietary concentrations of to 3000 ppm.

Two studies were performed in the mouse, as it became apparent after 6 months that the highest dietary concentration of 3000 ppm in the initial study would not meet the criteria for an MTD. Therefore the second study was initiated with dose levels of up 7000 ppm. The two studies are evaluated together and combined are considered to fulfil the guideline (OECD 451) criteria. In the first study, performed using dietary concentrations of 0, 10, 100, 1000 and 3000 ppm fludioxonil, mortality was unaffected by treatment. Survival rates at 18 months were 74-86% and 71-92% in males and females respectively.

A statistically significant increase in the incidence of lymphoma was seen in high dose females, the value is within the range of historical controls (13-32%; as the historical control data were presented in the original study report (conducted at Ciba Ceigy Environmental Health Center (EHC)), the dossier submitter assumes that it was conducted within the same time period and same strain, the testing facilities were the same. However the time interval needs to be confirmed by the original data owner of the study which is the obligation of the applicant to provide). Please refer to the position paper regarding origin of the HCD data provided Syngenta 2015.07.20

The incidence of lymphoma in females in the other dose groups was high but comparable to the incidence in control animals. Male mice had a lower incidence of lymphomas and did not show a similar increase as females did.

With respect to other neoplastic findings, the tumour types and incidences were within the normal range expected for an 18 month study in this strain of mice.

**Histopathological neoplastic findings in <u>male mice</u> throughout the two studies (Chang et al. 1993a) & (Chang et al. 1993b*)**

| **Sex** | **Males** | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| **Dose group ppm** | **0** | **0*** | **3*** | **10** | **30*** | **100** | **1000** | **3000** | **5000*** | **7000*** |
| No. of animals | 60 | 60 | 60 | 60 | 60 | 60 | 60 | 60 | 60 | 60 |
| No. of benign neoplasms | 28 | 22 | 26 | 39 | 29 | 39 | 24 | 34 | 24 | 19 |
| No. of malignant neoplasms | 19 | 10 | 9 | 17 | 10 | 10 | 20 | 18 | 5 | 6 |
| No. of metastatic multi-centric neoplasms | 6 | 84 | 7 | 47 | 51 | 30 | 74 | 65 | 40 | 0 |
| No. of total neoplasms | 53 | 116 | 42 | 103 | 90 | 79 | 118 | 117 | 69 | 25 |
| No. of animals with neoplasms | 33 | 25 | 29 | 34 | 30 | 40 | 33 | 38 | 23 | 20 |

**Histopathological neoplastic findings in <u>female mice</u> throughout the two studies (Chang et al. 1993a) & (Chang et al. 1993b*)**

| **Sex** | **Females** | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| **Dose group ppm** | **0** | **0*** | **3*** | **10** | **30*** | **100** | **1000** | **3000** | **5000*** | **7000*** |
| No. of animals | 60 | 60 | 60 | 60 | 60 | 60 | 60 | 60 | 60 | 60 |
| No. of benign neoplasms | 14 | 14 | 17 | 19 | 13 | 20 | 16 | 17 | 14 | 9 |
| No. of malignant neoplasms | 24 | 18 | 10 | 16 | 24 | 17 | 22 | 30 | 16 | 15 |
| No. of metastatic multi-centric neoplasms | 77 | 83 | 75 | 32 | 172 | 88 | 72 | 213 | 31 | 108 |
| No. of total neoplasms | 115 | 115 | 102 | 67 | 209 | 125 | 110 | 260 | 61 | 132 |
| No. of animals with neoplasms | 27 | 26 | 22 | 23 | 29 | 31 | 28 | 33 | 23 | 16 |

In the second mouse study, performed at dietary concentrations of 0, 3, 30, 5000 and 7000 ppm, survival to termination was markedly reduced in high dose animals (27% and 22% in males and females) and was associated with clinical signs including dyspnoea, hypothermia, pallor, hypoactivity, hunched posture and tremors. Increased mortality in this group became apparent from approximately one year and was largely attributable to nephropathy. There was no dose-related increase in the incidence of any specific neoplastic finding or total neoplasms at 5000 and 7000 ppm. This is in contrast to the high incidence of metastatic multi-centric neoplasms and total neoplasms observed in female mice administered 3000 ppm. A significantly increased incidence of lymphomas was observed in 3000 ppm females but was not apparent at 5000 or 7000 ppm. It cannot be excluded that the marked reduced survival in females at 7000 ppm may have influenced the lymphoma incidence the observed at the 18-month terminal sacrifice; however, both the lymphoma incidence and survival in the 5000 ppm dose group were comparable to controls.

**Result from the 18-month mice study; lymphoma incidences**

| | Incidence of lymphoma | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| **ppm** | **0 (1st)** | **0 (2nd)** | **3** | **10** | **30** | **100** | **1000** | **3000** | **5000** | **7000** |
| **Females** | | | | | | | | | | |
| un. deaths 0-12 m | 3/7 | 1/4 | 0/4 | 0/4 | 0/4 | 0/0 | 0/4 | 3/6 | 0/7 | 2/9 |
| 12 m sacrifice | 0/7 | 0/9 | 1/9 | 0/9 | 2/9 | 0/10 | 2/10 | 0/8 | 0/5 | 1/9 |
| un. deaths 13-18 m | 1/6 | 3/12 | 2/6 | 0/7 | 4/10 | 2/4 | 4/9 | 3/9 | 0/14 | 2/31 |
| 18 m sacrifice | 7/40 | 7/35 | 4/41 | 10/40 | 6/37 | 11/46 | 6/37 | 12/37 | 11/34 | 3/11 |
| total | 11/60 | 11/60 | 7/60 | 10/60 | 12/60 | 13/60 | 12/60 | 18/60 | 11/60 | 8/60 |
| Incidence [%] | 18 | 18 | 12 | 17 | 20 | 22 | 20 | 30 | 18 | 13 |
| **Males** | | | | | | | | | | |
| un. deaths 0-12 m | 0/3 | 0/1 | 0/5 | 0/2 | 1/8 | 1/3 | 1/3 | 0/0 | 1/3 | 0/9 |
| 12 m sacrifice | 0/9 | 0/10 | 0/8 | 0/10 | 0/7 | 0/9 | 0/10 | 0/10 | 1/10 | 0/9 |
| un. deaths 13-18 m | 0/5 | 2/12 | 0/9 | 1/11 | 1/13 | 1/6 | 2/10 | 2/11 | 1/10 | 0/28 |
| 18 m sacrifice | 2/43 | 1/37 | 1/38 | 0/37 | 0/32 | 0/42 | 4/37 | 0/39 | 1/37 | 0/14 |
| total | 2/60 | 3/60 | 1/60 | 1/60 | 2/60 | 2/60 | 7/60 | 2/60 | 4/60 | 0/60 |

| **Historical controls: incidence of thymus hyperplasia and malignant lymphoma# in female CD-1 mice** | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Studies | A | B | C | D | E | F | **Total** | **% (min-max)** |
| Thymus hyperpl. | 8 | 6 | 6 | 13 | 12 | - | | |
| Malign. lymphoma | 4 | 2 | 4 | 3 | 4 | 11 | | |
| combined | 12/50 | 8/60 | 10/60 | 16/50 | 16/50 | 11/60 | 73/330 | **22 (13-32)** |
| % | 24 | 13 | 17 | 32 | 32 | 18 | | |

# The incidence of malignant lymphoma and thymus hyperplasia were combined as thymus hyperplasia is often indistinguishable from thymus lymphoma.
un. = unscheduled, m = months, hyperpl. = hyperplasia, malign. = malignant

In the absence of a dose-response relationship, the increased incidences of lymphomas, metastatic multi-centric neoplasms and total neoplasms in females administered 3000 ppm is not considered to be related to the treatment with fludioxonil. It is therefore concluded that there is no evidence of carcinogenicity in the mouse.

#### 4.10.1.2 Carcinogenicity: inhalation

No data are available.

#### 4.10.1.3 Carcinogenicity: dermal

No data are available.

### 4.10.2 Human information

No human information is available.

### 4.10.3 Other relevant information

No other relevant information is available.

### 4.10.4 Summary and discussion of carcinogenicity

Studies performed with fludioxonil in the rat and mouse do not provide any clear evidence of carcinogenicity. A non-significantly increased incidence of hepatocellular tumours seen in the rat at an intermediate dietary concentration was without a dose-response relationship and was within the

historical control range; this finding is therefore not considered to be related to treatment with fludioxonil. Significantly increased incidences of lymphoma seen in female mice are within the laboratory's historical control range, are not apparent at the highest or higher tested dietary concentration (5000 and 7000 ppm) and are therefore not considered to be related to treatment with fludioxonil.

### 4.10.5 Comparison with criteria

Annex I Section 3.6.1.1 of the CLP Regulation defines a carcinogen as a substance which induces cancer or increase its incidence. Substances which have induced benign and malignant tumours in well-performed experimental studies on animals are considered also to be presumed or suspected human carcinogens unless there is strong evidence that the mechanism of tumour formation is not relevant for humans. Carcinogenic substances are allocated to Category 1 (known or presumed human carcinogens) or Category 2 (suspected human carcinogens).

A substance is classified in Category 1 for carcinogenicity on the basis of epidemiological and/or animal data. Substances known to have carcinogenic potential in humans (based largely on human evidence) are classified in Category 1A. Substances presumed to have carcinogenic potential for humans (based largely on animal evidence) are classified in Category 1B. A substance is classified in Category 1 for carcinogenicity on the basis of human and/or animal studies, but which is not sufficiently convincing to place the substance in Category 1A or 1B.

Studies performed with fludioxonil in the rat and mouse do not provide any clear evidence of carcinogenicity based on an overall weight and strength of evidence approach and in consideration of the important factors in Annex I: 3.6.2.2.6 of the CLP Regulation under section 3.6.2.3.2.

### 4.10.6 Conclusions on classification and labelling

Based on the available data, fludioxonil does not require classification for carcinogenicity according to Regulation (EC) No 1272/2008.

## 4.11 Toxicity for reproduction

**Table 20: Summary table of relevant reproductive toxicity studies**

| Method | Results<br>NOAEL & LOAEL<br>(mg/kg bw/day)<br>Maternal/ developmental | Remarks | Reference |
|---|---|---|---|
| **Developmental toxicity:**<br>OECD 414<br>GLP<br>Rat: Sprague Dawley<br>25 mated females /group<br>Fludioxonil (97.5%)<br>0, 10, 100 or 1000 mg/kg bw/d | NOAEL: 100 / 1000<br>LOAEL: 1000 / -<br>Dams: Reduced body weight gain and food consumption at 1000 mg/kg bw/d.<br>Foetuses: No effects. | 17 instead of 20 pregnant females at low dose | Savary, M.H. 1989a (IUCLID 8.10.1-01) |
| **Developmental toxicity:**<br>OECD 414<br>GLP<br>Rabbit NZW<br>16 inseminated females /group<br>Fludioxonil (97.5%)<br>0, 10, 100 or 300 mg/kg bw/d | NOAEL: 10 / 300<br>LOAEL: 100 / -<br>Dams: Reduced body weight gain at 100 mg/kg bw/d.<br>Foetuses: No effects. | None | Savary, M.H. 1989b (IUCLID 8.10.1-02) |
| **Fertility:**<br>OECD 416,<br>GLP<br>Rat Sprague Dawley<br>Fludioxonil (95.4%)<br>0, 30, 300, or 3000 ppm (0, 2.1, 21, or 212 mg/kg bw/d) | NOAEL: 21 / 21 / 212<br>LOAEL: 212 / 212 / -<br>Decreased body weight and body weight gain of parental rats and pups at 212 mg/kg bw/d.<br>No reproductive effects. | None | Singh *et al*., 1992 (IUCLID 8.10.2-01) |

### 4.11.1 Effects on fertility

#### 4.11.1.1 Non-human information

In a two-generation study (one litter per generation) in Sprague-Dawley rats performed according to OECD 416, males and females were administered fludioxonil at dietary concentrations of 0, 30, 300 and 3000 ppm (equivalent to an average of 0, 2.1, 21 or 212 mg/kg bw/d; the values were slightly lower in males compared to females, and slightly lower in the $F_0$ generation compared to the $F_1$ generation) continuously throughout a 10-week pre-mating period, mating, gestation and lactation of the resulting $F_1$ offspring (Singh *et al*, 1992). Litters were reduced to eight pups (4/sex where possible) at Day 4 *post partum*. Exposure of selected $F_1$ pups was continued following weaning for a 12-week pre-mating period and throughout mating, gestation and lactation of the resulting $F_2$ offspring.

There were no treatment-related mortalities in either of the parental generations. Discolouration of the penis/scrotum in males and perineum in females at 3000 ppm is attributable to the excretion of the blue-coloured metabolite SYN 518582 and is not considered to be of toxicological relevance. At the highest dose level of 3000 ppm fludioxonil caused treatment-related effects in the parental animals as well as in the pups.

Statistical significant reduced mean bodyweight in F1 males and F0 females (premating, gestation and lactation) correlated well with statistical significant reduced food consumption in F1 males and F0 females. Furthermore cumulative bodyweight gain was statistical significantly reduced in F0 females during premating and lactation at 3000 ppm.

Cumulative bodyweight gain was significantly reduced in $F_0$ females during premating and lactation at 3000 ppm. A marginal (but statistically significant) increase in relative testes weight in $F_1$ males at 3000 ppm and a marginal reduction in absolute ovary weight in $F_0$ females at 3000 ppm are considered to be secondary to lower terminal bodyweights in these animals. In the absence of a dose-response relationship, the marginal reduction in absolute ovary weight seen in $F_0$ females at 30 ppm is also considered to be without toxicological significance. Also at the high dose group of 3000 ppm statistically significant reductions in mean F1 and F2 pup body weight were observed in both sexes. There were no clinical signs that were attributable to treatment.

The numbers of implantation sites and litter sizes were slightly higher at 300 and 3000 ppm in the $F_2$ generation, but are considered to be within the normal biological variation and therefore unrelated to treatment. A marginally higher number of pups from these litters died within the first 4 days *post partum*, however findings are attributable to the larger litter sizes in these groups and are therefore also considered to be unrelated to treatment. Fludioxonil did not affect reproductive performance. No gross or histopathological changes of the examined organs were observed. Fludioxonil did not alter the litter parameters or cause gross changes in pups.

No treatment related effects were observed at the lower doses (30 and 300 ppm).

**2-generation study: organ weights**

| **group** | **1** | | **2** | | **3** | | **4** | |
|---|---|---|---|---|---|---|---|---|
| **ppm** | **0** | | **30** | | **300** | | **3000** | |
| | **F0** | **F1** | **F0** | **F1** | **F0** | **F1** | **F0** | **F1** |
| Terminal bw [g], males<br>females | 606.8<br>328.3 | 597.0<br>321.3 | 592.4<br>327.2 | 584.7<br>316.0 | 615.9<br>330.3 | 623.5<br>331.1 | 584.9<br>307.3* | 565.0<br>315.3 |
| Testes, absolute [g]<br>relative to bw [‰] | 3.45<br>5.7 | 3.74<br>6.3 | 3.52<br>6.0 | 3.81<br>6.6 | 3.54<br>5.8 | 3.78<br>6.1 | 3.52<br>6.1 | 3.80<br>6.8* |
| Ovaries, absolute [mg]<br>relative to bw [‰] | 120<br>0.4 | 110<br>0.3 | 100*<br>0.3 | 110<br>0.4 | 110<br>0.3 | 120<br>0.4 | 100*<br>0.3 | 110<br>0.4 |

* $p<0.05$ (Dunnetts's test)

## 2-generation study: parental body weight development

| group | 1 | | 2 | | 3 | | 4 | |
|---|---|---|---|---|---|---|---|---|
| ppm | 0 | | 30 | | 300 | | 3000 | |
| | F0 | F1 | F0 | F1 | F0 | F1 | F0 | F1 |
| **Males: bodyweight [g]° / [% of control]** | | | | | | | | |
| day 0 | 258 / 100 | 166 / 100 | 256 / 99 | 156 / 94 | 262 / 101 | 159 / 96 | 255 / 99 | 148** / 89 |
| day 14 | 355 / 100 | 296 / 100 | 352 / 99 | 281 / 95 | 361 / 102 | 288 / 97 | 345 / 97 | 267** / 90 |
| day 42 | 480 / 100 | 473 / 100 | 474 / 99 | 456 / 96 | 487 / 101 | 479 / 101 | 464 / 97 | 440** / 93 |
| day 70 / 84[§] | 558 / 100 | 583 / 100 | 547 / 98 | 570 / 98 | 567 / 102 | 601 / 103 | 540 / 97 | 551 / 95 |
| day 120-125[#] | 607 / 100 | 597 / 100 | 592 / 98 | 585 / 98 | 616 / 102 | 624 / 104 | 585 / 96 | 565 / 95 |
| **Females: bodyweight [g]° / [% of control]** | | | | | | | | |
| Premating: | | | | | | | | |
| day 0 | 187 / 100 | 145 / 100 | 184 / 98 | 138 / 95 | 184 / 98 | 143 / 99 | 182 / 98 | 136 / 94 |
| day 14 | 234 / 100 | 198 / 100 | 232 / 99 | 194 / 98 | 233 / 99 | 204 / 103 | 227 / 97 | 195 / 99 |
| day 42 | 283 / 100 | 271 / 100 | 281 / 99 | 268 / 99 | 285 / 101 | 279 / 103 | 268 / 95 | 265 / 98 |
| day 70 / 84[§] | 317 / 100 | 309 / 100 | 314 / 99 | 309 / 100 | 320 / 101 | 325 / 105 | 295* / 93 | 308 / 100 |
| Gestation: | | | | | | | | |
| day 0 | 315 / 100 | 309 / 100 | 13 / 100 | 311 / 101 | 319 / 101 | 324 / 105 | 290** / 92 | 312 / 101 |
| day 20 | 462 / 100 | 433 / 100 | 449 / 97 | 419 / 97 | 460 / 100 | 450 / 104 | 424** / 92 | 429 / 99 |
| Lactation: | | | | | | | | |
| day 0 | 362 / 100 | 342 / 100 | 359 / 99 | 339 / 99 | 369 / 102 | 352 / 103 | 333** / 92 | 331 / 97 |
| day 21 | 361 / 100 | 361 / 100 | 358 / 99 | 349 / 97 | 360 / 100 | 371 / 103 | 346 / 96 | 353 / 98 |
| **Males: cumulative bodyweight gain [g]° / [% of control]** | | | | | | | | |
| day 0 - 14 | 96.4 / 100 | 130 / 100 | 96.0 / 100 | 125 / 96 | 99.1 / 103 | 129 / 99 | 89.5 / 93 | 119 / 91 |
| day 0 - 42 | 222 / 100 | 307 / 100 | 218 / 98 | 300 / 98 | 225 / 101 | 320 / 104 | 209 / 94 | 292 / 95 |
| day 0 – 70/84[§] | 299 / 100 | 417 / 100 | 291 / 97 | 414 / 99 | 305 / 102 | 443 / 106 | 285 / 95 | 403 / 97 |
| day 70/84-120/5 | 49.2 / 100 | 43.1 / 100 | 45.2 / 92 | 39.0 / 91 | 48.5 / 99 | 47.4 / 110 | 44.9 / 91 | 38.9 / 90 |
| day 0 –120/125[#] | 349 / 100 | 431 / 100 | 336 / 97 | 429 / 100 | 354 / 101 | 465 / 108 | 330 / 95 | 417 / 97 |
| **Females: cumulative bodyweight gain [g]° / [% of control]** | | | | | | | | |
| Premating: | | | | | | | | |
| day 0 - 14 | 45.8 / 100 | 52.9 / 100 | 48.2 / 105 | 56.1 / 106 | 48.6 / 106 | 61.0 / 115 | 45.3 / 99 | 59.3 / 112 |
| day 0 - 42 | 94.0 / 100 | 126 / 100 | 97.4 / 104 | 130 / 103 | 101 / 107 | 136 / 108 | 85.9 / 91 | 129 / 103 |
| day 0 – 70/84[§] | 129 / 100 | 164 / 100 | 131 / 102 | 171 / 104 | 136 / 105 | 182 / 111 | 114* / 89 | 173 / 105 |
| Gestation: | | | | | | | | |
| day 0 - 20 | 147 / 100 | 124 / 100 | 136 / 93 | 109* / 88 | 141 / 101 | 126 / 102 | 135 / 92 | 118 / 95 |
| Lactation: | | | | | | | | |
| day 0 - 21 | -0.81 | 19.1 | -0.67 | 12.5 | -9.39 | 19.0 | 13.6* | 21.4 |

* $p<0.05$, ** $p<0.01$ (Dunnett's t-test); [#] terminal bodyweights, °rounded values, [§] end of premating period after 70 and 84 days for F0 and F1 animals, respectively

## 2-generation study: mating indices, survival, gestation and delivery

| | F0 | | | | F1 | | | |
|---|---|---|---|---|---|---|---|---|
| **group** | **1** | **2** | **3** | **4** | **1** | **2** | **3** | **4** |
| **ppm** | **0** | **30** | **300** | **3000** | **0** | **30** | **300** | **3000** |
| **Females** | | | | | | | | |
| placed with males and mated | 29 | 30 | 30 | 30 | 30 | 30 | 29 | 30 |
| inseminated | 29 | 30 | 30 | 29 | 29 | 30 | 29 | 29 |
| with defined day 0 p.c. | 29 | 30 | 28 | 29 | 29 | 30 | 29 | 29[#] |
| pregnant | 26 | 26 | 26 | 26 | 21 | 26° | 24 | 28 |
| mating after mating days | 2.6 | 3.2 | 3.5 | 2.7 | 3.3 | 2.2 | 3.8 | 2.4 |
| mating index [%] | 100 | 100 | 100 | 96.7 | 96.7 | 100 | 100 | 96.7 |
| fertility index [%] | 89.7 | 86.7 | 86.7 | 89.7 | 72.4 | 93.3 | 82.8 | 96.6 |
| no litter: non-pregnant | 3 | 4 | 4 | 3 | 8 | 2 | 5 | 1 |
| pregnant | 0 | 2 | 0 | 0 | 0 | 3 | 0 | 0 |
| delivering [number] | 26 | 24 | 26 | 26 | 21 | 25 | 24 | 28 |
| viable litters | 26 | 24 | 26 | 26 | 21 | 25 | 24 | 28 |
| gestation index [%] | 100 | 92.3 | 100 | 100 | 100 | 89.3 | 100 | 100 |
| parturition index [%] | 100 | 92.3 | 100 | 100 | 100 | 89.3 | 100 | 100 |
| duration of gestation [day] | 23.5 | 23.4 | 23.4 | 23.4 | 23.1 | 23.4 | 23.2 | 23.3 |
| **Males** | | | | | | | | |
| placed with females | 29 | 30 | 30 | 30 | 30 | 30 | 29 | 30 |
| mated | 29 | 30 | 26 | 29 | 29 | 30 | 29 | 29 |
| with females pregnant | 26 | 26 | 26 | 26 | 21 | 26 | 24 | 28 |
| mating index [%] | 100 | 100 | 86.7 | 96.7 | 96.7 | 100 | 100 | 96.7 |
| fertility index [%] | 89.7 | 86.7 | 100 | 89.7 | 72.4 | 93.3 | 82.8 | 96.6 |

[#] mating days were estimated for one female, ° two females with implants only

## 2-generation study: pup bodyweight

| | Males | | | | Females | | | |
|---|---|---|---|---|---|---|---|---|
| **group** | **1** | **2** | **3** | **4** | **1** | **2** | **3** | **4** |
| **ppm** | **0** | **30** | **300** | **3000** | **0** | **30** | **300** | **3000** |
| **Bodyweight [g] / [% of control]** | | | | | | | | |
| $F_1$ day 0 | 6.64 / 100 | 6.52 / 98 | 6.64 / 100 | 6.40 / 96 | 6.19 / 100 | 6.16 / 100 | 6.30 / 102 | 5.99 / 97 |
| day 4[#] | 10.18 / 100 | 9.88 / 97 | 10.47 / 103 | 9.28* / 91 | 9.59 / 100 | 9.40 / 98 | 10.02 / 104 | 8.80 / 92 |
| day 14 | 33.60 / 100 | 31.68 / 94 | 33.62 / 100 | 29.72** / 88 | 32.17 / 100 | 30.23 / 94 | 32.39 / 101 | 28.35** / 88 |
| day 21 | 56.87 / 100 | 54.34 / 96 | 56.68 / 100 | 50.27** / 88 | 53.73 / 100 | 51.55 / 96 | 54.28 / 101 | 47.62** / 89 |
| $F_2$ day 0 | 6.38 / 100 | 6.33 / 99 | 6.21 / 97 | 6.12 / 96 | 6.07 / 100 | 5.90 / 97 | 5.88 / 97 | 5.78 / 95 |
| day 4[#] | 9.15 / 100 | 9.64 / 105 | 9.03 / 99 | 8.67 / 95 | 8.89 / 100 | 8.96 / 101 | 8.76 / 99 | 8.16 / 92 |
| day 14 | 30.05 / 100 | 30.74 / 102 | 30.93 / 103 | 27.97* / 93 | 29.59 / 100 | 29.49 / 100 | 30.17 / 102 | 26.12** / 88 |
| day 21 | 48.67 / 100 | 50.53 / 104 | 51.07 / 105 | 44.62* / 92 | 47.51 / 100 | 47.88 / 101 | 49.43 / 104 | 41.69** / 88 |

* $p<0.05$, ** $p<0.01$ (Dunnett's t-test); [#] post culling

## 2-generation study: F1 litter data

| group<br>ppm | 1<br>0 | 2<br>30 | 3<br>300 | 4<br>3000 |
|---|---|---|---|---|
| Pregnant females | 26 | 26 | 26 | 26 |
| Viable litters | 26 | 24[#] | 26 | 26 |
| implantation sites [total/mean] | 421 / 16.2 | 384 / 14.8 | 391 / 15.0 | 397 / 15.3 |
| Pups delivered [total/mean] | 391 / 15.0 | 347 / 13.3 | 355 / 13.6 | 383 / 14.7 |
| Prenatal loss [%] | 7.1 | 9.6 | 9.2 | 3.5 |
| Pups liveborn [total/mean] | 383 / 14.7 | 339 / 14.1 | 343 / 13.2 | 381 / 14.7 |
| Live birth index [%] | 98.0 | 97.7 | 96.6 | 99.5 |
| Pups stillborn [total/mean/%] | 8 / 0.31 / 1.9 | 8 / 0.31 / 2.3 | 12 / 0.46 / 2.8 | 2 / 0.08 / 0.6 |
| Postimplant. loss [total/mean/ %] | 38 / 1.46 / 9.4 | 45 / 1.73 / 16.7 | 48 / 1.85 / 12.2 | 16 / 0.62 / 4.0 |
| Pups [number / %] died: day 0-21 | 15 / 3.9 | 5 / 2.7 | 8 / 2.3 | 12 / 3.1 |
| day 0 - 4 | 13 / 3.4 | 5 / 1.5 | 6 / 1.7 | 7 / 1.8 |
| day 5 - 7 | 2 / 0.5 | 3 / 0.9 | 1 / 0.3 | 1 / 0.3 |
| day 8-14 | 0 | 1 / 0.3 | 0 | 3 / 0.8 |
| day 15-21 | 0 | 0 | 1 / 0.3 | 1 / 0.3 |
| Pups culled day 4 [N / %] | 164 / 44.3 | 142 / 42.5 | 139 / 41.2 | 166 / 44.4 |
| Pups surviving day 0-4 | 370 / 96.9 | 334 / 98.5 | 337 / 98.3 | 374 / 98.3 |
| Pups surviving day 4-21 | 99.0 | 97.9 | 98.6 | 97.6 |
| Live pups per litter, day 0 | 14.7 | 14.1 | 13.2 | 14.7 |
| day 4 (pre-culling) | 14.2 | 13.9 | 13.0 | 14.4 |
| day 4 (post-culling) | 7.9 | 8.0 | 7.6 | 8.0 |
| day 21 | 7.8 | 7.8 | 7.5 | 7.8 |
| Sex ratio, day 0: % live males / females | 52.0 / 48.0 | 48.1 / 51.9 | 50.2 / 49.2 | 55.9 / 44.1 |
| Survival by sex [%] males day 0 - 4 | 96.8 | 98.5 | 98.1 | 98.5 |
| day 4 – 21 | 99.0 | 97.9 | 99.0 | 96.2 |
| females day 0 - 4 | 96.6 | 98.6 | 99.2 | 97.8 |
| day 4 – 21 | 99.0 | 97.9 | 98.4 | 99.0 |

[#] due to two females with implantations only, further two females excluded where sperm was not observed at vaginal washing but which delivered viable litters

**2-generation study: F2 litter data**

| group<br>ppm | 1<br>0 | 2<br>30 | 3<br>300 | 4<br>3000 |
|---|---|---|---|---|
| Pregnant females | 21 | 28 | 24 | 28 |
| Viable litters | 21 | 25[#] | 24 | 28 |
| implantation sites [total / group mean] | 314 / 14.9 | 342 / 12.7 | 394 / 16.4* | 457 / 16.3* |
| Pups delivered [total / group mean] | 299 / 14.2 | 312 / 12.4 | 368 / 15.3 | 440 / 15.7 |
| Prenatal loss [%] | 4.2 | 9.0 | 6.6 | 3.7 |
| Pups liveborn [total / group mean] | 297 / 14.1 | 305 / 11.3 | 363 / 15.1 | 433 / 15.5 |
| Live birth index [%] | 99.3 | 97.8 | 98.6 | 98.4 |
| Pups stillborn [total / group mean/ %] | 2 / 0.10 / 0.6 | 7 / 0.26 / 1.8 | 5 / 0.21 / 1.1 | 7 / 0.25 / 1.5 |
| Postimplant. loss [total/group mean/ %] | 17 / 0.81 / 5.6 | 37 / 1.41 / 16.3 | 31 / 4.29 / 7.3 | 24 / 0.86 / 5.4 |
| Pups [number / %] died: day 0-21 | 4 / 1.3 | 10[§] / 3.3 | 13 / 3.6 | 18 / 4.2 |
| day 0- 4 | 4 / 1.3 | 9 / 3.0 | 7 / 1.9 | 16 / 3.7 |
| day 5-7 | 0 | 1 / 0.3 | 2 / 0.6 | 1 / 0.2 |
| day 8-14 | 0 | 0 | 3 / 0.8 | 1 / 0.2 |
| day 15-21 | 0 | 0 | 1 / 0.3 | 0 |
| Pups culled day 4 [number / %] | 128 / 43.7 | 114 / 38.5 | 164 / 46.1 | 195 / 46.8 |
| Pups surviving day 0 - 4 [number / %] | 293 / 98.8 | 296 / 93.7 | 356 / 98.4 | 417 / 96.3* |
| Pups surviving day 4 - 21 [number / %] | 293 / 100 | 295 / 99.4 | 350 / 96.9 | 415 / 99.1 |
| Live pups per litter, day 0 | 14.1 | 12.2 | 15.1* | 15.5* |
| day 4 (preculling) | 14.0 | 11.8 | 14.8 | 14.9 |
| day 4 (postculling) | 7.9 | 7.6 | 8.0 | 7.9 |
| day 21 | 7.9 | 7.5 | 7.8 | 7.9 |
| Sex ratio, day 0: % live males / females | 49.8 / 50.2 | 48.9 / 51.1 | 51.5 / 48.5 | 47.1 / 52.9 |
| Survival by sex [%] males day 0 - 4 | 97.9 | 94.9 | 99.5 | 96.1 |
| day 4 – 21 | 100 | 100 | 96.9 | 100 |
| females day 0 - 4 | 100 | 92.7 | 97.5 | 96.8 |
| day 4 – 21 | 100 | 98.9 | 96.9 | 98.2 |

* $p<0.05$ (Dunnett's test), [#] due to two females with implantations, [§] including two pups from a female that had to be sacrificed during delivery

Statistical significantly reduced bodyweight and body weight gain were observed at the highest dose level in parental animals and offspring. Additionally, food consumption was occasionally decreased in parental animals at 3000 ppm. All reproductive and litter parameters were unaffected by treatment; treatment-related findings in offspring were limited to reduced weight at 3000 ppm.

A reproductive overall NOAEL of 3000 ppm (equivalent to 212 mg/kg bw day) was determined based on no treatment-related effects on reproductive parameters at the top dose level.

An overall NOAEL for parental and pup toxicity of 300 ppm (equivalent to 21 mg/kg bw/day) can be determined, based on the bodyweight effects in both sexes at 3000 ppm (equivalent to 212 mg/kg bw day).

#### 4.11.1.2 Human information

No human data are available.

### 4.11.2 Developmental toxicity

#### 4.11.2.1 Non-human information

Two guideline-compliant (OECD 414) developmental toxicity studies performed in rats and in rabbit are available for fludioxonil.

In the rat study (Savary, 1989a) groups of pregnant Sprague-Dawley rats were gavaged with fludioxonil at dose levels of 0, 10, 100, or 1000 mg/kg bw/d on Days 6-15 of gestation. Maternal toxicity was seen as slightly reduced food consumption (10% compared to controls) and mean bodyweight gain (21% compared to controls) on Days 6-11 at the highest dose level.

Significantly reduced weight gain by dams at 1000 mg/kg bw/d during the dosing phase resulted in significantly reduced corrected weight gain (without uterus weight) over the entire study period (day 0-20). Gravid uterus weights were unaffected by treatment. No treatment-related mortality occurred and no signs of toxicity were observed during the study period. Macroscopic examination revealed one female with a pale liver. No treatment-related effects were noted on the numbers of implantation sites, pre-implantation loss, early or late resorptions in any group. Total resorption was seen in two females at 10 and 100 mg/kg bw/d, these animals had few (1-3) implantation sites. The rate of resorption was not significantly different to the controls and is not considered to be treatment-related. There were no dead foetuses in any group. The numbers of live foetuses per litter, foetal sex ratio and foetal weights were not significantly different from the controls and did not show any dose-relationship. Significantly lower incidences of skeletal variations and anomalies were observed in the treated groups; findings are considered to represent normal biological variation and are therefore not of toxicological relevance. The incidence of foetuses with dilatation of the ureter and/or renal pelvis was slightly (but not significantly) increased at the highest dose level 1000 mg/kg bw/d compared to the concurrent controls. This finding is considered to be incidental as the foetal incidences in this group (4.0% and 5.6% respectively) are within the laboratory's historical control range (0.6-7.5%); it is additionally noted that the concurrent control incidences for these findings are at the lower end of the historical range. The validity of the historical control data has not been confirmed with respect to strain, time interval and laboratory. Please refer to the position paper regarding origin of the HCD provided Syngenta 2015.07.20 in which it is concluded "CiTox Lab may assume that HCD were generated in the CiTox Lab with the same source of rats (Charles River, France). "

Based on the effect on maternal toxicity (reduced body weight gain and food consumption) in the high-dose group a NOAEL for maternal toxicity is set to 100 mg/kg bw/day. The incidence of litters having foetuses with foetal soft tissue abnormalities (ureteral and/or pelvic dilations) is considered to be incidental and within the HCD of the laboratory and not considered to be treatment related. The NOAEL for developmental toxicity is set to 1000 mg/kg bw/day.

## Rat teratology study: maternal survival and pregnancy status

| | group<br>mg/kg bw/day | 1<br>0 | 2<br>10 | 3<br>100 | 4<br>1000 |
|---|---|---|---|---|---|
| Females | mated | 25 | 25 | 25 | 25 |
| | non pregnant | 5 | 8 | 4 | 5 |
| | pregnant | 20 | 17 | 21 | 20 |
| | accidental death (pregnant) | 1 | 0 | 0 | 0 |
| examined at scheduled necropsy: | pregnant | 19 | 17 | 21 | 20 |
| | with total resorptions | 0 | 2 | 2 | 0 |
| | with viable foetuses | 19 | 15 | 19 | 20 |

## Rat teratology study: maternal findings

| group<br>mg/kg bw/day | 1<br>0 | 2<br>10 | 3<br>100 | 4<br>1000 |
|---|---|---|---|---|
| **Bodyweight [g] / [% of control]** | | | | |
| day 0 | 256 / 100 | 267 / 104 | 254 / 99 | 265 / 104 |
| day 6 | 300 / 100 | 312 / 104 | 301 / 100 | 307 / 102 |
| day 15 | 356 / 100 | 373 / 105 | 353 / 99 | 351 / 99 |
| day 20 | 430 / 100 | 454 / 106 | 420 / 98 | 424 / 99 |
| **Bodyweight gain [g] / [% of control]** | | | | |
| day 0- 6 | 44 / 100 | 45 / 102 | 45 / 102 | 42 / 96 |
| day 6-15 | 56 / 100 | 61 / 109 | 52 / 93 | 44 / 79 |
| day 15-20 | 74 / 100 | 81 / 110 | 67 / 91 | 73 / 99 |
| day 0-20[#] | 98.3 / 100 | 103.3 / 105 | 102.1 / 104 | 82.2* / 84 |
| **Uterus weight** [g] / [% of control] | 76.0 / 100 | 83.8 / 110 | 63.4 / 83 | 77.2 / 102 |
| **Food consumption [g/animal/day] / [% of control]** | | | | |
| day 0- 5 | 28 / 100 | 32 / 114 | 31 / 111 | 31 / 111 |
| day 6-11 | 31 / 100 | 34 / 110 | 32 / 103 | 28* / 90 |
| day 12-15 | 33 / 100 | 34 / 103 | 34 / 103 | 31 / 94 |
| day 16-19 | 34 / 100 | 36 / 106 | 34 / 100 | 36 / 106 |

* $p<0.05$ (Student's t-test), [#] without uterus weight

## Rat teratology study: litter findings

| | group<br>mg/kg bw/day | 1<br>0 | 2<br>10 | 3<br>100 | 4<br>1000 |
|---|---|---|---|---|---|
| Corpora Lutea: | total / mean per animal | 342 / 17.1 | 302 / 17.8 | 326 / 15.5 | 347 / 17.4 |
| Implantation sites: | total / mean per animal | 286 / 14.3 | 233 / 13.7 | 224 / 10.7 | 273 / 13.7 |
| Implants[#]: | total / mean per animal | 270 / 14.2 | 229 / 15.3 | 221 / 11.6 | 273 / 13.7 |
| Preimplantation loss: | total / % per group | 56 / 16.4 | 69 / 22.8 | 102 / 31.3 | 74 / 21.3 |
| Live foetuses: | total / litters / mean per litter | 262 / 19 / 13.8 | 221 / 15 / 14.7 | 211 / 19 / 11.1 | 268 / 20 / 13.4 |
| Dead foetuses: | total | 0 | 0 | 0 | 0 |
| % live foetuses | | 97.0 | 96.5 | 99.5 | 98.2 |
| Early resorptions: | total / mean per animal | 8 / 0.4 | 11 / 0.6 | 12 / 0.6 | 5 / 0.3 |
| | % of implant. per group | 2.8 | 4.7 | 5.4 | 1.8 |
| Late resorptions: | total / mean per animal | 0 / 0 | 1 / 0.06 | 1 / 0.05 | 0 / 0 |
| | % of implant. per group | 0 | 0.4 | 0.4 | 0 |
| Total resorptions: | total / mean per animal | 8 / 0.4 | 12 / 0.7 | 13 / 0.6 | 5 / 0.3 |
| | % of implant. per group | 2.8 | 5.2 | 5.8 | 1.8 |
| Postimplantation loss: | total / mean per animal | 8 / 0.4 | 12 / 0.7 | 13 / 0.6 | 5 / 0.3 |
| | % of implant. per group | 2.8 | 5.2 | 5.8 | 1.8 |
| % male / % female foetuses | | 50.4 / 49.6 | 47.5 / 52.5 | 46.4 / 53.6 | 48.5 / 51.5 |
| mean foetal body weight [g] | | 3.73 | 3.86 | 3.91 | 3.95 |

[#] implants excluding females that died (one control animal) or had total resorptions (two group 2 and 3 animals)

**Rat teratology study with fludioxonil: foetal findings**

| group<br>mg/kg bw/day | 1<br>0 | 2<br>10 | 3<br>100 | 4<br>1000 |
|---|---|---|---|---|
| Foetuses / litters examined for **external** observations | 262 / 19 | 221 / 15 | 211 / 19 | 268 / 20 |
| External anomalies: total / % foetal incidence % litter incidence | 0 / 0 / 0 | 0 / 0 / 0 | 0 / 0 / 0 | 0 / 0 / 0 |
| Foetuses / litters examined for **skeletal** observations | 135 / 19 | 115 / 15 | 112 / 19 | 141 / 20 |
| Skeletal variations: total / % foetal incidence | 121 / 90 | 91* / 79 | 86** / 77 | 112* / 79 |
| litter / % litter incidence | 19 / 100 | 15 / 100 | 17 / 89 | 17 / 85 |
| Skeletal anomalies: total / % foetal incidence | 32 / 24 | 28 / 24 | 16 / 14 | 20* / 14 |
| litter / % litter incidence | 12 / 63 | 11 / 73 | 9 / 47 | 8 / 40 |
| Distorted rib: total / % foetal incidence / % litter incidence | 1 / 0.7 / 5.3 | 0 / 0 / 0 | 0 / 0 / 0 | 0 / 0 / 0 |
| unossified rib: total / % foetal incidence / % litter incidence | 2 / 1.5 / 11.0 | 0 / 0 / 0 | 0 / 0 / 0 | 0 / 0 / 0 |
| Skeletal malformations: total / % foetal incidence | 2 / 15 | 0 / 0 | 0 / 0 | 0 / 0 |
| litter / % litter incidence | 2 / 11 | 0 / 0 | 0 / 0 | 0 / 0 |
| Foetuses / litters examined for **visceral** observations | 127 / 19 | 106 / 15 | 99 / 17 | 126 / 20 |
| Dilated renal pelvis: total / % foetal incidence | 1 / 0.8 | 1 / 0.9 | 1 / 1.0 | 5 / 4.0 |
| litter / % litter incidence | 1 / 5.3 | 1 / 6.7 | 1 / 5.9 | 4 / 20 |
| Dilated ureter: total / % foetal incidence | 4 / 3.1 | 2 / 1.9 | 5 / 5.1 | 7 / 5.6 |
| litter / % litter incidence | 2 / 11 | 2 / 13 | 4 / 24 | 7 / 35 |
| Visceral anomalies: total / % foetal incidence | 4 / 3.1 | 2 / 1.9 | 5 / 5.1 | 9 / 7.1 |
| litter / % litter incidence | 2 / 11 | 2 / 13 | 4 / 24 | 8 / 40 |

* $p<0.05$, ** $p<0.01$ (Chi-Square t-test

In the second developmental toxicity study pregnant NZW rabbits (Savary, M.H. 1989b) were given daily doses of 0, 10, 100 or 300 mg/kg bw/day on day 6 through 18 of gestation which resulted in maternal toxicity in form of reduced body weight gain at the two highest doses (100 and 300 pmm) and food consumption in the high dose only.

At 100 ppm the reduction was less pronounced at not accompanied with a marked reduction in food consumption as at the top dose level. The reduction in body weight gain was however 17% lower than control and is still considered adverse by CA DK. Applicant argues that the finding is not clearly related to treatment due to the magnitude of change and that bodyweight values for individual animals in this group are within the concurrent control range and mean values are within the laboratory's historical control range. A reduction of 17% lower body weight gain compared with the concurrent control is however considered potentially adverse by CA DK and a NOAEL of 30 ppm for maternal toxicity is set; this value is in line with the EFSA conclusion.

The gravide uterine weight was comparable between the control and all dose groups.

No treatment-related mortality occurred and no treatment-related effects on pregnancy status were observed. Treatment-related clinical signs were limited to blue urine in animals at 100 and 300 mg/kg bw/d; this finding is due to the excretion of a blue metabolite and is not considered to be of toxicological significance.

No treatment-related effects were noted on the mean numbers of implantation sites, pre-implantation loss, early and late resorptions and on the incidences of external, skeletal or visceral abnormalities. Foetal weight was comparable in all groups. A marginal difference (however statistically significant) in the sex ratio of foetuses at 300 mg/kg bw/day were observed; the toxicological significance is unclear but is expected not to be related to treatment since the critical time of sexual differentiation is after birth and gender is primarily determined at fertilization by the genetic setup of the zygote Therefore the change in sex ratio (more females 52% versus 48% males)

is considered incidental. No differences in sex ratios were observed in the developmental rat study or in the two-generation study.

Based on effect on maternal toxicity (reduced body weight gain) in the two high dose groups the NOAEL for maternal toxicity is set to 100 mg/kg bw/day. The NOAEL for developmental toxicity is 300 mg/kg bw/day due to no treatment related foetotoxic effects at the highest dose.

**Rabbit teratogenicity study: maternal survival and pregnancy status**

| | **group**<br>**mg/kg bw/day** | **1**<br>**0** | **2**<br>**10** | **3**<br>**100** | **4**<br>**300** |
|---|---|---|---|---|---|
| Females | mated | 16 | 16 | 16 | 16 |
| | non pregnant | 2 | 1 | 1 | 0 |
| | pregnant | 14 | 15 | 15 | 16 |
| | death (pregnant) | 0 | 0 | 1 | 0 |
| examined at scheduled necropsy: | pregnant | 14 | 15 | 14 | 16 |
| | with total resorptions | 1 | 1 | 0 | 1 |
| | aborted | 1 | 0 | 0 | 1 |
| | with viable foetuses | 12 | 14 | 14 | 14 |

**Rabbit teratogenicity study: maternal findings**

| **group**<br>**mg/kg bw/day** | **1**<br>**0** | **2**<br>**10** | **3**<br>**100** | **4**<br>**300** |
|---|---|---|---|---|
| **Bodyweight [g] / [% of control]** | | | | |
| day 0 | 3763 / 100 | 3744 / 100 | 3716 / 99 | 3702 / 98 |
| day 6 | 3937 / 100 | 3946 / 100 | 3908 / 99 | 3861 / 98 |
| day 18 | 4089 / 100 | 4080 / 100 | 4034 / 99 | 3938 / 96 |
| day 28 | 4234 / 100 | 4205 / 99 | 4140 / 98 | 4061 / 96 |
| **Bodyweight gain [g] / [% of control]** | | | | |
| day 0- 6 | 173.3 / 100 | 140.7 / 81 | 191.4 / 110 | 159.3 / 92 |
| day 6-18 | 152.5 / 100 | 195.7 / 128 | 126.4 / 83 | 76.4 / 50 |
| day 18-28 | 145.0 / 100 | 125.0 / 86 | 105.7 / 73 | 122.9 / 85 |
| day 0-28 (individual range) | 470.8 / 100 | 461.4 / 98 | 423.6 / 90 | 358.6 / 76 |
| day 0-28# (individual range) | 63.6 (-514 – +431) | 47.3 (-302 – +287) | 9.6 (-393 - +374) | -70.2 (-413 - +201) |
| **Uterus weight** [g] / [% of control] | 407.2 / 100 | 414.1 / 102 | 414.0 / 102 | 428.8 / 105 |
| **Food consumption [g/animal/day] / [% of control]** | | | | |
| day 0- 6 | 209 / 100 | 193 / 92 | 198 / 95 | 189 / 90 |
| day 6-12 | 188 / 100 | 193 / 103 | 180 / 96 | 145* / 77 |
| day 12-19 | 191 / 100 | 189 / 99 | 194 / 102 | 164 / 86 |
| day 19-24 | 181 / 100 | 165 / 91 | 171 / 95 | 153 / 85 |
| day 24-28 | 138 / 100 | 127 / 92 | 148 / 107 | 110 / 80 |

* $p<0.05$ (Student's t-test), # without uterus weight

*Two other rabbit teratogenicity studies from the same test facility using the same strain of rabbits are available. In one range-finding study only 2 of 5 control females completed the pregnancy; their bodyweight gain (day 0-28) was 465 g (individual range 300-630g); the uterus weights were not given. In another regular study (12 control females completed the pregnancy) the bodyweight gain (day 0-28) of controls was 393 g (individual range 100-650 g); the respective bodyweight gain without uterus weight was 1.6 g (individual range –332 to +299 g). This also indicates that the bodyweight gain observed in group 3 females was unaffected by treatment.*

**Rabbit teratogenicity study: litter findings**

| group<br>mg/kg bw/day | | 1<br>0 | 2<br>10 | 3<br>100 | 4<br>300 |
|---|---|---|---|---|---|
| Corpora Lutea: | total / mean per animal | 138 / 10.6 | 161 / 10.7 | 145 / 10.4 | 152 / 10.1 |
| Implantation sites: # | total / mean per animal | 110 / 8.5 | 126 / 8.4 | 123 / 8.8 | 123 / 8.2 |
| Preimplantation loss: | total / % per group | 28 / 20.3 | 35 / 21.7 | 22 / 15.2 | 29 / 19.1 |
| | % per animal (mean) | 21.9 | 22.4 | 15.7 | 19.6 |
| Foetuses: | total / mean per animal | 99 / 7.6 | 111 / 7.4 | 109 / 7.8 | 116 / 7.7 |
| Alive foetuses: | total / % live foetuses | 99 / 100 | 109 / 98.2 | 108 / 99.1 | 115 / 99.1 |
| % of implantations per group/animal (mean) | | 90.0 / 84.5 | 86.5 / 83.0 | 87.8 / 88.3 | 93.5 / 88.8 |
| Dead foetuses: total / % dead foetuses | | 0 / 0 | 2 / 1.8 | 1 / 0.9 | 1 / 0.9 |
| % of implantations per group/animal (mean) | | 0 / 0 | 1.6 / 1.2 | 0.8 / 0.7 | 0.8 / 0.7 |
| Early resorptions: | total / mean per animal | 7 / 0.5 | 5 / 0.3 | 6 / 0.4 | 6 / 0.4 |
| % of implantations per group/animal (mean) | | 6.4 / 12.2 | 4.0 / 9.2 | 4.8 / 5.1 | 4.9 / 9.6 |
| Late resorptions: | total / mean per animal | 4 / 0.3 | 10 / 0.7 | 8 / 0.6 | 1 / 0.1 |
| % of implantations per group/animal (mean) | | 3.6 / 3.3 | 7.9 / 6.6 | 6.5 / 5.9 | 0.8 / 0.8 |
| Total resorptions: | total / mean per animal | 11 / 0.8 | 15 / 1.0 | 14 / 1.0 | 7 / 0.5 |
| % of implantations per group/animal (mean) | | 10.0 / 15.5 | 11.9 / 15.7 | 11.4 / 11.0 | 5.7 / 10.5 |
| Postimplantation loss: total / mean per animal | | 11 / 0.8 | 17 / 1.1 | 15 / 1.1 | 8 / 0.5 |
| % of implantations per group/animal (mean) | | 10.0 / 15.5 | 13.5 / 17.0 | 12.2 / 11.7 | 6.5 / 11.2 |
| Viable male foetuses: | total / % | 62 / 63 | 61 / 56 | 57 / 53 | 55* / 48 |
| Viable females foetuses: | total / % | 37 / 37 | 48 / 44 | 51 / 47 | 60* / 52 |
| mean foetal body weight [g] | | 34.4 | 36.1 | 36.6 | 36.5 |

# excluding females that had died or aborted
* $p<0.05$ (Dunnett's test)

**Rabbit teratogenicity study: foetal findings**

| group<br>mg/kg bw/day | | 1<br>0 | 2<br>10 | 3<br>100 | 4<br>300 |
|---|---|---|---|---|---|
| Foetuses / litters examined | | 99 / 12 | 109 / 14 | 108 / 14 | 115 / 14 |
| External observations: total / % foetal incid. / % litter incidence | | 0 / 0 / 0 | 0 / 0 / 0 | 0 / 0 / 0 | 0 / 0 / 0 |
| Skeletal variations: | total / % foetal incidence | 40 / 40 | 40 / 37 | 39 / 36 | 52 / 45 |
| | litter / % litter incidence | 10 / 83 | 12 / 86 | 13 / 93 | 13 / 93 |
| Skeletal anomalies: | total / % foetal incidence | 62 / 63 | 70 / 64 | 60 / 56 | 63 / 55 |
| | litter / % litter incidence | 12 / 100 | 14 / 100 | 13 / 93 | 14 / 100 |
| Fused sternebra: total / % foetal incidence / % litter incidence | | 0 / 0 / 0 | 1 / 0.9 / 7.1 | 3 / 2.8 / 14 | 0 / 0 / 0 |
| Skeletal malformations: | total / % foetal incidence | 0 / 0 | 1 / 0.9 | 3 / 2.8 | 0 / 0 |
| | litter / % litter incidence | 0 / 0 | 1 / 7.1 | 2 / 14 | 0 / 0 |
| Visceral observations: | total / % foetal incidence | 0 / 0 | 0 / 0 | 0 / 0 | 0 / 0 |
| | litter / % litter incidence | 0 / 0 | 0 / 0 | 0 / 0 | 0 / 0 |

#### 4.11.2.2 Human information

No human data are available.

### 4.11.3 Other relevant information

Effects on or via lactation:

There is no evidence for specific effect on or via lactation from the 2-generation rat study.

### 4.11.4 Summary and discussion of reproductive toxicity

No evidence of reproductive toxicity was seen in two-generation rat study at dietary concentrations of up to 3000 ppm (equivalent to 212 mg/kg bw/d), at which parental toxicity was observed. There is no evidence from repeated dose toxicity studies performed with fludioxonil of effects on the reproductive system of either sex.

Developmental toxicity studies in the rat and rabbit do not show any effects on the developing foetus at the highest dose levels used (1000 and 300 mg/kg bw/d, respectively), which were sufficient to cause maternal toxicity.

There is no evidence from the multi-generation study of any effects of fludioxonil treatment on lactation or via lactation on offspring. Offspring effects in this study were limited to reduced weight gain at the highest dietary concentration; findings are associated with reduced maternal weight gain and are not considered to be a direct effect of fludioxonil exposure via lactation.

### 4.11.5 Comparison with criteria

The definition of reproductive toxicity in the CLP Regulation (Annex I: 3.7.1.1) includes adverse effects on sexual function and fertility in adult males and females, as well as developmental toxicity in the offspring.

Adverse effects on sexual function and fertility are defined (Annex I: 3.7.1.3) as any effect of a substance that has the potential to interfere with sexual function and fertility including, but not limited to, alterations to the female and male reproductive system, adverse effects on onset of puberty, gamete production and transport, reproductive cycle normality, sexual behaviour, fertility, parturition, pregnancy outcomes, premature reproductive senescence, or modifications in other functions that are dependent on the integrity of the reproductive system.

Adverse effects on development of the offspring (Annex I: 3.7.1.4) includes any effect which interferes with normal development of the conceptus, either before or after birth, and resulting from exposure of either parent prior to conception, or exposure of the developing offspring during prenatal development, or post-natally, to the time of sexual maturation. As classification for developmental toxicity is primarily intended to provide a hazard warning for pregnant women and for men and women of reproductive capacity, for pragmatic purposes, classification for developmental toxicity is essentially intended to encompass adverse effects induced during pregnancy, or as a result of parental exposure.

Adverse effects on or via lactation are included under reproductive toxicity, but for classification purposes such effects are treated separately (Annex I: 3.7.1.5).

For the purpose of classification for reproductive toxicity, substances are allocated to one of two categories. Within each category, effects on sexual function and fertility and on development are considered separately. Effects on lactation are allocated to a separate hazard category. Substances are allocated to Category 1 (substances that are a known or presumed human reproductive toxicant) or to Category 2 (substances that are a suspected human reproductive toxicant). Substances are classified in Category 1 when they are known to have produced an adverse effect on sexual function and fertility or development in humans; or when there is evidence from animal studies providing a strong presumption that the substance has the capacity to cause effects in humans. Classification is further distinguished on the basis of whether the evidence for classification is primarily from human data (Category 1A) or from animal data (Category 1B). Substances are classified in Category 2

when there is some evidence from experimental animals of an adverse effect on sexual function and fertility or on development; and where the evidence is not sufficiently convincing to place the substance in Category 1. Effects are relevant for classification where these have been observed in the absence of other toxicity, if the adverse effect on reproduction is considered not to be a secondary non-specific consequence other toxicity.

### 4.11.6 Conclusions on classification and labelling

Fludioxonil was not toxic to reproduction in the two-generation study in rats at daily dietary doses up to 212 mg/kg bw/day, a dose level at which parental toxicity was observed. In the developmental toxicity studies in rats and rabbits, no effects on the foetuses were observed at any dose level even though maternal toxicity occurred at the high dose levels (1000 mg/kg bw/day in rats, 100 mg/kg bw/day in rabbits).

In the absence of any effects on sexual function or fertility, on developmental toxicity, on or via lactation, fludioxonil is not classified for reproductive toxicity according to Regulation (EC) No 1272/2008.

## 4.12 Other effects

### 4.12.1 Non-human information

#### 4.12.1.1 Neurotoxicity

No specific data are available. Fludioxonil has no structural relationship to known neurotoxic substances. There is no evidence of specific neurotoxicity or neuropathology from the standard toxicological studies.

#### 4.12.1.2 Immunotoxicity

No specific data are available. There is no evidence of immunotoxicity from the standard toxicological studies. The repeated dose dermal toxicity study (Schneider, 1990) notes histopathological changes of enlarged cortical macrophages, often revealing lymphophagocytosis, in the thymus of females at 1000 mg/kg bw/d. Although these findings are considered to be potentially treatment-related in the context of this study, it is notable, that no relevant thymus effects were observed in other studies (oral) with fludioxonil in rats (or in any other species).

#### 4.12.1.3 Specific investigations: other studies

No data are available.

#### 4.12.1.4 Human information

The routine surveillance of plant personnel involved in the manufacture and formulation of fludioxonil has not revealed any health effects attributable to exposure to fludioxonil. No poisoning cases have been reported to the manufacturer and no cases are reported in the scientific literature. No epidemiological studies relating to fludioxonil exposure are reported in the literature.

### 4.12.2 Summary and discussion

There is no evidence of any toxicological effects of fludioxonil.

### 4.12.3 Comparison with criteria

Not relevant.

### 4.12.4 Conclusions on classification and labelling

There are no additional effects of fludioxonil triggering classification for effects on human health according to the criteria of Regulation (EC) No 1272/2008.

# 5 ENVIRONMENTAL HAZARD ASSESSMENT

## 5.1 Degradation

**Table 21: Summary of relevant information on degradation**

| Method | Results | Remarks | Reference |
|---|---|---|---|
| Fludioxonil: hydrolysis.<br>US EPA subdivision N, para. 161-1 | No degradation observed after 30 days. | Fludioxonil hydrolytically stable | Hawkins, D.R. *et a*l., 1991a<br>(IUCLID 10.1.1.1-01) |
| CGA 339833: hydrolysis.<br>OECD 111 | $DT_{50}$: 597 days at pH 7 and 25°C | No major metabolites observed | Van der Gaauw, A., 2002<br>(IUCLID 10.1.1.1-02) |
| Fludioxonil: aqueous photolysis<br>US EPA subdivision N, para. 161-2 | $DT_{50}$: 9.9 days at 25°C<br>Photolysis rate constant ($k^c_p$): 0.0699 days | [pyrrole-$^{14}$C]-fludioxonil tested | Kirkpatrick, D., 1994a<br>(IUCLID 10.1.1.1-03) |
| Fludioxonil: aqueous photolysis<br>US EPA subdivision N, para. 161-2 | $DT_{50}$: 8.7 days at 25°C<br>Photolysis rate constant ($k^c_p$): 0.0795 days | [phenyl-$^{14}$C]-fludioxonil tested | Kirkpatrick, D., 1994b<br>(IUCLID 10.1.1.1-04) |
| Fludioxonil: ready biodegradation<br>92/69/EEC C.4-C | 7% biodegradation in 29 days | Fludioxonil not biodegradable according to the test ($CO_2$ evolution test) | Baumann, W., 1993<br>(IUCLID 10.1.1.2-01) |
| Fludioxonil: soil photolysis.<br>US EPA subdivision N, para. 161-3 | $DT_{50}$: 10 days at 25°C<br>95.7-96.5% AR recovered | [pyrrole-$^{14}$C]-fludioxonil was tested. 11.7% CGA 192155, 9.1% CGA 339833 and 12.3% CGA 265378 were formed | Kirkpatrick, D., 1994d,f<br>(IUCLID 10.2.1-14)<br>(IUCLID 10.2.1-16) |
| Fludioxonil: soil photolysis.<br>US EPA subdivision N, para. 161-2 | $DT_{50}$: 9 days at 25°C<br>94.7-96.9% AR recovered | [phenyl-$^{14}$C]-fludioxonil was tested. 10% CGA 192155, 6% CGA 339833 and 2% CGA 265378 were formed | Kirkpatrick, D., 1994e<br>(IUCLID 10.2.1-15) |
| Fludioxonil: aerobic testing according to various methods in 8 different soils. | $DT_{50}$: 143-482 days at 20°C<br>Geomean $DT_{50}$: 265 days (n=8) at 20°C | No major metabolites observed | Hawkins, D.R. et al., 1999b<br>(IUCLID 10.2.1-01)<br>Abildt, U., 1991<br>(IUCLID 10.2.1-02)<br>Ellgehausen, H., 1992a<br>(IUCLID 10.2.1-03)<br>Ellgehausen, H., 1992b<br>(IUCLID 10.2.1-04)<br>Minet, U., 1994a<br>(IUCLID 10.2.1-05)<br>Minet, U., 1994b<br>(IUCLID 10.2.1-06)<br>Minet, U., 1994c<br>(IUCLID 10.2.1-07) |

| Method | Results | Remarks | Reference |
|---|---|---|---|
| | | | Reischmann, F.J., 1994 (IUCLID 10.2.1-08) |
| CGA 192155:<br>Dutch registration guidelines, Jan 1987, section G 1, Section 6.2.C | $DT_{50}$: 16-24 days at 20°C<br>Worst case $DT_{50}$: 24 days at 20°C | No major metabolites observed | Ulbrich, R., 1998<br>(IUCLID 10.2.1-09) |
| CGA 339833:<br>Dutch registration guidelines, Jan 1987, section G 1, Section 6.2.C | $DT_{50}$: 9.3-16 days at 20°C<br>Worst case $DT_{50}$: 16 days at 20°C | No major metabolites observed | Ulbrich, R., 1999<br>(IUCLID 10.2.1-10) |
| Fludioxonil: anaerobic testing.<br>US EPA subdivision N, para. 162-1 and 162-2 | <1% AR as $CO_2$ after 392 days.<br>$DT_{50}$: > 392 days | No major metabolites observed. | Adam, D., 1998<br>(IUCLID 10.2.1-11) |

### 5.1.1 Stability

Hydrolysis

Investigation of the hydrolysis of fludioxonil at pH 5, 7 and 9 indicated no degradation over the 30 day test period. Hence, hydrolysis is not considered a relevant degradation pathway for fludioxonil. Hydrolysis was investigated for the major photoproduct CGA 339833. The results indicated that CGA 339833 has a $DT_{50}$ of 597 days at pH7 and 25°C. This indicates that hydrolysis will not be a significant degradation pathway for CGA 339833 at environmental temperatures.

Photolysis

Fludioxonil is quickly degraded by photolysis in sterile aqueous solutions. The first order half-life is equivalent to approximately 10 days natural sunlight at latitude of 30°N and approximately 9 days of natural sunlight at 40°N, assuming 12 hours sunlight. A number of major photo-products were identified (CGA 339833, CGA 344623, and A5) (Kirkpatrick, 1994a; Kirkpatrick, 1994b). In laboratory tests with thin layer soil plates, fludioxonil was degraded with a half-life of approximately 9.5 days at 25°C. Major photolysis products were identified (CGA 339833, CGA 192155, and CGA 265378) (Kirkpatrick, 1994d,f; Kirkpatrick 1994e). Consequently, photolysis has been identified as a major degradation pathway for fludioxonil in the environment.

### 5.1.2 Biodegradation

#### 5.1.2.1 Biodegradation estimation

As experimental data on biodegradation is available, an estimation of biodegradation is not considered to be necessary.

#### 5.1.2.2 Screening tests

Fludioxonil was 7% degraded after 29 days in a $CO_2$ evolution test (Baumann, 1993). The test was conducted using activated sewage sludge from a municipal sewage treatment plant as the inoculum. The inoculum was not adapted to the test substance. Fludioxonil was added to the inoculum at a concentration of *ca* 27 mg/L (*ca* 16 mg ThOC/l) and incubated at 22 ± 2°C for 29 days. $CO_2$ evolution was measured at intervals by passing air through the test vessels and through traps containing sodium hydroxide. Validation of the test system and viability of the inoculum was

confirmed using sodium benzoate (15 mg DOC/L) as a positive control. The test results conclude that fludioxonil is not classified as readily biodegradable.

#### 5.1.2.3 Simulation tests

Water/sediment

Fludioxonil was found to rapidly dissipate from the water phase in water/sediment studies ($DT_{50}$ = 1-2 days) due to adsorption to the sediment. Whole system degradation of fludioxonil is slow, with a first-order $DT_{50}$ of 451-699 days (855-1326 days at 12°C, Arrhenius equation). Mineralisation to $CO_2$ accounted for less than 2% AR (Gonzalez-Valero, 1992).

Soil

In aerobic degradation studies fludioxonil was found to be slightly degradable in soil with $CO_2$ (0.6-11.1% AR (pyrrole labelled) and 10.8-20.5% AR (phenyl-labelled) after 90 days at 20°C) and bound residues (2.4 - 18.0% AR (pyrrole-labelled) and 17.3 - 19.4% AR (phenyl-labelled) after 90 days at 20°C) observed as the principal degradates in all studies. Soil metabolites of fludioxonil were observed only in small amounts (total 0.3-8.4% AR). The resulting experimental $DT_{50}$ values for degradation of fludioxonil were in the range of 143 to 482 days (geometric mean 265 days, n=8, 20°C). When recalculated to 12°C using the Arrhenius equation the $DT_{50}$ is 502 days (Hawkins *et al*, 1999b; Abildt, 1991; Ellgehausen, 1992a; Ellgehausen, 1992b; Minet, 1994a; Minet, 1994b; Minet, 1994c; Reischmann, 1994).

For the photo degradation product CGA 192155, experimental aerobic $DT_{50}$ values ranged from 16-24 days in three soils at 20°C. When recalculated to 12°C using the Arrhenius equation the highest $DT_{50}$ is 46 days (Ulbrich, 1998).

For the photo degradation product CGA 339833 experimental $DT_{50}$ values ranged from 9.3-16 days in three soils at 20°C (Ulbrich, 1999). When recalculated to 12°C using the Arrhenius equation the highest $DT_{50}$ is 30 days.

In an anaerobic test with fludioxonil at 25°C degradation was found to be slow with a $DT_{50}$ of > 1 year with metabolites, including $CO_2$, forming less than 1% AR after 392 days. Consequently, fludioxonil is expected to degrade slowly in anaerobic soils.

### 5.1.3 Summary and discussion of degradation

Fludioxonil is not readily biodegradable and has been shown to degrade slowly in water/sediment studies with a whole system $DT_{50}$ of 451-699 days. Fludioxonil was also shown to degrade slowly in soil with $DT_{50}$ values ranging 143-482 days at 20°C (geometric mean of 265 days). Degradation of fludioxonil in soil under anaerobic conditions was slow with a $DT_{50}$ of >1 year.

Fludioxonil is hydrolytically stable. However, fludioxonil is subject to photolytic degradation in both water and soil. The first order half-life in water is equivalent to approximately 10 days natural sunlight at latitude of 30°N and approximately 9 days of natural sunlight at 40°N, assuming 12 hours sunlight.

The photoproducts of fludioxonil, CGA 192155 and CGA 339833, degraded relatively quickly in aerobic soils with $DT_{50}$ values of 16-24 days and 9.3-16 days respectively. In soil fludioxonil was photolytically degraded with a half-life of approximately 9.5 days at 25°C.

Based on the information above, fludioxonil is not considered to be rapidly degradable for the purposes of environmental classification according to guidance on Regulation (EC) 1272/2008.

## 5.2 Environmental distribution

### 5.2.1 Adsorption/Desorption

A batch equilibrium adsorption/desorption study conducted with 5 different soils indicated that fludioxonil has a low potential for mobility in soils. The normalized Koc values were between 12,000-385,000 L/kg with an arithmetic mean Koc of 145,000 L/kg (n=5). The pH of soils was not observed to influence sorption. Consequently, fludioxonil is not expected to be mobile in soils.

### 5.2.2 Volatilisation

Fludioxonil has a low vapour pressure of 3.9 $x10^{-7}$ Pa (at 25°C) and a low Henry's law constant of approximately 5.4 x $10^{-5}$ $m^3$ Pa $mol^{-1}$. Therefore, volatilisation from soil or water is not expected to be a significant entry route into air for fludioxonil.

### 5.2.3 Distribution modelling

The main route of environmental exposure for the envisaged usage is via the sewage treatment plant (STP) resulting from down the drain emissions of fludioxonil. Following emission to the STP, freshwater and freshwater sediment compartments may be exposed to fludioxonil. In addition to this it is likely that soil will be exposed due to spreading of STP sludge to soil. Fludioxonil is of low mobility in soils and as such exposure of groundwater is unlikely. No significant emissions to air are expected due to the low vapour pressure and Henry's law constant of fludioxonil.

## 5.3 Aquatic Bioaccumulation

Fludioxonil has a log Kow of 4.12, which indicates that there may be potential for bioaccumulation. However, as an experimental whole-body BCF value derived with bluegill sunfish (*Lepomis macrochirus*) is relatively low and as depuration following transfer to water was rapid, it is considered that fludioxonil has limited potential for bioaccumulation (Gonzalez-Valero, 1994).

**Table 22: Summary of relevant information on aquatic bioaccumulation**

| Method | Results | Remarks | Reference |
|---|---|---|---|
| US EPA subdivision N, para. 165-4, following GLP, 2 | Measured BCF: 366 L/kg wet fish<br>Uptake rate constant ($K_1$): 83<br>Depur. Rate constant ($K_2$): 0.227<br>Depur. Time ($DT_{90}$) <2 days | Relatively low whole-body BCF and rapid depuration indicate low potential for bioaccumulation. | Gonzales-Valero, J.F., 1994<br>(IUCLID 9.1.7-02) |

### 5.3.1 Aquatic bioaccumulation

Based on an experimental BCF for fish of 366 L/kg, it is considered that fludioxonil has a low potential for bioaccumulation.

#### 5.3.1.1 Bioaccumulation estimation

Estimation of bioaccumulation has not been performed as experimental data are available.

#### 5.3.1.2 Measured bioaccumulation data

Investigation of bioaccumulation with bluegill sunfish indicates that fludioxonil is rapidly concentrated in fish tissues, reaching 95% of steady-state within two weeks. At steady-state the bioconcentration factor was 58 L/kg for the edible portions of the fish and 741 L/kg for the non-edible portions of the fish. The whole fish bioconcentration factor was 366 L/kg. Depuration was rapid ($DT_{90}$ <2 days) following transfer of fish to fresh water.

Consequently, fludioxonil has limited potential for bioconcentration and is unlikely to bioaccumulate in the environment.

### 5.3.2 Summary and discussion of aquatic bioaccumulation

Estimation methods have not been considered for bioaccumulation as experimental data is available. Experimental data indicates that fludioxonil is not likely to bioaccumulate.

## 5.4 Aquatic toxicity

**Table 23: Summary of relevant information on aquatic toxicity**

| Method | Results | Remarks | Reference |
|---|---|---|---|
| **Acute toxicity** | | | |
| **Fish** | | | |
| *Oncorhynchus mykiss*<br>US EPA 72-1<br>GLP | 96 h $LC_{50}$ = 0.47 mg/L | Flow-through, mean measured exposure concentrations. | Holmes CM & Swigert JP, 1993a (IUCLID 9.1.1-01) |
| *Oncorhynchus mykiss*<br>US EPA 72-1<br>GLP | 96 h $LC_{50}$ = 0.23 mg/L | Flow-through, mean measured exposure concentrations. | Biever, R.C., 1997a (IUCLID 9.1.1-02) |
| *Lepomis macrochirus*<br>US EPA 72-1<br>GLP | 96 h $LC_{50}$ = 0.74 mg./L | Flow-through, mean measured exposure concentrations. | Biever, R.C., 1997b (IUCLID 9.1.1-03) |
| *Cyprinodon variegatus*<br>US EPA 72-1<br>GLP | 96 h $LC_{50}$ = 1.2 mg/L | Marine<br>Flow-through, mean measured exposure concentrations. | Holmes CM & Swigert JP, 1993b (IUCLID 9.1.1-04) |
| **Invertebrates** | | | |
| *Daphnia magna*<br>US EPA 72-2<br>GLP | 48 h $EC_{50}$ = 0.40 mg/L | Flow-through, mean measured exposure concentrations. | Surprenant, D.C., 1990 (IUCLID 9.1.2-01) |
| *Daphnia magna*<br>US EPA 72-2<br>GLP | 48 h $EC_{50}$ = 0.90 mg/L | Flow-through, mean measured exposure concentrations. | Holmes CM & Swigert JP, 1993b (IUCLID 9.1.2-02) |
| **Algae** | | | |

| Method | Results | Remarks | Reference |
|---|---|---|---|
| *Desmodesmus subspicatus*[1]<br>OECD 201<br>GLP | 48h $ErC_{50}$ >0.926 mg/L<br>48h $ErC_{10}$ = 0.09 mg/L | Static, geometric mean measured concentrations. Endpoint is calculated from the area under the curve. | Rufli H, 1989a<br>(IUCLID 9.1.3-01) |
| *Pseudokirchneriella subcapitata*[2]<br>FIFRA 122-2 & 123-2<br>GLP | 48h $ErC_{50}$ = 0.21 mg/L<br>48h NOEC = 0.027 mg/L | Static, geometric mean measured concentrations. Endpoint is calculated from growth rate | Hoberg, J.R., 1992, 2005<br>(IUCLID 9.1.3-02) |
| **Chronic toxicity** | | | |
| **Fish** | | | |
| *Oncorhynchus mykiss*<br>OECD 204<br>GLP | 21 day NOEC (mortality) = 0.014 mg/L* | Flow-through, arithmetic mean measured concentrations | Grade R. (1993a)<br>(IUCLD 9.1.6.1-01 |
| *Oncorhynchus mykiss*<br>OECD 204<br>GLP | 21 day NOEC (mortality) = 0.011 mg/L* | Flow-through, arithmetic mean measured concentrations | Grade R. (1993b)<br>(IUCLD 9.1.6.1-02 |
| *Oncorhynchus mykiss*<br>OECD 204<br>GLP | 21 day NOEC (mortality) >0.01 mg/L* | Flow-through, nominal concentrations | Grade R. (1993c)<br>(IUCLD 9.1.6.1-03 |
| *Oncorhynchus mykiss*<br>OECD 215<br>GLP | 28 day NOEC (growth rate) = 0.04 mg/L | Flow-through, arithmetic mean measured concentrations | Maynard , S. J. (2005)<br>(IUCLD 9.1.6.1-05 |
| *Pimephales promelas*<br>US EPA FIRFA 540/9-82-024, US EPA-OPP 540/9-86-138, ASTM 1241-88 (OECD 210)<br>GLP | 28 day early life stage NOEC = 0.039 mg/L | Flow-through, mean measured exposure concentrations. | Graves, C.W. *et al*., 1994<br>(IUCLID 9.1.6.1-05) |
| **Invertebrates** | | | |
| *Daphnia magna*<br>US EPA 5401-85-024, OECD 211<br>GLP | 21 day reproduction NOEC = 0.019 mg/*L* | Flow-through, mean measured exposure concentrations. | Putt, A.E., 1991<br>(IUCLID 9.1.6.2-01) |
| *Daphnia magna*<br>OECD 202 (Part II)<br>GLP | 21 day reproduction NOEC = 0.005 mg/L* | Semi-static, mean measured exposure concentrations. | Rufli, H., 1989c<br>(IUCLID 9.1.6.2-02) |
| *Daphnia magna*<br>OECD 211<br>GLP | 21 day reproduction NOEC = 0.035 mg/L | Semi-static, mean measured exposure concentrations | Fournier, A.E., 2014<br>(IUCLID 9.1.6.2-03) |

[1] *formerly known as Scenedesmus subspicatus*

[2] *formerly known as Selenastrum capricornutum*

**study result not considered to be reliable and therefore not used in the overall assessment (see annotations in IUCLID)*

### 5.4.1 Fish

#### 5.4.1.1 Short-term toxicity to fish

The acute toxicity of fludioxonil to fish was investigated in laboratory tests conducted under flow-through conditions with two freshwater (*O. mykiss* and *L. macrochirus*) and one marine fish species (*C. variegatus*). The 96-hour $LC_{50}$ values were found to be in a range of 0.23-1.2 mg/L. The lowest endpoint submitted was the $LC_{50}$ value of 0.23 mg/L (95% confidence interval: 0.18-0.33 mg/L) provided by an acute toxicity test with *O. mykiss* (Biever, 1997a (IUCLID 9.1.1-02)).

#### 5.4.1.2 Long-term toxicity to fish

A NOEC of 0.04 mg/L is provided by a 28-day OECD 215 study of the effects of fludioxonil on the growth of *O. mykiss* (Maynard, 2005 (IUCLID 9.1.6.1-04)). In a 28-day early life stage (ELS) toxicity test with *P. promelas*, mean length and weight and survival at 28 days post-hatch were the most sensitive biological parameters. Based on these indicators in the ELS study, the overall chronic NOEC for fish was determined to be 0.039 mg/L (Graves *et al*, 1994 (IUCLID 9.1.6.1-05)). A series of earlier studies (Grade, 1993a, b, c; IUCLID 9.1.6.1-01, -02, -03) provide lower endpoints for long-term toxicity to fish (*O. mykiss*). These studies were conducted in a GLP-certified laboratory according to an appropriate OECD guideline; however difficulty with the formulation of the stock solutions (flocculation of the test substance) was experienced and therefore difficulty dosing into the tanks was experienced. The highest concentrations used in these studies are therefore uncertain. During the EU registration process of fludioxonil as a plant protection product under Directive 91/414/EEC, these studies were noted to be outdated and were also considered to contain significant technical shortcomings (notably the failure to maintain correct dosing during the test period). These deficiencies led to the conduct of a juvenile growth test with rainbow trout according to the most recent OECD Guideline 215 (Maynard 2005). This new study therefore supersedes the earlier studies (OECD 204) with rainbow trout (Grade 1993 a-c).

### 5.4.2 Aquatic invertebrates

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

The acute toxicity of fludioxonil to aquatic invertebrates was investigated in two laboratory tests conducted under flow-through conditions with the freshwater crustacean species *Daphnia magna*. The 48-hour $EC_{50}$ values for *D. magna* were found to be in a range of 0.40 – 0.90 mg/L. The lowest $EC_{50}$ endpoint for *D. magna* was 0.40 mg/L (Surprenant, 1990 (IUCLID 9.1.2-01)).

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

Three 21-day chronic toxicity studies were conducted with *Daphnia magna*. In a study by Putt (1991 (IUCLID 9.1.6.2-01)), the most sensitive biological parameters indicating effects of fludioxonil were the number of offspring/female and the mean adult body length. The overall NOEC of this study was established to be 0.019 mg/L. In a second test, the fraction of dead young and the time for appearance of first brood were the most sensitive biological parameters. The overall NOEC of this test was determined to be 0.005 mg/L (Rufli, 1989c (IUCLID 9.1.6.2-02)).

Although it complied with the guideline requirement current at the time it was performed, the Rufli (1989c) study failed to meet the reproductive performance criteria for studies of long-term toxicity to *Daphnia magna* according to the current state-of-the-art as embodied in OECD Guideline 211 (1998 *et seq*.). Hence the untreated control organisms each produced an average of only 55 live juveniles, whereas the current validity requirement is for at least 60, and even this level is normally easily exceeded. It is also notable that adult mortalities were recorded in the latter days of the study, with the numbers of immobilised parent animals unrelated to dose. Taken together, these findings are indicative of sub-optimal test conditions that limited reproductive output as well as survival. The presence of the solvent system comprising acetone and an unidentified alkylphenol-based surfactant reduced juvenile production still further. The NOEC of 0.005 mg fludioxonil/L obtained in this study is considered to have been influenced by a combination of stress factors in addition to the presence of the test substance and **for that reason is considered to be invalid**. In the newest study by Fournier (2014 (IUCLID 9.1.6.2-03)) performed according to OECD 211, an overall NOEC of 0.035 mg/L (mean measured concentration) was found for reproduction, mortality and growth. This study is given a reliability factor of 1 as it is fully compliant with and fulfils all the validity criteria given in the OECD 211 guideline and, moreover, measured concentrations are quite stable during the test period whereas there is a high variation in the measured concentrations of the two other tests.

### 5.4.3 Algae and aquatic plants

The effects of fludioxonil on the growth of primary producers were determined under static conditions in two species of green algae: *Pseudokirchneriella subcapitata* (formerly known as *Selenastrum capricornutum*) and *Desmodesmus subspicatus* (formerly *Scenedesmus subspicatus*). *P. subcapitata* was the more sensitive of the two species and presented the lower $E_rC_{50}$ value of 0.21 mg/L and a NOEC of 0.027 mg/L after 48 hours of exposure (Hoberg, 1992 (IUCLID 9.1.3-02)).

The study in *Desmodesmus subspicatus* yielded a 48h $ErC_{50}$ of >0.926 mg/L and a 48h $ErC_{10}$ of 0.09 mg/L Results for algae were all based on 48-hour exposure and geomean measured concentrations due to excessive pH variation at 72 and 120 hours of exposure.

### 5.4.4 Other aquatic organisms (including sediment)

A test of the toxicity of fludioxonil spiked into artificial sediment to the sediment-dwelling larval stages of *Chironomus riparius* provided a NOEC of 40 mg/kg dry sediment (8.70 mg/kg wet sediment), based on the low survival of emaciated emerged midges at higher concentrations (Grade, 1998 (IUCLID 9.1.9-01)). Emergence and larval development rates were unaffected relative to the control at concentrations up to and including 160 mg a.s./kg dry sediment.

### 5.4.5 Higher tier studies on aquatic organisms

An outdoor aquatic microcosm study (Giddings, 1993; IUCLID 9.1.8-01) was conducted to study the environmental fate and ecological effects of fludioxonil on aquatic organisms following five repeated applications at 14-day intervals. Nominal concentrations were: 0 (control), 3.0, 8.2, 16.4, 32.8 µg/L.

Periphyton abundance and taxonomic richness were significantly reduced at all treatment levels on one occasion near the end of the treatment period. Periphyton data were highly variable, and the effects did not appear on later sampling events. Chrysophytes and diatoms in the phytoplankton

were affected by fludioxonil at the highest single treatment level of nominally 32.8 µg/L. Green and blue green algae were not reduced (blue-green algae may have increased at the highest treatment level). Among the zooplankton, only the rotifer *Keratella* showed consistent effects of treatment with fludioxonil at the highest single treatment level of nominally 32.8 µg/L. Macro invertebrates may have been reduced at the highest treatment level near the end of the study, but there were few statistically significant differences in macroinvertebrate abundance. Bluegill sunfish survival and growth were unaffected by fludioxonil treatment.

Overall, the impact of fludioxonil on the microcosm communities was minor, and occurred mainly at the lowest trophic level (phytoplankton and periphyton). Secondary effects may have occurred on the rotifer *Keratella*. Densities of benthic macroinvertebrates might have been reduced near the end of the study at the highest single treatment level of nominally 32.8 µg/L. Fish were unaffected.

The study is found to meet the reliability criteria according to paragraph 3.1.2 in the draft Guidance Document for the use of aquatic model ecosystem studies for biocides (2013). However, only few effects were seen during the study, possibly due to rapid degradation of fludioxonil by photolytic decomposition while the sunlight penetrated to the bottom of the tanks during most of the study. he mesocosm study address effects on a broad range of species. Based on laboratory effect data, algae (*P. subcapitata*) and fish (*O. mykiss*) seems to be the most sensitive species regarding short-term effects, and crustaceans (*Daphnia magna*) seem to be the most sensitive species regarding chronic effects. Both groups of sensitive organisms were represented in the mesocosm study. The study was found to be acceptable (reliability factor 2), but does not influence the classification of fludioxonil; it is mentioned here for completeness.

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

The criteria for assessing environmental hazards state the following:

In deciding whether a substance should be classified, a search of appropriate databases and other sources of data should be made for at least the following substance properties: water solubility; octanol/water partition coefficient (log $K_{OW}$); bioaccumulation; bioconcentration factor in fish (BCF); acute aquatic toxicity (L(E)$C_{50}$s); chronic aquatic toxicity (NOECs or equivalent L(E)$C_X$s: e.g. $EC_{10}$); and degradation (evidence of rapid degradability, hydrolysis).

Although not used directly in the criteria, the water solubility and stability data are important since they are a valuable help in the data interpretation of the other properties. However, water solubility may be difficult to determine and is frequently recorded as simply being low, insoluble or less than the detection limit. This may create problems in interpreting aquatic toxicity and bioaccumulation studies. Hydrolysis data (according to Test Methods Regulation (EC) No 440/2008; OECD Test guideline 111) and information on the hydrolysis products as well as their behaviour in water might be helpful as well. As an example, for substances where the dissipation half-life ($DT_{50}$) is less than 12 hours, environmental effects are likely to be attributed to the hydrolysis products rather than to the parent substance itself. Preferably data shall be derived using the standardised test methods referred to in Article 8 (3). In practice data from other standardised test methods such as national methods shall also be used where they are considered as equivalent. Where valid data are available from non-standard testing and from non-testing methods, these shall be considered in classification provided they fulfil the requirements specified in section 1 of Annex XI to Regulation (EC) No 1907/2006. In general, both freshwater and marine species toxicity data are considered suitable for use in classification provided the test methods used are equivalent. Where such data are not available classification shall be based on the best available data.

Assignment of aquatic hazard classification categories

The assessment criteria were applied in the evaluation of available environmental toxicity data for fludioxonil, by considering the key acute and chronic endpoints contained according to the scheme presented below. **Based on the information presented at Point 5.1.3, fludioxonil is considered to be not rapidly degradable for the purposes of environmental classification according to guidance on Regulation (EC) 1272/2008.**

**Aquatic hazard classification categories applicable to fludioxonil**

| **Annex I: Table 4.1.0 Classification categories for hazardous to the aquatic environment** | |
|---|---|
| **(a) Acute (short-term) aquatic hazard** | |
| **Acute Category 1** (*Note 1*)<br>96 hr $LC_{50}$ (for fish)<br>48 hr $EC_{50}$ (for Crustacea)<br>72 or 96 hr $E_rC_{50}$ (for algae or other aquatic plants) | <br>≤ 1 mg/L and/or<br>≤ 1 mg/L and/or<br>≤ 1 mg/L (*Note 2*) |
| **(b) Chronic (long term) aquatic hazard**<br>**(i) Non-rapidly degradable substances (*Note 3*) for which there are adequate chronic toxicity data available** | |
| **Chronic Category 1**<br>Chronic NOEC or $EC_x$ (for fish)<br>Chronic NOEC or $EC_x$ (for Crustacea)<br>Chronic NOEC or $EC_x$ (for algae or other aquatic plants) | <br>≤ 0.1 mg/L and/or<br>≤ 0.1 mg/L and/or<br>≤ 0.1 mg/L |

*Note 1 When classifying substances as Acute Category 1 and/or Chronic Category 1 it is necessary at the same time to indicate the appropriate M-factor(s).*

*Note 2 Classification shall be based on the $E_rC_{50}$ [= $EC_{50}$ (growth rate)]. In circumstances where the basis of the $EC_{50}$ is not specified or no $ErC_{50}$ is recorded, classification shall be based on the lowest $EC_{50}$ available.*

*Note 3 When no useful data on degradability are available, either experimentally determined or estimated data, the substance should be regarded as not rapidly degradable.*

Further possible outcomes are available for substances that are rapidly degradable or substances for which no reliable chronic toxicity endpoints are available. Since these categories are not relevant to fludioxonil, they have not been presented or considered further.

Derivation of the M-factor for highly toxic substances

Substances with acute toxicities below 1 mg/L or chronic toxicities below 0.1 mg/L (if non-rapidly degradable) and 0.01 mg/L (if rapidly degradable) contribute as components of a mixture to the toxicity of the mixture even at a low concentration, and shall normally be given increased weight in applying the summation of classification approach.

The relevant outcomes for fludioxonil are identified in the table below, based on the lowest reliable short term $L/EC_{50}$ and chronic NOEC values given in Section 5.6.

**Multiplying (M) factors for highly toxic components of mixtures**

| Acute toxicity | M factor | Chronic toxicity | M factor | |
|---|---|---|---|---|
| **L/$EC_{50}$ value** | | **NOEC value** | **NRD** | **RD** |
| 0.1 < $L(EC)_{50}$ ≤ 1 | 1 | 0.01 < NOEC ≤ 0.1 | 1 | - |
| 0.01 < $L(EC)_{50}$ ≤ 0.1 | 10 | 0.001 < NOEC ≤ 0.01 | 10 | 1 |
| 0.001 < $L(EC)_{50}$ ≤ 0.01 | 100 | 0.0001 < NOEC ≤ 0.001 | 100 | 10 |
| 0.0001 < $L(EC)_{50}$ ≤ 0.001 | 1000 | 0.00001 < NOEC ≤ 0.0001 | 1000 | 100 |
| 0.00001 < $L(EC)_{50}$ ≤ 0.0001 | 10000 | 0.000001 < NOEC ≤ 0.0001 | 10000 | 1000 |
| (continue in factor 10 intervals) | | (continue in factor 10 intervals) | | |

*NRD: Not rapidly degradable*

*RD: Rapidly degradable*

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

The lowest reliable short term L/$EC_{50}$ endpoints for the three main trophic groups of aquatic organisms (fish, invertebrates and algae) are all < 1 mg/L and > 0.1 mg/L. The lowest reliable L/EC50 endpoints for algae is 0.21 mg/L, for invertebrates is 0.40 mg/L and fish is 0.23 mg/L. **Classification in Acute Category 1 therefore applies to fludioxonil with an M-factor of 1.**

Fludioxonil is not rapidly degradable and adequate chronic toxicity endpoints are available.

The lowest reliable chronic NOEC endpoints for fish (0.039 mg/L), algae (0.027 mg/L) and invertebrates (0.019 mg/L) are all < 0.1 mg fludioxonil/L. **Therefore, according to the criteria outlined above, classification as Chronic Category 1 applies to fludioxonil**. Due to the NOEC from the test with Daphnia magna (0.019 mg/L) and the fact that fludioxonil is not rapidly degradable **an M-factor of 1 is applied.**

Based on these findings, fludioxonil should be classified in the following categories:

Aquatic environmental hazard acute category 1, H400.
Aquatic environmental hazard chronic category 1, H410.

For labelling purposes, H400 is subsumed by H410 and the environmental hazard classification shall be indicated by H410 alone.

The following Multiplying Factors apply to fludioxonil when it is present as a component of a mixture:

Acute toxicity M factor: 1.
Chronic toxicity M factor: 1.

## 6 OTHER INFORMATION

No information available

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# 8 ANNEXES

The following human toxicology studies were not submitted for the approval of fludioxonil under Regulation EC 528/2012. The studies were however submitted for the approval of fludioxonil under Regulation EC 1107/2009. Study summaries are available in Volume 3, Annex B, B.6, Part 1 and Part 2 of the non-confidential DAR (February 2006). For completeness, the results from these studies are included in Section 4 of this CLH report.

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## Historical control data (HCD data):

**Syngenta Position on Historical Control Data:**

It is widely accepted by the scientific community that historical controls are only of value when the data can be comparable. In this respect, information about strain and source is essential. According to the OECD Guidance Document 116, historical control data submitted for consideration should be taken from the same laboratory, utilizing the same strain, age and sex of animals obtained from the same supplier, and only include those studies ideally conducted within a five years span on either side of the study under review. (OECD Guidance Document 116, April 2010). Although the OECD Guidance Document 116 was issued in 2010, it can be confirmed that the basic requirements of historical control data was already established and widely accepted in early 1980's (Haseman, Toxicologic Pathology, 12 126-135, 1984). In fact, the OECD guidelines for chronic toxicity/carcinogenicity (OECD 453) and for teratogenicity (OECD 414) studies from 1981 the following recommendations were given:

*"The incidence of lesions normally occurring in the strain of animals used (under the same laboratory conditions, i.e. historical control) is indispensable for correctly assessing the significance of changes observed in exposed animals" (OECD guideline 453, May 1981).*

*"The findings of a teratogenicity study should be evaluated in terms of the observed effects and the dose levels producing effects. It is necessary to consider the historical teratogenicity data on the species/strain tested" (OECD guideline 414, May 1981).*

Those older versions from the OECD guidelines clearly state that the same strain of animals had to be used for the historical control data.

In addition to that, normally a testing laboratory has got one preferred strain in use for a given species for each type of toxicology study and therefore the HCD will be built on the same strain.

Unfortunately, it is not possible to get detail information about historical control data for toxicology studies with fludioxonil which were conducted during late 1980's and early 1990's. Syngenta, however, believes the data quoted as historical control data in the studies can be qualified.

**Study 8.9.1.1-01**

**Fankhauser H (1990)**

**28-Day oral cumulative toxicity study in rats (gavage) - CGA 173506 tech (Fludioxonil)**

**Ciba-Geigy Experimental Toxicology, Stein, Switzerland. Report #881492**

The study was performed in Tif:RAIf (SPF) rats, sourced in-house. In-life dates August-October 1989.

Historical control data are provided for serum cholesterol concentration and ketonuria (below). Rat strain and date ranges are acceptable.

- Danish EPA request conformation that historical data are for the performing laboratory (i.e. Ciba-Geigy, Stein).

Historical controls: Cholesterol levels in female Tif:RAIf (SPF) rats

| | Females | | | |
|---|---|---|---|---|
| | **N** | **Median** | **5%** | **95%** |
| Chol [mmol/L], age 14-24 weeks (1984/85) | 99 | 1.720 | 1.070 | 2.750 |
| Chol [mmol/L], age 14-24 weeks (1985/86) | 80 | 1.720 | 1.150 | 2.570 |
| Chol [mmol/L], age 16-26 weeks (1991-93) | 258 | 2.105 | 1.550 | 2.940 |
| Chol [mmol/L], age 9-12 weeks (1988-92) | 89 | 2.07 | 1.29 (min) | 3.02 (max) |

*N = number of animals*

Historical controls: Ketonuria in Tif:RAIf (SPF) rats (1988-1993)

| | Males | | | | Females | | | |
|---|---|---|---|---|---|---|---|---|
| | **n** | **Incidence (%)** | | | **n** | **Incidence (%)** | | |
| | | **total** | **min** | **max** | | **total** | **min** | **max** |
| Ketonuria, (age 9-11 weeks) | 11 | 53/75 (71) | 1/5 (20) | 10/10 (100) | 11 | 37/74 (50) | 0/5 (0) | 10/10 (100) |

*n = number of studies*

**Answer from Syngenta:**

**Tif:RAIf is an in-house strain of Ciba-Geigy, Stein, Switzerland. As long as Tif:RAIf rats were used for the studies quoted as historical control data (HCD), the study laboratory was Ciba-Geigy, Stein.**

**Study 8.9.1.1-05**

**Chang CF & Wynand DS (1993a)**

**18-Month dietary oncogenicity study with CGA-173506 in mice**

**Ciba-Geigy Environmental Health Centre, Farmington, CT, USA**

**Report F-00019**

**Study 8.9.1.1-06**

**Chang CF & Wynand DS (1993b)**

**18-Month dietary oncogenicity study with CGA-173506 in mice**

**Ciba-Geigy Environmental Health Centre, Farmington, CT, USA**

**Report F-00071**

Studies were performed at Ciba-Geigy EHC in August 1989-March 1991 (F-00019) or March 1990-October 1991 (F-00071) using CD-1 mice sourced from Charles River (Raleigh, NC, USA).

The study report for F-00019 includes tabulated results for the incidences of lymphoma in this study and the additional mouse carcinogenicity study performed by the same authors (F-00071), with reference to historical control data (studies A-F). No further information is provided.

- Danish EPA has requested information on the date range of the historical studies and confirmation that the referenced studies were performed in the Ciba-Geigy Laboratory using the same source of mice (Charles River, USA).

**Answer from Syngenta:**

**Syngenta checked the raw data of the studies. However, we could not identify which study data were quoted as HCD in the study.**

**CD-1® is a trademark of Swiss albino mouse (ICR) of Charles River. Thus, at least, we believe that the mouse strains were consistent in all HCD studies supplied from Charles River USA.**

| | Incidence of lymphoma | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| **ppm** | **0 (1st)** | **0 (2nd)** | **3** | **10** | **30** | **100** | **1000** | **3000** | **5000** | **7000** |
| **Females** | | | | | | | | | | |
| un. deaths 0-12 m | 3/7 | 1/4 | 0/4 | 0/4 | 0/4 | 0/0 | 0/4 | 3/6 | 0/7 | 2/9 |
| 12 m sacrifice | 0/7 | 0/9 | 1/9 | 0/9 | 2/9 | 0/10 | 2/10 | 0/8 | 0/5 | 1/9 |
| un. deaths 13-18 m | 1/6 | 3/12 | 2/6 | 0/7 | 4/10 | 2/4 | 4/9 | 3/9 | 0/14 | 2/31 |
| 18 m sacrifice | 7/40 | 7/35 | 4/41 | 10/40 | 6/37 | 11/46 | 6/37 | 12/37 | 11/34 | 3/11 |
| total | 11/60 | 11/60 | 7/60 | 10/60 | 12/60 | 13/60 | 12/60 | 18/60 | 11/60 | 8/60 |
| Incidence [%] | 18 | 18 | 12 | 17 | 20 | 22 | 20 | 30 | 18 | 13 |
| **Males** | | | | | | | | | | |
| un. deaths 0-12 m | 0/3 | 0/1 | 0/5 | 0/2 | 1/8 | 1/3 | 1/3 | 0/0 | 1/3 | 0/9 |
| 12 m sacrifice | 0/9 | 0/10 | 0/8 | 0/10 | 0/7 | 0/9 | 0/10 | 0/10 | 1/10 | 0/9 |
| un. deaths 13-18 m | 0/5 | 2/12 | 0/9 | 1/11 | 1/13 | 1/6 | 2/10 | 2/11 | 1/10 | 0/28 |
| 18 m sacrifice | 2/43 | 1/37 | 1/38 | 0/37 | 0/32 | 0/42 | 4/37 | 0/39 | 1/37 | 0/14 |
| total | 2/60 | 3/60 | 1/60 | 1/60 | 2/60 | 2/60 | 7/60 | 2/60 | 4/60 | 0/60 |

**Historical controls: incidence of thymus hyperplasia and malignant lymphoma# in female CD-1 mice**

| Studies | A | B | C | D | E | F | **Total** | **% (min-max)** |
|---|---|---|---|---|---|---|---|---|
| Thymus hyperpl. | 8 | 6 | 6 | 13 | 12 | - | | |
| Malign. lymphoma | 4 | 2 | 4 | 3 | 4 | 11 | | |
| combined | 12/50 | 8/60 | 10/60 | 16/50 | 16/50 | 11/60 | 73/330 | **22 (13-32)** |
| % | 24 | 13 | 17 | 32 | 32 | 18 | | |

# The incidence of malignant lymphoma and thymus hyperplasia were combined as thymus hyperplasia is often indistinguishable from thymus lymphoma.

un. = unscheduled, m = months, hyperpl. = hyperplasia, malign. = malignant

**Appendix 10.16 Historical Control Data - Lymphoma in Female CD-1 Mice**

**Environmental Health Center**

| Study | Week | Finding | Incidence | Combined* | % |
|---|---|---|---|---|---|
| A | 78 | Thymus Hyperplasia | 8 | 12/50 | 24 |
| | | Malignant Lymphoma | 4 | | |
| B | 78 | Thymus Hyperplasia | 6 | 8/60 | 13.3 |
| | | Malignant Lymphoma | 2 | | |
| C | 78 | Thymus Hyperplasia | 6 | 10/60 | 16.7 |
| | | Malignant Lymphoma | 4 | | |
| D | 78 | Thymus Hyperplasia | 13 | 16/50 | 32 |
| | | Malignant Lymphoma | 3 | | |
| E | 78 | Thymus Hyperplasia | 12 | 16/50 | 32 |
| | | Malignant Lymphoma | 4 | | |
| F | 78 | Malignant Lymphoma | 11 | 11/60 | 18.3 |

* The incidences of malignant lymphoma and thymus hyperplasia were combined for consideration as thymus hyperplasia often is indistinguishable from thymus lymphoma.

**Study 8.9.1.1-07**

**Chang JC & Richter AG (1993)**

**First addendum to the final report: 2-Year chronic toxicity/oncogenicity study with CGA-173506 in rats**

**Ciba-Geigy Environmental Health Centre, Farmington, CT, USA**

**Report F-00018.**

The study report includes reference to historical control data for liver tumour incidences in the rat (Studies A-G). The study was performed using Sprague-Dawley Crl: CD(SD)BR rats sourced from Charles River (Raleigh, NC, USA): in-life phase September 1989-September 1991.

- Danish EPA has requested information on the date range of the historical studies and confirmation that the referenced studies were performed in the Ciba-Geigy Laboratory using the same source of rat (Charles River, USA).

**Answer from Syngenta:**

**Syngenta checked the raw data of the study. However, we could not identify which study data were quoted as HCD in the study.**

**Syngenta may assume that HCD were generated in Ciba-Geigy EHC with the same source of rats (Charles River, USA).**

CIBA-GEIGY CORPORATION -2322-

F-00018: 2-YEAR CHRONIC TOXICITY/ONCOGENICITY STUDY WITH CGA-173506 IN RATS

TABLE 10.15.9 HEPATOCELLULAR TUMOR INCIDENCES IN HISTORICAL CONTROL STUDIES (CONT'D.)

HISTORICAL CONTROL LIVER TUMOR INCIDENCE IN INDIVIDUAL EHC STUDIES

**MALES**

| STUDY | ADENOMA | CARCINOMA | ADENOMA + CARCINOMA |
|---|---|---|---|
| A | 1/60 (1.7%) | 0/60 (0%) | 1/60 (1.7%) |
| B | 3/60 (5%) | 0/60 (0%) | 3/60 (5%) |
| C | 0/60 (0%) | 3/60 (5%) | 3/60 (5%) |
| D | 8/60 (13.3%) | 1/60 (1.7%) | 9/60 (15%) |
| E | 1/70 (1.4%) | 0/70 (0%) | 1/70 (1.4%) |
| F | 0/70 (0%) | 1/70 (1.5%) | 1/70 (1.4%) |
| G | 2/60 (3.3%) | 0/60 (0%) | 2/60 (3.3%) |

**FEMALES**

| STUDY | ADENOMA | CARCINOMA | ADENOMA + CARCINOMA |
|---|---|---|---|
| A | 3/60 (5%) | 1/60 (1.7%) | 4/60 (6.7%) |
| B | 6/60 (10%) | 0/60 (0%) | 6/60 (10%) |
| C | 0/60 (0%) | 0/60 (0%) | 0/60 (0%) |
| D | 2/60 (3.3%) | 0/60 (0%) | 2/60 (3.3%) |
| E | 0/70 (0%) | 1/70 (1.4%) | 1/70 (1.4%) |
| F | 1/70 (1.4%) | 0/70 (0%) | 1/70 (1.4%) |
| G | 1/60 (1.7%) | 0/60 (0%) | 1/60 (1.7%) |

**Study 8.10-1-01**

**Savary MH (1989)**

**Assessment of possible embryotoxic or teratogenic effects in rats by oral route.**

**CIT, France**

**Report No. 8811777 / CIT Study #4517 RSR**

Reference is made in the study report to the historical control range for the incidence of dilated ureter and dilated renal pelvis in examined foetuses (0.6-7.5%). No further details are reported.

The study was performed in Sprague-Dawley rats sourced from Charles River (France), in October 1988.

- Danish EPA has requested full information on the date range of the historical studies and confirmation that the referenced studies were performed in the CIT using the same source of rat (Charles River, France).

**Answer from Syngenta:**

**The raw data of the study were not returned to Syngenta and a company who received the raw data already destroyed it. Syngenta contacted CiTox Lab (former laboratory name, CIT), France, to identify the HCD studies and the information requested. However, CiTox Lab does not keep the any data for this study. Therefore, it is not possible to get any information about the studies which were referred to in this report.**

**CiTox Lab may assume that HCD were generated in the CiTox Lab with the same source of rats (Charles River, France).**