Committee for Risk Assessment

RAC

Opinion

proposing harmonised classification and labelling
at EU level of

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

EC Number: -
CAS Number: 131341-86-1

CLH-O-0000001412-86-162/F

Adopted

9 June 2017

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9 June 2017

CLH-O-0000001412-86-162/F

OPINION OF THE COMMITTEE FOR RISK ASSESSMENT ON A DOSSIER PROPOSING HARMONISED CLASSIFICATION AND LABELLING AT EU LEVEL

In accordance with Article 37 (4) of Regulation (EC) No 1272/2008, the Classification, Labelling and Packaging (CLP) Regulation, the Committee for Risk Assessment (RAC) has adopted an opinion on the proposal for harmonised classification and labelling (CLH) of:

Chemical name: **fludioxonil (ISO); 4-(2,2-difluoro-1,3-benzodioxol-4-yl)-1H-pyrrole-3-carbonitrile**

EC Number: **-**

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

The proposal was submitted by **Denmark** and received by RAC on **20 June 2016.**

In this opinion, all classification and labelling elements are given in accordance with the CLP Regulation.

PROCESS FOR ADOPTION OF THE OPINION

Denmark has submitted a CLH dossier containing a proposal together with the justification and background information documented in a CLH report. The CLH report was made publicly available in accordance with the requirements of the CLP Regulation at ***http://echa.europa.eu/harmonised-classification-and-labelling-consultation/*** on **19 July 2016**. Concerned parties and Member State Competent Authorities (MSCA) were invited to submit comments and contributions by **2 September 2016**.

ADOPTION OF THE OPINION OF RAC

Rapporteur, appointed by RAC: **Christine Hölzl**

Co-Rapporteur, appointed by RAC: **Pietro Paris**

The opinion takes into account the comments provided by MSCAs and concerned parties in accordance with Article 37(4) of the CLP Regulation and the comments received are compiled in Annex 2.

The RAC opinion on the proposed harmonised classification and labelling was adopted on **9 June 2017** by **consensus**.

Classification and labelling in accordance with the CLP Regulation (Regulation (EC) 1272/2008)

	Index No	International Chemical Identification	EC No	CAS No	Classification		Labelling			Specific Conc. Limits, M-factors	Notes
					Hazard Class and Category Code(s)	Hazard statement Code(s)	Pictogram, Signal Word Code(s)	Hazard statement Code(s)	Suppl. Hazard statement Code(s)		
Current Annex VI entry	No current Annex VI entry										
Dossier submitters proposal	608-RST-VW-Y	fludioxonil (ISO); 4-(2,2-difluoro-1,3-benzodioxol-4-yl)-1H-pyrrole-3-carbonitrile		131341-86-1	Aquatic Acute 1 Aquatic Chronic 1	H400 H410	GHS09 Wng	H410		M=1 M=1	
RAC opinion	608-RST-VW-Y	fludioxonil (ISO); 4-(2,2-difluoro-1,3-benzodioxol-4-yl)-1H-pyrrole-3-carbonitrile		131341-86-1	Aquatic Acute 1 Aquatic Chronic 1	H400 H410	GHS09 Wng	H410		M=1 M=10	
Resulting Annex VI entry if agreed by COM	608-RST-VW-Y	fludioxonil (ISO); 4-(2,2-difluoro-1,3-benzodioxol-4-yl)-1H-pyrrole-3-carbonitrile		131341-86-1	Aquatic Acute 1 Aquatic Chronic 1	H400 H410	GHS09 Wng	H410		M=1 M=10	

GROUNDS FOR ADOPTION OF THE OPINION

RAC evaluation of physical hazards

Summary of the Dossier Submitter's proposal

The Dossier Submitter (DS) did not propose classification for physico–chemical hazards for fludioxonil, based on the negative results of the studies.

Comments received during public consultation

Two experts from industry agreed with the proposed no classification for physical hazards. One Member State Competent Authority (MSCA) pointed out that the data on the substance solubility in an organic solvent is available in the DAR (2005).

Assessment and comparison with the classification criteria

The tests conducted according to guidelines EEC A.14 (explosive), EEC A.10 (flammability of solids), EEC A.17 (oxidising) and EEC A.16 (auto-ignition) showed that fludioxonil is not explosive, highly flammable, oxidising or auto-flammable.

RAC agrees with the DS, that **no classification is warranted on the basis of its physico-chemical properties**.

HUMAN HEALTH HAZARD EVALUATION

RAC evaluation of acute toxicity

Summary of the Dossier Submitter's proposal

The DS presented three studies performed in accordance with OECD Test Guidelines (TG) and GLP for acute toxicity, one for each route of exposure.

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 in an acute toxicity test conducted according to OECD TG 401, using Sprague Dawley rats (5/sex). Clinical signs were limited to soft stool in half of the animals on the day of dosing (5000 mg/kg bw).

The acute dermal LD_{50} of fludioxonil was found to be > 2000 mg/kg bw. The test was carried out according to OECD TG 402, in which rats were exposed to a limit dose of 2000 mg/kg bw. Clinical signs of toxicity, including piloerection, hunched posture, ventral recumbency and dyspnoea, were observed in all animals and persisted for up to six days.

The acute inhalation LC_{50} of fludioxonil was found to exceed the maximum achievable concentration of 2.636 mg/L. The test has been carried out according to OECD TG 403, in which rats have been exposed for 4h (nose only) to 2.636 mg/L fludioxonil. Signs of toxicity in exposed rats included piloerection, hunched posture and dyspnoea and these disappeared in all animals by day 5 after treatment. Reduced weight gain was apparent in exposed males.

Comments received during public consultation

Four comments from MSCA and one from industry supported the DS proposal for no classification for acute toxicity via oral, inhalation and dermal routes.

Assessment and comparison with the classification criteria

Oral

Classification is required where the LD_{50} is ≤ 2000 mg/kg bw. Based on the acute oral LD_{50} for fludioxonil of > 5000 mg/kg bw in the rat, RAC agrees with the DS that **no classification is warranted for acute oral toxicity**.

Dermal

Classification is required where the LD_{50} is ≤ 2000 mg/kg bw. The LD_{50} in rats was > 2000 mg/kg bw. RAC agrees with the DS that **no classification is warranted for acute dermal toxicity**.

Inhalation

Classification is required where the LC_{50} value of ≤ 5 mg/L (dusts and mists). The highest achievable concentration (2.636 mg/L) did not cause mortality in rats. Thus, the 4h LC_{50} (dust/solid aerosols) to rats for fludioxonil is > 2.636 mg/L, which is reported to be the maximum technically achievable concentration. RAC agrees with the DS that **no classification is warranted for acute inhalation toxicity**.

RAC evaluation of specific target organ toxicity – single exposure (STOT SE)

Summary of the Dossier Submitter's proposal

Relevant data for fludioxonil are limited to acute oral, dermal and inhalation toxicity. No toxicity to a specific organ was observed in those toxicity studies in animals. Additionally, no acute organ toxicity was observed in short-term or long-term studies. No effects that could lead to classification as STOT SE where reported.

Comments received during public consultation

Four comments from MSCA and one from industry supported the DS proposal for no classification for STOT SE.

Assessment and comparison with the classification criteria

Classification with STOT SE is appropriate for substances showing clear evidence of toxicity to a specific organ following a single exposure. No effects that could lead to classification as STOT SE were reported.

RAC thus agrees with the DS that **no classification for STOT SE** is warranted.

RAC evaluation of skin corrosion/irritation

Summary of the Dossier Submitter's proposal

Two GLP-compliant studies for skin irritation conducted in New Zealand White (NZW) rabbits according to OECD TG 404 with fludioxonil have been summarised by the DS. In both studies no vehicle has been used (gauze patch moistened with distilled water and 0.5 g fludioxonil/animal have been applied). In the first study, findings were limited to slight erythema (mean score of 0.22 for 24-72 hours) in two out of three male rabbits. In a second study no signs of local irritation were observed at any time point in any of the three male or three female rabbits following a four-hour semi-occluded application of undiluted fludioxonil.

Fludioxonil was shown to be a non-irritant or a minimal irritant and was not considered to require classification as a skin irritant based on comparison with the criteria detailed in Regulation (EC) No 1272/2008.

Comments received during public consultation

Four MSCA and one comment from industry supported the DS proposal for no classification for skin corrosion/irritation.

Assessment and comparison with the classification criteria

Classification of a substance for skin irritation Category 2 is required based on animal data, where a) the mean score (from gradings over 24-72 hours after patch removal) from 2/3 animals is ≥ 2.3 - ≤ 4 for erythema/eschar or for oedema or b) where inflammation persists to the end of the observation period (generally 14 days) in at least 2 animals or c) if there is pronounced variability amongst animals with a very definite response related to exposure to the substance in a single animal (even if criteria (a) and (b) are not met).

In one of the skin irritation studies in rabbits with fludioxonil mean scores of 0.22 for erythema/eschar and 0 for oedema were obtained. Irritation has been observed at one hour post application, which persisted up to 48 hours in one animal. In the second study, no signs of irritation have been observed and the mean scores for 24-72 hours were 0. Fludioxonil has been shown to be non-irritant or a very mild irritant. The erythema scores are below the mean values of the criteria for skin irritation Category 2 and no persistency of inflammation has been observed.

RAC concurs with the DS that fludioxonil **does not warrant classification for skin irritation** properties based on the criteria in the CLP Regulation.

RAC evaluation of serious eye damage/irritation

Summary of the Dossier Submitter's proposal

The eye irritation potential of fludioxonil was tested in a standard guideline and GLP-compliant study (OECD TG 405) in rabbits. Fludioxonil (0.1 mL, or 31 mg) was applied to the eyes of three female NZW Rabbits. The observation period after instillation was 72 hours.

Fludioxonil was found to be a mild eye irritant under the conditions of the 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.

The findings were limited to conjunctival erythema in 2 out of 3 female NZW rabbits while no effects were observed on the cornea or iris. Thus, the DS concluded that fludioxonil does not require classification for serious eye damage or for eye irritation based on the criteria laid down in Regulation (EC) No 1272/2008.

Comments received during public consultation

Four MSCA and one comment from industry supported the DS proposal for no classification for eye damage/irritation.

Assessment and comparison with the classification criteria

Under CLP, a substance should be classified for irreversible eye effects (Category 1) if it produces in at least one animal effects on the cornea, iris or conjunctiva that are not expected to reverse or have not fully reversed within an observation period of normally 21 days, and/or it produces at least in two of three tested animals scores for corneal opacity ≥ 3 and/or iritis > 1.5.

Since conjunctival erythema was mild and reversible within 48 hours, fludioxonil does not meet the criteria for classification into Category 1 (serious eye damage).

Under CLP, a substance should be classified for reversible eye effects (Category 2) if, in at least two of three tested animals, a positive response is observed of corneal opacity ≥ 1 and/or iritis ≥ 1 and/or conjunctival redness ≥ 2 and/or conjunctival oedema ≥ 2; calculated as mean score following grading at 24, 48 and 72 hours and which are fully reversible.

Only one study is available, the findings from which demonstrate that only the conjunctival erythema score is slightly above zero (mean score: 0.22 for 24-72 hours, individual scores: 0.3 for erythema). The findings were reversible within 48 hours and no further negative findings were observed.

RAC concurs with DS´s proposal, that fludioxonil **does not require classification for serious eye damage or for eye irritation** according to Regulation (EC) No 1272/2008.

RAC evaluation of respiratory sensitisation

Summary of the Dossier Submitter's proposal

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.

Comments received during public consultation

Four MSCA and two comments from industry supported the DS proposal for no classification for respiratory sensitisation.

Assessment and comparison with the classification criteria

A respiratory sensitiser is described as a substance that will lead to hypersensitivity of the airways. **In the absence of any data, fludioxonil cannot be classified for respiratory sensitisation.**

RAC evaluation of skin sensitisation

Summary of the Dossier Submitter's proposal

Fludioxonil and a product containing fludioxonil (Celest 025 FS) were tested in a guideline-compliant skin sensitisation maximisation tests (OECD TG 406) following GLP.

In the first study, fludioxonil was applied to skin of ten test animals per sex (five control animals were included). Fludioxonil (97.5%) of 1% and 30% were used for intradermal and topical induction exposures, respectively. Local and 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 by the DS, since it was not the highest non-irritant dose (since a concentration of even 30% did not induce mild-to moderate skin irritation) and partially invalidated the study.

No dermal reactions were observed in the test or the control animals after challenge exposures. Thus, no evidence of sensitisation was seen under the conditions of this study. The DS considered that further confirmatory data were needed to draw the final conclusion regarding skin sensitisation.

A further test was conducted with the product Celest 025 FS (containing 26 g fludioxonil/L). Delayed contact hypersensitivity was investigated using 10 test animals per sex and 5 control animals per sex. Preliminary testing demonstrated that after dermal induction with concentrations from 0.5-5% in physiological saline and after topical application of concentrations from 10-100% (with physiological saline as the vehicle) irritation was produced 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. Due to the colour of the test material, it was not possible to document the irritation potential by the test material during topical induction. 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 were detected. The product Celest 025 FS was not considered to be a skin sensitiser under the conditions of the performed study.

The DS took into consideration that the challenge concentration of 10% in the first study can be regarded to be too low and the OECD guideline was not fulfilled referring to the level of doses selected. However, a second study with a formulation containing only one active substance also showed negative results.

The DS concluded that since there are no positive reactions seen in either maximisation study performed with fludioxonil, the substance does not require classification for skin sensitisation according to Regulation (EC) No 1272/2008.

Comments received during public consultation

Five MSCA and two industry comments supported no classification for skin sensitisation. One MSCA raised the concern that the applied fludioxonil concentrations, both the topical induction and the challenge concentrations, were too low and thus the reliability of the results obtained are questionable. The second test carried out with the product cannot support the absence of a sensitising potential of fludioxonil. This MSCA stated that the tested doses for topical induction and the challenge concentrations of fludioxonil are 2% and 0.6% respectively and the irritation potential of the product could not be measured due to the red colour of the product. The MSCA asked for further available information on skin sensitisation. As a result of this request, more information on skin sensation tests (GMPT, LLNA, Buehler) with different fludioxonil technical and

formulations containing fludioxonil were provided by industry after the public consultation. In total 19 studies have been performed including Buehler tests, GPMT and LLNA tests. Out of those 19 studies, only one study indicated a weak positive response.

In the Buehler assay (25%) with A8240B (500 g/L) – the induction concentration of the formulation applied was 50% and the challenge concentration was 10% (equivalent to 0.5% induction/5% challenge fludioxonil). A more robust study with the same formulation, a guinea pig maximization test (induction concentration 1%), in which the challenge concentration applied was 100% of the formulation (equivalent to 0.5% induction/50% challenge fludioxonil), was clearly negative. Industry stated, that that additional information do not support skin sensitisation potential.

According to industry, there is a further maximisation test available with fludioxonil solo formulation A8240B (GEOX WG50 - concentration of fludioxonil 49.6%); the concentration at induction was 1% and the challenge concentrations were 25% and 50% (appr. 12.5% and 25% fludioxonil, respectively). There were no signs of a reaction after challenge and thus the product was considered to be a non-sensitiser. No study report or study summaries have been provided for the test.

Assessment and comparison with the classification criteria

No signs of sensitisation were seen in the two M&K studies carried out with fludioxonil and with a product containing fludioxonil.

According to the OECD TG 406, in the guinea pig maximization test method, "*the concentration of test substance used for each induction exposure should be well-tolerated systemically and should be the highest to cause mild-to-moderate skin irritation. The concentration used for the challenge exposure should be the highest non-irritant dose.*"

RAC takes into account that there are still uncertainties on the intrinsic sensitising properties of fludioxonil since the concentrations applied for induction and challenge were considered too low in the presented study carried out with pure fludioxonil.

However, there are no human data indicating a possible sensitising potential attributable to fludioxonil. In conclusion, since no signs of sensitisation have been detected in the provided inadequate maximisation tests, and in the absence of other reliable studies, the criteria for classification are not met.

RAC evaluation of specific target organ toxicity– repeated exposure (STOT RE)

Summary of the Dossier Submitter's proposal

Fludioxonil was tested for repeated dose toxicity via the oral route in the rat (28 days, 20 days, 90 days, 2 years, dietary application), the mouse (90 days, two 18 month studies) and the dog (28 days range finding study, 90 days, and 1 year) and a dermal 28 day study in the rat. All studies followed appropriate TG protocols and were conducted according to GLP.

In the following section the summary of the DS´s proposal is presented. Further detailed information (e.g. on incidences), if considered relevant, has been extracted from the DAR.

Rat

In all oral repeated dose toxicity studies (28d, 20d, 90d, 2y), treatment related adverse effects were found in the liver and kidneys, characterised mainly as increased organ weights, hepatocyte

hypertrophy and liver degeneration and necrosis (long term study), chronic nephropathy, renal tubular casts and tubular necrosis. Body weight and body weight gain was also affected, mainly in the highest dose groups.

In a **28 day study** 10, 100 or 1000 mg/kg bw/d fludioxonil were administrated to 10 animals per sex. Fludioxonil, applied via gavage to Tif:RAIf (SPF) rats, induced reduced cumulative body weight gain only at the highest dose (F: 12%, M: 9%). Clinical chemistry revealed statistically significantly reduced glucose concentrations (F: 18%, M: 17%) at the highest dose level and in males at 100 mg/kg bw/d (M: 13%). Plasma cholesterol levels were increased significantly in both sexes (F: 36%, M: 27.5%) at the highest dose level and at 100 mg/kg bw/d in females (29%). The plasma cholesterol levels were within the historical control data (HCD) provided by industry. Ketones were present in all dose groups in males (no clear dose-response relationship) and in females at 100 and 1000 mg/kg bw/d. The incidences in the study were stated to be within the HCD. Treatment related increases in relative kidney weight (F: 7 %, M: 7%) were observed in both sexes at the high dose level and in females at 100 mg/kg bw/d (6.5%). Absolute kidney weight was not changed. Relative liver weight was increased (8%) at the highest dose level in males. In females, relative and absolute liver weight was increased at 100 mg/kg bw/d (in both cases by 14%) and at 1000 mg/kg bw/d (rel. 24%, abs. 16%). Histopathology revealed increased incidences of hepatocyte hypertrophy in the centrilobular region of the liver in both sexes (F: 4/10, M: 2/10) at the top dose level and renal tubular casts in males at 1000 mg/kg bw/d. The LOAEL is set at 1000 mg/kg bw/d since the increase in liver weight in females (14%) at 100 mg/kg bw/d is not considered to be adverse (absence of histopathological correlates or clinical chemistry parameters).

In a **20 day study,** Sprague Dawley (SD) rats were exposed to 1000 ppm (124/133 mg/kg bw/d, M/F), 5000 ppm (624/698 mg/kg bw/d, M/F), 10000 ppm (1260/1407 mg/kg bw/d, M/F), and 20000 ppm (2493/2823 mg/kg bw/d, M/F) of fludioxonil. Body weight was affected only in high dose males after three weeks (12% decrease). Some clinical chemistry parameters were slightly decreased (such as plasma concentration of sodium and chloride) in groups given ≥ 5000 ppm; these changes are potentially related to tubular nephrosis. Relative liver weights have been increased statistically significantly (18-24%) in the two highest dose groups. Relative and absolute kidney weights were statistically significantly increased only in high-dose males (rel. 65%, abs. 32%) and in females only at 1000 ppm (rel. 13%, abs. 15%). Microscopic observations included tubular nephrosis, for which incidences and severity were increased in a dose-dependent manner (see table below). Other organ weight changes were not associated with histopathological or macroscopic findings. A NOAEL is set at 124 mg/kg bw/d (1000 ppm) for males and 698 mg/kg bw/d (5000 ppm) for females. The NOAEL of 124 mg/kg bw/d for males is based on only one male out of 6 showed slight nephrosis. However, this is justified because other studies have indicated kidney as target organ and the occurrence of nephrosis at that time point is not a normal finding in this strain of rats.

Table: Incidence of tubular nephrosis

Dose group ppm	Female	Male	Severity
1000	0	0	
5000	0	1/6	Slight
10000	1/6	4/6	Minimal to marked
20000	3/6	6/6	Severe

In the **90 day** oral toxicity study, Sprague Dawley rats were exposed to 10 ppm (0.8/1.0 mg/kg bw/d, M/F), 100 ppm (6.6/7.1 mg/kg bw/d, M/F), 1000 ppm (64/70 mg/kg bw/d, M/F), 7000 ppm (428/462 mg kg bw/d, M/F), 20000 ppm (1283/1288 mg/kg bw/d, M/F) of fludioxonil. Body weight gains were negatively affected at the high dose groups in both sexes. Some clinical parameters were altered in the highest dose groups (7000 and 20000 ppm), indicative of liver

and kidney toxicity (e.g., increased cholesterol, and 5´nucleotidase, increased bilirubin). Relative kidney and liver weights were statistically significantly increased in the two highest dose groups. Organ weight changes were consistent with the microscopically identified changes in the liver (centrilobular hepatocyte hypertrophy) and kidneys (chronic nephropathy with a prominent active inflammatory component, see table below). An increased incidence (5/10) (not statistically significant) of slight centrilobular hepatocyte hypertrophy was also seen in males at 1000 ppm (64 mg/kg bw/d). The finding is not considered relevant since no relevant clinical chemistry parameters have been altered at that concentration. Furthermore, at 1000 ppm no histopathological findings were observed in the liver in the 2 year study. The NOAEL was set at 1000 ppm (corresponding to 64 and 70 mg/kg bw/d in males and females).

Table: Major histopathological non-neoplastic findings (extracted from DAR)

Sex	**Males**						**Females**					
Dose group (ppm)	0	10	100	1000	7000	20000	0	10	100	1000	7000	20000
Number of rats	10	10	10	10	10	10	10	10	10	10	10	10
Chronic nephropathy	3	3	2	4	10*	10	0	0	0	0	0	10
Chronic nephropathy with active inflammation	0	0	0	1	6	9	0	0	0	0	0	9
Centrilobular hepatocyte hypertrophy	0	0	0	5	5	9	0	0	1	1	6	10

In the **12 month combined toxicity/carcinogenicity** study, SD rats were exposed to 0, 10 ppm (0.37/0.44 mg/kg bw, M/F), 30 ppm (1.1/1.3 mg/kg bw/d, M/F), 100 ppm (3.7/4.4 mg/kg bw/d, M/F), 1000 ppm (37/44 mg/kg bw, M/F) or 3000 ppm (113/141 mg/kg bw, M/F) fludioxonil. Signs of toxicity included reduced body weights and reduced cumulative body weight gain (F: 16%, M: 10%) at the highest dose levels, transient changes in red blood cell parameters indicating slight anaemia (at the high dose in females). Clinical chemistry parameters and organ weights are largely considered not to be related to the treatment. Relevant findings during gross necropsy were enlarged livers in high dose males and kidneys with cysts in males at doses ≥ 1000 ppm. Histopathology, however, reported reduced incidences of kidneys with cysts (M: 1000 and 3000 ppm) and thus the macroscopic findings in the kidneys are considered toxicologically not relevant. Non-neoplastic histological findings were observed in the liver (degeneration, atrophy, inflammation, and necrosis) of high dose animals and in the kidneys (cysts, progressive nephropathy) of high dose males. Toxicologically relevant adverse effects have been observed only at the highest dose, thus the NOAEL was set at 37 mg/kg bw/d for males and 44 mg/kg bw/d for females, corresponding to 1000 ppm.

In a **dermal** 28 day toxicity study (OECD TG 410, GLP), albino rats were exposed to 0, 40, 200 or 1000 mg/kg bw/d for 6 hours, 5 days per week. No treatment related effects were observed on mortality, clinical signs (local or systemic), bodyweights, food consumption, haematology or organ weights. The only treatment-related change was enlarged cortical macrophages, often revealing lymphophagocytosis in the thymus of females at 1000 mg/kg bw/d. The NOAEL is therefore considered to be 200 mg/kg bw/d for females and 1000 mg/kg bw/d for males.

Mouse

The three oral repeated dose toxicity studies in mice (90 day and two 18 month studies) also indicated adverse effects on liver and kidney.

In a **90 day** oral toxicity study, mice (CD-1) were exposed to 10 ppm (1.3/1.9 mg/kg bw/d, M/F), 100 ppm (13.9/17 mg/kg bw/d, M/F), 1000 ppm (144/ 178 mg/kg bw/d, M/F), 3000 ppm (445/559 mg/kg bw, M/F), 7000 ppm (1052/1307 mg/kg bw, M/F) of fludioxonil. Body weight and weight gain were only affected in high-dose females. 5´nucleotidase was increased in high dose males and females, other changes in clinical parameters were without a dose-response

relationship or were within the HCD. Relative liver weights were increased in high dose males and from 3000 ppm in females. Relative kidney weights were slightly increased in all dose groups, but did not reach statistical significance. Histopathological findings included nephropathy (significantly higher incidences) at the highest dose groups (F: 9/10, M: 10/10) and significantly higher incidences of centrilobular hepatocyte hypertrophy at the highest dose (F: 8/10, M: 7/10), non-significant in females in the 3000 ppm group (F: 3/10). Increase of 5´nucleotidase (significant for both sexes at 7000 ppm) indicated cholestasis, which might have resulted from the liver cell hypertrophy (higher increase in females than in males). A NOAEL was set at 445 mg/kg bw/d for males and 559 mg/kg bw/d for females (corresponding to 3000 ppm).

Two **18 month carcinogenicity** studies were performed with mice exposed to up to 7000 ppm of fludioxonil. In the first study, mice were exposed to 0, 10 ppm (9.78 mg/kg bw/d), 100 ppm (98.9 mg/kg bw/d), 1000 ppm (1003 mg/kg bw/d) or 3000 ppm (3030 mg/kg bw/d) and in the second study to 0, 3 (3.25 mg/kg bw/d), 30 (30.5 mg/kg) bw/d, 5000 ppm (5021 mg/kg bw/d) and 7000 ppm (7111 mg/kg bw/d). Nephropathy was observed in the kidneys from 5000 ppm on (590/715 mg/kg bw/d, M/F) in both sexes (M: 53/60 at 5000 ppm and 59/60 at 7000 ppm, F: 21/60 at 5000 ppm and 58/60 at 7000 ppm). The findings were *inter alia* characterised by glomerular atrophy, hyaline change, tubular dilatation and protein casts. A marked reduction in survival, associated with nephropathy was seen at the highest dietary concentration of 7000 ppm (exceeded the MTD). Red blood cell parameters were affected in the highest dose group. Liver weights were increased at ≥ 5000 ppm in both sexes and increased hepatic cysts have been observed in males in the highest dose group. Increased incidences of hepatocellular necrosis (no incidences provided in the DAR) and bile duct hyperplasia (23/60 vs 0/60 in the control) were observed in males in the highest dose group. An overall NOAEL of 112/133 kg bw/d (M/F) has been set based on effects such as reduced weight gain, increased liver weight, liver and kidney pathology seen at dose levels of 360/417 mg/kg bw/d and higher.

Dog

In a **90 day** dog study incorporating a 28-day recovery period, Beagle dogs were initially given 0, 200 (6.2/5.9 mg/kg bw/d, M/F), 2000 (60/59 mg/kg bw/d, M/F) and 15000 ppm fludioxonil in the diet. The highest dose was reduced to 10000 ppm (299/351 mg/kg bw/d, M/F) due to marked weight loss in both sexes. Body weight was reduced in males at the highest dose from week 4; in high dose females bodyweights were only reduced at week 8 and 14. Increased frequency of diarrhoea was observed in both sexes at 2000 ppm (60/59 mg/kg bw/d, M/F) and 10000 ppm (299/351 mg/kg bw/d, M/F). This effect is considered to be treatment related but not adverse, since the incidences were episodic and transient and the same effect was not seen in a one year dog study. Increased cholesterol concentrations in high dose animals were reversible during the recovery period. Absolute and relative liver weights were increased in the high dose groups. Histopathological findings were limited to an increased incidence in bile duct hyperplasia in both sexes at the highest dose level. These findings correspond to increased liver weights. The NOAEL is considered to be 60 and 59 mg/kg bw/d for males and females, corresponding to 2000 ppm.

In a further dog study in which fludioxonil was administered for **12 months** at dietary concentrations of 0, 100, 1000 (33.1/35.3 mg/kg bw/d, M/F), 8000 ppm. Body weight was reduced in the highest dose groups for males; for females, non statistically significantly decreased body weight gains of 43% (1000 ppm) and 51% (8000 ppm) were observed. Total cholesterol was increased in males at 8000 ppm. Furthermore, absolute (14%) and relative (36%) liver weights were increased in females of the highest dose group and the relative liver weight (28%) was also increased in high dose males. The findings correlate with the macroscopic observation of liver enlargement (2/4 high-dose females). A single incidence of biliary epithelial cell proliferation in females at 8000 ppm was found. The NOAEL was set at 33.1 and 35.5 mg/kg bw/d in males and females, corresponding to 1000 ppm.

Conclusion of the DS

The outcome of repeated oral toxicity studies performed with fludioxonil in the rat, mouse and dog demonstrated that the liver and kidney are the main target organs. Adverse effects have been seen generally at high dose levels, but these were accompanied by adaptive findings and at lower dose levels the effects are considered to be not of toxicological significance.

Overall, the DS concluded that classification of fludioxonil for STOT RE is not warranted.

Comments received during public consultation

Four MSCA and one comment from industry supported the DS proposal for no classification for STOT RE.

Assessment and comparison with the classification criteria

In total, seven oral repeated dose toxicity studies in rats, mice and dogs have been considered. A summary of the established NOAELs and LOAELs and the guidance dose/concentration values for different study durations are provided below in order to guide classification as STOT RE 2:

Table: Summary of established NOAELs and LOAELs comparison with guidance values (STOT RE, oral rat)[1]

Study	**NOAEL mg/kg bw/d**	**LOAEL mg/kg bw/d**	**Guidance values, oral rat mg/kg bw/d***
RAT			
20 day study, oral	**124 (M), 689 (F)**	**624 (M), 1407 (F)**	**45 < C ≤ 450**
28 day study, oral	**100 (M/F)**	**1000 (M/F)**	**30 < C ≤ 300**
90 day study, oral	**64 (M), 70 (F)**	**428 (M), 462 (F)**	**10 < C ≤ 100**
2 year study	37 (M), 44 (F)	113 (M), 141 (F)	1.25 < C ≤ 12.5
MOUSE			
90 day study, oral	445 (M), 559 (F)	1052 (M), 1307 (F)	10 < C ≤ 100
18 month study	112 (M), 133 (F)	360 (M), 417 (F)	~ 1.25 < C ≤ 12.5[2]
DOG			
90 day study, oral	**60(M), 58.5 (F)**	**299 (M), 351 (F)**	**10 < C ≤ 100**
1 year study, oral	33 (M), 33 (F)	298 (M), 331 (F)	2.5 < C ≤ 25

[1] bold: studies in which the NOAEL was within the guidance values, but the effective dose (LOAEL) was above the guidance values. [2] Guidance value for 2 year study. *Values adjusted using Haber's rule, where necessary.

Studies demonstrate, that for the **rat** the main targets of fludioxonil's toxicity are the kidneys and liver.

In the 20 day rat toxicity study, the NOAEL for females (689 mg/kg bw/d) is far above the GV values and the NOAEL for males (124 mg/kg bw/d) is considered to be conservative, since only 1 out of 6 males showed nephrosis characterised as being slight at the next dose level. RAC agrees that the outcome of the study does not indicate a need for classification.

In the 28 day rat toxicity study at a dose level 1000 mg/kg bw/d (=LOAEL), relevant toxic effects were increased liver weights in females (rel. 24%, abs. 16%), increased incidences of hepatocyte hypertrophy in both sexes (F: 4/10, M: 2/10) and renal tubular casts in males. Increased liver weights in females (14%) were already observed at a dose level of 100 mg/kg bw/d, but no histopathological correlates were detected and the increased cholesterol levels were within the HCD. Thus, the effect seen at 100 mg/kg bw/d can be considered as non-adverse effect. Based on the described effects and the fact that the LOAEL is more than 3 fold higher than the upper

level of the guidance values, no adverse effects are expected in the range of guidance values for classification.

In the 90 day rat toxicity study, a NOAEL was established at 64/70 mg/kg bw/d (M/F), based on effects on the liver (centrilobular hepatocyte hypertrophy, M: 5/10, F: 6/10) and on the kidneys in females (chronic nephropathy with active inflammation, M: 6/10) at 428/626 mg/kg bw/d (M/F). At the established LOAEL (428/626 mg/kg bw/d, M/F) cholesterol levels were increased (M: 49%, F: 50%) and also absolute and relative liver weights were increased (M: abs. 7.5%, rel. 21%, F: abs. 16%, rel. 41%), indicating severe adverse effects at that dose level. A slight, but not statistically significantly increased incidence of centrilobular hepatocyte hypertrophy (M: 5/10, F: 0/10) was also seen at 64 mg/kg bw/d in males. There were no further confirmatory clinical chemistry data and no liver weight changes were found at that concentration. The LOAEL is 4 fold higher as the upper level of the guidance value (GV) and the NOAEL is close to the upper level of GV. Thus, it can be reasonably assumed that there are no adverse effects at dose levels which are relevant for classification.

In the 90 day study carried out with **dogs,** at 60/58.5 mg/kg bw/d increased incidences of diarrhoea were observed; however, these findings were transient and episodic and they have not been observed in a further one year rat study. RAC agrees with the DS that those findings are treatment related but do not trigger classification. Further effects at the LOAEL of 299/351 mg/kg bw/d included increased cholesterol levels (not present after recovery period) in high dose animals, higher absolute and relative liver weights (M: abs. 6%, rel.19%, F: abs. 30%, rel. 44%). Liver weights were also altered at 60/58.5 mg/kg bw/d (M: abs. 10%, rel. 6%, F: abs. 17%, rel. 9%). In the DAR, it is stated that there is an indication of reversibility with respect to changes in absolute and relative organ weights. Histopathological findings have been observed only at the LOAEL, which included higher incidences of bile duct proliferation (no incidences provided) and portal fibrosis (3/4 males) at the highest dose group. According to the DAR, there was indication of reversibility at the end of the recovery period.

In conclusion, the studies carried out with rats, mice and dogs indicate that liver and kidney are the main target organs. However, effects have only been observed at doses which are not relevant for classification. At moderate doses no adverse effects are expected.

A 28 day dermal toxicity study with albino rats was also performed. The study outcome demonstrated that the observed effects are well above the guidance values for STOT RE 2 classification ($20 < C \leq 200$ mg/kg bw/d).

In conclusion, RAC agrees with the DS that **no classification for STOT RE is warranted** based on the study outcomes of the available repeated dose toxicity studies carried out with rats, mice and dogs.

RAC evaluation of germ cell mutagenicity

Summary of the Dossier Submitter's proposal

The mutagenicity potential of fludioxonil was tested in an appropriate battery of studies *in vitro* and *in vivo*, covering gene mutation and chromosomal damage endpoints.

In vitro

Fludioxonil did not induce gene mutations in *in vitro* studies on different strains of bacterial cells (OECD TG 471, GLP) or mammalian cells (V76 cells, OECD TG 476, GLP). The substance was clastogenic and aneugenic in Chinese Hamster ovary cells (OECD TG 473, GLP) where specific 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 in this test system. Fludioxonil also had DNA damaging potential in a UDS test (OECD TG 482: the guideline was deleted in 2014, GLP) in rat hepatocytes.

In vivo

The CLH report contained 7 studies evaluating the mutagenic potential of fludioxonil *in vivo*: 3 micronucleus tests (1 in mouse bone marrow, OECD TG 474, Hertner, 1990; 2 in hepatocytes, non-guideline studies, all GLP (Meyer, 1991, and Ogorek, 1999), 2 bone marrow chromosome aberration tests, 1 in rats (similar to OECD TG 475, GLP, Myhr, 1999) and 1 in Chinese hamsters (OECD TG 475, GLP, Hertner, 1993a), 1 UDS assay in the rat (OECD TG 486, GLP, Hertner, 1993b), and 1 mouse dominant lethal test (OECD TG 478, GLP, Hertner, 1992).

All studies gave negative results, except for one equivocal result in a non-guideline micronucleus test assessing fludioxonil potential to induce micronuclei in rat hepatocytes (Meyer, 1991). The first part of this test, in which fludioxonil was administered 3 days prior to a mitogenic stimulus (4-acetylaminofluorene), did not induce the formation of micronuclei in rat hepatocytes. However, in the second part of the experiment, fludioxonil was administered 29h after the mitogenic stimulus and in this experiment a slight, but significant increase of micronucleated hepatocytes in the low (1250 mg/kg bw) and mid dose (2500 mg/kg bw) were reported. No statistically significant increase was seen in the high dose group (5000 mg/kg bw). All results, except from 1 animal in the low dose group, were within the historical control range. Only few animals were investigated (3 males per dose). While the positive control produced a clear increase in micronucleated hepatocytes, the results of fludioxonil, though statistically significant, were not dose-dependent and were almost within historical control values. Therefore, the DS concluded that the results were equivocal and the test should have been repeated with a larger number of animals.

A newer study (Ogorek, 1999) with a similar protocol testing doses up to 1250 mg/kg bw was negative. There were no statistically significant increases in the number of micronucleated hepatocytes. Individual animals in the fludioxonil treated groups and in the positive control group showed marked increases in the frequency of hepatocytes in apoptosis, but the group averages did not show statistically significant differences when compared to the negative control. This effect was thought to be due to synergistic effects of treatment with fludioxonil or positive control substance with the hepatocyte necrogenic agent 4-acetylaminofluorene used in the assay.

The DS concluded that fludioxonil was not clastogenic or aneugenic in rat hepatocytes *in vivo*, under the conditions of the study.

Three *in vivo* tests investigated the clastogenic and aneugenic potential of fludioxonil in bone marrow. Although no cytotoxicity was reported in the bone marrow (PCE/NCE ratio not affected, and where investigated, the mitotic index was not affected), which would be an indication, that fludioxonil reached the bone marrow, the DS concluded that it is likely that the substance was distributed to the bone marrow. Toxicokinetic data showed that fludioxonil administered orally is rapidly and widely distributed in blood and various organs and tissues including bone. After 0.5 h, 0.41% and 0.58% of the applied dose were found in the bone tissue in males and females, respectively. An initial C_{max} 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 12h showing rapid distribution and within the time frame of the micronucleus study. Given the presence of fludioxonil in the blood and detection in the bone together with a high level of absorption, the DS concluded that it can be assumed that the bone marrow was adequately exposed in the tests.

The negative UDS test (OECD TG 486) in rat liver cells (Hertner, 1993b) was negative.

The mouse dominant lethal test (OECD TG 478) was also negative. There was no evidence for cytotoxic effects on the pre-implantation stages; the post-implantation mortality of embryos was slightly increased, but not statistically significant, within historical control values and with no evident dose-response. The DS concluded that fludioxonil is not genotoxic in germ cells of the mouse *in vivo* under the conditions of the assay.

The DS concluded that fludioxonil did show clastogenic potential *in vitro* and an equivocal result for clastogenicity/aneugenicity in rat hepatocytes in 1 of 7 negative *in vivo* studies. However, this study was not well performed and a newer test following a similar protocol was negative. The available *in vivo* studies covered all relevant endpoints to assess the mutagenic potential of fludioxonil.

Comments received during public consultation

Four MSCA and one industry comment supported the conclusion of the DS for no classification. Furthermore, industry provided two additional bacterial reverse mutation tests supporting the negative results of the already available gene mutation tests in bacteria.

Assessment and comparison with the classification criteria

According to the CLP Regulation classification as a germ cell mutagen in Category 1A is based on positive evidence from human epidemiological studies. No such evidence exists for fludioxonil, therefore classification in Cat. 1A is not supported.

Classification in either Category 1B or 2 is not supported for fludioxonil.

There is no evidence for positive effects in the single available *in vivo* heritable germ cell mutagenicity test in mice (OECD TG 478).

Two out of four *in vitro* tests gave positive results indicating clastogenic and aneugenic potential *in vitro* (positive results in 1 OECD TG 473 study and in 1 OECD TG 482 study – protocol now deleted).

However, six out of seven *in vivo* studies testing fludioxonil's mutagenic potential in somatic cells gave negative results. Only one study, a non-guideline study assessing the induction of micronuclei in rat hepatocytes after fludioxonil treatment gave equivocal results. However, in this study only three animals per dose were investigated, there was no clear dose response and the results were comparable with data from historical control. With the exception of one animal the results were within the range of historical control data. Additionally, a later study using a comparable test protocol gave negative results.

The available chromosome aberration tests (a mouse and a Chinese hamster OECD TG 475 study) and a mouse micronucleus test (OECD TG 474) in bone marrow did not show any indication for mutagenic potential. RAC agrees with the DS's conclusion that the target tissue was reached, based on the presence of fludioxonil in the blood and detection in the bone together with a high level of absorption. Additionally, RAC notes that fludioxonil has a log Kow of 4.12, indicating lipophilicity. Affinity to the bone marrow which is high in lipids can therefore be assumed.

The *in vivo* UDS test in rat hepatocytes (OECD TG 486) was also negative.

These results indicate that fludioxonil is not mutagenic *in vivo*. RAC agrees with the DS proposal for **no classification for mutagenicity**.

RAC evaluation of carcinogenicity

Summary of the Dossier Submitter's proposal

In the following section the summary of the DS's proposal is presented. Further detailed information (e.g. general toxicity, study quality) if considered relevant has been extracted from the DAR.

The carcinogenic potential of fludioxonil has been investigated in three dietary guideline carcinogenicity studies, one in rats (OECD TG 453) and two in mice (OECD TG 451), all were GLP-compliant.

Rat

Animals were tested at six doses up to 3000 ppm (113/141 mg/kg bw/d in males and females, respectively). The study has some deficiencies, as the animals were too old at the start of the study (7 weeks instead of ≤ 6 weeks), there was a dosing error (the 30 ppm females once were given phosphamidon for 24 hours instead of fludioxonil) and the survival after 24 months was too low to fulfil the OECD TG 453 criteria (not < than 50%); however, survival was in line with the requirements of the relevant EPA guideline no. 83-5 (≥ 50% at 18 months and ≥ 25% at 24 months). There were no treatment related effects on survival, but survival was low in both the control and treated groups (< 50%).

Food and water consumption were similar across groups, including the controls. Body weights of high dose females were reduced statistically significantly during most of the study, body weight reduction of high dose males reached statistically significance after the first year, and was lower than in controls during most of the remainder of the study. The cumulative body weight gain was statistically significantly reduced to ~90% of the controls in high dose males and females. Cumulative weight gain was also decreased statistically significantly in the 1000 ppm males during the first 13 weeks. No effects on body weight gain were seen in the recovery groups.

For details on haematology, clinical chemistry parameters, effects on organ weights, gross necropsy findings and non-neoplastic findings, see the section on STOT RE. In short, it can be summarised that changes in red blood cell parameters were primarily observed at the two higher dose levels at 12 months. The other findings were mainly related to kidney and liver toxicity and included increased relative liver weight in high dose males and females at 12 and 13 months respectively and increased relative kidney weight in high dose animals at 13 months, which correlated with enlarged livers (high dose males), kidneys with cysts (in males from 1000 ppm) and kidneys with discoloured foci or general discolouration (from 1000 ppm). Nephropathy increased in frequency and severity in high dose males compared to the controls. In the livers of high dose animals degeneration, atrophy, inflammation, and necrosis were recorded at increased frequency and severity compared to controls.

Except for liver tumours, there were no differences between the controls and any of the dosed groups with respect to benign or malignant neoplasms or on the number of animals with neoplasms. The numbers of metastatic neoplasms and total neoplasms were high in both sexes of almost all groups. No statistics were performed on these data.

In males a higher incidences of liver adenoma, carcinoma and combined adenoma/carcinoma were observed in the low dose (10 ppm) and the high dose groups (3000 ppm). However, the incidences did not exhibit a monotonic dose-response relationship and were within historical control values. The incidence of hepatocellular tumours (adenoma and adenoma & carcinoma combined) were slightly increased in females at 3000 ppm. However, this increase was not statistically significant after Bonferroni adjustment of the tail probabilities. The carcinoma incidence of 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 (no tumour multiplicity) and were not associated with other evidence of a proliferative hepatocellular response such as increases in foci of altered cells.

Table: Incidence (% of animals examined) of hepatocellular tumours in Sprague Dawley rats

Sex	**Males**							**Females**						
Dose group ppm	0	10	30	100	1000	3000	HCD	0	10	30	100	1000	3000	HCD
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

HCD: historical control data

Table: 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

In the absence of a dose-response relationship, coupled with the fact that the tumour incidences fall within the historical control range, the DS considered the hepatocellular tumours seen in high dose females as non-treatment related.

As the HCD were presented in the original study report and consisted of seven studies conducted at Ciba Ceigy Environmental Health Center, the DS assumed that they were conducted within the same time period and the testing facilities were the same. This conclusion is supported by a statement on the available HCD, submitted by industry; however, confirmation from the original data owner is missing.

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

Mouse

Two mouse carcinogenicity studies have been performed. The studies have been evaluated together and in combination they are considered to fulfil the OECD TG 451 criteria; studies were carried out according to GLP. In the first study 0, 10, 100, 1000, 3000 ppm were applied with the diet. Survival rates at 18 months were 74-86% and 71-92% in males and females, respectively. In the second study 0, 3, 30, 5000, 7000 ppm were applied and the survival rates at the highest dose were markedly reduced (27% and 22% in males and females). In the highest dose group clinical signs observed included dyspnoea, hypothermia, pallor, hyperactivity, hunched posture and tremors. The increased mortality became apparent at one year and was

attributable to nephropathy (M: 31/60, F: 32/60). RAC takes into account that the highest dose group exceeded the MTD and thus results at that dose level need to be interpreted with caution.

Lymphomas were statistically significantly increased at 3000 ppm only in females in the first study. According to the DAR there is also a statistical significant difference for lymphomas considered to be the cause of death in females in the first study but not in the second. In the second study at 5000 and 7000 ppm, no increased lymphoma incidences are described (see Table, combined results from both studies). It is noted, that the markedly reduced survival in females at 7000 ppm may have influenced the lymphoma incidence observed at the 18-month terminal sacrifice; however, both the lymphoma incidence and survival in the 5000 ppm dose group were comparable to controls.

Table: Lymphoma incidences from 18 month mice studies (combined results)[1]

	ppm	**0 (1st)**	0 (2nd)	3	**10**	30	**100**	**1000**	**3000**	5000	7000
FEMALES	No. of animals	**60**	60	60	**60**	60	**60**	**60**	**60**	60	60
	Total	**11**	11	7	**10**	12	**13**	**12**	**18**	11	8
	% incidence	**18**	18	12	**17**	20	**22**	**20**	**30**	18	13
	Lymphoma considered to be cause of death	**1**	3	1	**0**	3	**2**	**4**	**6**	0	3
MALES	No. of animals	**60**	60	60	**60**	60	**60**	**60**	**60**	60	60
	Total	**2**	3	1	**1**	2	**2**	**7**	**2**	4	0
	% incidence	**3**	5	2	**2**	3	**3**	**12**	**3**	7	0

[1] bold text: outcome of first carcinogenicity study

The lymphoma incidences at 3000 ppm are considered to be within the HCD for females (13-32%, combined thymus hyperplasia and malignant lymphoma). According to industry (additional information on the use of HCD) the studies considered for historical control were performed at the same laboratory, in the same time period, and using the same mice strain.

The information on HCD is summarised below:

Historical control data are available from six studies, the incidence of malignant lymphoma and thymus hyperplasia were combined as thymus hyperplasia is often indistinguishable from thymus lymphoma.

Table: HCD: Incidence of thymus hyperplasia and malignant lymphoma in female CD-1 mice

Studies	A	B	C	D	E	F	Total	% min-max
Thymus hyperplasia	8	6	6	13	12	-	45/330	13 (6-13%)
Malign. lymphoma	4	2	4	3	4	11	28/330	8.4 (2-11%)
Total Combined %	**12/50 24**	**8/60 13**	**10/60 17**	**16/50 32**	**16/50 32**	**11/60 18**	**73/330**	**22 (13-32%)**

According to the DAR in the second mouse study the only increased incidence of neoplastic findings in dosed animals in relation to control group was hepatocellular adenoma/carcinoma in males (0 ppm: 6/60, 3 ppm: 14/60, 30 ppm: 9/16, 5000 ppm: 10/60, 7000 ppm: 9/60); however ,no dose-relationship was observed.

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

The DS concluded that in the absence of a dose-response relationship, the increased incidences of lymphomas observed at 3000 ppm are not considered to be related to the treatment with fludioxonil.

The DS concluded that studies performed with fludioxonil in the rat and mice do not provide any clear evidence of carcinogenicity based on an overall weight and strength of evidence approach and thus no classification is proposed.

Comments received during public consultation

Four MSCA and two comments from industry supported the DS proposal for no classification for carcinogenicity.

Assessment and comparison with the classification criteria

According to CLP Regulation (Annex I, Section 3.6.1.1) a carcinogen is defined as a substance which induces cancer or increases its incidence.
Classification in Category 1A is based on positive evidence from human epidemiological studies. No such evidence exists for fludioxonil, therefore classification in Category 1A is not supported.

Classification in Category 1B, presumed or suspected human carcinogen, is supported by animal experiments which demonstrate sufficient evidence for animal carcinogenicity, unless there is strong evidence that the mechanism of tumour formation is not relevant for humans.
Classification in Category 2, suspected human carcinogen, is supported by either limited evidence of carcinogenicity in human studies (not available for fludioxonil) or from limited evidence of carcinogenicity in animal studies.

For fludioxonil three carcinogenicity studies with rats and mice according to OECD TG and GLP are available.

In the rat, a higher incidence of liver adenoma, carcinoma and combined adenoma/carcinoma were observed in the low dose (10 ppm) group and the high dose (3000 ppm) group males. However, the incidences did not exhibit a monotonic dose-response relationship and were within historical control values. The incidence of hepatocellular tumours (adenoma and adenoma & carcinoma combined) were slightly increased in females at 3000 ppm. However, this increase was not statistically significant after Bonferroni adjustment of the tail probabilities. The carcinoma incidence of 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 (no tumour multiplicity) and were not associated with other evidence of a proliferative hepatocellular response such as increases in foci of altered cells. The tumorigenic observations in treated rats were within the historical control range.

A mechanistic investigation as part of the carcinogenicity study assessed fludioxonil's effect on cell proliferation in the rat liver (PCNA staining), in order to further analyse the observed hepatocellular adenomas and carcinomas. The marginally increased labelling indices for female rats administered ≥ 1000 ppm is considered incidental due to a lack of statistical significance and dose-response relationship.

Studies carried out with mice do indicate a higher number of lymphomas in females which received 3000 ppm (first study); however, at 5000 ppm and 7000 ppm (second study) no increased incidences were seen. Since the two studies were performed at the same time and in the same laboratory, a combined interpretation of results is justified. According to information

extracted from the DAR, statistical analysis of lymphoma data with Fisher's Exact test with Bonferroni correction did not indicate any significant differences in lymphoma incidence in any treated group when compared to the controls. Furthermore, the increased incidence rate at 3000 ppm (30%) is within the historical control range of 13-32%. RAC notes, that incidence of historical control range includes thymus hyperplasia and malignant lymphoma thymus, since thymus hyperplasia is often indistinguishable from thymus lymphoma. No information is provided if thymus hyperplasia was also considered in the evaluation of total lymphoma incidence in the carcinogenicity study with fludioxonil.

Fludioxonil is not considered mutagenic. A comprehensive test battery including *in vitro* and *in vivo* genotoxicity tests gave mainly negative results, except for an *in vitro* chromosome aberration tests and an *in vitro* UDS test. However, as the *in vivo* tests including micronucleus tests, chromosome aberration tests and an UDS tests gave negative results (except for a non-standard micronucleus test in rat hepatocytes, which was overruled by a second negative micronucleus study with negative result), no classification for germ cell mutagenicity was proposed (see section on germ cell mutagenicity).

In summary, RAC concludes that the available carcinogenicity studies do not give sufficient evidence to support a classification of fludioxonil as carcinogen.

In conclusion, RAC supports the DS's proposal that **classification for carcinogenicity is not warranted**.

RAC evaluation of reproductive toxicity

Summary of the Dossier Submitter's proposal

One two generation reproduction study in rats with doses of 0, 2.1, 21, or 212 mg/kg bw/d (corresponding to 0, 30, 300 or 3000 ppm) in the diet and two prenatal developmental toxicity studies with rats exposed to 0, 10, 100 or 1000 mg/kg bw/d (gavage) and rabbits exposed to 0, 10, 100, 300 mg/kg bw/d are summarised in the CLH dossier. The studies were performed according OECD test guidelines and under GLP.

Two-generation reproduction study

Statistically significant 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 the high dose range. A marginal (but statistical significant) increase in relative testes weight in F1 males and a marginal reduction in absolute ovary weight in F0 females at 3000 ppm are considered to be secondary to lower terminal bodyweights in these animals. A reduction in absolute ovary weight was also seen in F0 females at 30 ppm; however, there was no dose-relationship and thus the findings are considered not to be toxicologically relevant. Numbers of implantation sites and litter sizes were slightly higher at 300 and 3000 ppm (appr. 10%) in the F2 generation, but are considered to be within normal biological variation. A marginally higher number of pups from these litters died four days *post partum*. However, the findings are attributable to the larger litter sizes in these groups and are therefore considered to be unrelated to treatment. There were no clinical signs attributable 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. A reproductive NOAEL of 3000 ppm (equivalent to 212 mg/kg bw/d) was set.

Prenatal development toxicity studies

Rat

In the rat study 0, 10, 100 or 1000 mg/kg bw/d of fludioxonil was applied via gavage on gestation days 6-15. Maternal toxicity was found in terms of lower food consumption (10% vs control) and decreased main bodyweight gain (21% compared to control). Furthermore, macroscopic examination revealed one female with a pale liver. No treatment-related effects were noted on the numbers of implantation sites, pre-implantation loss or early or late resorptions.

Effects on development were observed in terms of significantly lower incidences of skeletal variations and anomalies in the treated groups. These findings are considered to represent normal biological variation and were therefore not considered to be of toxicological relevance.

Further effects on development were limited to higher incidences of foetuses with dilatation of the ureter (5.6 % vs 3.1% control) and/or renal pelvis (4% vs 0.8%) at the highest dose of 1000 mg/kg bw/d. The incidence was slightly but not statistically significant increased. These findings were considered incidental, since the foetal incidences in this group (5.6% and 4%) were within the laboratory's historical control range (0.6-7.5%). It is noted, that concurrent control incidences (dilated ureter: 3.1, deleted renal pelvis: 0.8) for these findings were at the lower end of the historical range. RAC notes, that the validity of the HCD could not be confirmed by the DS with respect to strain, time interval and laboratory. Industry provided an explanatory comment in relation to HCD, presumably by the conducting laboratory, that same strains have been used in the same laboratory.

The DS concluded that the incidences of litters having foetuses with foetal soft tissue abnormalities (ureteral and/or pelvic dilations) were incidental and within the HCD of the laboratory. A NOAEL for developmental toxicity was set at 1000 mg/kg bw/d and for maternal toxicity at 100 mg/kg bw.

Rabbit

In the second developmental toxicity study, maternal toxicity was found in terms of reduced body weight in the two highest dose groups. A NOAEL for maternal toxicity was set at 100 ppm. No treatment-related effects were noted on the mean numbers of implantation sites, pre-implantation loss, early and late resorptions or on the incidences of external, skeletal or visceral abnormalities. A marginal difference in the sex ratio (F: 52%, M: 48%) of foetuses at 300 mg/kg bw/d was observed, but the toxicological significance of the finding is unclear, so the change in sex ratio is considered incidental. Furthermore, no differences in sex ratios were observed in the developmental rat study or in the two generation toxicity study. The NOAEL for developmental toxicity is 300 mg/kg bw/d, since no treatment related foetotoxic effects have been observed at the highest dose.

Comments received during public consultation

One MSCA commented that further details on the validity of the HCD in relation to the visceral anomalies (foetal and litter incidences of dilated renal pelvis and dilated ureter) need to be checked. According to the DS and information from industry, it seems plausible that the same laboratory and strain have been used. It needs to be noted, that industry provided more information on HCD data after the PC. The information confirms the validity of considering the HCD for hazard assessment. Further data on visceral anomalies were also provided by industry.

Four MSCA and two experts from industry supported the DS proposal for no classification as a reproductive toxicant.

Assessment and comparison with the classification criteria

According to the CLP Regulation, reproductive toxicity includes adverse effects on sexual function and fertility in adult males and females, as well as developmental toxicity in the offspring.

Study outcomes demonstrate that there are no treatment related effects on fertility. Findings on development were limited to reduced skeletal variations and to visceral anomalies (dilated renal pelvis, dilated ureter) in the rat study at 1000 mg/kg bw/d. The visceral anomalies expressed in % foetal incidence were within the historical control range and there were no statistically significant differences between treated and control groups. Furthermore, at 1000 mg/kg bw/d maternal toxicity (reduced food consumption, body weight) needs to be considered. Further information on the HCD has been provided by industry at a late stage, i.e. after public consultation. The same strain at the same laboratory has been used, thus the validity of the HCD was confirmed.

RAC concludes, that the observed non-significant changes are not related to substance administration and were not severe enough to warrant classification.

Minor changes in the sex ratio have been observed in the rabbit study, which can be considered incidental.

In conclusion, RAC agrees with the DS that **no classification for reproductive toxicity is warranted**.

ENVIRONMENTAL HAZARD EVALUATION

RAC evaluation of aquatic hazards (acute and chronic)

Summary of the Dossier Submitter's proposal

Degradation

A hydrolysis study conducted according to U.S. EPA, Pesticide Assessment Guidelines, Subdivision N, Section 161-1 and in compliance with GLP was run at pH 5, 7 and 9 at 25 °C for 30 days and at pH 1 and 13 at 70 °C for up to 10 days. No degradation and consequently no hydrolysis products were observed. Hence, fludioxonil is stable to hydrolysis at environmentally relevant pH values. Hydrolysis is therefore not expected to be a significant degradation route for fludioxonil. Additionally, hydrolysis was investigated for the major aquatic photoproduct CGA 339833. The results of the study, carried out according to OECD TG 111 and in compliance with GLP, show that CGA 339833 is stable to hydrolysis at most environmentally relevant pH values. DT_{50} at 25° C is extrapolated to be 597 days at pH 7 and 53 days at pH 9. At pH 9 and 50 °C, 75% of CGA 339833 degraded to M4, an unidentified hydrolysis product.

The photodegradation of radio-labelled fludioxonil in water at pH 7 was studied according to US EPA subdivision N, Section 161-2 guideline. The study, in compliance with GLP, was carried out at 25 °C with continuous artificial light. Fludioxonil is degraded with a first order half-life equivalent to 10 days of natural sunlight at latitude 30°N, assuming 12 hours of daylight. Three degradation products which account for > 10% were not identified in this study, but were identified in another study as CGA 339833, CGA 344623 and A5. Consequently, aquatic photolysis is considered to be an important transformation route for fludioxonil in the environment.

The ready biodegradability was investigated following the method described in the 92/69/EEC C.4-C (carbon dioxide evolution test) and in compliance with GLP. Fludioxonil was added to the non pre-adapted inoculum (activated sewage sludge from a municipal sewage treatment plant)

at a concentration of *ca* 27 mg/L (*ca* 16 mg ThOC/L) over a period of 29 days at 22 ± 2°C. The degree of biodegradation was 7% (mean) within 29 days, in contrast to the reference substance, sodium benzoate, which degraded to 93%. Consequently, fludioxonil is not considered readily biodegradable.

A water/sediment simulation study, carried out according to U.S. EPA, Pesticide Assessment Guidelines, Subdivision N, Section 162-4 and in compliance with GLP, was run for 177 days using two water/sediment systems (one from a pond near Tugbach, BL, Switzerland, and another from the river Rhine). Due to its high adsorptivity, fludioxonil rapidly disappeared from the water, showing half-life times of about 1 day and 2 days for the Pond and Rhine sediment/water systems, respectively. However, the analysis of water and sediment extracts showed that fludioxonil was very slowly degraded with estimated half-lives of about 700 and 450 days in Pond and Rhine systems, respectively, assuming first order kinetics for the degradation (1326 and 855 days at 12 °C, Arrhenius equation). Non-extractables were the major degradation products of the [^{14}C]-pyrrole labelled fludioxonil. A few minor metabolite fractions, accounting for 0.1 to 5% of the radioactivity applied, were observed in the sediment and water extracts, but they were not identified. Mineralisation to CO_2 accounted for less than 2% AR.

In aerobic soil degradation studies, carried out according to various methods in 8 different soils, fludioxonil was found to be slightly degradable with CO_2 (0.6-20.5% AR after 90 days at 20°C) and bound residues (2.4 - 19.4% AR after 90 days at 20°C) observed as the principal degradants 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. In an anaerobic test, performed according to U.S. EPA, Pesticide Assessment Guidelines, Subdivision N, Section 162-1 and 162-2, 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.

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

Bioaccumulation

Based on experimental data, fludioxonil (a surface active substance) has a measured log K_{ow} of 4.12 (method OECD 107, 25 °C). This value is not considered valid since it is obtained from the shake flask method that is not applicable to surfactants.

In the bioaccumulation study, carried out according to U.S. EPA, Pesticide Assessment Guidelines, Subdivision N, Section 165-4 and in compliance with GLP, bluegill sunfish (*Lepomis Macrochirus*) were continuously exposed to radio-labelled fludioxonil at a concentration of 0.01 mg/L for 28 days in a dynamic flow-through system and thereafter the depuration of radioactivity followed in untreated water for 14 days. 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 the transfer of fish to fresh water.

Based on this information, the DS concluded that fludioxonil has a low potential for bioaccumulation.

Aquatic toxicity

In the following table, the results of the provided ecotoxicology tests from acute and chronic studies for three trophic levels are summarised.

Test organism / guideline, test method	Short-term result	Long-term result	Reference
Toxicity to fish			
Oncorhynchus mykiss US EPA 72-1 Flow-through	96h LC_{50} = 0.47 mg/L (meas)	-	Holmes and Swigert, 1993a
Oncorhynchus mykiss US EPA 72-1 Flow-through	96h LC_{50} = 0.23 mg/L (meas)	-	Biever, 1997a
Lepomis macrochirus US EPA 72-1 Flow-through	96h LC_{50} = 0.74 mg./L (meas)	-	Biever, 1997b
Cyprinodon variegatus (Marine) US EPA 72-1 Flow-through	96h LC_{50} = 1.2 mg/L (meas)	-	Holmes and Swigert, 1993b
Oncorhynchus mykiss OECD TG 215 Flow-through	-	28 day NOEC (growth rate) = 0.04 mg/L (meas)	Maynard, 2005
Pimephales promelas US EPA FIRFA 540/9-82-024, US EPA-OPP 540/9-86-138, ASTM 1241-88 (OECD TG 210) Flow-through	-	28 day early life stage NOEC = 0.039 mg/L (meas)	Graves *et al.*, 1994
Toxicity to aquatic invertebrates			
Daphnia magna US EPA 72-2 Flow-through	48h EC_{50} = 0.40 mg/L (meas)	-	Surprenant, 1990
Daphnia magna US EPA 72-2 Flow-through	48h EC_{50} = 0.90 mg/L (meas)	-	Holmes and Swigert, 1993c
Daphnia magna US EPA 5401-85-024, OECD TG 211 Flow-through	-	21 day reproduction NOEC = 0.019 mg/L (meas)	Putt, 1991
Daphnia magna OECD TG 211 Semi-static	-	21 day reproduction NOEC = 0.035 mg/L (meas)	Fournier, 2014
Daphnia magna OECD TG 202 (Part II) Semi-static	-	21 day reproduction NOEC = 0.005 mg/L* (meas)	Rufli, 1989c
Toxicity to algae and aquatic plants			
Desmodesmus subspicatus OECD TG 201 Static	48h E_rC_{50} > 0.926 mg/L (meas)	48h E_rC_{10} = 0.09 mg/L (meas)	Rufli, 1989a
Pseudokirchneriella subcapitata FIFRA 122-2 & 123-2 Static	48h E_rC_{50} = 0.21 mg/L (meas)	48h NOEC = 0.027 mg/L (meas)	Hoberg, 1992, 2005

*study result not considered to be reliable by DS

The studies are considered to be acceptable and valid. For acute toxicity, the most sensible specie was found to be the algae *Pseudokirchneriella subcapitata*, with an EC_{50} in the range 0.1-1 mg/L.

The lowest NOEC was obtained in a chronic test with *Daphnia magna*, the value was between 0.01 and 0.1 mg/L.

Comments received during public consultation

Regarding bioaccumulation in fish, one MSCA asked if the presented BCF was lipid normalised. The DS stated that there has been no correction/normalisation of BCF according to fish with 5% lipid content as it is found that the tested fish *Lepomis macrochirus* has a lipid concentration that do not deviate significantly from the recommended 5%. However, the actual lipid concentration of the tested fish was not reported.

Eight comments were received on environmental classification: 6 expressed agreement with the proposal, albeit noting the lack of detailed information, the other two are discussed below. An MSCA required further information to confirm appropriate M factor for the Aquatic Chronic classification, providing detailed comments on specific endpoints. Another MSCA did not support the chronic M-factor of 1 and it proposed M factor = 10 for aquatic chronic classification, based on the 21d NOEC = 0.005 mg/L with Daphnia (Rufli, 1989c). RAC and DS provided a different opinion on the validity of this chronic test. The test was performed according to the OECD TG 202, part II, the results were based on survival of juveniles per parent and time to first brood. Taking into account the available information reported in the summary of the study, RAC considered the study acceptable and the results reliable.

Regarding the toxicity to algae, two MSCA commented on the duration endpoints related to pH drift. Additional information are required to justify the proposed non-standards duration endpoints (48h instead of standard 72h). According to the DS, pH drift is too high after 72 and 120 hours and therefore 48h is the more suitable endpoint with exponential growth occurring at this time.

Assessment and comparison with the classification criteria

Degradation

RAC agrees with the DS proposal to consider fludioxonil as not rapidly degradable. The substance is hydrolytically stable under acidic, neutral and alkaline conditions and it is not readily biodegradable. In addition, fludioxonil is demonstrated to be very persistent in water/sediment and soil simulation tests. Although the study of aqueous photolysis suggests that fludioxonil undergoes photodegradation, the actual degree of photodegradation in the aquatic environment is uncertain and not relevant for classification purposes.

Bioaccumulation

The measured BCF of 366 L/kg is below the CLP criterion (BCF ≥ 500). Therefore, RAC agrees with the DS proposal to consider that the bioaccumulation potential of fludioxonil is low.

Aquatic toxicity

Acute toxicity data are available for three trophic levels. All the acute endpoints are in the range of 0.1 < L(E)C_{50} ≤ 1 mg/L. According to the DS, the lowest value is from a 48h E_rC_{50} for algae *Pseudokirchneriella subcapitata* of 0.21 mg/L.

According to RAC, the pH drift from 0h to 120h for the Hoberg (1992) study does not justify the deviation from standard 72h E_rC_{50} endpoint.

In the following table from the Addendum 1 to DAR (October 2006) and from further information provided by DS, values at 48, 72, 96 and 120h are reported for the relevant endpoint E_rC_{50} and NOE_rC

Table: Growth inhibition of *Selenastrum capricornutum* by fludioxonil (Hoberg, 1992, 2005)

Time interval	E_rC_{50} (mg/L) [95% conf. int.] Based on mean measured conc.*	NOEC
0-48	0.21 [0.074-0.46]	0.027[a]
0-72	0.41 [0.32-0.48]	0.014[b]
0-96	0.31 [0.30-0.33]	0.014[b]
0-120	0.33 [0.32-0.34]	0.014[b]

[a] The NOEC was determined by Williams' Test.
[b] Kruskal-Walls' Test did not determine a reasonable NOEC. Therefore, the NOEC was empirically estimated to be the highest concentration with less than 10% inhibition.

The 72h EC_{50} = 0.41 mg/L is considered the suitable endpoint by RAC, therefore the lowest 96h LC_{50} = 0.23 mg/L (meas) value results from the fish *Oncorhynchus mykiss* (Biever, 1997a). However the classification proposed by DS is not affected.

Chronic toxicity data are available for all three trophic levels. The lowest chronic endpoint is reported for invertebrates with a 21d NOEC value for *Daphnia magna* of 0.005 mg/L. This chronic endpoint is in the range of 0.001 < NOEC ≤ 0.01 mg/L.

Conclusion on the classification

Fludioxonil is considered not rapidly degradable and has a low potential for bioaccumulation. The lowest acute toxicity value falls in the range of 0.1 < $L(E)C_{50}$ ≤ 1 mg/L mg/L and the lowest chronic toxicity value lies in the toxicity range of 0.001 < NOEC ≤ 0.01 mg/L.

RAC concludes that fludioxonil fulfils the CLP criteria for classification as **Aquatic Acute 1 - H400** with an **M-factor of 1** and **Aquatic Chronic 1 – H410** with an **M-factor of 10**.

ANNEXES:

Annex 1 The Background Document (BD) gives the detailed scientific grounds for the opinion. The BD is based on the CLH report prepared by the Dossier Submitter; the evaluation performed by RAC is contained in 'RAC boxes'.

Annex 2 Comments received on the CLH report, response to comments provided by the Dossier Submitter and RAC (excluding confidential information).