

FLUDIOXONIL

First draft prepared by

A. Bartholomaeus

Therapeutic Goods Administration, Canberra, Australia

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Explanation

Fludioxonil is the International Organization for Standardization (ISO) approved name for a new phenylpyrrole fungicide, 4-(2,2-difluoro-1,3-benzodioxol-4-yl)pyrrole-3-carbonitrile (International Union of Pure and Applied Chemistry, IUPAC), that interferes with glucose transport across fungal membranes. Fludioxonil has not been evaluated previously by the JMPR.

Evaluation for acceptable daily intake

Unless otherwise stated, the studies evaluated in this monograph were performed by laboratories that were certified for good laboratory practice (GLP) and complied with the relevant Organisation for Economic Co-operation and Development (OECD) test guideline(s) or similar guidelines of the European Union or United States Environmental Protection Agency. As these guidelines specify the tissues normally examined and the clinical pathology tests normally performed, only significant exceptions to these guidelines are reported here, to avoid repetitive listing of study parameters.

1. Biochemical aspects

1.1 Absorption, distribution and excretion

Rats were treated by gavage with [¹⁴C]pyrrole fludioxonil (purity, 99%), which was supplemented with unlabelled fludioxonil (purity, 99%) at the highest dose. Groups of five male and five female rats were given a single dose of [¹⁴C]fludioxonil at 0.5 mg/kg bw, or

Figure 1. Chemical structure of fludioxonil

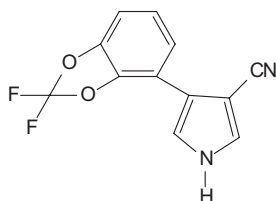


Table 1. Excretion of radiolabel (% of administered dose) in rats 7 days after oral treatment with fludioxonil

Excreta	Dose					
	0.5 mg/kg bw, single dose		0.5 mg/kg bw per day, for 14 days		100 mg/kg bw, single dose	
	Males	Females	Males	Females	Males	Females
Urine	16.2	16.9	13.4	14.6	16.8	19.5
Faeces	81.2	79.1	82.8	81.5	77.6	77.6
Air	—	—	—	—	<0.01	<0.01

From Bissig (1990)

were pretreated with unlabelled fludioxonil for 14 days and then given a single dose of [^{14}C]fludioxonil at 0.5 or 100 mg/kg bw. Urine and faeces and, at 100 mg/kg bw, expired air were collected over the subsequent 7 days, after which tissues were analysed for radiolabel. An additional group of three females was treated with [^{14}C]fludioxonil at 0.5 mg/kg bw and blood was collected over the subsequent 48 h. An additional group of 10 females was treated with [^{14}C]fludioxonil at 0.5 mg/kg bw and tissues were collected after either 0.5 h (t_{cmax}) or 9 h ($t_{\text{cmax}/2}$). Another group of five bile-duct cannulated females was treated with [^{14}C]fludioxonil at 100 mg/kg bw and urine, faeces and bile were collected over the next 48 h. In bile-duct cannulated rats, approximately 67% of the administered dose was excreted in the bile, 10% in the urine and 14% in the faeces over the first 48 h, with at least 77% of the administered dose being absorbed from the intestinal tract. In intact animals, faecal excretion was of the order of 80% of the administered dose. Blood kinetic investigations demonstrated rapid absorption of an oral dose of 0.5 mg/kg bw from the intestinal tract into the general circulation, with maximum blood concentration (t_{cmax}) reached within 0.5 h, declining to half this concentration after 9 h ($t_{\text{cmax}/2}$). Rate and route of excretion were independent of both sex and dose. Approximately 96% of the administered dose was excreted within 7 days. Most of this was excreted within the first 24 h. Seven days after a single oral dose of [^{14}C]fludioxonil at 0.5 mg/kg bw, in rats that had or had not been pretreated with 14 daily oral doses of unlabelled fludioxonil at 0.5 mg/kg bw per day, concentrations of radiolabel in most tissues were below the limit of detection. Fludioxonil equivalents were detected in the liver (0.002 ppm), kidneys (0.004 ppm), blood (0.0007 ppm) and lungs (0.0012 ppm). After a dose of 100 mg/kg bw, tissue residues other than in the liver, kidneys, blood and lungs were low, but concentrations in the tissues of females were generally twice those found in males. The total amount of radioactivity recovered in tissues was approximately 0.17% of the administered dose. Elimination of radiolabelled fludioxonil from tissues ($t_{1/2}$) was biphasic; between 2 and 6 h for phase 1 and between 30 and 60 h for phase 2 (Bissig, 1990).

Groups of three rats were treated with single doses of ^{14}C -labelled fludioxonil at 0.5 or 100 mg/kg bw by gavage and blood was collected for 48 h. Additional groups were treated

at the same doses and sacrificed for analysis of tissues at t_{cmax} , $t_{\text{cmax}/2}$ and two other time-points. At 0.5 mg/kg bw, maximum blood concentrations (fludioxonil equivalents: males, 0.065 mg/l; and females, 0.027 mg/l) were achieved after 15 min. Biphasic elimination was observed, with a rapid first phase and slow second phase. Blood concentrations declined to half C_{max} within 1 h. At 100 mg/kg bw, maximum blood concentrations (fludioxonil equivalents: males, 4.5 mg/l; and females, 3.2 mg/l) were achieved between 4 and 8 h. Concentrations again declined in a biphasic manner reaching half C_{max} within a further 8 h. The area under the curve of concentration–time (AUC) was approximately proportional to the dose administered and was similar in males and females. Thus, the proportion of a dose absorbed was independent of both sex and dose. At 0.5 mg/kg bw at t_{cmax} , all residues contained fludioxonil equivalents at ≤ 1 ppm and most were < 0.1 ppm, except in the liver, kidneys, lungs and plasma in all animals and blood and heart in females. The distribution pattern was similar at the highest and lowest doses. At the highest dose, residues were < 13 mg of fludioxonil equivalents per kg at t_{cmax} . Tissue depletion kinetics were biphasic for both doses. As fludioxonil is rapidly cleared from both the blood and the tissues, there appears to be little potential for bioaccumulation of either unchanged fludioxonil or its metabolites (Müller & Thanei, 1995).

Fludioxonil (purity, 97%) labelled with ^{14}C in the phenyl ring and unlabelled fludioxonil (purity, 99.9%), in a wettable granule formulation (62.5 g/kg), diluted in water, was applied to the shaved skin (10 cm^2) of male rats (body weight, approximately 250 g) at a concentration of $4.5\text{ }\mu\text{g/cm}^2$ (reflecting a typical concentration recommended for application in the field) or at $560\text{ }\mu\text{g/cm}^2$ for an exposure period of 6 h. After exposure, the application site was washed three times with a mild soap solution. The depletion of the remaining radioactivity at the application site, after washing, was determined at 24 and 48 h after application. Urine and faeces were collected from animals individually at 0–6, 6–24 and 24–48 h after application. For four animals at each dose, blood was collected at 0.5, 1, 2, 4, 6, 8, 24 h and 48 h after application by cutting the tip of the tail. At sacrifice, the amount of radiolabel was measured in the blood, urine, faeces, cage wash, skin wash, at the application site, in control skin, extracts of the cover and residual carcass. At $4.5\text{ }\mu\text{g/cm}^2$, blood radioactivity was below the limit of detection at all time-points and absorption was low, reaching 5.1% at 24 h and 7.4% of the applied dose at 48 h after application. Elimination was mainly in the faeces (3.78% and 5.78% at 24 h and 48 h, respectively) and to a lesser degree in the urine (0.69% and 1.01%, respectively). At $560\text{ }\mu\text{g/cm}^2$, the concentration of radiolabel in the blood reached a maximum of 0.017 mg of fludioxonil equivalents/l at 0.5 h after application and decreased rapidly thereafter to below the limit of detection at 1 h and all following sampling points. Absorption was lower than that at the lowest dose, 0.32% of the applied dose after 24 h and 0.61% after 48 h, reflecting a saturation of the rate of dermal flux. The bulk of the applied doses (76.5–90.5%) was removed with the skin wash at 6 h after application. The amount of fludioxonil remaining in the skin at 6, 24 and 48 h was almost constant (6.0, 8.5 and 5.7% at $4.5\text{ }\mu\text{g/cm}^2$; and 3.1, 4.3 and 2.7% at $560\text{ }\mu\text{g/cm}^2$) indicating little mobility of fludioxonil in the skin depot. Assuming that a steady state for percutaneous penetration is rapidly attained and maintained during the first 6 h after application, the flux rate at $560\text{ }\mu\text{g/cm}^2$ ($0.0774\text{ }\mu\text{g/cm}^2 \cdot \text{per h}$) was more than three times that at $4.5\text{ }\mu\text{g/cm}^2$ ($0.0284\text{ }\mu\text{g/cm}^2 \cdot \text{per h}$), despite the dose being 120-fold greater, indicating saturation of the rate of dermal flux at the highest dose. Although a proportion of the fludioxonil remaining in the skin 24 h after application is available for subsequent movement into the systemic circulation, for human risk assessment purposes the appropriate dose metric is the amount reaching the systemic circulation during 24 h, as it is this amount that can reasonably be compared with appropriate no-observed-adverse-effect levels (NOAELs).

Table 2. *In vivo* percutaneous absorption/excretion of radiolabel (% of applied dose) in rats treated with fludioxonil

Dose Sacrifice time (h)	4.5 µg/cm ²			560 µg/cm ²		
	6	24	48	6	24	48
Total excretion (urine, faeces, cage wash)	0.21	4.5	6.84	0.01	0.18	0.49
Residues (whole blood, non-treated skin, carcass)	3.41	0.64	0.53	0.07	0.14	0.12
Total systemic absorption	3.6	5.1	7.4	0.08	0.32	0.6
Treated skin	6.0	8.5	5.7	3.1	4.33	2.7

From Mewes (1999)

On this basis the dermal absorption factors for fludioxonil are approximately 5 and 0.5% of the applied dose at exposures of 4.5 and 560 µg/cm² respectively (Mewes, 1999).

Fludioxonil (purity, 99%) labelled with ¹⁴C in the phenyl ring and unlabelled fludioxonil (99.8%), in a wettable granule formulation (62.5 g/kg), diluted in water, was applied at a concentration of 3.6 µg/cm² to rat skin or at a concentration of 3.4 µg/cm² to human skin (these values reflecting a typical concentration recommended for application in the field—300 g of active ingredient/800 l per 10 000 m²) and at 690 µg/cm² to rat skin and at 720 µg/cm² human skin for 6 h, without occlusion. Rat epidermis was prepared from male rats aged approximately 23 days, and human cadaver abdominal epidermal skin was obtained from Caucasian donors (one male, aged 68 years, and one female, aged 86 years). Circles of epidermal membranes (approximately 1.8 cm in diameter) were cut and mounted in the diffusion cells between the donor and receptor chamber. The receptor fluid was ethanol/water (1:1 v/v) delivered at a flow rate of about 3 ml/h. Perfusates were collected hourly for the first 6 h after application, then every 2 h until 48 h. The penetration of fludioxonil through human skin was markedly lower than through rat skin (1% to 2% of the values in rats).

For rat skin, the flux, which reflects the rate of penetration under steady-state conditions, amounted to 0.075 µg/cm²/h at the lowest dose (steady-state conditions from about 1 h to 19 h). At the highest dose, the flux was 0.52 µg/cm² per h (steady-state conditions from about 1 h to 48 h). With the human epidermis, the majority of the individual values determined in the perfusate were below the limit of determination (LQ). However, based on the determined values, 0.2% and 0.01% of the applied dose penetrated the epidermis at the lowest and highest dose within 12 h, corresponding to 0.01 and 0.08 µg/cm². The total penetration at the two doses was 0.5% and 0.02% of the applied dose within 24 h. Since the individual values were mainly below the limit of determination, the flux constants were calculated based on the corresponding LQ values accounting for 0.0007 and 0.008 µg/cm² per h at the lowest and highest dose, respectively (Hassler, 1999).

1.2 Biotransformation

Pooled urine, bile and faeces collected from animals given ¹⁴C-labelled fludioxonil in a previous kinetic study (Bissig, 1990) were used to investigate the metabolic transformation of fludioxonil. No unchanged fludioxonil was detected in the urine or bile. Seven metabolites were detected in the bile. Four metabolites were separated and identified. Two of the metabolites were glucuronic acid derivatives of fludioxonil, with one of these being the major metabolite (56% of the administered dose). Twenty metabolites were detected in

Table 3. Penetration of rat and human epidermis by fludioxonil

	% of administered dose	Flux ($\mu\text{g}/\text{cm}^2$)	% of administered dose	Flux ($\mu\text{g}/\text{cm}^2$)
Rat epidermis				
Dose administered	3.6 $\mu\text{g}/\text{cm}^2$		687 $\mu\text{g}/\text{cm}^2$	
12 h	23.91 ^a	0.86 ^a	0.69	4.72
24 h	37.67 ^a	1.35 ^a	1.35	9.72
48 h	50.73 ^a	1.82 ^a	2.69	18.47
Flux rate ($\mu\text{g}/\text{cm}^2$ per h)	0.075		0.518	
Human epidermis				
Dose administered	3.4 $\mu\text{g}/\text{cm}^2$		718 $\mu\text{g}/\text{cm}^2$	
12 h	0.24 ^b	0.01 ^b	0.01 ^b	0.08 ^b
24 h	0.50 ^b	0.02 ^b	0.02 ^b	0.16 ^b
48 h	1.03 ^b	0.03 ^b	0.05 ^b	0.33 ^b
Flux rate ($\mu\text{g}/\text{cm}^2$ per h)	0.0007 ^c		0.008 ^c	

From Hassler (1999)

^aThese values were calculated based on an interpolation of the corresponding mean values of 11–13 h and 23–25 h and an extrapolation of the values at 47 h

^bMost of the individual values are below the limit of determination

^cFlux calculated based on the corresponding LQ values

Table 4. Metabolites in rats given a single oral dose of fludioxonil at 100 mg/kg bw

Metabolite	Chemical name	% of administered dose (urine, faeces, and bile) ^a
SYN 518577	4-(2,2-difluoro-benzo[1,3]dioxol-4-yl)-2-hydroxy-1H-pyrrole-3-carbonitrile	0
Met 1G (SYN518577 glucuronyl conjugate)	2- β -D-glucuronyl-4-(2,2-difluoro-benzo[1,3]dioxol-4-yl)-1H-pyrrole-3-carbonitrile	56.0 (0.5, 0, 55.5)
Met 2G (SYN518577 sulfate conjugate)	4-(2,2-difluoro-benzo[1,3]dioxol-4-yl)-1H-pyrrole-3-carbonitrile-2-hydrogen sulfate	<0.7 (<0.5, 0, <0.2)
SYN 518578	4-(2,2-difluoro-benzo[1,3]dioxol-4-yl)-5-hydroxy-1H-pyrrole-3-carbonitrile	0
MET 3G (SYN 518578 glucuronyl conjugate)	4-(2,2-difluoro-benzo[1,3]dioxol-4-yl)-5- β -D-glucuronyl-1H-pyrrole-3-carbonitrile	2.8 (0.7, 0, 2.1)
MET 1U (SYN 518578 sulfate conjugate)	4-(2,2-difluoro-benzo[1,3]dioxol-4-yl)-1H-pyrrole-3-carbonitrile-5-hydrogen sulfate	1.1 (1.1, 0, 0)
SYN 518576	4-(2,2-difluoro-7-hydroxy-benzo[1,3]dioxol-4-yl)-1H-pyrrole-3-carbonitrile	0
MET 4G (SYN 518576 glucuronyl conjugate)	4-(2,2-difluoro-7- β -D-glucuronyl-benzo[1,3]dioxol-4-yl)-1H-pyrrole-3-carbonitrile	2.2 (0.5, 0, 1.7)
Fludioxonil (unchanged)		11.6 (0, 11.6, 0)

From Thanei (1992)

^aPercentage of the administered dose found in all samples combined; in parentheses, % of the administered dose that was found in the urine, faeces, and bile, respectively

the urine; four of these were identical to metabolites in the bile. The predominant radio-labelled substance in the faeces (2–11% of the administered dose) was unchanged fludioxonil. The metabolism of fludioxonil was extensive, involving primarily oxidation of the pyrrole ring leading to a major (57% of the administered dose) and a minor (4% of the administered dose) oxo-pyrrole metabolite; hydroxylation of the phenyl ring yielded the corresponding phenol metabolite, representing 2% of the administered dose. These phase I metabolites were subsequently excreted as glucuronyl- and sulfate conjugates, and, together with unabsorbed and unchanged fludioxonil excreted in the faeces, accounted for approximately 75% of the administered dose. Investigation of a blue metabolite of fludioxonil is discussed further in section 2.6 (Thanei, 1992).

Table 5. Distribution of metabolites in rats given a single oral dose of fludioxonil at 100 mg/kg bw

Metabolite ^a	Fludioxonil equivalents (% of administered dose)			
	Urine	Faeces	Bile	Total
MET 1G	0.5	—	55.5	56.0
MET 2G	<0.5	—	<0.2	<0.7
<i>Total conjugates of SYN 518577</i>				about 56.7
MET 3G	0.7	—	2.1	2.8
MET 1U	1.1	—	—	1.1
<i>Total conjugates of SYN 518578</i>				3.9
MET 4G (conjugate of SYN 518576)	0.5	—	1.7	2.2
CGA 173506 (fludioxonil)	—	11.6	—	11.6
Coloured	0.4	—	—	0.4
Total	3.2–3.7	11.6	59.3–59.5	74.1–74.8

From Thanei (1992)

^aSee Figure 2 for structures associated with the codes for metabolites in this table

Table 6. Acute toxicity of fludioxonil

Species ^b	Strain	Route	LD ₅₀ (mg/kg bw)/LC ₅₀ (mg/l)	Reference
Mouse	Hsd:ICR	Oral	>5000; no deaths	Glaza (1991c)
Rat	Hsd:Sprague-Dawley	Oral	>5000; no deaths	Glaza (1991a)
Rat	Tif:RAIf	Dermal	>2000; no deaths	Hartmann (1988)
Rat	Tif:RAIf	Inhalation	>2.6; no deaths ^a	Hartmann (1989)
Rat	Hsd:Sprague-Dawley	Inhalation	>0.54; no deaths	Holbert (1992)

^aPiloerection, hunched posture and dyspnoea were noticed for up to 7 days

^bAll values are for both sexes combined

2. Toxicological studies

2.1 Acute toxicity

(a) Lethal doses

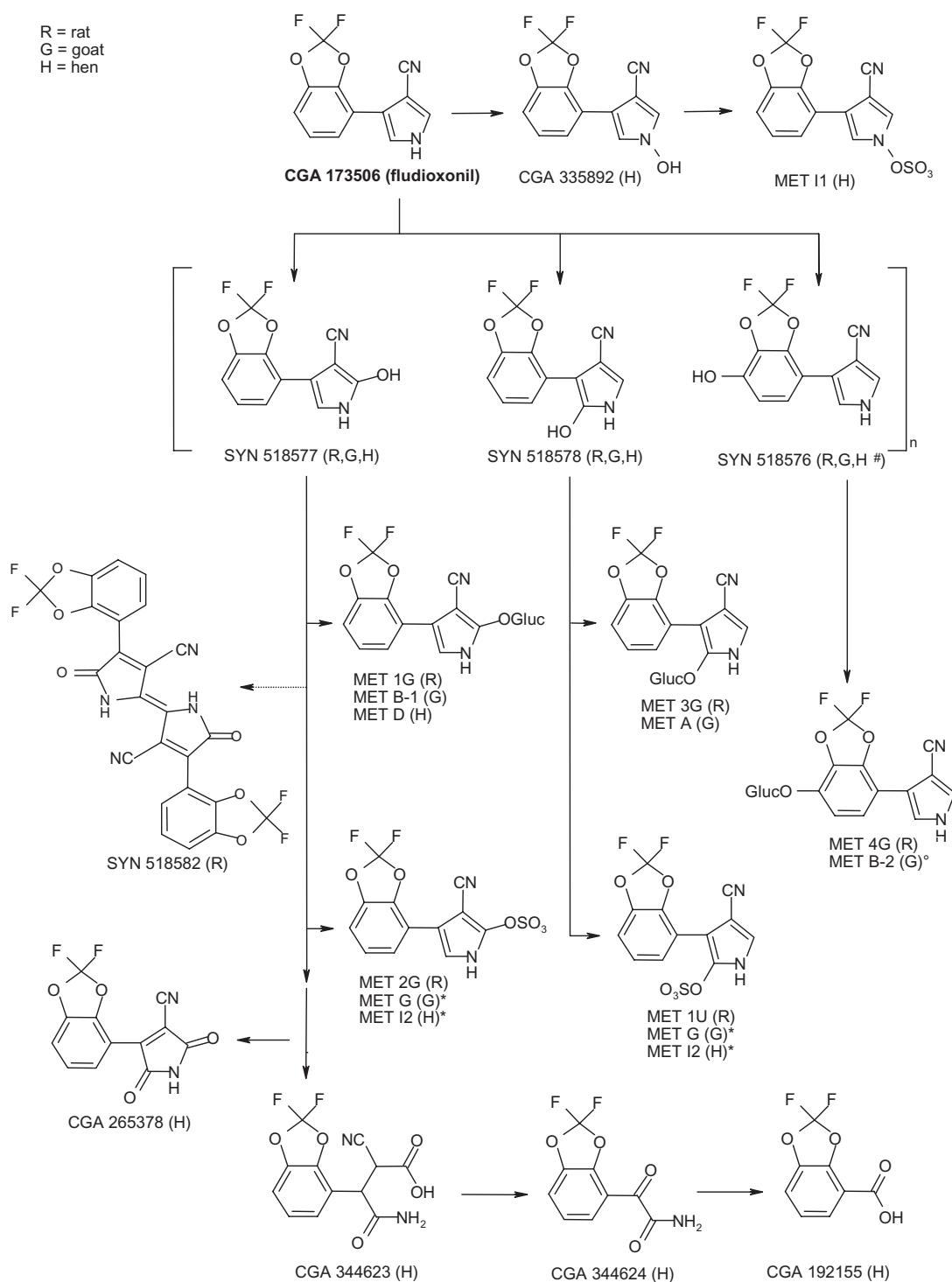
Table 6 summarizes the results of studies to establish the acute toxicity of fludioxonil.

(b) Dermal and ocular irritation and dermal sensitization

In two studies, New Zealand white rabbits were exposed dermally to 0.5 g of fludioxonil (purity, 97.5%) under a semi-occlusive dressing moistened with water or 0.9% saline. No deaths occurred, and no effects on body weights or clinical signs were noted. In the first study, erythema (grade 1) that persisted for up to 48 h was noted in two animals. Grade 1 oedema was also noted in one animal 1 h after removing the bandage. The mean irritation score for 24 to 48 h was 0.66 (Schneider, 1988a). In the second study there were no signs of erythema or oedema (Glaza, 1991b). Fludioxonil is not an irritant to rabbit skin.

Fludioxonil (purity, 97.5%), 0.1 ml, was placed in the left eye of three female rabbits. The lids were held closed for 1 s. The right eye remained untreated and served as a control. Slight conjunctival redness was seen in three animals at 1 h; this had regressed in one animal by 24 h, and by 48 h in the other two animals. Chemosis (grade 1) of the conjunctiva was noted in one animal at 1 h, clearing by 24 h. No effects on the cornea or iris were noted. Fludioxonil was at most a slight eye irritant in female rabbits (Schneider, 1988b).

Figure 2. Comparative metabolism of fludioxonil in rats, goats and hens



* no differentiation was made in hens and goats concerning 2-or 5-position of the conjugate

isolated in hens; intermediate in rats and goats

° tentative identification in goats

Fludioxonil (95.4%, 0.1 ml) was placed in the left eye of New Zealand white rabbits and the lids were held closed for 1 s. Three rabbits had the test materials flushed from the treated eye with lukewarm tap water for 60 s, 30 s after exposure. The right eye remained untreated and served as a control. No corneal damage was observed. In unwashed eyes, iridial irritation (grade 1) was seen at 1 h, continuing for up to 48 h, conjunctival redness (grade 2–3) was seen at 1 h decreasing in severity over the next 72 h and chemosis (grade 1–3) was noted in all eyes at 1 h decreasing in severity over the next 48 h. A clear discharge of the conjunctivae (grade 2) was noted in one animal at 1 h. In the washed eyes, irritation of the iris was noted in one eye 1 h after exposure (grade 1), conjunctival redness (grade 2) in all animals at 1 h and in two animals at 48 h, and chemosis (grade 1) in all eyes at 1 h persisting in one animal for 24 h. Fludioxonil caused slight ocular irritation in rabbits. Washing the eye immediately with water reduced the severity of the irritation. Average irritation scores for unwashed/washed eyes at 1, 24, 48, 72 and 96 h respectively were; 13.7/7.7, 6.8/2.7, 3.7/1.3, 0.7/0, 0/0 (Glaza, 1991d).

In a Magnusson & Kligman maximization assay, Pirbright white guinea-pigs were given 0.1 ml of fludioxonil (purity, 97.5%) by intradermal injection into the shaved neck. One week later, a second induction dose was applied topically by incorporation in vaseline onto a patch and placed on the neck. The exposed region was covered with an occlusive dressing for 48 h. On the day before this treatment, the application sites were pretreated with 10% sodium lauryl sulfate. Two weeks later, fludioxonil in vaseline was applied under an occlusive dressing to the flank for 24 h. Control animals were treated with the vehicle only during the induction period and exposed to fludioxonil in the challenge phase. The doses of fludioxonil used were: intradermal induction, 1%; epidermal induction, 30%; epidermal challenge, 10%. Skin reactions were evaluated after the induction doses and 24 and 48 h after removing the challenge dressing. Body weights were recorded at the start and end of the study. Food, water and the housing environment were controlled and monitored. No signs of irritation were noted for control or test animals after the induction period. No signs of irritation were noted after the challenge dose for either control or test animals. There was no apparent affect on body weight. Fludioxonil did not cause skin sensitization in guinea-pigs (Schneider, 1988c).

2.2 *Short-term studies of toxicity*

Mice

Groups of 10 male and 10 female CD-1 mice were fed diets containing fludioxonil (purity, 96%) at a concentration of 0, 10, 100, 1000, 3000 or 7000 ppm for 90 days. Actual fludioxonil intakes were 0, 1.3, 14, 140, 450 or 1050 mg/kg bw per day for males and 0, 1.9, 17, 180, 560 or 1310 mg/kg bw per day. Food, water and the housing environment were controlled and monitored. The amount of fludioxonil in the diet was analysed regularly. Toxicity was evaluated through: mortality, daily clinical observation, body weight and food consumption measurements, ophthalmology, haematology (not including mean cell haemoglobin concentration or prothrombin time), serum biochemistry (including measurements of direct bilirubin, 5' nucleotidase and sorbital dehydrogenase activity), urine analysis, organ weights, gross pathology and histopathology (including thigh muscle).

There were no deaths and no treatment-related ophthalmological changes. Haematology parameters were unaffected and clinical signs were limited to discoloured urine and blue staining of the pelvic region at 1000, 3000 and 7000 ppm in males. Significantly lower body-weight gains were noted in females at 7000 ppm (73% of control) throughout the study.

Table 7. Key findings in a 90-day study in mice given diets containing fludioxonil

Finding	Sex (historical control range)	Dietary concentration (ppm)					
		0	10	100	1000	3000	7000
<i>Serum biochemistry</i>							
Potassium (mg/dl)	Males (3.8–5.1)	4.8	4.6	4.5	4.5	4.4	4.7
	Females (3.7–5.3)	4.8	4.6	4.7	4.7	4.5	3.9**
5′Nucleotidases (IU/l)	Males (10–21)	14	15	13	13	15	18**
	Females (25–53)	33	33	31	35	39	50**
<i>Organ weights</i>							
Liver, absolute (g)	Males	1.455	1.446	1.387	1.483	1.437	1.613
	Females	1.225	1.172	1.217	1.245	1.324	1.375*
Liver, relative to body weight	Males	4.044	3.890	3.981	4.106	4.205	4.745*
	Females	4.166	4.178	4.074	4.283	4.749*	5.065*
Thymus, absolute (g)	Males	0.023	0.027	0.023	0.023	0.019	0.022
	Females	0.033	0.027	0.032	0.027	0.028	0.022*
Thymus, relative to brain weight	Males	4.730	5.670	4.712	4.544	3.802	4.503
	Females	6.524	5.371	6.461	5.449	5.484	4.474*
<i>Pathology</i>							
Kidneys, nephropathy	Males	2	2	2	2	1	10**
	Females	2	3	1	2	2	9**
Liver, hypertrophy	Males	0	0	0	0	0	7**
	Females	0	0	0	0	3	8**

From Chang & Morrissey (1990); Chang (1990b)

* $p < 0.05$, ** $p < 0.01$

Body weights in males and food intake in both sexes were unaffected. The concentration of 5'nucleotidase in serum was significantly increased in mice at 7000 ppm. Females exposed to fludioxonil at 7000 ppm had significantly reduced concentrations of potassium. These variations are within the range for historical controls. Discolouration of the urine, due to the presence of a coloured metabolite, and the presence of bilirubin was noted in males at 1000, 3000 and 7000 ppm and discolouration of the kidneys and digestive tract was noted in males and females at 7000 ppm. Liver weights, absolute and relative to body weight, were increased in mice of both sexes at 7000 ppm and correlated with centrilobular hypertrophy in females at 3000 ppm and in both sexes at 7000 ppm. In the absence of clinical chemistry reflecting liver damage, and as these liver changes are likely to reflect an adaptive response to the high xenobiotic load, it was concluded that they were not adverse effects. Thymus weights, both absolute and relative to brain weight, were significantly decreased in females at 7000 ppm. The incidence of chronic nephropathy was increased in mice of both sexes at 7000 ppm. The NOAEL for this study was 3000 ppm (450 mg/kg bw per day) on the basis of nephropathy and elevated serum 5'nucleotidase activity in both sexes and reduced weight gains in females at 7000 ppm (Chang & Morrissey, 1990; Chang, 1990b).

Rats

In a range-finding study, groups of six male and six female Sprague Dawley Crl: CD(SD)Br rats received diets containing fludioxonil (purity not reported) at a concentration of 0, 1000, 5000, 10 000 or 20 000 ppm (equal to 120, 620, 1300 or 2500 mg/kg bw per day for males and 130, 700, 1400 or 2800 mg/kg bw per day for females) for 20 days (males) or 21 days (females). The homogeneity and stability of fludioxonil in the diet was checked before the study. Mortality was checked twice per day on working days and once on weekends. Clinical signs were checked daily. Body weight, and food (three animals per group) and water consumption (three animals per group) were recorded weekly. At the end of treatment, all animals were subjected to the following examinations: haematology (in four to six animals of each group; reticulocyte counts were not performed, and differential leukocyte

count was carried out only for animals in the control group and at the highest dose), clinical chemistry (except phospholipids, bilirubin, creatinine, creatinine kinase), urine analysis (except specific gravity and examination of sediment). At sacrifice, the adrenals, brain, heart, kidneys, liver, spleen, thymus, ovary and testes were weighed and the adrenals, heart, kidneys, liver, spleen and thymus were examined histologically (for animals in the control group and at the highest dose; kidneys were also examined at the intermediate doses).

There were no mortalities. Clinical signs were limited to black faeces in animals treated with fludioxonil at ≥ 5000 ppm during the last week of treatment, which may have been due to the presence of the blue-coloured metabolite SYN 518582 formed by non-enzymatic autoxidation at high doses. This effect is not considered to be toxicologically relevant. In males at 20 000 ppm, body weights at the end of the treatment period were 88% of those of the controls, food consumption was 90% of that of the controls and water consumption was increased by approximately 20% (also at 10 000 ppm). Haematological and urine analysis parameters were unaffected by treatment, although individual males at 10 000 and 20 000 ppm had slightly lower urine volumes with a slightly increased specific gravity. A slight ($\leq 5\%$) but highly statistically significant reduction in concentrations of sodium and chloride in both sexes at ≥ 5000 ppm appears to be treatment related, and correlates with tubular nephrosis, although similar findings were not obtained in any other studies of repeated dose in rats, including the 28-day study, reported below, which used comparable doses. A dose-related increase in cholesterol levels was seen in both sexes, which was significant at 20 000 ppm only. A slight decrease in concentrations of glucose was seen in females at $\geq 10 000$ ppm. No toxicological or biological relevance was attributed to the variations in α -1 globulins in the males as a dose-response relationship was not apparent and a similar effect was not seen in females. Slight decreases in alkaline phosphatase and alanine aminotransferase activities, while likely to be treatment related, were discounted as not being adverse. Alkaline phosphatase and alanine transferase serum activities in rats are affected by nutritional status and are commonly found to be lower in rats gaining less weight and/or eating less food.

Increased absolute and relative weights of the kidney were observed at $\geq 10 000$ ppm and were associated with tubular nephrosis. In addition, paleness of the kidneys and blackish and punctiform foci were seen in one to two males at 10 000 and 20 000 ppm. A single male at 5000 ppm also had tubular nephrosis. Increased relative weights of the liver were observed at 10 000 and 20 000 ppm, but were not associated with any clinical chemistry or histopathological changes consistent with liver damage and were therefore considered to be likely to reflect an adaptive response. Further variations in the absolute and/or relative weight of some organs (heart, spleen, brain) were not associated with any histopathological changes (heart, spleen), considered to be secondary to the reduced body weights for males at 20 000 ppm (increased relative brain weights, reduced absolute heart and spleen weight) and/or without a dose-response relationship (heart and spleen weights in females). The NOAEL was 1000 ppm, equal to 120 and 130 mg/kg bw per day in males and females respectively, on the basis of altered kidney histology at higher doses (Courcy di Rosa, 1988).

Groups of 10 male and 10 female Tif:RAIf rats were given fludioxonil (purity, 97.5%) at a dose of 0, 10, 100 and 1000 mg/kg bw per day by gavage in distilled water containing 0.5% carboxymethylcellulose and 0.1% Tween 80, daily for 28 days. Food, water and the housing environment were controlled and monitored. Toxicity was evaluated through: mortality, daily clinical observation, body weight, measurements of food and water con-

Table 8. Clinical pathology, organs weights and histopathological changes in 20-day study in rats given diets containing fludioxonil

	Dietary concentration (ppm)									
	Males					Females				
	0	1000	5000	10 000	20 000	0	1000	5000	10 000	20 000
<i>Clinical pathology</i>										
Cl ⁻ (mmol/l)	107.8	109.4	103.6**	104.5*	103.0**	103.7	104.3	100.4**	98.3**	101.0**
Na ⁺ (mmol/l)	142.3	142.9	138.8**	137.9**	138.0**	136.9	138.2	133.2**	133.4**	133.5**
Glucose (mmol/l)	5.34	6.54	4.89	6.33	5.10	5.87	5.69	5.36	4.78*	4.59**
Cholesterol (mmol/l)	1.9	2.2	2.3	2.5	2.7*	2.3	2.2	2.4	2.5	3.2**
Alkaline phosphatase (U/l)	563	472	421	417	424	382	397	313	265**	333
Alanine transferase (U/l)	27	21*	20*	21	18**	25	24	21	20	18*
α -1 Globulin (g/l)	4.3	2.5**	2.6**	2.7**	3.1*	1.8	2.5	1.9	2.4	1.7
<i>Organ weights and histology</i>										
Carcass (g)	249	238	242	231	202**	159	156	149	161	149
Kidney:										
Absolute weight (g)	2.274	2.227	2.364	2.384	3.017**	1.571	1.642	1.505	1.811*	1.528
Relative weight (%)	9.14	9.34	9.75	10.27	15.10**	9.91	10.55	10.12	11.25*	10.27
Paleness	0/6	0/6	0/6	1/6	2/6	0/6	0/6	0/6	0/6	0/6
Foci	0/6	0/6	0/6	0/6	1/6	0/6	0/6	0/6	0/6	0/6
Tubular nephrosis	0/6	0/6	1/6	4/6	6/6	0/6	0/6	0/6	1/6	3/6
Liver:										
Absolute weight (g)	8.235	7.981	8.559	9.050	8.113	5.879	5.915	5.901	7.323**	6.853
Relative weight (%)	33.13	33.59	35.39	39.16**	40.10**	37.20	37.92	39.79	45.44*	46.23**

Courcy di Rosa (1988)

* $p < 0.05$, ** $p < 0.01$

sumption, ophthalmology, haematology (including thrombocyte count, but not mean cell haemoglobin concentration or platelet count), serum biochemistry (except gamma glutamyl transferase), urine analysis (except microscopic examination), organ weights, gross pathology and histopathology (spleen, heart, adrenals, kidney, liver, lungs). No treatment-related mortality, ophthalmological changes or haematological alterations were observed and the only clinical sign noted was a blue discolouration of the tail in all animals at 1000 mg/kg bw per day; this was also noted at necropsy. Body-weight gains at this dose were 60% and 91% those of the controls for females and males respectively. Food consumption of the females at 1000 mg/kg bw per day was up to 20% less than that of the controls throughout the first 3 weeks of the study. Concentrations of glucose were reduced in males at 100 and 1000 mg/kg bw per day and in females at 1000 mg/kg bw per day. Concentrations of cholesterol were elevated in males at 1000 mg/kg bw per day and in females at 100 and 1000 mg/kg bw per day. Bilirubin and calcium concentrations were elevated in females at 1000 mg/kg bw per day. Significant increases in albumin and globulin concentrations and resultant total protein concentrations measured in males at 10 and 1000 mg/kg bw per day, were without a dose-response relationship, were small in magnitude and were consequently concluded to be incidental. The incidence of ketonuria and a yellow-green discolouration of the urine were noted in rats at 100 and 1000 mg/kg bw per day together with an increase in bilirubin concentration in females at 1000 mg/kg bw per day. The discolouration is likely to be due to the presence of a coloured metabolite. Occult blood in the urine was seen in two out of ten males at 1000 mg/kg bw per day. Liver weights relative to body weights were significantly increased at 1000 mg/kg bw per day in both sexes and at 100 mg/kg bw per day in females. The absolute weights of the liver and relative weights of the kidney were significantly increased at 100 and 1000 mg/kg bw per day in females. Females at 1000 mg/kg bw per day also had significantly elevated brain weights relative to body weights, secondary to reduced body-weight gains. Histopathological changes were confined to animals treated

at 1000 mg/kg bw per day and consisted of hepatocellular hypertrophy of the centrilobular region in two males and four females, pale casts in the renal tubules of two males, foreign material in the epidermis of the tail in two males and seven females, a fatty liver in one female, a fatty adrenal cortex in one female and lymphohistiocytic infiltration of the renal pelvis in one female. The NOAEL was 10 mg/kg bw per day on the basis of altered clinical chemistry at higher doses. The slight effects seen at 100 mg/kg bw per day suggest that this dose is a borderline NOAEL/lowest-observed-effect level (LOAEL) (Fankhauser, 1990).

Groups of 10 male and 10 female Sprague-Dawley rats were fed diets containing fludioxonil (purity, 96%) at a concentration of 0, 10, 100, 1000, 7000 or 20000 ppm for 90 days. Actual achieved doses were 0, 0.8, 6.6, 64, 430, 1300 mg/kg bw per day for males and 0, 1.0, 7.1, 70, 460 or 1300 mg/kg bw per day for females. Food, water and the housing environment were controlled and monitored. The amount of fludioxonil in the diet was analysed regularly. Toxicity was evaluated through: mortality, daily clinical observation, measurements of body weight and food consumption, ophthalmology, haematology (including erythrocyte volume fraction, clotting time, activated thromboplastin time, fibrinogen), serum biochemistry (including direct bilirubin, 5' nucleotidase and sorbitol dehydrogenase activity), urine analysis, organ weights, gross pathology and histopathology (including mammary glands and thigh muscle, but not gall bladder).

Rates of mortality were unaffected by treatment, although one male at 20000 ppm died of unknown causes on day 36 and another male at 7000 ppm was terminated in a moribund condition with a pituitary adenoma on day 50. The excretion of a blue coloured metabolite caused the urine of animals at 1000, 7000 and 20000 ppm to be coloured green-blue; at 7000 and 20000 ppm, there was blue discolouration of the tail with discolouration of the abdomen and scrotum in the males, and blue or black discolouration of the tail and kidneys in nearly all rats and of the digestive tract in a few animals. Significantly lower body-weight gains were noted in females at 7000 ppm and in both sexes at 20000 ppm, with a slight, non-significant, decrease in weight gains of males at 1000 ppm. Corresponding reductions in food intakes were noted. Ophthalmology in both sexes and haematology in males were unaffected. In females at 20000 ppm, significant reductions were noted in the

Table 9. Clinical chemistry and organ weight changes in a 28-day study in rats treated with fludioxonil by gavage

Parameter	Dose (mg/kg bw per day)							
	Males				Females			
	0	10	100	1000	0	10	100	1000
<i>Clinical chemistry</i>								
Glucose (mmol/l)	7.866	7.875	6.794*	6.546*	6.576	6.968	6.074	5.459*
Bilirubin (µmol/l)	2.413	2.226	2.267	2.391	2.657	2.808	2.744	3.453*
Protein (g/l)	64.54	66.91	65.05	66.71*	66.75	66.38	64.63	65.78
Albumin (g/l)	36.05	37.34*	36.74	37.12*	37.71	37.48	36.68	37.50
Globulin (g/l)	28.49	29.57*	28.31	29.65*	29.04	28.90	27.95	28.28
Cholesterol (mmol/l)	1.640	1.757	1.638	2.040**	1.594	1.792	2.053*	2.171*
Calcium (mmol/l)	2.675	2.652	2.598	2.696	2.570	2.555	2.539	2.597*
<i>Organ weight changes</i>								
Liver, absolute (g)	18.14	17.93	19.09	18.78	10.55	11.30	12.04*	12.32*
Liver, relative to body weight	48.81	49.10	52.11	52.92*	42.40	45.59	48.33*	52.94**
Kidney, relative to body weight	6.943	6.871	7.002	7.438*	7.486	7.167	7.978*	8.003
Brain, relative to body weight	6.377	6.426	6.358	6.613	8.635	8.701	8.675	9.169*

From Fankhauser (1990)

* $p < 0.05$, ** $p < 0.01$

following parameters: concentration of haemoglobin (also noted at 7000 ppm), erythrocyte volume fraction, mean corpuscular volume and mean corpuscular haemoglobin. At 20000 ppm, the following alterations in serum biochemistry were observed: decreased concentration of glucose, increased concentration of blood urea nitrogen, increased concentration of total bilirubin (also noted in males at 7000 ppm), increased cholesterol (also noted at 7000 ppm), and increased activity of gamma-glutamyl transferase. Females at 20000 ppm also had significantly increased alkaline phosphatase activity and decreased 5' nucleotidase activity (also noted at 7000 ppm). An increase in the presence of bilirubin in urine was noted at 7000 and 20000 ppm. The volume of urine was significantly decreased in females at 10, 7000 and 20000 ppm. Given the absence of a significant effect at 100 and 1000 ppm, the effect at 10 ppm was concluded to be incidental.

Increased relative (to body) weights were observed at 7000 and 20000 ppm for the adrenals (males only), brain, kidneys, liver, spleen and testes. The increase in relative weights of the brain and testes is likely to be secondary to reduced body weights, as the absolute weights of these organs tend to be conserved to some extent with reduced body-weight gains. Nonetheless, at 20000 ppm absolute weights of the brain were decreased in females and absolute heart weights were decreased in both sexes. Absolute and relative weights of the thymus were significantly decreased at 7000 and 20000 ppm. Five females and one male at 20000 ppm were emaciated. Chronic nephropathy with active inflammation was noted in females at 20000 ppm and in males at ≥ 1000 ppm. The incidence of chronic nephropathy was increased at 7000 ppm in males and at 20000 ppm in both sexes. Hypertrophy of the centrilobular region of the liver was noted in one female at 100 ppm and in both sexes at ≥ 1000 ppm. Given the lack of statistical significance or a dose-response relationship in females at 100 and 1000 ppm, the single incidence at 10 ppm was discounted as incidental. As this is likely to reflect an adaptive response to the high xenobiotic load, it was also not considered to be an adverse effect at 1000 ppm, at which dose no evidence of liver damage from histology or clinical chemistry was observed. One male at 7000 ppm had a benign neoplasm in the pituitary and another had an adenoma of the pituitary. The NOAEL was 1000 ppm (64 mg/kg bw per day) on the basis of evidence of damage to the kidneys and liver at higher doses (Chang, 1990a).

Groups of five male and five female Tif:RAIf rats were dermally exposed to fludioxonil (purity, 97.5%) at a dose of at 0, 40, 200 or 1000 mg/kg bw per day, in 0.5% (w/v) carboxymethylcellulose in 0.1% (w/v) aqueous polysorbate 80, 5 days per week for 4 weeks. Fludioxonil was applied under an occlusive dressing that remained in place for 6 h and the application area was subsequently cleaned with lukewarm water. Control rats were exposed to a dressing containing the carrier under the same regime. Food, water and the housing environment were controlled and monitored. Toxicity was evaluated through: mortality, daily clinical observation, measurements of body weight and food consumption, skin irritation, haematology (including thrombocyte count, but not mean cell haemoglobin concentration or platelet count), serum biochemistry (except gamma-glutamyltransferase), organ weights, gross pathology and histopathology (skin at application site, skin at remote site, kidney, liver, thymus).

There were no deaths, no treatment-related clinical signs, no significant differences in body weights and, in males, no alteration in food consumption. For females at 1000 mg/kg bw per day, a slight increase in food intake was noted in the last 2 weeks. Organ weights, haematological and clinical biochemistry parameters were unaffected. Testicular atrophy was noted in one male at 1000 mg/kg bw per day, both macroscopically and microscopically.

Table 10. Principal observations in a 90-day study in rats fed diets containing fludioxonil

Parameter	Sex	Historical control range	Dietary concentration (ppm)					
			0	10	100	1000	7000	20000
<i>Haematology</i>								
Haemoglobin (g/dl)	Males	14.5–17.3	15.3	15.7	15.5	15.6	15.5	14.9
	Females	13.5–16.9	15.6	15.7	15.9	15.2	14.7*	14.1**
Erythrocyte volume fraction (%)	Males	42.9–52.2	46.7	47.7	47.4	47.1	47.1	45.5
	Females	40.4–49.6	45.7	45.8	46.7	44.3	43.3	41.7**
Mean corpuscular volume (fl)	Males	46–57	54	54	54	53	53	53
	Females	53–60	55	55	55	54	54	52**
Mean corpuscular haemoglobin (per g)	Males	15.6–18.9	17.5	17.8	17.6	17.5	17.5	17.3
	Females	17.9–20.6	18.8	18.7	18.8	18.7	18.3	17.6**
<i>Serum biochemistry</i>								
Glucose (mg/dl)	Males	60–201	137	136	148	142	114	64**
	Females	49–183	123	98	121	93	84	63**
Blood urea nitrogen (mg/dl)	Males	12–22	16	15	16	15	15	20**
	Females	12–23	17	18	16	17	18	21*
Bilirubin (mg/dl)	Males	0.1–0.3	0.2	0.2	0.2	0.2	0.3*	0.4**
	Females	0.0–0.3	0.2	0.2	0.2	0.2	0.2	0.3*
Cholesterol (mg/dl)	Males	40–99	69	60	74	61	103*	109**
	Females	59–147	79	83	79	80	119*	122**
Gamma-glutamyl transferase (IU/l)	Males	4–6	5	5	4	4	5	10**
	Females	4–7	5	5	5	4	5	15**
Alkaline phosphatase (IU/l)	Males	68–145	91	92	98	111	92	110
	Females	20–119	54	54	64	54	59	97**
5’Nucleotidases (IU/l)	Males	11–43	16	13	16	13	12	11
	Females	11–25	21	24	21	18	14*	12**
<i>Body and organ weights</i>								
Body weight (g)	Males	—	577	601	591	546	518	418**
	Females	—	327	303	324	308	273**	227**
Adrenals, absolute (mg)	Males	—	57.5	59.9	65.6	57.6	69.9	63.9
	Females	—	59.9	62.1	84.3	64.0	60.0	55.1
Adrenals, relative to body weight	Males	—	0.010	0.010	0.011	0.011	0.0141*	0.0161**
	Females	—	0.020	0.022	0.027	0.023	0.0246	0.0283
Brain, absolute (g)	Males	—	2.145	2.273	2.160	2.195	2.245	2.090
	Females	—	1.966	1.980	1.962	1.965	1.934	1.858**
Brain, relative to body weight	Males	—	0.384	0.399	0.321	0.419	0.453**	0.541**
	Females	—	0.678	0.721	0.655	0.719	0.782*	0.950**
Heart, absolute (g)	Males	—	1.893	1.773	1.847	1.704	1.619	1.347**
	Females	—	1.074	1.110	1.125	1.053	1.029	0.816*
Heart, relative to brain weight	Males	—	88.25	78.10	85.61	77.70	72.660*	64.730**
	Females	—	54.66	56.02	57.36	53.67	52.320	43.913**
Kidneys, relative to body weight	Males	—	0.615	0.614	0.608	0.678	0.738*	0.857**
	Females	—	0.651	0.660	0.638	0.694	0.714	0.876**
Liver, relative to body weight	Males	—	2.734	2.644	2.817	2.827	3.323**	3.798**
	Females	—	2.596	2.624	2.603	2.791	3.667**	4.434**
Liver, relative to brain weight	Males	—	717.9	670.6	749.2	676.2	737.47	714.53
	Females	—	395.3	364.7	400.9	393.7	462.05	470.78*
Spleen, absolute (g)	Males	—	0.878	0.961	0.863	0.895	0.836	0.749
	Females	—	0.526	0.490	0.551	0.525	0.495	0.409**
Spleen, relative to body weight	Males	—	0.158	0.167	0.151	0.170	0.169	0.191**
	Females	—	0.171	0.178	0.184	0.191	0.202	0.210*
Thymus, absolute (g)	Males	—	0.339	0.315	0.366	0.358	0.227*	0.160**
	Females	—	0.226	0.216	0.263	0.263	0.205	0.124*
Thymus, relative to body weight	Males	—	0.059	0.054	0.064	0.067	0.046	0.040*
	Females	—	0.066	0.079	0.087	0.096*	0.083	0.063
Thymus, relative to brain weight	Males	—	15.86	13.72	16.94	16.28	10.191*	7.659*
	Females	—	11.41	11.00	13.35	13.34	10.508	6.663*
Testes, relative to body weight	Males	—	0.642	0.644	0.622	0.698	0.775*	0.944**
<i>Histology</i>								
Kidneys, inflammation	Males	—	0	0	0	1	6**	9**
	Females	—	0	0	0	0	0	9**
Kidneys, nephropathy	Males	—	3	3	2	4	10**	10**
	Females	—	0	0	0	0	0	10**
Kidneys, pelvis dilatation	Males	—	1	0	0	0	0	0
	Females	—	0	0	0	1	1	2
Liver, hypertrophy	Males	—	0	0	0	5	5	9**
	Females	—	0	0	1	1	6*	10**

From Chang (1990a)

 $p < 0.05$, ** $p < 0.001$

One male from each of the groups at 40 and 200 mg/kg bw per day had mottled lungs that, after microscopic examination, were described as indicating chronic bronchopneumonia. Given the isolated occurrence and lack of a dose-response relationship, these changes in the lung were concluded to be incidental to treatment. At 1000 mg/kg bw per day, all females displayed phagocytic cells in the thymus, one had lymphohistiocytic infiltration of remote skin and one had extramedullary haematopoiesis of the liver. Given the absence of such findings in studies in animals treated orally, the presence of phagocytic cells in the thymus is of uncertain relationship to treatment, but cannot be entirely discounted as incidental. Renal lymphohistiocytic infiltration was noted in one female from each of the groups at 200 and 1000 mg/kg bw per day. The NOAEL was 200 mg/kg bw per day on basis of the appearance of phagocytic cells in the thymus of females at 1000 mg/kg bw per day (Schneider, 1990).

Dogs

Groups of four male and four female beagle dogs (six males and six females in the control group and the group receiving a dose of 15 000/10 000 ppm) were fed diets containing fludioxonil (purity, 97.5%) at a concentration of 0, 200, 2000 or 15 000 ppm (0, 6.2, 59 and 290 mg/kg bw per day for males and 0, 6.2, 60 and 340 mg/kg bw per day for females) for 90 days. Owing to marked body-weight loss and reduced food consumption at 15 000 ppm in the first 3 weeks, the dose was reduced to 10 000 ppm. The highest dose is referred to hereafter as 10 000 ppm. At the end of the 90-day period of treatment, two dogs of each sex in the control group and at 10 000 ppm were allowed to recover on control diets for 4 weeks. Food, water and the housing environment were controlled and monitored. The amount of fludioxonil in the diet was analysed regularly. Toxicity was evaluated through: mortality, daily clinical observation, measurements of body weight and food consumption, ophthalmology, haematology (including erythrocyte volume fraction and thrombocyte count, but not platelet count or prothrombin), serum biochemistry, urine analysis (including nitrites), organ weights (including pituitary, thyroid, parathyroid, but not thymus), gross pathology and histopathology (including mammary glands, but not gall bladder).

No deaths occurred during the study and ophthalmology and gross pathology were normal. Green discolouration of the digestive tract was noted in one male at 2000 ppm and three dogs of each sex at 10 000 ppm. Blue faeces were noted in dogs at 2000 and 10 000 ppm. This discolouration was attributed to a coloured metabolite and was not considered to be toxicologically relevant. Three dogs of each sex at 2000 ppm and all dogs at 10 000 ppm had intermittent diarrhoea. Within 2 days of cessation of treatment, faeces were normal for all animals. All other effects reported below occurred at 10 000 ppm only. Body weights of both sexes were reduced and food intake was markedly reduced, by up to 50%, during the first 3 weeks; after the dose was reduced no significant variations were noted. Erythrocyte counts were lower for both sexes at week 13 and recovered only partially on cessation of treatment. Reduced concentrations of haemoglobin were also noted at weeks 8 and 13, but not week 4. Mean cell haemoglobin concentration was reduced and thrombocyte count and fibrinogen concentrations were elevated, and the mean corpuscular volume was elevated in females; however, these effects were reversible. A significant increase in serum cholesterol concentrations was noted in females, but was reversible. Liver weight, both absolute and relative to body weight, was increased in both sexes and may have been related to increased severity, but not incidence, of bile duct proliferation, found in all groups at this dose. The NOAEL was 2000 ppm (59 mg/kg bw per day) on the basis of anaemia and increased serum cholesterol concentrations at 10 000 ppm. As the intermittent diarrhoea did

Table 11. Key findings of a 90-day study in dogs fed diets containing fludioxonil

Parameter	Dietary concentration (ppm)							
	Male				Female			
	0	200	2000	10 000	0	200	2000	10 000
Erythrocyte count ($10^6/\text{mm}^3$)	6.89	6.95	6.45	6.48	6.53	6.79	6.6	5.46*
Haemoglobin (g/dl)	16.2	16.7	15.2	15.2	15.4	16.1	15.8	12.9*
Fibrin (g/l)	1.93	1.53	1.63	2.12	1.72	1.42	1.4	2.28**
Platelets (g/l)	310	325	337	440**	397	336	432	527*
Cholesterol (mmol/l)	4.2	4.6	4.2	4.7	4.4	3.3	3.7	6.9**

From Moysan (1990)

 $p < 0.05$, ** $p < 0.001$

not affect food consumption or the condition of the animals, was readily reversible and was not observed in a 1-year study at doses of up to 8000 ppm, it was considered unlikely to reflect systemic toxicity and was not therefore considered in relation to the the NOAEL (Moysan, 1990).

Groups of four male and four female beagle dogs were fed diets containing fludioxonil (purity, 97.5%) at a concentration of 0, 100, 1000 or 8000 ppm (0, 3.1, 33 or 300 mg/kg bw per day in males and 0, 3.3, 36 or 330 mg/kg bw per day in females) for 52 weeks. Food, water and the housing environment were controlled and monitored. The amount of fludioxonil in the diet was analysed regularly. Toxicity was evaluated through: mortality, daily clinical observation, measurements of body weight and food consumption, ophthalmology, haematology (including thrombocyte count, fibrinogen, activated thromboplastin time, erythrocyte volume fraction and quick time), serum biochemistry (including creatine kinase), urine analysis (including nitrites), organ weights (including pituitary, thyroids and parathyroids, but not thymus), gross pathology and histopathology (including mammary glands).

There were no deaths and food consumption, urine analysis parameters and ophthalmology were unaffected. Blue faeces were noted in all dogs at 1000 and 8000 mg/kg and those at 8000 ppm had discolouration of the digestive tract. Animals receiving fludioxonil at a dietary concentration of 8000 ppm weighed considerably less than control animals throughout the study. A single female at 1000 ppm lost weight from week 22 to week 32 and intermittently subsequently, and finished the study with a lower body weight than any animal at 8000 ppm. The remaining animals at this dose had body weights comparable to those of the controls. Given the pattern of weight change in this individual animal, it was concluded to be incidental to treatment. One male at 8000 ppm displayed alopecia from week 44. In males at 8000 mg/kg, the thrombocyte count and fibrinogen concentrations were consistently elevated throughout the study, and prothrombin time was reduced at week 52. These values were all within the ranges for historical controls. Platelet counts were increased significantly in males and non-significantly in females at 8000 ppm. Although the haematology changes are small in magnitude, they are generally consistent with those seen in the 90-day study, including slight non-significant decreases in erythrocyte and haemoglobin values, and it was therefore concluded that they were related to treatment. Significant increases in serum cholesterol concentrations, above the range for historical controls, were noted in males at 8000 ppm and total bilirubin concentrations were slightly elevated in all dogs at 8000 ppm throughout the study. At 8000 ppm, increased liver weights (correlating with visibly enlarged livers in two females) and decreased spleen weights were noted in both sexes and heart weights in males were reduced. Two females at 8000 ppm had enlarged

Table 12. Key findings of a 12-month study in dogs fed diets containing fludioxonil

Parameter	Sex	Dietary concentration (ppm)			
		0	100	1000	8000
Cholesterol (mmol/l)	Male	2.8	3.2	3.0	4.7*
	Female	4.8	4.1	4.0	4.8
Total bilirubin (µmol/l)	Male	2	2	1	3
	Female	2	2	2	4
Prothrombin time (s)	Male	7.0	6.6	6.8	6.3*
	Female	6.5	6.3	6.6	6.4
Fibrin (g/l)	Male	1.87	2.27	2.02	2.78*
	Female	2.04	1.89	2.06	2.11
Platelets (10 ³ /mm ³)	Male	316	324	368	413*
	Female	402	395	442	500
<i>Body and organ weights</i>					
Body weight (kg)	Male	11.5	12.4	12.5	9.7
	Female	12.3	12.0	11.1	10.1
Liver	Male	288.2	265.5	297.0	305.6
	Female	285.7	266.3	267.8	324.9*
Spleen	Male	25.03	24.39	25.20	18.07
	Female	29.24	21.26	24.33	24.08
Heart	Male	95.87	91.87	96.95	72.37**
	Female	85.46	83.09	84.47	81.30

From Vallet (1992)

 $p < 0.05$, ** $p < 0.001$

livers. The size of the spleen was reduced in two males and one female at 8000 ppm and in one male at 100 ppm. The size of the thymus was reduced in two dogs of each sex at 8000 ppm and in one male at 1000 ppm. Other than biliary epithelial cell proliferation observed in one female at 8000 ppm, no histological abnormalities were noted. The NOAEL was 1000 ppm (33 mg/kg bw per day) on the basis of reduced body-weight gains, increased cholesterol concentrations and altered haematology at 8000 ppm. Given the mild degree of the effects observed at 8000 ppm and the wide dose spacing between the NOAEL and LOAEL, the Meeting concluded that the NOAEL is conservative (Vallet, 1992).

2.3 Long-term studies of toxicity and carcinogenicity

Mice

Groups of 60 male and 60 female CD-1 mice were fed diets containing fludioxonil (purity, 95.4%) at a concentration of 0, 10, 100, 1000 or 3000 ppm (0, 1.1, 11.3, 112 or 360 mg/kg bw per day for males and 0, 1.4, 16.5, 133 or 417 mg/kg bw per day for females) for 18 months. Ten mice of each sex per group were terminated after 12 months. Food, water and the housing environment were controlled and monitored. The amount of fludioxonil in the diet was analysed regularly. Toxicity was evaluated through: mortality, daily clinical observation, body weight, measurements of food and water consumption, ophthalmology, haematology (including reticulocyte time), organ weights, gross pathology and histopathology. The survival patterns for control and treated groups were similar. Pelvic staining, blue discolouration of the skin, stomach and urine seen in some animals at 1000 and 3000 ppm were attributed to a coloured metabolite in the urine and discounted as toxicologically irrelevant. A slight decrease (statistically significant from week 10 to 78) in body-weight gain was noted in males at 3000 ppm; at various stages throughout the study these males consumed significantly more food than did the controls. Water consumption, only measured for weeks 50 and 51, was increased for males at 3000 ppm.

Haematology and ophthalmology were unaffected by treatment, with the exception of females at 1000 and 3000 for males at 3000ppm for whom a slightly, but significantly, reduced mean corpuscular haemoglobin concentration (controls, 34.52%; 1000ppm, 33.6%**; 3000ppm, 33.6%**; where ** $p < 0.01$) was observed at 18 months. Because of the small magnitude of the effect in this study, the absence of correlating haematology findings and the lack of a dose-response relationship, it was concluded that the reduced mean cell haemoglobin concentration, in isolation, was of equivocal relationship to treatment and was not considered in the identification of a NOAEL for this study, despite the statistical significance and the fact that it was consistent with weak anaemia at 7000ppm in a second study in mice. At 3000ppm, liver weights, absolute and/or relative, were elevated. The incidences of enlarged spleen (males and females), liver (females), thymus (females) and lymph nodes (males and females) were increased at 3000ppm. There were no significant variations noted in the incidence of non-neoplastic lesions. There was a significant increase in the incidence of lymphoma in females at 3000ppm (11, 10, 13, 12, 18 for the control group to the group receiving a dose of 3000ppm, respectively). Lymphoma is a common finding in ageing female CD-1 mice and the historical incidence at the conducting laboratory is 13–32%, which is comparable to the incidence noted in females at 3000ppm. Lymphoma was the cause of death of more females at 3000ppm than in any other group (1, 0, 2, 4, 6 for the control group to the group receiving a dose of 3000ppm, respectively). Given the high background rate of this finding and the absence of any increase in another study by the same authors using higher doses, the apparent increase in lymphomas observed in this study was concluded to be incidental. No significant variations in the incidence of neoplastic lesions were noted. The NOAEL was 1000ppm (112mg/kg bw per day) on the basis of increased liver weights, and thymic and splenic enlargement at 3000ppm (Chang & Wyand, 1993a).

Groups of 60 male and 60 female CD-1 mice were fed diets containing fludioxonil (purity, 95.4%) at a concentration of 0, 3, 30, 5000 or 7000ppm (0, 0.33, 3.3, 590 or 850mg/kg bw per day for males, and 0, 0.41, 4.1, 715 or 1000mg/kg bw per day for females) for 18 months. Ten mice of each sex per group were terminated after 12 months. Food, water and the housing environment were controlled and monitored. The amount of fludioxonil in

Table 13. Key findings of an 18-month study in mice given diets containing fludioxonil

Parameter	Sex	Dietary concentration (ppm)				
		0	10	100	1000	3000
Body weight	Male	40.8	42.3	43.1	42.0	40.0
	Female	32.0	34.8	34.3	34.5	33.4
Liver weight, absolute	Male	1.674	1.663	1.728	1.763	1.822
	Female	1.382	1.454	1.487	1.476	1.612**
Liver weight, relative to body weight	Male	4.168	4.031	4.084	4.183	4.518
	Female	4.366	4.262	4.441	4.282	4.887*
Liver weight, relative to brain weight	Male	325.151	324.139	330.177	338.419	361.290*
	Female	272.003	285.332	292.107	286.882	317.480**
Enlarged spleen	Male	14	16	9	18	22
	Female	19	22	21	26	28
Enlarged liver	Male	1	2	2	2	3
	Female	1	2	1	3	6
Enlarged thymus	Male	3	0	1	4	0
	Female	4	8	7	10	13
Enlarged lymph nodes	Male	1	2	3	3	6
	Female	0	3	4	3	6

From Chang & Wyand (1993a)

$p < 0.05$, ** $p < 0.001$

the diet was analysed regularly. Toxicity was evaluated through: mortality, daily clinical observation, measurements of body weight and food consumption, ophthalmology, haematology (including reticulocyte time), organ weights, gross pathology and histopathology.

At 7000 ppm, survival was markedly decreased (control: males, 76% and females, 71%; 7000 ppm: males, 27% and females, 22%) and animals displayed rough coat, hypothermia, reduced activity, pallor, distended abdomen, loose stools, dehydration, dyspnoea, discoloured stools, stained anus, discoloured urine and stained pelvic region. At 5000 ppm, rough coat, pallor and loose stools, were observed. Weight gains in males were 8% and 25% below those of controls for males at 5000 and 7000 ppm and 20% below those of controls for females at these doses. Discolouration of the urine, anus, pelvic region and some internal organs seen at 5000 and 7000 ppm were attributed to a coloured metabolite of fludioxonil and discounted as toxicologically irrelevant. Slight anaemia was observed in both sexes at 7000 ppm from 12 months. At 7000 ppm, absolute and relative weights of the kidney were significantly reduced in males (also at 5000 ppm) and elevated in females. Despite the unusual pattern of this change in the two sexes, as it correlates with nephropathy accompanied by calcification of renal tubules at 5000 and 7000 ppm, which was implicated in the death of 31 males and 32 females at 7000 ppm, it was considered to be a reflection of treatment. Absolute and relative weights of the liver in both sexes were elevated at 5000 and 7000 ppm. Rough coats, an increased incidence of liver cysts, renal pitting and renal cysts were seen in both sexes at 7000 ppm, and an elevated incidence of liver necrosis and bile duct hyperplasia was seen in males and a slight increase in the incidence of haemorrhage of the lungs was seen in females at this dose. There was no increase in the number or type of neoplastic lesions. The incidence of lymphomas, which appeared to be increased in the previous study, in females was similar to that in controls (control, 11; 3 ppm, 7; 30 ppm, 12; 5000 ppm, 11; 7000 ppm, 8). The NOAEL was 30 ppm (3.3 mg/kg bw per day) on the basis of decreased body-weight gains, increased liver weights and nephropathy at the next higher dose (Chang & Wyand, 1993b).

The two 18-month studies used the same strain and source of mice, the same batch of fludioxonil and were conducted in the same laboratory with only a difference of 6 months between the commencement of the first and second study. The overall NOAEL for these two studies was 1000 ppm (112 mg/kg bw per day).

Rats

Groups of 50 male and 50 female Sprague-Dawley rats were fed diets containing fludioxonil (purity, 95.4%) at a concentration of 0, 10, 30, 100, 1000 or 3000 ppm (0, 0.37, 1.1, 3.7, 37 or 110 mg/kg bw per day for males, and 0, 0.44, 1.3, 4.4, 44 or 140 mg/kg bw per day) for up to 24 months. Twenty rats of each sex per group were maintained for clinical laboratory tests. An additional ten rats of each sex per group were terminated after 12 months, and a further ten rats of each sex from the control group and the group at 3000 ppm were treated for 12 months, removed from the test diet for 1 month and then terminated. A further nine males from each group were maintained for studies of metabolism. Food, water and the housing environment were controlled and monitored. The amount of fludioxonil in the diet was analysed regularly. Toxicity was evaluated through: mortality, daily clinical observation, measurements of body weight, food and water consumption, ophthalmology, haematology, serum biochemistry (including creatine kinase, 5-nucleotidase, sorbitol dehydrogenase), organ weights (except thymus), gross pathology and histopathology (including cervix).

Table 14. Key observations in an 18-month study in mice given diets containing fludioxonil

Parameter	Sex	Dietary concentration (ppm)				
		0	3	30	5000	7000
<i>Haematology</i>						
Erythrocytes (10 ⁶ /µl)	Male	8.83	8.72	8.34	8.00	7.27
	Female	8.39	8.60	8.74	8.32	5.90**
Haemoglobin (g/dl)	Male	13.6	13.1	12.7	11.9	10.9*
	Female	12.7	12.4	13.2	12.5	8.9**
Erythrocyte volume fraction (%)	Male	37.9	36.8	35.2	33.1	30.5*
	Female	36.4	35.4	37.6	35.4	26.0**
Reticulocytes (%)	Male	2.1	2.3	2.0	2.9	3.4
	Female	1.8	1.9	1.4	2.5	2.7
<i>Body and organ weights</i>						
Body weights	Male	42	42.5	42.6	41.2	38.2*
	Female	37.7	36.0	35.9	34.5**	35.0
Kidneys, absolute (g)	Male	0.664	0.667	0.680	0.597*	0.527**
	Female	0.429	0.450	0.441	0.428	0.497*
Kidneys, relative to body weight	Male	1.763	1.724	1.774	1.628	1.524**
	Female	1.280	1.391	1.354	1.356	1.570**
Liver, absolute (g)	Male	1.583	1.588	1.631	1.789*	1.696
	Female	1.385	1.481	1.376	1.652**	1.852**
Liver, relative to body weight	Male	4.253	4.186	4.336	4.843*	4.978
	Female	4.119	4.562	4.221	5.240**	5.798**
<i>Gross pathology</i>						
Rough coat	Male	1	4	6	5	27
	Female	1	2	4	4	21
Liver cysts	Male	0	2	1	2	8
	Female	1	1	0	0	1
Kidneys, rough pitted surface	Male	0	2	1	4	29
	Female	1	0	1	5	21
Kidney cysts	Male	8	18	11	9	17
	Female	8	3	5	10	17
<i>Histopathology</i>						
Nephropathy	Male	0	0	0	53	59
	Female	0	0	0	21	58
Liver necrosis	Male	2	3	4	7	13
	Female	5	5	3	1	5
Bile duct, hyperplasia	Male	0	0	1	4	23
Kidneys, calcification	Male	1	5	5	13	45
	Female	2	2	1	18	56
Lungs, haemorrhage	Male	1	1	2	2	1
	Female	0	1	2	3	8

From Chang & Wyand (1993b)

 $p < 0.05$, ** $p < 0.001$

Treatment did not affect survival rates, ophthalmology, food and water consumption or clinical signs, other than the observation of some diarrhoea at 3000 ppm. Blue staining of the urine, stools and fur were attributed to a coloured metabolite and discounted as toxicologically irrelevant. Final body weights were lower in both sexes at 3000 ppm, although this did not reach statistical significance. Body-weight gains were significantly reduced at 3000 ppm (by 11%) in both sexes. Slight anaemia and slight decreases in leukocyte counts were noted at 3000 ppm. For males, statistical variations in bilirubin, creatine, gamma-glutamyl transferase and creatinine kinase activities were noted at 6 months, however these trends were not noted at 12, 18 and 24 months. In treated females, gamma-glutamyl transferase activities were statistically, but not toxicologically, significantly increased at 6, 18 and 24 months. The values were generally quite low in absolute terms and comparable to the range for historical controls. Although absolute organ weights were comparable to those of the controls, slightly elevated weights of kidneys and livers relative to body weight were noted in males at 3000 ppm. Increased relative weight of the testes at 24 months reflected

lower body weight and is not toxicologically significant. Males at 3000 ppm had increased incidences of enlarged liver, hepatocellular hypertrophy, nephropathy and renal cysts (also observed at 1000 ppm, but not confirmed histologically). Histologically, the incidence of renal cysts was increased in males at 3000 ppm only. Increased incidences of centrilobular necrosis and basophilic foci of the liver were noted at 3000 ppm in both sexes. An increase in the number of hepatocellular tumours (benign plus malignant) were also noted in females at 3000 ppm, but the incidence was within the historical range (adenoma, 0–10%; carcinoma, 0–1.7%; adenoma plus carcinoma, 0–10%), there were no histological changes indicative of a proliferative response (e.g. foci of cellular alteration) and the tumors were discrete nodules. The overall incidence of neoplasms and the number of animals with neoplasms were similar for control and treated groups. The NOAEL was 1000 ppm (37 mg/kg bw per day) on the basis of an increased incidence of renal cysts and nephrosis in males and decreased body-weight gains in both sexes at the next higher dose (Chang & Richter, 1993).

A subsequent re-examination of the slides of livers from this study (Eneigh Hart, 1994) was performed in order to determine whether the slight increase in hepatocellular tumours in females at 3000 ppm was attributable to a compound-related mitogenic effect and, if so, to identify a no-observed-effect level (NOEL) for this effect. An immunohistochemical method was used to detect proliferating cell nuclear antigen (PCNA). A slight, dose-related but not statistically significant, increase in the labelling index was noted in females at 12 months (labelling index: 0.35, 0.37, 0.49, 0.67, 0.56 for dietary concentrations from 0 to 3000 ppm respectively), but not at 13 months (i.e. after fludioxonil had been withdrawn for 1 month) indicating rapid reversibility of cell proliferation on compound withdrawal. Males were unaffected. The overall NOAEL of 1000 ppm was confirmed.

2.4 Genotoxicity

The results of studies of genotoxicity with fludioxonil are summarized in Table 16.

2.5 Reproductive toxicity: multigeneration studies

Rats

In a two-generation study, groups of 30 Sprague-Dawley rats were given diets containing fludioxonil (purity, 95.4%) at a concentration of 0, 30, 300 or 3000 ppm. Actual intakes of fludioxonil were calculated (weighted average) as 0, 2.2, 22 or 230 mg/kg bw per day for males and 0, 2.0, 21 or 210 mg/kg bw per day for females, for the control group to the group receiving 3000 ppm respectively. Treatment of the parental (F_0) rats commenced 70 days before cohabitation, continued through cohabitation (up to 21 days) and was subsequently continued for females until pups were weaned at day 21 of lactation. Parental males and females, and unmated females were terminated after weaning of the pups. Pups from the F_0 generation were culled to four males and four females on postnatal day 4 where possible. At postnatal day 21, pups of the F_0 dams (one male and one female from each litter where possible) were randomly selected for the F_1 generation and the remainder were terminated. Food, water and the housing environment were controlled and monitored. The amount of fludioxonil in the diet was analysed regularly. Toxicity was evaluated through: mortality, daily clinical observations, measurements of body weight and food consumption, gross pathology, litter parameters, mating performance, fertility indices, reproduction parameters, survival indices, sex ratio, organ weights (testes and ovaries only) and histopathology (uterus and gross lesions).

Table 15. Key findings of a 2-year study in rats given diets containing fludioxonil

Parameter	Sex	Time-point (months)	Dietary concentration (ppm)							
			0	10	30	100	1000	3000		
<i>Haematology</i>										
Leukocytes (10 ³ /μl)	Male	12	13.6	11.8	11.1	12.3	11.9	10.9		
		24	12.2	13.0	13.1	14.3	13.0	10.4		
	Female	12	7.1	6.6	6.6	7.3	6.1	6.6		
		24	9.4	9.0	8.4	10.2	9.5	8.5		
Erythrocytes (10 ⁶ /μl)	Male	12	9.14	9.26	9.07	9.25	8.98	9.04		
		24	8.34	8.11	8.27	8.21	8.12	8.04		
	Female	12	8.32	8.46	8.43	8.28	8.46	7.91*		
		24	7.54	7.13	7.57	6.95	6.77	6.72		
Haemoglobin (g/dl)	Male	12	15.9	16.0	16.1	16.0	15.7	15.7		
		24	14.7	14.4	14.6	14.3	14.4	14.1		
	Female	12	15.3	15.6	15.6	15.2	15.4	14.4**		
		24	14.0	13.5	14.3	12.9	13.0	12.5		
Erythrocyte volume fraction (%)	Male	12	47.5	17.9	47.6	47.8	47.4	48.0		
		24	43.6	42.9	43.1	42.8	43.1	42.3		
	Female	12	45.4	46.2	46.5	45.5	45.9	45.1		
		24	41.6	39.7	41.8	38.2	38.2	37.1		
<i>Serum biochemistry</i>										
Gamma-glutamyl transferase (μg/l)	Male	18	5	7*	7*	5	7*	(Historical controls)		
		24	3	6**	6**	5	6**	7** (1–7)		
	Female	18	2	3	4**	4**	4**	4** (0–8)		
		24	5	7*	7*	7*	7*	7* (0–6)		
		<i>Body and organ weights</i>								
		Body weight (g)	Male	24	733	791	794	828	757	697
Female	24		564	595	561	552	583	518		
Liver, relative	Male	12	2.383	2.371	2.429	2.329	2.497	2.704*		
		24	2.614	2.625	2.756	2.443	2.658	2.868		
	Female	12	2.423	2.334	2.528	2.412	2.632	2.507		
		24	3.083	4.643	3.193	3.345	4.023	4.387		
Kidneys, relative	Male	12	0.551	0.545	0.538	0.552	0.518	0.547		
		24	0.766	0.679	0.716	0.680	0.727	0.806		
	Female	12	0.580	0.510	0.570	0.567	0.637	0.559		
		24	0.557	0.563	0.530	0.615	0.524	0.577		
Testes, relative	Male	12	0.525	0.499	0.526	0.499	0.519	0.502		
		24	0.473	0.478	0.487	0.434	0.479	0.557*		
<i>Gross pathology</i>										
Kidney cysts	<i>n</i>		70	60	60	60	60	70		
	Male	24	6	7	4	9	12	11		
	Female	24	0	1	4	2	1	3		
<i>Histology</i>										
Enlarged liver	<i>n</i>		70	60	60	60	60	70		
	Male	24	2	4	3	3	3	12		
	Female	24	6	3	1	4	0	3		
Liver, degeneration/atrophy/ centrilobular necrosis	Male	24	4	8	4	5	9	16		
	Female	24	8	5	9	9	13	21		
Liver, hepatocellular hypertrophy	Male	24	17	16	15	21	16	27		
	Female	24	19	13	4	5	13	15		
Hepatocellular carcinoma ^a	Female	24	0	0	0	0	0	1		
Hepatocellular adenoma	Female	24	0	2	0	0	0	4		
Kidney cysts	Male	24	18	6	5	7	11	28		
	Female	24	0	1	2	1	0	3		
Nephrosis	Male	24	59	45	47	49	51	67		

From Chang & Richter (1993)

^aIn males, the historical incidence of hepatocellular tumours (adenomas plus carcinomas) ranges from 1.4% to 15%. In females the historical incidence of hepatocellular tumours ranges from 0% to 10%**p* < 0.05, ***p* < 0.001

Table 16. Results of studies of genotoxicity with fludioxonil

End-point	Test object	Concentration or dose	Purity (%)	Result	Reference
<i>In vitro</i>					
Reverse mutation	<i>S. typhimurium</i> , plate incorporation	0, 200, 780, 3130, 12 500 or 50 000 µg/ml ±S9, in acetone	NS	Negative	Ogorek (1987) ^a
Reverse mutation	<i>S. typhimurium</i> , broth culture	0, 0.976, 3.91, 16.5, 250 or 1000 µg/ml ±S9, in DMSO	NS	Negative	Deparade (1987)
Reverse mutation	<i>S. typhimurium</i> , <i>E. coli</i> , ±S9, plate incorporation	0, 200, 780, 3130, 12 500 or 50 000 µg/ml ±S9, in acetone	97.5	Negative	Ogorek (1989)
Gene mutation	Chinese hamster V79 cells	0, 0.5, 1.0, 2.0, 4.0, 8.0, 10.0, 12.0, 16.0 or 20.0 µg/ml –S9, in DMSO	97.5	Negative	Dollenmeier (1989)
		0, 1.5, 3.0, 6.0, 12.0, 18.0, 24.0, 30.0, 36.6, 48.0 or 60.0 µg/ml +S9, in DMSO		Negative	
Chromosomal aberration	Chinese hamster ovary CCL 61 cells	0, 10.94, 21.88 or 43.75 µg/ml (3 h) –S9, in DMSO	97.5	Positive	Strasser (1989)
		0, 2.73, 5.47 or 10.94 µg/ml (24 h) –S9, in DMSO		Negative	
		0, 5.74, 10.94, 21.88, 43.75, 87.5, 175.0 or 350.0 µg/ml +S9, in DMSO		Weak	
		0, 4.1, 12.3, 37, 111, 333, 1000, 2500 or 5000 µg/ml		Positive ^a	
Unscheduled DNA synthesis	Rat hepatocytes	0, 4.1, 12.3, 37, 111, 333, 1000, 2500 or 5000 µg/ml	97.5	Negative	Hertner (1989)
<i>In vivo</i>					
Micronucleus formation	Tif:MAGf mice	0, 1250, 2500 or 5000 mg/kg bw in aqueous carboxy methyl cellulose	97.5	Negative	Hertner (1990)
Micronucleus formation	Tif:RAIf rats	0, 1250, 2500, 5000 mg/kg bw in aqueous carboxy methyl cellulose	97.5	Negative	Meyer (1991), Ogorek (1999)
Chromosomal aberration	Chinese hamster	0, 1250, 2500 or 5000 mg/kg bw in aqueous carboxy methyl cellulose	96.4	Negative	Hertner (1993a)
Dominant lethal mutation	Tif:MAGf mice	0, 1250, 2500 or 5000 mg/kg bw in aqueous carboxy methyl cellulose	96.4	Negative	Hertner (1992a)
DNA repair synthesis	Tif:RAIf rats	0, 2500 or 5000 mg/kg bw in aqueous carboxy methyl cellulose	96.4	Negative	Hertner (1993c)
Replicative DNA synthesis				Negative	
Bone marrow aneuploidy	CrI:CD(SD)BR rats	1250, 2500 or 5000 mg/kg bw in aqueous carboxy methyl cellulose	97.5	Negative	Myhr (1999)
Dominant lethal mutation	Tif:MAGf mice	1250, 2500, 5000 mg/kg bw per day	96.4	Negative	Hertner (1992b)

DMSO, dimethyl sulfoxide; NS, not stated

^aGLP status unknown^bLess than a doubling of the control value, but statistically significantly higher (aberration rates: negative control, 6%; positive control, 84%; 87.5 and 175 µg/ml, 11%) at non-cytotoxic concentrations

Parental F₀ males at 3000 ppm ate, and weighed (<5% reduction in weight gains), slightly less than the controls, and F₁ males had slightly but significantly reduced food consumption and weight gains (<10%). Weight gains in F₀ parental females at 3000 ppm were generally lower than those of controls, reaching significance at some time-points, but were largely unaffected in F₁ females. Food consumption was reduced in the F₀ but not F₁ females. Absolute weights of the testes were similar in the control and treated groups, however, F₁ males at 3000 ppm had significantly higher testicular weights relative to body weights (control, –0.63; 3000 ppm, –0.68, $p < 0.05$) reflecting the lower body weights (testes weights tend to be conserved where weight gain is reduced). Treatment did not affect the number of females mating within 21 days, fertility or reproduction parameters, or the length of gestation. The number of implantation sites per dam was significantly higher for F₁ groups at 300 and 3000 ppm compared with controls, which is clearly not an adverse effect and is likely to be an incidental finding. Absolute weights of the ovary were significantly lower in F₀ females at 30 and 3000 ppm than in controls, although no dose-related response was noted (control, –0.12 g; 30 ppm, –0.10* g; 300 ppm, –0.11 g; 3000 ppm, –0.10* g; where

* is $p < 0.05$) and no effect was seen in F_1 females. It was consequently concluded that this finding was incidental. No significant differences in survival indices were noted. One F_0 dam treated with fludioxonil at 3000ppm had 13 pups with no milk in their stomachs. F_1 and F_2 pups at 3000ppm weighed significantly less than controls (8–12% by day 14 and similar difference at day 21). This effect is not attributable to increased litter size or effects on parental weight gains as these were not consistent across the two generations of pups. The effects are also not attributable to consumption of maternal diet by the pups as the extent of the effect was maximal on day 14 with no progression to day 21. Consumption of maternal diet increased substantially between days 14 and 21. No treatment-related gross pathological changes were noted in culled pups and weanlings. The mean litter size was significantly increased in F_2 pups at 300 and 3000ppm; this is probably an incidental finding and is clearly not adverse. The mean proportion of pups surviving to day 4 was significantly decreased for the F_2 generation at 3000ppm, but was similar to the F_1 control value for this parameter. Also the number of pup deaths in the control group on postnatal days 0–21 was 3.9 per litter for F_1 pups and 1.3 per litter for F_2 pups, compared with 3.1 per litter and 4.2 per litter for the F_1 and F_2 pups at 3000ppm, respectively, suggesting that the apparent decrease in survival rate in the F_2 pups at 3000ppm was artifactual. The NOAEL for parental toxicity was 300ppm (21 mg/kgbw per day) on the basis of slightly lower body weights and food intake in males at the next higher dose. The NOAEL for pup development was 300ppm (21 mg/kgbw per day) on the basis of lower body weights at the next higher dose. There were no effects on the reproductive success of parental animals at the highest dose (Singh, 1992).

Sprague-Dawley rats were mated, with the day on which a sperm-positive vaginal smear was obtained being designated day 0. Groups of 25 successfully mated females were then treated with fludioxonil (purity, 97.5%) at a dose of 0, 10, 100 or 1000mg/kgbw per day by gavage on days 6–15 of gestation. Maternal toxicity was evaluated using: rate of mortality, daily clinical observations, measurements of body weight and food consumption, and dissection and examination of the reproductive tract. Fetuses were removed by caesarean section on day 20 of gestation, and toxicity was determined by external, visceral and skeletal examination, and measurement of body weight. Food, water and the housing environment were controlled and monitored. One female in the control group died during the study and two dams in the groups receiving a dose of 10 or 100mg/kgbw per day had total resorptions. No treatment-related clinical signs were noted. Females treated with fludiox-

Table 17. Body weights (g/% of that of controls) of pups in a two-generation study of reproductive toxicity in rats fed diets containing fludioxonil

Generation	Postnatal day	Dietary concentration (ppm)							
		Males				Females			
		0	30	300	3000	0	30	300	3000
F_1	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
	4 ^a	10.18/100	9.88/97	10.47/103	9.28*/91	9.59/100	9.40/98	10.02/104	8.80/92
	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
	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	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
	4 ^a	9.15/100	9.64/105	9.03/99	8.67/95	8.89/100	8.96/101	8.76/99	8.16/92
	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
	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

From Singh (1992)

^aWeight of remaining pups after culling

* $p < 0.5$, ** $p < 0.01$

Table 18. Litter parameters in a two-generation study of reproductive toxicity in rats fed diets containing fludioxonil

Parameter	Dietary concentration (ppm)			
	0	30	300	3000
<i>F₁ generation</i>				
No. of implantation sites	16.2	14.8	15.0	15.3
Mean litter size (day 0)	15.0	13.3	13.6	14.7
Mean % of pups that survived until day 4	96.9	98.5	98.3	98.3
<i>F₂ generation</i>				
No. of implantation sites	14.86	12.68	16.42*	16.32*
Mean litter size (day 0)	14.1	12.2	15.1*	15.5*
Mean % of pups that survived until day 4	98.8	93.7	98.4	96.3*

From Singh (1992)

* $p < 0.5$, ** $p < 0.01$ **Table 19. Findings in fetuses in a study of developmental toxicity in rats given fludioxonil by gavage**

Parameter	Dose (mg/kg bw per day)			
	0	10	100	1000
Body weight (g)	3.73 ± 0.39	3.86 ± 0.50	3.91 ± 0.45	3.95 ± 0.41
Dilated renal pelvis	1 (0.8%)	1	1	5 (4.0%)
Dilated ureter	4 (3.1%)	2	5	7 (5.6%)

From Savary (1989a)

onil at 1000 mg/kg bw per day had significantly lower body-weight gains during gestation (control, -98.3 g; 1000 mg/kg bw per day, -82.2 g). A slight decrease in food consumption at 1000 mg/kg bw per day was noted from day 6 to day 11 of gestation. Treatment had no effect on the number of live implants per dam, rate of resorptions, pup sex ratio, or fetal external or skeletal alterations. There was a very slight, non-significant increase in pup weight with dose. Slight increases in the incidence of dilated renal pelvis and dilated ureter were noted in fetuses at 1000 mg/kg bw per day, but remained well within the range for historical controls (0.6–7.5%) and the pattern of the dose–response relationship suggests that this is an incidental finding. The NOAEL for fetal development was 1000 mg/kg bw per day, the highest dose tested. The NOAEL for maternal toxicity was 100 mg/kg bw per day on the basis of lower body-weight gains at the next higher dose. Fludioxonil was not teratogenic in this study (Savary, 1989a).

Rabbits

Groups of 16 female rabbits were artificially inseminated and then treated with fludioxonil (purity, 97.5%) at a dose of 0, 10, 100 or 300 mg/kg bw per day by gavage in 0.5% methylcellulose solution in distilled water during days 6–18 of gestation. Maternal toxicity was evaluated using: rate of mortality, daily clinical observations, measurements of body weight and food consumption, and dissection and examination of the reproductive tract. Fetuses were removed by caesarean section on day 28 of gestation and toxicity was determined by external, visceral and skeletal examination, and measurement of body weight. Food, water and the housing environment were controlled and monitored. One female at 100 mg/kg bw per day died during the study and one dam in the control group, and in the groups receiving a dose of 10 and 300 mg/kg bw per day had total resorptions. One female in the control group and one female at 300 mg/kg bw per day aborted. Blue-coloured urine,

Table 20. Key observations in a study of developmental toxicity in rabbits given fludioxonil by gavage

Observation	Dose (mg/kg bw per day)			
	0	10	100	300
<i>Skeletal variation</i>				
Reduced ossification of the fifth sternebra	22	20	30	33
Total skeletal variations	40	40	39	52
<i>Body and organ weights</i>				
Body weight (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
Body-weight gain (g/[% of control])				
Days 0–6	173.3/100	140.7/81	191.4/110	159.3/92
Days 6–18	152.5/100	195.7/128	126.4/83	76.4/50
Days 18–28	145.0/100	125.0/86	105.7/73	122.9/85
Days 0–28 (individual range)	470.8/100	461.4/98	423.6/90	358.6/76
Days 0–28 ^a (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
<i>Food consumption</i> ([g/animal per day]/ [% of control])				
Days 0–6	209/100	193/92	198/95	189/90
Days 6–12	188/100	193/103	180/96	145*/77
Days 12–19	191/100	189/99	194/102	164/86
Days 19–24	181/100	165/91	171/95	153/85
Days 24–28	138/100	127/92	148/107	110/80

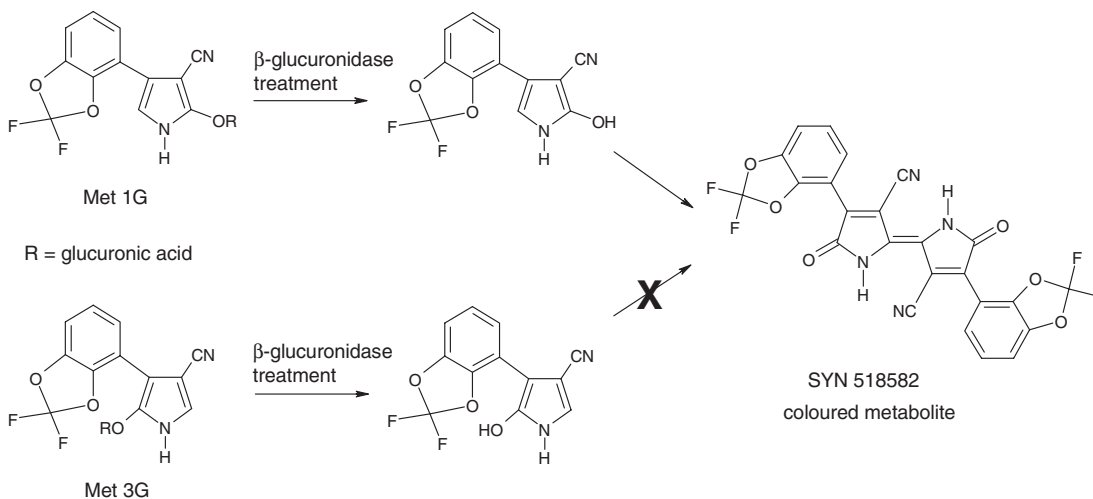
From Savary (1989b)

* $p < 0.05$ (Student's t-test)^a Without uterus weight

attributable to the presence of a blue metabolite of fludioxonil, was noted in four dams at 100 mg/kg bw per day and 12 dams at 300 mg/kg bw per day, but was considered to be of no toxicological relevance. Females treated with fludioxonil at 300 mg/kg bw had significantly lower body-weight gains during gestation (471, 424 and 359 g at 0, 100 and 300 mg/kg bw per day respectively), and reduced food consumption over the period of the study, and those at 100 mg/kg bw per day weighed slightly less than the controls from day 18. The equivocally lower net body-weight gain, without reduced food consumption, observed at 100 mg/kg bw per day was at least partially due to lower body weights in this group at the start of dosing. The individual values were well within the range of those for the controls and the final body weights expressed as a percentage of those of the controls were comparable at doses of up to 100 mg/kg bw per day (100%, 99%, 97% and 95% at 0, 100 and 300 mg/kg bw per day respectively). Consequently, the slightly lower body-weight gain at 100 mg/kg bw per day was considered to be not adverse and was probably incidental.

The sex ratio was statistically different in the control group (63% male) and at 300 mg/kg bw (48% male), but is unlikely to be attributable to treatment. Treatment did not affect the number of resorptions, fetal loss, fetal weights, or external, visceral and skeletal anomalies. There was a non-significant increase in the incidence of reduced ossification of the fifth sternebra and overall incidence of skeletal variations at 300 mg/kg bw, which is likely to reflect delayed development secondary to maternal nutritional stress manifested as reduced weight gains and food consumption. The NOAEL for fetal development was 300 mg/kg bw per day. The NOAEL for maternal toxicity was 100 mg/kg bw per day on the basis of lower body-weight gains and reduced food consumption at the next higher dose (Savary, 1989b).

Figure 3. Mechanism of production of the coloured metabolite of fludioxonil



2.6 Special studies: mechanisms

To investigate the cause of the blue colouration seen in the urine and some organs, the metabolites of fludioxonil in pooled samples of urine collected at 1, 3, 6, 12, 18 and 24 months during the long-term study in rats given diets containing fludioxonil at a concentration of 0, 10, 30, 100, 1000 or 3000 ppm were investigated. Male rats given diets containing fludioxonil at 3000 ppm for 7, 13 and 24 months were given a pulse dose of [4-¹⁴C]pyrrole fludioxonil by gavage. Urine was collected from these animals for the next 72 h. From the original study, animals at 1000 and 3000 ppm displayed coloured urine with an absorption maximum of 594 ± 2 nm. The absorption spectrum was between 450 nm and 650 nm. Two of the original samples had a further peak at 650 nm. For this peak, absorption increased with storage, indicating that it is derived from a degradation product. The extent of colouration of the urine reached a plateau after 3 months. The proportion of the administered radiolabel that was recovered in the urine was similar at 7, 13 and 24 months (24–33% over 24 h and 30–34% over 72 h). In a previous study of metabolism, 16.5% of the administered radiolabel was collected in the urine over 72 h after a single dose of fludioxonil. The pattern of metabolites was determined for the 0–24 h after the pulse dose. For all samples, a pronounced absorption maximum was noted between 593 nm and 596 nm. The pattern of urinary metabolites was similar to that noted in toxicokinetic studies. One nonpolar fraction was revealed to be the coloured component and represented between 3.9 and 6.4% of the radiolabelled dose, which corresponds to 1–2% of a daily dose at a dietary concentration of 3000 ppm. When one of the other metabolites was treated with β-glucuronidase, the solution turned blue immediately. The relative molecular mass was determined to be 538 and the proposed structure is presented in Figure 3 (Thanei, 1994).

2.7 Studies on metabolites

Studies were conducted on the acute oral toxicity and genotoxicity of several metabolites of fludioxonil that are formed in plants, soil, and/or chickens. These metabolites have the company codes of SYN 518579/CGA 308565 (a tautomeric pair; structures are given in Figure 4), CGA 265378, CGA 339833, CGA 308103, and CGA 192155. Their structures are provided in Figure 5, and the results of these studies are summarized in Table 21. In addition, a 90-day dietary study with CGA 339833 was conducted in rats.

Figure 4. Structures of some key plant and animal metabolites of fludioxonil that are not found in rats

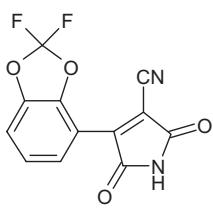
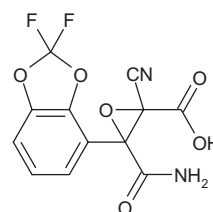
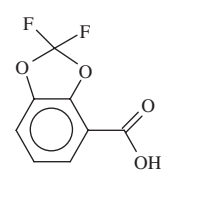
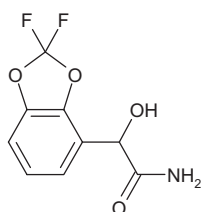
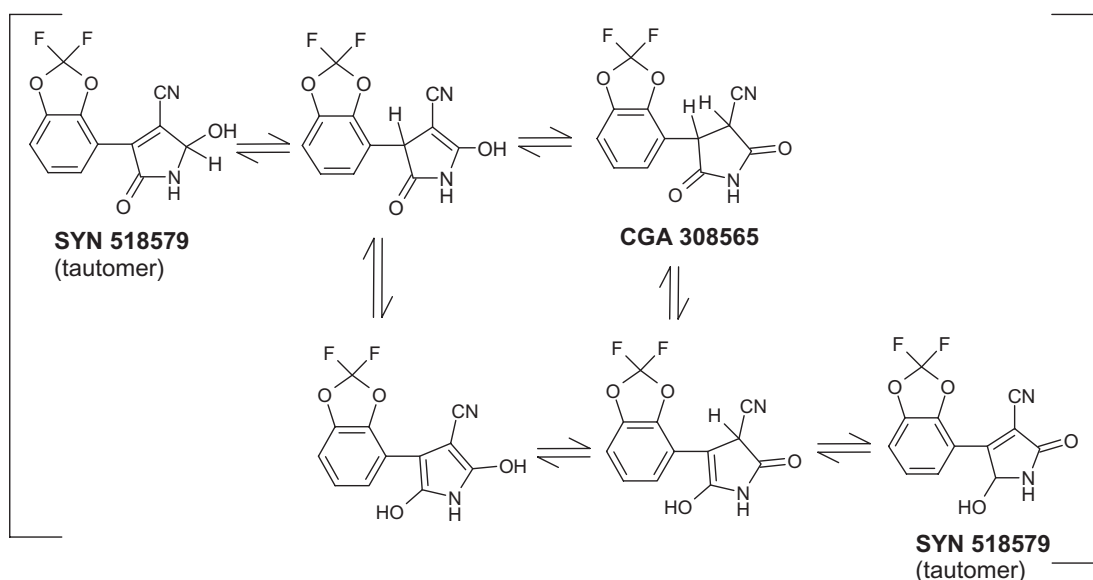
			
CGA 265378	CGA 339833	CGA 192155	CGA 308103

Figure 5. Tautomerism of SYN 51879 and CGA 308565



CGA 339833 is formed from fludioxonil by photolysis, hydroxylation, and consequent opening of the pyrrol ring. The compound was identified in soil and water (photolysis studies at 15–31%) and in plants (wheat, peach, green onions, lettuce and in studies of rotational crops with wheat, radish and mustard).

Groups of 10 male and 10 female HanBrl:WIST(SPF) rats were given diets containing CGA 339833 (purity, 96%) at a concentration of 0, 10, 100, 800, 2500 or 7000 ppm for 90 days. Actual achieved doses of CGA 339833 were 0.7, 7.1, 58, 190, 510 mg/kg bw per day for males and 0.9, 8.7, 67, 210, 600 mg/kg bw per day. Stability and homogeneity of the test article in the diet were checked before treatment and at intervals during the study. Mortality was checked twice per day and clinical signs were checked daily. Body weight, food and water consumption were recorded before the start of treatment and once per week thereafter. Ophthalmological examinations were performed for all animals before treatment, and for animals in the control group and in the group receiving the highest dose in week 13. A functional observational battery (activity and overexcitation of the central nervous

Table 21. Acute oral toxicity and genotoxicity of metabolites of fludioxonil

Metabolite	End-point and test object	Dose	Result	Reference
Syn 518579/CGA 308565	LD ₅₀ , rat	2000 mg/kg bw	LD ₅₀ > 2000 mg/kg bw (no deaths)	Hartmann (1993a)
	Ames test, <i>S. typhimurium</i> and <i>E. coli</i>	312.5–5000 µg/plate ±S9	Negative	Hertner (1992c)
CGA 265378	LD ₅₀ , rat	2000 mg/kg bw	LD ₅₀ > 2000 mg/kg bw (no deaths)	Hartmann (1992)
	Ames test, <i>S. typhimurium</i> and <i>E. coli</i>	312.5–5000 µg/plate ±S9	Negative	Hertner (1992a)
CGA 339833	LD ₅₀ , rat	2000 mg/kg bw	LD ₅₀ > 2000 mg/kg bw (no deaths)	Sommer (2000)
	Ames test, <i>S. typhimurium</i> and <i>E. coli</i>	312.5–5000 µg/plate ±S9	Negative	Deparade (2000)
	Gene mutation, mouse lymphoma L5178Y cells	4 h: 400–3200 µg/ml (–S9); 200–3400 µg/ml (+S9); 24 h: 400–3200 µg/ml (–S9)	Negative	Wollny (2001)
	<i>In vitro</i> Chromosomal aberration, Chinese hamster V79 cells	–S9: 800–2400 µg/ml (4 h + 14 h), 200–1600 µg/ml (18 h), 800 µg/ml (28 h); +S9: 200–2400 µg/ml (4 h + 14 h), 200–800 µg/ml (4 h + 24 h)	Positive: –S9: 18 h Negative –S9: 4 h (+14 h) and 28 h +S9: 4 h (+14 h) and 4 h (+24 h)	Schulz (2002)
	<i>In vivo</i> Micronucleus formation, rat bone marrow	500, 1000 or 2000 mg/kg bw	Negative	Fox (2002)
CGA 308103	LD ₅₀ , rat	200, 500, 1000 or 2000 mg/kg bw	1000 < LD ₅₀ < 2000 mg/kg bw (Deaths: 0/5, 0/5, 0/3, 5/5)	Hartmann (1993c)
	Ames test, <i>S. typhimurium</i> and <i>E. coli</i>	312.5–5000 µg/plate	Negative	Hertner (1994)
CGA 192155	LD ₅₀ , rat	2000 mg/kg bw	LD ₅₀ > 2000 mg/kg bw	Hartmann (1993b)
	Ames test, <i>S. typhimurium</i> and <i>E. coli</i>	78.1–1250 µg/plate (+S9 <i>S. typhimurium</i> first experiment) 312.5–5000 µg/plate (all other experiments)	Negative	Hertner (1993b)

system, sensorimotor, autonomic and physiological functions and motor activity tests) was performed towards the end of treatment. At the end of treatment, haematology, clinical chemistry, urine analysis, organ weights, gross pathology and histopathology were performed.

There were no treatment-related effects on mortality, clinical signs, the functional observation battery, food and water consumption, haematology, ophthalmological parameters, or gross pathology. Body-weight gain was slightly reduced in animals at the highest dose, resulting in final body weights that were 10% and 5% lower than those of the controls for males and females respectively. Apparently lower body-weight gain in males at 100 ppm was at least partly due to single animals in the control group and in the group receiving fludioxonil at 10 ppm with cumulative weight gains that were >50% greater than the average for those groups. If body-weight gains are calculated without these two outliers, then values for groups of males treated at ≤100 ppm are similar to those of the controls. Weight gains for groups treated with fludioxonil at 800 to 7000 ppm remain approximately 10–13% below that of the control group, but with a flat dose–response

relationship particularly at 800 and 2500 ppm. Consequently, lower body-weight gains in males treated at 100 ppm are considered to be incidental to treatment and those observed at 800 and 2500 ppm to be of equivocal relationship to treatment. Significantly lower triglyceride and higher phosphorus values were observed in males at 7000 ppm. Males at 7000 ppm excreted slightly larger volumes of a more dilute urine. A slightly increased absolute weight of the liver was noticed in females at 7000 ppm and relative weights were increased in both sexes at this dose. The weight changes correlated with centrilobular hepatocellular hypertrophy. Relative weight of the kidney was increased for males at 7000 ppm and slight non-significant increases were seen in males at 2500 ppm and in females at 7000 ppm. Histopathologically, a slightly increased incidence and severity of tubular casts was seen in males at 800 to 7000 ppm. The tubular casts were found in the proximal tubuli and consisted of eosinophilic formations in the tubular lumen and walls without causing tubular distention. As tubular casts were present in more than half of the control males, the slight increase in incidence and grading was not considered to be adverse—especially for males at 800 ppm where this finding was not accompanied by any change in kidney weight or other indications of kidney damage in clinical pathology parameters. Increased relative weights of the testes in males at 7000 ppm were considered to be secondary to reduced weight gains in this group, as absolute testes weights tended to be preserved. Increased weights of the ovaries in females treated at 100 ppm were attributable to the presence of watery cysts in one animal, and increased adrenal weight in this group was discounted as incidental owing to the lack of a dose–response relationship. Histopathology revealed an increased incidence of minimal to slight atrophy in the olfactory epithelium at 2500 and 7000 ppm, which was characterized by the disorderly arrangement of the olfactory epithelium, retention of secretion in the olfactory mucosal glands, and foamy appearance of sub-mucosal supporting cells. A slightly increased incidence of follicular cell hypertrophy in the thyroid gland was seen in females at 7000 ppm.

The NOAEL for CGA 339833 was 800 ppm (equal to 58 and 67 mg/kg bw per day in males and females) on the basis of increased relative weight of the liver correlating with hepatocellular hypertrophy, increased relative weight of the kidney and tubular casts at ≥ 2500 ppm (males only), and minimal to slight atrophy of the olfactory epithelium at ≥ 2500 ppm (Sommer, 2001).

3. Observations in humans

Medical surveillance of employees engaged in the manufacture of fludioxonil or its formulation into products since 1992 has not revealed any adverse health effects. Medical surveillance included, at some sites, anamnesis, physical examination, haematology clinical chemistry and urine analysis (Lorez & Ledgerwood, 2003).

Comments

After oral administration of radiolabelled fludioxonil, the radiolabel is rapidly and extensively (approximately 80% of the administered dose) absorbed, widely distributed, extensively metabolized and rapidly excreted, primarily in the faeces (approximately 80%) via the bile (approximately 70%), with a small amount being excreted in the urine (approximately 20%). The maximum blood concentration is reached within 1 h after administration. Elimination is biphasic, with half-lives of between 2 h and 5 h for the first phase, and between 30 and 60 h for the second phase. Fludioxonil is rapidly cleared from the blood and tissues, and there is consequently negligible potential for accumulation. The metabo-

Table 22. Principle findings of a 90-day study with CGA 339833 in rats

	Dietary concentration (ppm)											
	Males						Females					
	0	10	100	800	2500	7000	0	10	100	800	2500	7000
Terminal body weight (g)	403.5	398.5	374.0	371.6	374.1	359.8	215.6	222.4	216.4	220.6	212.1	205.4
Body weight (% of control), week 13	100	99	94	93	94	91	100	103	101	102	98	95
Body-weight gain (g), weeks 1–13	227	221	203	197	199	188**	93	98	97	93	86	81
Triglycerides (mmol/l)	0.84	0.79	0.71	0.62	0.54	0.45**	0.391	0.417	0.399	0.466	0.384	0.367
PO ₄ ³⁻ (mmol/l)	1.56	1.45	1.45	1.46	1.57	1.81**	1.27	1.22	1.54*	1.35	1.44	1.49
<i>Urine</i>												
Volume (ml)	2.98	3.66	2.63	3.67	4.02	4.69*	3.80	3.91	3.26	4.03	4.46	4.16
Relative density (1)	1.072	1.063	1.078	1.066	1.053	1.046*	1.050	1.048	1.051	1.036	1.040	1.046
<i>Liver</i>												
Absolute weight (g)	14.6	13.91	13.38	12.67	13.57	14.23	7.66	7.86	8.13	7.79	7.76	8.66
Relative to body weight (%)	36.2	35.1	35.8	34.1	36.4	39.5*	35.5	35.4	37.5	35.4	36.6	42.3*
Relative to body weight (% of control)	100	97	99	94	101	109	100	100	106	100	103	119
Hepatocyte hypertrophy [grade]	0/10 [—]	0/10 [—]	0/10 [—]	0/10 [—]	7/10 [1.0]	10/10 [2.4]	0/10 [—]	0/10 [—]	0/10 [—]	0/10 [—]	1/10 [1.0]	7/10 [1.9]
<i>Kidney</i>												
Absolute weight (g)	2.519	2.427	2.373	2.343	2.524	2.492	1.630	1.683	1.657	1.584	1.551	1.615
Relative to body weight (%)	6.25	6.14	6.35	6.30	6.78	6.93**	7.54	7.58	7.66	7.21	7.32	7.88
Relative to body weight (% of control)	100	98	102	101	109	111	100	101	102	96	97	105
Tubular casts [grade]	6/10 [1.3]	7/10 [1.3]	7/10 [1.0]	8/10 [1.8]	10/10 [1.6]	10/10 [1.8]	0/10 [—]	0/10 [—]	1/10 [2.0]	1/10 [2.0]	0/10 [—]	0/10 [—]
<i>Nasal cavity</i>												
Atrophy of olfactory epithelium [grade]	0/10 [—]	0/10 [—]	0/10 [—]	0/10 [—]	4/10 [1.0]	10/10 [1.6]	0/10 [—]	0/10 [—]	0/10 [—]	0/10 [—]	1/10 [1.0]	7/10 [1.9]
<i>Thyroid gland</i>												
Follicular cell hypertrophy [grade]	10/10 [1.2]	9/10 [1.3]	8/10 [1.3]	7/10 [1.0]	5/10 [1.0]	10/10 [1.4]	2/10 [1.0]	3/10 [1.0]	1/10 [1.0]	3/10 [1.0]	1/10 [1.0]	5/10 [1.0]

From Sommer (2001)

* $p < 0.05$, ** $p < 0.01$ (Dunnett's test)

lism of fludioxonil proceeds primarily through oxidation of the pyrrole ring, leading to one major (57% of the administered dose) and one minor (4% of the administered dose) oxo-pyrrole metabolite. Hydroxylation of the phenyl ring yields the corresponding phenol metabolite, which represents 2% of the administered dose. These phase I metabolites are subsequently excreted as glucuronyl and sulfate conjugates and, together with unabsorbed and unchanged fludioxonil excreted in faeces, account for approximately 75% of the administered dose. The dimerization of the hydroxy pyrrole metabolite produces a metabolite of an intense blue colour.

The dermal absorption of fludioxonil, excluding material bound to the skin, is low in rats in vivo (<5%) and in human skin in vitro (<0.5%). In a study of dermal penetration in rats in vitro, values for dermal absorption at low levels of application were comparable to those obtained in a study performed in vivo (<2%), but at higher levels these values significantly overestimated absorption in vivo (38%).

Fludioxonil has low acute toxicity in rats when administered by oral, dermal or inhalation routes, producing no deaths at 5000 and 2000 mg/kg bw and 2.6 mg/l of air, respectively, the highest doses tested. There were also no deaths in mice given fludioxonil at 5000 mg/kg bw by gavage. Fludioxonil is a slight ocular irritant in rabbits, but is neither a dermal irritant in rabbits nor a dermal sensitizer in guinea-pigs (Magnusson & Kligman maximization assay).

In studies of repeated doses in mice and rats, the liver (necrosis, centrilobular hypertrophy, increased serum cholesterol and 5' nucleotidase), the kidneys (nephropathy, inflammation, cysts) and haematopoietic system (mild anaemia) were the principle targets. Such effects often set the LOAELs for these studies, together with reduced body-weight gains. In mice, these effects were observed after 90 days of treatment at 450 mg/kg bw per day and at 590 mg/kg bw per day in one 18-month study, but not at 360 mg/kg bw per day in another such study. In rats, effects were seen at doses of ≥ 400 mg/kg bw per day in short-term studies and at 110 mg/kg bw per day in a 2-year study; lower body-weight gains were also observed at these doses. Liver toxicity was generally manifested by increased concentrations of serum cholesterol and bilirubin, and centrilobular hypertrophy and/or necrosis. Anaemia in mice (at > 590 mg/kg bw per day for 18 months) and rats (at 1300 mg/kg bw per day for 3 months) was seen at doses greater than the LOAEL. In dogs, anaemia was observed at the LOAEL (at 290 mg/kg bw per day for 3 months, but only after 4 weeks of treatment; and at a dose of 300 mg/kg bw per day for 12 months). No haematological effects were observed in shorter studies in mice (at ≤ 1050 mg/kg bw per day for 90 days) or rats (at ≤ 2500 mg/kg bw per day for 20 days, and at ≤ 1000 mg/kg bw per day for 28 days).

Blue discolouration of the urine, perineal fur, kidneys and gastrointestinal tract were common observations in all species. These effects were secondary to the formation of the blue metabolite in quantities that were sufficient, at high doses, to stain the various tissues. The effect is not toxicologically significant and was disregarded in identifying NOAELs from studies in which it was observed.

Fludioxonil gave negative results in assays for reverse mutation in *S. typhimurium* and *E. coli*, gene mutation in Chinese hamster V79 cells, unscheduled DNA synthesis in rat hepatocytes, micronucleus formation in bone marrow of rats and mice in vivo, and chromosome aberration in Chinese hamsters in vivo. Fludioxonil was clastogenic in Chinese hamster ovary cells (CCL61) in vitro at non-cytotoxic concentrations. There was no evidence of heritable genetic damage in an assay for dominant lethal mutations in mice.

The Meeting concluded that fludioxonil is unlikely to be genotoxic in vivo.

The carcinogenicity potential of fludioxonil was examined in a study in rats and in two studies in mice. While the incidence of lymphomas was slightly increased in females in one study in mice receiving diets containing fludioxonil at a concentration of 3000 ppm (equal to 360 mg/kg bw per day), no increase was observed in a concurrent life-time study in mice given diets containing fludioxonil at dietary concentrations of up to 7000 ppm (1000 mg/kg bw per day). Lymphoma is a common finding in ageing female CD-1 mice, and the historical incidence at the laboratory conducting these studies was 13–32%, which encompasses the incidence noted in females at 3000 ppm (30%). Given the high background rate of this finding and the lack of any increase in the other study by the same authors using higher doses, the Meeting concluded that the apparent increase in lymphomas observed in one study in mice was incidental. There was no evidence of carcinogenic potential

with fludioxonil in the study in rats. The overall NOAELs in the long-term studies were 112 mg/kg bw per day and 37 mg/kg bw per day in mice and rats respectively.

On the basis of the above consideration and on the lack of genotoxic potential in vivo, the Meeting concluded that fludioxonil is unlikely to pose a carcinogenic risk to humans.

In a two-generation study of reproductive toxicity in rats, at a dose of 210 mg/kg bw per day, adult males had reduced body-weight gains and food consumption and pups had lower body-weight gains than did the controls. The NOAEL for parental and pup toxicity was 21 mg/kg bw per day. The NOAEL for effects on reproductive performance was 21 mg/kg bw per day on the basis of reduced pup weights. As no effects on body-weight gain, or any other parameter, were seen in adult rats at 37 mg/kg bw per day in a 2-year study, the NOAEL for parental animals in the study of reproductive toxicity can also be considered to be >37 mg/kg bw per day. The NOAEL of 21 mg/kg bw per day for pup toxicity was based on effects observed at 210 mg/kg bw per day. These effects were relatively mild and the dose-response relationship appears to be shallow (12% decrease in body-weight gain over the dose range of 190 mg/kg bw per day, or a 1%, or less, decrease in body-weight gain per dose increment of 16 mg/kg bw per day). Assuming a linear dose-response relationship between 21 and 210 mg/kg bw per day, then at the proposed overall NOAEL for rats of 37 mg/kg bw per day, a decrease in body-weight gain of $\leq 1\%$ would be predicted in pups; this would not be interpreted as being an adverse effect. Consequently, the use of an overall NOAEL of 37 mg/kg bw per day is also appropriate for pup toxicity. In a study of developmental toxicity in rabbits and another in rats, fludioxonil was neither teratogenic nor fetotoxic and fetal weights were unaffected at doses of up to 1000 mg/kg bw per day and 300 mg/kg bw per day, respectively. Maternal toxicity in these studies was limited to reduced body-weight gain at 1000 mg/kg bw per day and 300 mg/kg bw per day in rats and rabbits, respectively.

The Meeting concluded that the existing database on fludioxonil was adequate to characterize the potential hazards to fetuses, infants and children.

Studies of acute oral toxicity and genotoxicity with a range of plant metabolites of fludioxonil demonstrated that these metabolites were of low acute oral toxicity and were not genotoxic. A 90-day study in rats given diets containing a photolytic/hydrolytic degradation product of fludioxonil found in soil and water yielded a NOAEL of 800 ppm (equal to 58 mg/kg bw per day), on the basis of increased relative weight of the kidney and tubular casts at ≥ 2500 ppm (in males), and minimal to slight atrophy of the olfactory epithelium at ≥ 2500 ppm.

Toxicological evaluation

The Meeting established an ADI of 0–0.4 mg/kg bw based on a NOAEL of 37 mg/kg bw per day in a 2-year dietary study in rats, and a 100-fold safety factor.

Although effects on the kidneys occurred after relatively short periods of exposure, the Meeting concluded that such effects were unlikely to result from a single exposure. Consequently, the Meeting concluded that an ARfD for fludioxonil was unnecessary.

Levels relevant to risk assessment

Species	Study	Effect	NOAEL	LOAEL
Mouse	18-month study of toxicity and carcinogenicity ^a	Toxicity	1000 ppm, equal to 112 mg/kg bw per day	3000 ppm, equal to 360 mg/kg bw per day
		Carcinogenicity	3000 ppm, equal to 360 mg/kg bw per day ^b	—
Rat	2-year study of toxicity and carcinogenicity ^a	Toxicity	1000 ppm, equal to 37 mg/kg bw per day	3000 ppm, equal to 110 mg/kg bw per day
		Carcinogenicity	3000 ppm, equal to 110 mg/kg bw per day ^b	—
	Two-generation study of reproductive toxicity ^a	Parental toxicity	300 ppm, equal to 21 mg/kg bw per day ^d	3000 ppm, equal to 210 mg/kg bw per day
		Embryo- and fetotoxicity	300 ppm, equal to 21 mg/kg bw per day ^d	3000 ppm, equal to 210 mg/kg bw per day
	Developmental toxicity ^c	Maternal toxicity	100 mg/kg bw per day	1000 mg/kg bw per day
		Embryo- and fetotoxicity	1000 mg/kg bw per day ^b	—
Rabbit	Developmental toxicity ^c	Maternal toxicity	100 mg/kg bw per day	300 mg/kg bw per day
		Embryo- and fetotoxicity	300 mg/kg bw per day ^b	—
Dog	12-month study of toxicity ^{a,e}	Toxicity	1000 ppm, equal to 33 mg/kg bw per day	8000 ppm, equal to 300 mg/kg bw per day

^a Diet^b Highest dose tested^c Gavage

^d The NOAEL of 21 mg/kg bw per day in this study was adjusted upwards to 37 mg/kg bw per day on the basis of an absence of effects in 90-day and 2-year studies in rats at 64 and 37 mg/kg bw per day, respectively. Additionally, interpolation of the reduced weight gain in pups in the study of reproductive toxicity indicated a likely, non-adverse, reduction in weight gain of 1% or less at the higher NOAEL of 37 mg/kg bw per day

^e The LOAEL for this study was 300 mg/kg bw per day. Owing to the wide dose spacing used for this study, the NOAEL is conservative and hence the slightly higher NOAEL obtained in the 2-year study in rats was selected for establishment of the ADI

Estimate of acceptable daily intake for humans

0–0.4 mg/kg bw

Estimate of acute reference dose

Unnecessary

Studies that would provide information useful for continued evaluation of the compound

Further observations in humans

Summary of critical end-points for fludioxonil

Absorption, distribution, excretion and metabolism in animals

Rate and extent of oral absorption	Rapid, approximately 80%
Dermal absorption	Poor, <10% in the rat in vivo; ≤1% in human skin in vitro
Distribution	Extensive
Rate and extent of excretion	Largely complete within 24 h; approximately 10% in urine and 80% in the faeces; 70% of the administered dose was excreted in the bile
Potential for accumulation	Low, no evidence of accumulation
Metabolism in mammals	Extensively metabolized, involving primarily oxidation of the pyrrole ring leading to a major (57% of the administered dose) and a minor (4% of the administered dose) oxo-pyrrole metabolite, followed by glucuronyl- and sulfate conjugation
Toxicologically significant compounds (animals, plants and the environment)	Parent compound and metabolites

Acute toxicity

Rat, LD ₅₀ , oral	>5000 mg/kg bw (no deaths)
Rat, LD ₅₀ , dermal	>2000 mg/kg bw (no deaths)
Rat, LC ₅₀ , inhalation	>2.6 mg/l of air (no deaths)
Rabbit, dermal irritation	Not irritating
Rabbit, ocular irritation	Slight irritant
Skin sensitization	Not sensitizing (Magnusson & Kligman test)

Short-term studies of toxicity

Target/critical effect	Damage to liver (rats and dogs) and kidney (mice and rats)
Lowest relevant oral NOAEL	1000 ppm, equal to 33 mg/kg bw per day (12-month study in dogs)
Lowest relevant dermal NOAEL	200 mg/kg bw per day (rats)
Lowest relevant inhalation NOAEC	—

Genotoxicity

Unlikely to pose a genotoxic risk in vivo

Long-term studies of toxicity and carcinogenicity

Target/critical effect	Reduced body-weight gains and liver necrosis in rats, liver and kidney damage in mice
Lowest relevant NOAEL	1000 ppm, equal to 37 mg/kg bw per day, in rats
Carcinogenicity	Not carcinogenic in rats or mice; unlikely to pose a carcinogenic risk to humans

Reproductive toxicity

Reproductive target/critical effect	Reduced pup weight gains in rats at parentally toxic doses
Lowest relevant reproductive NOAEL	300 ppm, equal to 21 mg/kg bw per day
Developmental target/critical effect	None
Lowest relevant developmental NOAEL	300 mg/kg bw per day (the highest dose tested in rabbits)

Neurotoxicity

No evidence of neurotoxicity or delayed neurotoxicity in any study conducted

Other toxicological studies

Studies on plant metabolites and a photolytic/hydrolytic degradation product of fludioxonil indicated that these were of no greater toxicity than the parent compound

Medical data

Medical monitoring since 1992 of employees engaged in the manufacture of fludioxonil, or its formulation, into products has not revealed any adverse health effects

Summary

	Value	Study	Safety factor
ADI	0–0.4 mg/kg bw	2-year study in rats (liver effects and reduced body-weight gains)	100
ARfD	Unnecessary	—	—

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