B.6.3.2.2 Ninety-day oral dietary toxicity study in mice

Author(s):	Chang, J.F.C. and Morrissey, R.L.
Study title:	CGA-173506 – 90-day oral toxicity study in mice. EPA guideline no. 82-1.
Testing facility:	[redacted]
Report number:	Laboratory study number: F-00017.
Study duration:	In life: 6 December 1988 – 10 March 1989.
Date of report:	31 January 1990.
Test substance:	Fludioxonil (CGA-173506).
Batch no.:	Lot number FL-881677.
Purity:	96 %
Test animals:	Male and female Swiss mice of the strain Crl:CD - 1® (ICR) BR.
Origin:	Charles River Laboratories, Kingston, NY.
Body weight:	Males: 23-29 g; females: 19-24 g. 6 weeks old at study start.
Groups:	10/sex/dose group.
Husbandry:	Standard conditions.
Study design:	In accordance with OECD guideline no. 408, Repeated dose 90-day oral toxicity study in rodents, 1981. 13-15 days acclimatisation and feeding for 90 days.
Dose:	Dietary concentrations were 0, 10, 100, 1000, 3000, and 7000 ppm.
Vehicle/solvent:	Diet (Purina Certified Rodent Chow #5002 ground meal).
Route:	Oral, dietary.
Statistics/ measurements:	One-way analysis of variance followed by Dunnett's t-test for most data (body weights, body weight gains, food consumption, feed efficiency, haematology, clinical biochemistry, organ weights); besides Fisher's exact test with Bonferroni correction (treatement-related non-neoplastic lesions).
GLP:	EPA-FIFRA GLP Standards (40 CFR part 160) except that some historical control data were obtained from unaudited studies. Comparable with OECD and Japanese MAFF GLP standards.
Guideline:	EPA-FIFRA guidelines (40 CFR part 158, Section 82-1); OECD guideline no. 408, 1981; Japanese MAFF guidelines on subchronic oral toxicity testing.
Deviation:	None.
Acceptability:	Acceptable.

Results:

Analytical results for individual samples for each dietary concentration revealed that the concentration as well as the homogeneity was within the specifications (15 % of nominal value), except for the 10 ppm and the 100 ppm preparations which differed on one occasion up to 20 % from nominal content.

No deaths occurred in any of the groups.

Clinical observations included blue coloured urine (from 1000 ppm) and blue stains on the pelvis (from 100 ppm) and were first noted in males at 7-14 after treatment started; similar findings were not noted in females of any dose group or at any time during the study. Other clinical observations, which were noted in both sexes, included cloudy eyes, thin hair, scab on pinnae, and swollen or torn pinnae.

No treatment-related eye changes were recorded.

The body weights or body weight gains were not affected in males at any time during the study. High-dose females had statistically significantly decreased body weight and cumulative body weight gain at several weeks, especially late in study (weeks 9, 11, and 12).

There were no significant differences between control and treatment groups with respect to food consumption.

The feed efficiency was not affected in males or females of any dose group.

Table 6.3-8 shows the intake of the test substance expressed in mg/kg bw/day for the various dose groups.

Table 6.3-8
Achieved dosages (mg/kg bw/day)

Dose group	0 ppm	10 ppm	100 ppm	1000 ppm	3000 ppm	7000 ppm
Males	0	1.3	13.9	144	445	1052
Females	0	1.9	17	178	559	1307

With respect to haematology no statistically significant differences were observed between control and treated groups for any of the parameters examined.

Regarding clinical biochemistry the statistically significant changes included increased activity of 5'-nucleotidase in high-dose males and females (129 % and 152 % of control group values, respectively), increased gamma-glutamyl transferase in males of the two lowest dose groups (values within the historical control range and without dose relation), increased total bilirubin in 1000 and 3000 ppm females (within historical control values and without dose relation), and decreased potassium in high-dose females (within historical values).

The urinalysis revealed green-, blue- or brown-coloured specimens containing detectable amounts of bilirubin in males from 1000 ppm; no significant findings were noted in urine samples from females.

Selected organ weights are presented in Table 6.3-9. The relative liver weights were statistically significantly increased in high-dose males and from 3000 ppm in females. The relative kidney weights were slightly increased at most dose levels but without achieving statistical significance changes from the control group. Furthermore, the weight of the adrenals in relation to brain weight was statistically significantly increased in the 100 ppm dose group females.

Table 6.3-9
Mean absolute (abs) (g) and relative (rel) (g/100 g animal) organ weights

Sex	Males						Females					
Dose group ppm	0	10	100	1000	3000	7000	0	10	100	1000	3000	7000
Body weight abs	36.300	37.200	34.900	36.100	34.200	34.000	29.400	28.100	29.900	28.778	27.900	27.200
Thymus abs	0.023	0.027	0.023	0.023	0.019	0.022	0.033	0.027	0.032	0.027	0.028	0.022*
rel	0.064	0.074	0.067	0.063	0.054	0.064	0.112	0.096	0.106	0.089	0.099	0.080
Kidneys abs	0.589	0.591	0.575	0.601	0.547	0.599	0.391	0.391	0.390	0.399	0.385	0.372
rel	1.639	1.595	1.648	1.668	1.604	1.763	1.330	1.402	1.309	1.400	1.383	1.373
Liver abs	1.455	1.446	1.387	1.483	1.437	1.613	1.225	1.172	1.217	1.245	1.324	1.375*
rel	4.044	3.890	3.981	4.106	4.205	4.745**	4.166	4.178	4.074	4.283	4.749**	5.065**

Dunnett's test: * $p \leq 0.05$; ** $p \leq 0.01$.

At necropsy male animals had blue stains on trunk, thorax and abdomen from 1000 ppm and above (9/10 high-dose males), discoloured aglandular mucosa in the stomach (6/10 high-dose males), kidneys with depressed focal discoloration (6/10 high-dose males), discoloured urine in the urinary bladder from 1000 ppm, and discoloured caecum content (4/10 high-dose males). High-dose females had general discoloration of the aglandular mucosa of the stomach (5/10), caecum (3/10) and gall bladder (2/10), kidneys with focal discoloration (1/10), whereas stained fur or discoloured urine were not recorded in females. All other recorded observations (alopecia, lungs that failed to open, enlarged spleen, enlarged or discoloured lymph nodes, discoloured prostate, raised focus of the duodenum, cysts in ovaries or uterus, and opaque eyes) occurred as single incidents scattered over the control and treated groups.

Major histopathological non-neoplastic findings are presented in Table 6.3-10. Histopathological examinations revealed a statistically significant increased incidence of nephropathy in high-dose animals, which was graded 1&2 (up to 6 foci of tubular basophilia sometimes along with eosinophilic casts) in all animals except for 3/10 males and 1/10 females where grading 3 was used (7 or more foci of tubular basophilia, many associated with eosinophilic casts, tubular necrosis or lymphocytic infiltration of the interstitium). The incidence of centrilobular hepatocyte hypertrophy was also statistically significantly increased in the high-dose group.

Table 6.3-10
Major histopathological non-neoplastic findings.

Sex	Males						Females					
Dose group ppm	0	10	100	1000	3000	7000	0	10	100	1000	3000	7000
Number of mice examined	10	10	10	10	10	10	10	10	10	10	10	10
Nephropathy	2	2	2	2	1	10**	2	3	1	2	2	9**
Centrilobular hepatocyte hypertrophy	0	0	0	0	0	7**	0	0	0	0	3	8**

Statistically significant: * = $p \leq 0.05$; ** = $p \leq 0.01$

Discussion:

The body weight or body weight gain were not affected significantly in male mice whereas in female mice decreased body weight and body weight gain was observed primarily towards the end of the feeding period in high dose animals. Blue coloured urine and blue stains on the pelvis occurred in male mice only from 1000 ppm whereas blue discoloration of the content and aglandular mucosa of the gastrointestinal tract was seen in both sexes at 7000 ppm; however, these findings were not accompanied by histopathological changes and the toxicological significance of it is unclear. The relative liver weights were significantly increased in high-dose males and from 3000 ppm in females whereas the relative kidney weights were only slightly increased (non-significantly). The histopathological changes included nepropathy with significantly increased incidence in high-dose animals and centrilobular hepatocyte hypertrophy, which was recorded with significantly increased incidence in high-dose animals and with a non-significantly increase in females at 3000 ppm. The increase in serum 5' nucleotidase indicates a cholestasis, which might result from the liver cell hyperthrohy (much higher increase in females than in males). The target organs were the kidneys and the liver in both sexes of the CD-1 mice.

Conclusion:

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

B.6.3.3 Other routes and duration

B.6.3.3.1 Oral dietary 20-day study in rats

Author(s):	Courcy di Rosa, J.
Study title:	CGA 173506 tech. Toxicity by oral administration to rats (admixture with the diet) for 20 days.
Testing facility:	[redacted]
Report number:	Study No. 3548 TSR/CGA 173506 tech./Test No. 87 1518.
Study duration:	In life: 18 November 1987 – 15 December 1987.
Date of report:	30 June 1988.
Test substance:	CGA 173506 (Fludioxonil) tech.
Batch no.:	PA-2882/9-16
Purity:	Not given.
Test animals:	Sprague Dawley Crl:CD (SD) Br, males and females.
Origin:	Centre d'Elevage Charles River (76410 Saint-Aubin-les-Elbeuf, France).
Body weight:	Average males: 150 g; average females: 105 g at study start.
Groups:	6/sex/dose group.
Husbandry:	Standard conditions.
Study design:	Similar to OECD guideline no. 407 except for the number of days the test diets were administered and that haematology, blood biochemistry examinations, and urinalysis were performed after 14 days on test diet rather than at study end. Dietary administration for 20 days to male rats and for 21 days to female rats.
Dose:	0, 1000, 5000, 10000 or 20000 ppm (mg/kg diet).
Vehicle/solvent:	Diet: "Aliment Composé – complet, Entretien Rat et Souris A 04 C" (Villemoisson-sur-Orge)
Route:	Oral – dietary.
Statistics/ measurements:	Comparison between control and treated groups was made by: Dunnett's test (if no significant heterogeneity of variance) or Mann-Whitney's test (if heterogeneity of variance established).
GLP:	OECD Principles of Good Laboratory Practice, 1981
Guideline:	Protocol based on OECD guideline no. 407 but with the changes noted above.
Deviation:	Not a guideline study.
Acceptability:	Acceptable.

Results:

The only clinical sign recorded was black coloured faeces during the last study week in all animals (both males and females) from the 5000, 10000 and 20000 ppm dose groups.

No deaths were observed during the study period.

The body weight was decreased about 12 % (not statistically significant) in high-dose males after 3 weeks whereas the body weights of all other treated groups and at all other time points were similar to control group body weights.

Food consumption was similar in control and treated animals of both sexes during the first two weeks of the study (not recorded for the last week). The food conversion ratio was similar between control and treated animals of both sexes. The achieved dosages were calculated over the two first weeks of dosing only.

Table 6.3-13 shows the intake of the test substance expressed in mg/kg bw/day for the various dose groups at week 1 and 2 as well as the mean intake.

Water consumption was increased (but not statistically significantly) in both males (about 20 %) and females (about 28 %) of the two highest dose groups for the two first weeks of the study when compared to control groups. The water consumption was not recorded for the last week of the study.

Table 6.3-13
Achieved dosages (mg/kg bw/day)

Dose group	Males				Females			
Week of treatment	1000	5000	10000	20000	1000	5000	10000	20000
1	132.0	648.3	1275.4	2564.4	147	750.0	1472.5	2854.7
2	116.1	599.6	1244.2	2421.6	119	645.3	1341.9	2791.6
Mean	124.1	624.0	1259.8	2493.0	133.2	697.7	1406.7	2823.2

There were no statistically significant differences between dosed and control group animals with respect to haematology except that MCHC was slightly increased in females of the two mid-dose groups after two weeks on test diets.

Clinical biochemistry revealed a number of statistically significantly differences between control and dosed groups including decreased sodium and chloride in both sexes from 5000 ppm, decreased cholesterol in high-dose animals, decreased glucose in females from 10000 ppm, decreased alkaline phosphatase in females at 10000 ppm, decreased alanine aminotransferase in males from 1000 ppm and in high-dose females, decreased alpha-1-globulin in males from 1000 ppm (both on a percentage and a g/L basis), and increased albumin in males at 1000 and 5000 ppm (on a g/L basis only).

Urinalysis after two weeks of treatement revealed changes between control and dosed groups only for male animals and included a statistically significantly increase (1-1.5 %) in mean specific gravity and decreased (non-significant) mean volume in the 10000 ppm (25 % reduction) and in the 20000 ppm (38 % reduction) dose groups. The lack of statistical significance was due to that only half of the animals had reduced volume.

Selected organ weights are presented in Table 6.3.14. The relative liver weights were statistically significantly increased in both sexes from 10000 ppm. The relative kidney weights were statistically significantly increased in high-dose males but in females only at 10000 ppm.

Table 6.3-14
Mean absolute (abs) (g) and relative (rel) (g/100 g animal) organ weights

Dose group		Males					Females				
Organ		0	1000	5000	10000	20000	0	1000	5000	10000	20000
Body weight	abs	249	238	242	231	202**	159	156	149	161	149
Heart	abs	1.130	1.087	0.962	1.055	0.857*	0.863	0.740	0.666**	0.795	0.714*
	rel	0.455	0.457	0.398	0.456	0.423	0.546	0.475	0.449*	0.493	0.479
Spleen	abs	0.677	0.527	0.507*	0.624	0.467**	0.669	0.491*	0.430**	0.518	0.526
	rel	0.275	0.223	0.210	0.271	0.232	0.435	0.315*	0.291*	0.321	0.354
Kidneys	abs	2.274	2.227	2.364	2.384	3.017**	1.571	1.642	1.505	1.811*	1.528
	rel	0.914	0.934	0.975	1.027	1.510**	0.991	1.055	1.012	1.125*	1.027
Liver	abs	8.223	7.981	8.559	9.050	8.113	5.879	5.915	5.901	7.323**	6.853
	rel	3.313	3.359	3.539	3.916**	4.010**	3.720	3.792	3.979	4.544**	4.623**
Testes	abs	2.663	2.753	2.578	2.770	2.522					
	rel	1.076	1.168	1.072	1.194	1.261					

Statistically significant: * = $p \leq 0.05$; ** = $p \leq 0.01$

The macroscopic changes recorded were primarily among high-dose animals and included greyish or blackish colour of the stomach, paleness, and punctiform foci.

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

Conclusion:

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

B.6.4.3. *In vivo* studies in germ cells

B.6.4.3.1 Dominant lethal test in Mouse

Author(s):	Hertner, T.
Study title:	Dominant lethal test, Mouse, 8 weeks
Testing facility:	[redacted]
Report number:	CGA173506/0228
Study duration:	May 21, 1992 to October 8, 1992
Date of report:	November 16, 1992
Test substance:	Fludioxonil, CGA173506 tech.
Batch no.:	P.910007
Purity:	96.4%.
Test systems:	Mouse (Tif:MAGf(SPF))
Positive controls:	Cyclophosphamide, 133 mg/kg bw.
Study design:	30 males/dose and 60 females/dose/mating period. All doses of Fludioxonile were administered once by oral gavage. Each male were mated to two virgin females per mating period. There were established 8 mating periods with a total length of 8 weeks.
Test substance concentrations:	1250, 2500 and 5000 mg/kg bw
Vehicle/solvent control:	Carboxymethylcellulose (CMC, 0.5%).
Statistics:	Linear-to-Linear Association tests were used, either with linear scores or with mean rank scores.
GLP:	U.S. EPA, June, 1988; U.S. FDA, April, 1985; Swiss Federal Department of the Interior, March, 1986; Japan MAFF, August, 1984; OECD, July, 1983.
Guideline:	OECD TG 478, 1984; EPA, 52 FR 19081; EEC, L333, 1988
Deviation:	None
Acceptability:	Acceptable

Results:

There was no effect of Fludioxonil on the mating frequency or on the implantation frequency.

In some of the mating periods (I, IV and VIII) the post-implatation mortality of embryos was slightly increased compared to the negative control group (Table 6.4-7). However, the effect was not statistical significant, there was no clear dose-response and the values are within the range of historical negative control data for embryonic deaths reported from two other studies (4.6-11.5% and 2.4-11.1%).

Table 6.4-7
Summary table – Effects of Fludioxonil on post-implantation mortality in mouse

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

*$p<0.05$

of 2).
However, as the result expressed as gross nucleus grain count revealed a positive effect of Fludioxonil, and since there is no evidence that the net grain count should be a more reliable expression of data compared to the gross grain count, the results from the UDS test revealed evidence of induction of DNA damage by Fludioxonil.

The positive control chemicals used in the test induced significant increase in the number of silver grains per nucleus and confirmed adequacy of the experimental conditions for detecting unscheduled DNA synthesis.

Table 6.4-4
Effects of Fludioxonil on rat hepatocyte Unscheduled DNA Synthesis (UDS)

Treatment	Concentration	Grains/nucleus mean ± s.d.		Net nuclear grains[1] mean ± s.d.	
		Original experiment	Confirmatory experiment	Original experiment	Confirmatory experiment
Negative control	0 µg/mL	2.29 ± 1.96	2.64 ± 1.57	0.33 ± 2.18	-1.03 ± 2.10
Fludioxonil	4.1µg/mL	5.31 ± 2.74	4.81 ± 2.95	0.15 ± 2.99	-0.41 ± 3.19
	12.3 µg/mL	6.07 ± 3.36	5.79 ± 4.32	0.02 ± 3.19	-0.02 ± 3.41
	37 µg/mL	7.44 ± 3.42	5.63 ± 3.47	1.39 ± 3.16	-1.17 ± 3.67
	111 µg/mL	7.58 ± 3.87	5.14 ± 3.06	0.51 ± 3.79	0.01 ± 3.09
	333 µg/mL	7.29 ± 3.62	5.71 ± 2.58	1.75 ± 3.90	-0.16 ± 2.82
	1000 µg/mL	7.33 ± 4.10	6.03 ± 3.04	0.30 ±3.74	-0.28 ± 3.63
	2500 µg/mL	6.94 ± 3.19		0.59 ±3.60	
	5000 µg/mL	7.65 ± 3.60		-0.44 ± 3.97	
2-Acetylaminofluorene	45 µM	14.27 ± 5.49	20.73 ± 6.48	9.35 ± 5.64	12.44 ± 6.12
4-Aminobiphenyl	25 µM	13.76 ±5.66	17.71 ±5.77	7.71 ± 4.57	9.82 ±5.84

[1] Net nuclear grains are calculated as the mean number of grains over the nucleus minus the mean number of grains over a nucleus-equivalent area of cytoplasm

Conclusion:

Fludioxonil (CGA173506) was tested at concentrations from 4.1-5000 µg/mL in an unscheduled DNA synthesis in primary rat hepatocytes. Fludioxonil excerted a DNA damaging effect, as there was a dose-related increase in the gross number of silver grains per nucleus.
It can therefore be concluded that under the experimental conditions used in the experiment Fludioxonil showed DNA damaging potential.

B.6.4.1.4. Unscheduled DNA Synthesis in rat hepatocytes

Author(s):	Hertner, T.
Study title:	Autoradiographic DNA repair test on rat hepatocytes (OECD conform)
Testing facility:	[redacted]
Report number:	CGA173506/0031
Study duration:	August 9, 1988 to October 27, 1988
Date of report:	June 19, 1989
Test substance:	Fludioxonil, CGA173506 tech.
Batch no.:	P.805002
Purity:	97.5%.
Test systems:	Primary rat hepatocytes freshly isolated from male rat.
S9 Fraction (metabolic activation):	Not applicable.
Positive controls (+ S9-mix):	Not applicable.
Positive controls (– S9-mix):	2- Acetylaminofluorene (2-AAF) (25µM) and 4-Aminobiphenyl (4-ABP) (25 µM).
Study design:	Fludioxonil was evaluated in the DNA-repair test in rat hepatocytes in accordance with the OECD TG 482 (1987). A preliminary cytotoxicity test was performed followed by two - one original and one confirmatory – experiment both with and without metabolic activation. The number of silver grains/nucleus was evaluated in 150 cells. Both nuclear gross numbers and nett nuclear grains were calculated.
Test substance concentrations:	Cytotoxicity test: 5-5000 µg/ mL. DNA repair test: 4.1, 12.3, 37, 111, 333, 1000, 2500 and 5000 µg/mL. The 2 highest concentrations were only evaluated in the original experiment and not in the confirmatory experiment.
Vehicle/solvent control:	Dimethylsulfoxide (DMSO).
Statistics:	Mean number of silver grains ± S.D.
GLP:	U.S. EPA, June 2, 1988; U.S. FDA, April 20, 1985; Swiss Federal Department of the Interior, March, 1986; Japan MAFF, 10 August 1984; OECD, July 26, 1983.
Guideline:	OECD Guideline 482, 1987; EPA 798.5550, 1987; EEC L133, 1984.
Deviation:	None
Acceptability:	Acceptable

Results:

No sign of toxicity was observed at the highest concentration in the preliminary cytotoxicity test. Therefore 5000 µg/mL was chosen as the highest concentration to be tested. Percipitation of the test substance was observed at concentrations higher than 20 µg/mL and due to strong precipitation at the highest concentrations 1000 µg/mL were the highest concentration tested in the confirmatory experiment.

As can be seen in Table 6.4-4, the gross number of grains over the nucleus increased with increasing concentrations of Fludioxonil in both experiments. Also, in both experiments the percental distribution range of silver grains per nucleus was significantly shifted to higher values when the cells were treated with Fludioxonil compared to the range in the vehicle control.
The number of grains over the cytoplasm increased when the cells were exposed to Fludioxonil compared to vehicle treated cells. When considering the net nuclear grain counts (mean number of grains over the nucleus minus the mean number of grains over a nucleus-equivalent area of cytoplasm) none of the values fullfilled the criteria for a positive result (the mean net nuclear grain count at any concentration should be 2 as well as a difference to the vehicle control

300 ppm dose group: Fludioxonil did not induce any changes related to treatment at this dose.

30 ppm dose group: Fludioxonil did not induce any changes related to treatment at this dose.

Conclusion:

In this two-generation study in Sprague-Dawley rats performed according to OECD TG 416, males and females were fed Fludioxonil at dietary doses of 0, 2.1, 21 or 212 mg/kg bw/day. Fludioxonil did not affect the ability of the parental animals to mate, produce a litter of normal size and number, and to raise a litter. The NOAEL for reproductive effects is therefore the highest dose level of 212 mg/kg bw/day. The overall NOAEL for parents and offspring is 21 mg/kg bw/day based on decreased body weight of parental rats and pups at 212 mg/kgbw/day.

B.6.4.2.5 Test of aneuploidy in rat bone marrow

Author(s):	Myhr, B.C.
Study title:	Evaluation of aneuploidy in Rat Bone Marrow Cells
Testing facility:	[redacted]
Report number:	CGA173506/5139
Study duration:	September 21, 1998 to January 18, 1999
Date of report:	December 30, 1999
Test substance:	Fludioxonil, CGA173506 tech.
Batch no.:	P.910007
Purity:	97.5%.
Test systems:	Bone marrow cells from Rat (Crl:CD®(SD)BR)
Positive controls:	Vinblastine sulfate, 1 mg/kg bw, i.p.
Study design:	5 animals/sex/group All animals were subcutaneously implanted with BrdUrd pellets (50 mg, paraffin-coated) prior to dosing. All doses of Fludioxonile were administered once by oral gavage. The animals were sacrificed 30 hours after treatment (1.5-2 hours after i.p. injection of colchicines (2 mg/kg)). If possible, 100 metaphases were scored per animal for the incidence of numerical chromosome aberrations. Approximately 500 cells were scored for mitotic index.
Test substance concentrations:	1250, 2500 and 5000 mg/kg bw.
Vehicle/solvent control:	Carboxymethylcellulose (CMC, 0.5%).
Statistics:	Analysis of Variance on rank transformed proportions of cells with numerical aberrations. Dunnets test were used to determine which, if any, dose groups were significantly different from the negative control.
GLP:	U.S. EPA; Japan MAFF; OECD;
Guideline:	No guideline on evaluation of aneuploidy in bone marrow.
Deviation:	Not applicable
Acceptability:	Acceptable

Results:

The original positive control group receiving 9 mg vinblastine sulfate/kg bw showed excessive mortality for both male and female rats. Therefore, in a separate experiment two new positive control groups were set up receiving 1 and 5 mg vinblastine sulfate/kg bw. Based on the toxicity observation the dose level of 1 mg/kg bw were chosen as a positive control for the original study (no signs of toxicity).

There was no sign of a cytotoxic effect in bone marrow as there were no differences in mitotic index between the treated and the negative control animals.

In both female and male rats especially at the lowest dose, there was a slight but not statistically significant increase in the number of bone marrow cells with numerically aberrations in Fludioxonil treated animals compared to control animals. The effect was not dose-dependent. The author has not supplied the study report with historical data of numerical aberrations in vehicle treated animals, and it is therefore not possible to judge whether the figures for the treated animals are within historical vehicle control data.

The positive control chemical tested in a separate test induced significant increase in the number of aneuploid metaphases and confirmed adequacy of the experimental conditions for detecting induction of aneuploidy.

Table 6.4-6
Effects of Fludioxonil on the number of cells with numerical aberrations

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

** P<0.01

Conclusion:

Fludioxonil (CGA173506) was tested at doses from 1250 to 5000 mg/kg bw for its potential to induce aneuploidy in rat hepatocytes. A slight increase in number of aberrant cells was observed. The increase was statistically insignificant and not dose-dependent. Therefore, it is concluded that in the tissue investigated and under the particular conditions used in the experiment Fludioxonil did not show an aneugenic potential *in vivo*.

B.6.4.2.4 Micronucleus test on rat hepatocytes

Author(s):	Ogorek, B.
Study title:	*In vivo* Micronucleus test on rat hepatocytes
Testing facility:	[redacted]
Report number:	CGA173506/5055
Study duration:	October 28, 1998 to August 10, 1999
Date of report:	September 28, 1999
Test substance:	Fludioxonil, CGA173506 tech.
Batch no.:	P.910007
Purity:	96.4%.
Test systems:	Hepatocytes from male Rat (Tiflbm: RAI (SPF))
Positive controls:	Cyclophosphamide, 20 mg/kg bw, i.p.
Study design:	5 males/group. All doses were administered once by oral gavage. 4-acetylaminofluorene (4-AAF) was used as an agent stimulating the mitotic acticity in the liver and was also administered via oral gavage (1000 mg/kg bw). Fludioxonil was administered 29 hours after the mitogenic stimulus. The animals were sacrificed 3 days after treatment. 1000 hepatocytes were scored per animal for the incidence of micronuclei.
Test substance concentrations:	50, 125 and 1250 mg/kg bw
Vehicle/solvent control:	Carboxymethylcellulose (CMC, 0.5%)
Statistics:	Analysis of Variance on square root transformed data (Freeman-Tukey transformed).
GLP:	U.S. EPA, August, 1989; U.S. FDA, August, 1989; Swiss Federal Department of the Interior, March, 1986; Japan MAFF, 10 August 1984; OECD, October, 1989.
Guideline:	No guideline on micronucleus test in hepatocytes.
Deviation:	Not applicable
Acceptability:	Acceptable

Results:

There was a slight but insignificant dose-response relationship in the number of micronucleated hepatocytes in rat treated with Fludioxonil. The mean percentage of micronucleated hepatocytes in the negative control group was 0.82±0.26 compared to 0.88±0.49, 1.04±0.70 and 1.46±0.63 for the animals treated with 50, 125 and 1250 mg/kg bw, respectively. Mean (±s.d) historical data on micronucleated hepatocytes in negative control rats (CMC, 0.5%) range from 0.07±0.12 to 1.38±0.53 (the figures are group means (3-4 animal/group) of 12 groups). As the increase was not significant and as it was close to historical control values the observation is not considered as test substance induced chromosome damage.

The positive control chemical used in the test induced significant increase (3.52%) in the number of micronucleated hepatocytes and confirmed adequacy of the experimental conditions for detecting induction of micronuclei.

Conclusion:

Fludioxonil (CGA173506) was tested at doses from 50 to 1250 mg/kg bw in a micronucleus test in rat hepatocytes. There was a slight but insignificant increase in the number of micronucleated hepatocytes and the values were almost within historical control values. Therefore, it is concluded that in the tissue investigated and under the particular conditions used in the experiment Fludioxonil did not show a clastogenic or an aneugenic potential *in vivo*.

but significant increase in the number of micronucleated hepatocytes in animals treated with the lowest and intermediate dose of Fludioxonil (Table 6.4-5). However, all results except from one animal in the lowest dose group are within the reported historical data of micronucleated hepatocytes.
Only three animals per dose group were investigated. According to the authors, the reason for the relatively small treatment groups was that the isolation of rat hepatocytes was time consuming and it was important to ensure a manageable number of animals.

The positive control chemicals used in the test induced significant increases in the number of micronucleated hepatocytes and confirmed adequacy of the experimental conditions for detecting induction of micronuclei.

Table 6.4-5
Effects of Fludioxonil on micronucleated rat hepatocytes when administered after a mitogenic stimulus

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

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

Conclusion:

Fludioxonil (CGA173506) was tested at doses from 1250-5000 mg/kg bw in an *in vivo* micronucleus test in rat hepatocytes.
The result of the test was equivocal as a slight but significant positive result was obtained in the experiment where Fludioxonil was administered after treatment with a mitogenic stimulus. The increase was not dose-dependent as the increase was only observed at the lowest and intermediate dose levels. However, the results were except from one animal within the set of historical data. In the light of the equivocal result and the relatively small treatment groups (3 animal/group), the second experiment should have been repeated. But a newer study is submitted which is negative, 6.4.2.4.

Conclusion:

Fludioxonil (CGA 173506) was tested at doses from 1250-5000 mg/kg bw in an *in vivo* micronucleus test in mouse. In the tissue investigated and under the particular conditions used in the experiment Fludioxonil did not show a clastogenic potential *in vivo*.

B.6.4.2.3 Micronucleus test on rat hepatocytes

Author(s):	Meyer, A.
Study title:	*In vivo* Micronucleus test on rat hepatocytes
Testing facility:	[redacted]
Report number:	CGA173506/0089
Study duration:	May 16, 1990 to January 22, 1991
Date of report:	February 21, 1991
Test substance:	Fludioxonil, CGA173506 tech.
Batch no.:	P.805002
Purity:	97.5%.
Test systems:	Hepatocytes from Rat (Tif: RAIf (SPF))
Positive controls:	Part 1: Dimethylnitrosamine, 10 mg/kg bw, i.p. Part 2: Cyclophosphamide, 20 mg/kg bw, i.p.
Study design:	4 males/group (only slides from 3 males/group were investigated). All doses were administered once by oral gavage. 1000 hepatocytes were scored per animal for the incidence of micronuclei. 4-acetylaminofluorene (4-AAF) was used as an agent stimulating the mitotic acticity in the liver either after (exp. 1) or before (exp. 2) treatment with test substance . 4-AAF was administered via oral gavage (100 mg/kg bw). Experiment 1: Fludioxonil was administered 3 days prior to the mitogenic stimulus. The animals were sacrificed 3 days after mitogenic stimulus. Experiment 2: Fludioxonil was administered 29 hours after the mitogenic stimulus. The animals were sacrificed 3 days after treatment.
Test substance concentrations:	Part 1: 1250, 2500 and 5000 mg/kg bw. Part 2: 1250, 2500 or 5000 mg/kg bw.
Vehicle/solvent control:	Carboxymethylcellulose (CMC, 0.5%)
Statistics:	The significance of differences was analysed by Analysis of Variance and the Cochran-Armitage trend test.
GLP:	U.S. EPA, June 2, 1988; U.S. FDA, April 20, 1985; Swiss Federal Department of the Interior, March, 1986; Japan MAFF, 10 August 1984; OECD, July 26, 1983.
Guideline:	No guideline on micronucleus test in hepatocytes.
Deviation:	Not applicable
Acceptability:	Acceptable

Results:

There is no information of cytotoxicity. The doses were selected in a preliminary tolerability test.

In the first experiment, where Fludioxonil was administered prior to the mitotic stimulus (4-AAF), there was no significant increase in the number of micronucleated hepatocytes in animals treated with Fludioxonil compared to the negative control.

In the second experiment, where Fludioxonil was administered after the mitotic stimulus (4-AAF), there was a slight

The positive control chemical used in the test induced a marked increase in the number of post-implantation mortality of embryos and confirmed adequacy of the experimental conditions for detecting induction of genetic damage to germ cells.

Conclusion:

Fludioxonil (CGA173506) was tested at 1250, 2500 and 5000 mg/kg bw in a dominant lethal test in mouse.
Under the particular conditions used in the experiment Fludioxonil did not show a potential for inducing dominant lethal mutations in germ cells.