CANCER BRIEFING PACKAGE

PC CODE: 027412 (Fluopicolide)

DATE OF PACKAGE: 11/2/2006

SUBMITTED BY: Jessica Kidwell
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UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

OFFICE OF
PREVENTION, PESTICIDES, AND

MEMORANDUM

DATE: November 2, 2006

SUBJECT: Cancer Assessment Review Committee Meeting on FLUOPICOLIDE

FROM: Jessica Kidwell, Executive Secretary
Cancer Assessment Review Committee
Health Effects Division (7509C)

TO: Addressees

Attached for your review is a package on FLUOPICOLIDE prepared by Myron Ottley.

A meeting to review the carcinogenicity classification of this chemical is scheduled for Wednesday, November 15, 2006 at 9:30 am in Room S-10100, PY1.

Addressees:

L. Brunsman
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M. Copley
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K. Farwell
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Fuopicolide CARC Package

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CANCER ASSESSMENT DOCUMENT

FOR COMMITTEE DELIBERATION

EVALUATION OF THE CARCINOGENIC POTENTIAL OF ***FLUOPICLOLIDE***

Date of the Report
Submitted by: Myron Ottley

CANCER **A**SSESSMENT **R**EVIEW **C**OMMITTEE
HEALTH EFFECTS DIVISION
OFFICE OF PESTICIDE PROGRAMS

CONTENTS

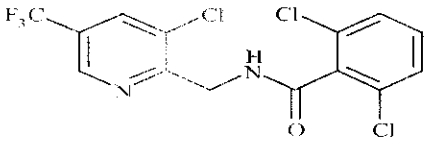
EXECUTIVE SUMMARY

I. INTRODUCTION

On November 15, 2006, the Cancer Assessment Review Committee of the Health Effects Division of the Office of Pesticide Programs met to evaluate the carcinogenic potential of Fluopicolide.

II. BACKGROUND INFORMATION

Fluopicolide (AE C638206 or 2,6-dichloro-*N*-[[3-chloro-5-(trifluoromethyl)-2-pyridinyl]methyl]benzamide) is a fungicide which belongs to the benzamide class and the pyridine class of pesticides. It controls a wide range of *Oomycete* (Phycomycete) diseases including downy mildews (*Plasmopara*, *Pseudoperonospara*, *Peronospora*, *Bremia*), late blight (*Phytophthora*), and some *Pythium* species. Fluopicolide is a new chemical proposed for use on imported grapes. PC Code: 027412; CAS Registry No.: 239110-15-7.



III. EVALUATION OF CARCINOGENICITY STUDIES

1. Combined Chronic Toxicity/Carcinogenicity Study with Fluopicolide in F-344 Rats

Reference: Cooper, S. 2003. AE C638206: Combined carcinogenicity and toxicity study by dietary administration to CD rats for 104 weeks. Huntingdon Life Sciences, Ltd., England. Laboratory Project ID No. AES 024/032124 and Project No. AES/024. November 18, 2003. MRID 46474139. Unpublished.

A. Experimental Design

AE C638206 (Fluopicolide, 95.9%, a.i.; Batch No. OP2050046) was administered to 60 Crl:CD (SD) IGS BR rats/sex/dose in the diet at concentrations of 0 (controls), 50, 200, 750 or 2500 ppm (equivalent to 0, 2.1, 8.4, 31.5 or 109.4 mg/kg bw/day in males and 0, 2.8, 10.8, 41.0 or 142.2 mg/kg bw/day in females) for up to 104 weeks. An additional 20 animals/sex/dose were administered the same concentration and sacrificed after 52 weeks of treatment for a interim sacrifice. A third set of 10 animals/sex/dose were fed the treated diet at the same concentrations for 52 weeks followed by 13 weeks of being fed basal diet prior to sacrifice in a recovery study.

B. Discussion of Tumor Data

At the doses tested, there was not a treatment-related increase in tumor incidence of any type in animals dosed with up to 2500 ppm AE C638206 for up to 104 weeks.

C. Non-Neoplastic Lesions

Males had a statistically significant increase in the incidence and severity of non-neoplastic microscopic lesions in the thyroid, kidney and liver in the main study. A corresponding increase ($p < 0.05$) in the incidence of enlarged kidneys and thyroids were present in the males at 2500 ppm compared to controls on gross observation. Histopathological examination showed an increased incidence of thyroid cystic follicular hyperplasia in the males. This was observed in 0/60, 1/37, 0/37, 4/35 and 7/60 ($p < 0.05$) of the dosing regimen (control, 50, 200, 750 or 2500 ppm). During the recovery period, all lesions present were reversed except for a slight increase in the severity of the renal cortical tubular basophilia in the males. Females had no statistically significant differences in lesions in any of the dose groups in either the toxicity or the main study.

Table 1. Non-Neoplastic Lesions in F344 Rats Fed Fluopicolide

Organ/Lesion	Severity	Dietary concentration (ppm)				
		0	50	200	750	2500
Males (52 weeks)						
KIDNEY		n = 20	n = 20	n = 15	n = 20	n = 20
Cortical tubular basophilia	total (avg. severity)[a]	7 (1.3)	10 (1.2)	9 (1.3)	20** (1.3)	20** (2.1)
Medulla granular casts	total (avg. severity)	0	0	0	0	7** (1.6)
Males (104 weeks)						
THYROID		n = 60	n = 37	n = 37	n = 35	n = 60
cystic follicular hyperplasia	total	0	1	0	4	7**
KIDNEY		n = 60	n = 60	n = 60	n = 60	n = 60
Tubular casts	total (avg. severity)	24 (1.6)	30 (1.5)	32 (1.4)	32 (1.5)	45** (2.0)
Cortical tubular dilatation	total (avg. severity)	10 (1.8)	9 (2.0)	9 (2.0)	8 (2.0)	27** (2.0)
Cortical cysts	total (avg. severity)	3 (2.0)	2 (2.0)	6 (2.3)	9 (2.1)	11* (2.5)
Papilla mineralization	total (avg. severity)	0	1 (1.0)	2 (2.0)	2 (1.0)	12** (1.4)

Data obtained from MRID 46474139, Text Tables 5, 6, 9, and 10, pp. 41-45 and Table 13H, p. 446-466.

[A] Severity is as follows: (1) = minimal, (2) = slight, (3) = moderate and (4) = marked

* statistically different from controls, $p < 0.05$

** statistically different from controls, $p < 0.01$

D. Adequacy of the Dosing for Assessment of Carcinogenicity

Dosing was considered adequate based on the decreased body weight gain in the male and female rats at 2500 ppm (the highest dose level), and the non-neoplastic lesions observed at 2500 ppm in males. Effects were minimal in the female. However, a reproductive study, (MRID 46474124), and a companion, supplemental study providing histopathological evaluation of liver and kidneys (MRID 46474125), indicated kidney toxicity (microscopic lesions) in male and female rats in

both parental generations and decreased body weight gain in the F_0 females treated with AC638206 for 16 weeks at 2000 ppm. These results indicate that dosing in the chronic rat study at 2500 ppm was adequate.

2. Carcinogenicity Study in Mice

Reference: Chevalier, G. (2003) AE C638206: Carcinogenicity study by oral route (Dietary admixture) in C57BL/6 mice. Centre International de Toxicologie, Evreux Cedex, France. Laboratory project ID 21557 TCS; Bayer Report no. C038732, November 20, 2003. MRID 46474130. Unpublished.

A. Experimental Design

AE C638206 (Fluopicolide) (95.9% a.i., batch #OP2050046) was administered to 50 C57BL/6 mice/sex/dose in the diet at dietary levels of 0, 50, 400, or 3200 ppm (equivalent to 0, 7.9, 64.5, 551.0 mg/kg bw/day for males, and 0, 11.5, 91.9, and 772.3 mg/kg bw/day for females) for 18 months. Satellite groups of 10 C57BL/6 mice/sex/dose were similarly treated for 12 months.

B. Discussion of Tumor Data

As shown in Table 2, There was a treatment related increase in the incidence of hepatocellular adenoma when compared to controls. The 3200 ppm animals had statistically significant increases in hepatocellular adenoma in both sexes after 78 weeks, and a small increase after 52 weeks. The adenoma incidence after 78 weeks of the dosing regimen (0, 50, 400, 3200 ppm) was 5/50, 0/50, 5/50, and 11/50 in males, respectively, and 1/50, 2/50, 0/50, and 16/50 in females, respectively. After 52 weeks, hepatocellular adenoma was found in 3/10 high-dose females but no males. The adenomas were correlated with an increased incidence of liver masses and nodules at necropsy.

TABLE 2: Incidence of neoplastic liver lesions in mice treated 52 weeks (satellite study) or 78 weeks (main study) with AE C638206.

Hepatocellular lesion	0 ppm	50 ppm	400 ppm	3200 ppm	0 ppm	50 ppm	400 ppm	3200 ppm
	Males				Females			
Adenoma:								
52 weeks	0/10	0/10	0/10	0/10	0/10	0/10	1/10	3/10†
(%)	(0)	(0)	(0)	(0)	(0)	(0)	(10)	(30)
78 weeks	5/50	0/50	5/50	11/50†	1/49	2/50	0/50	16/50**‡
	(10)	(0)	(10)	(22)	(2)	(4)	(0)	(32)
Carcinoma:								
52 weeks	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10
	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)
78 weeks	3/50	1/50	0/50	2/50	0/49	0/50	2/50	0/50
	(6)	(2)	(0)	(4)	(0)	(0)	(4)	(0)
Adenoma + carcinoma								
52 weeks	0/10	0/10	0/10	0/10	0/10	0/10	1/10	3/10
	(0)	(0)	(0)	(0)	(0)	(0)	(10)	(30)
78 weeks	8/50	1/50	5/50	13/50	1/49	2/50	2/50	16/50**‡
	(40)	(2)	(10)	(26)	(2)	(4)	(4)	(32)

Data obtained from pages 40, 162, 164, and 167 of MRID 46474130.
*p <0.05, **p <0.01: Statistically different from the control group, determined by the reviewer using the Fisher exact test.
†p <0.05, ‡p <0.01: Statistically different from the control group, determined by the study author.

When compared to historical controls of the testing facility (Table 3), the incidence of hepatocellular adenoma in the current study (Table 2) exceeded that of the historical control in both males and females. The incidence of carcinomas was well within that observed in the historical control, but when combined with the adenomas, the incidence exceeded the historical controls in both sexes. The historical controls come from two studies conducted concurrently between 1998 and 2000, in the same laboratory, using the same strain of mouse as the current study (MRID 46745702).

Table 3. Incidence of Tumors in Historical Controls C57B1/6 Mice

	Males (%)		Females (%)	
	Study 1	Study 2	Study 1	Study 2
Adenoma	5/49 (10)	6/100 (6)	0/50 (0)	2/100 (2)
Carcinoma	0/49 (0)	1/100 (1)	0/50 (0)	1/100 (1)
Adenoma + Carcinoma	5/49 (10)	7/100 (7)	0/50 (0)	3/100 (3)

Tables 5a and 5b present the results of qualitative statistical analysis of the tumors.

Table 5a.

Fluopicolide – C57BL/6 N Crl:BR SPF VAF Mouse Study (MRID 46474130)

Male Liver Tumor Rates+ and Fisher's Exact Test and Exact Trend Test Results

	Dose (ppm)			
	0	50	400	3200
Adenomas (%)	5[a]/47 (11)	0/49 (0)	5/48 (10)	11/49 (22)
p =	0.0018**	1.0000[n]	0.6435	0.0999
Carcinomas (%)	3/47 (6)	1/49 (2)	0/48 (0)	2[b]/49 (4)
p =	0.4738	0.9463[n]	1.0000[n]	0.8319[n]
Combined (%)	7[c]/47 (15)	1/49 (2)	5/48 (10)	13/49 (27)
p =	0.0019**	0.9976[n]	0.8326[n]	0.1244

+Number of tumor bearing animals/Number of animals examined, excluding those that died or were sacrificed before week 53.

[a]First adenoma observed at week 66, dose 0 ppm.

[b]First carcinoma observed at week 71, dose 3200 ppm.

[c]One animal in the control group had both an adenoma and a carcinoma.

[n]Negative change from control.

Note: There were no tumors observed in interim sacrifice animals.
Significance of trend denoted at control.
Significance of pair-wise comparison with control denoted at dose level.
If *, then $p < 0.05$. If **, then $p < 0.01$.

Table 5b.

Fluopicolide – C57BL/6 N Crl:BR SPF VAF Mouse Study (MRID 46474130)

Female Liver Tumor Rates[+] and Fisher's Exact Test
and Exact Trend Test Results

	Dose (ppm)			
	0	50	400	3200
Adenomas (%)	1/58 (2)	2/60 (3)	1/60 (2)	19[a]/57 (33)
p =	0.0000**	0.5128	0.7605	0.0000**
Carcinomas (%)	0/58 (0)	0/60 (0)	2[b]/60 (3)	0/57 (0)
p =	0.5729	1.0000	0.2564	1.0000
Combined (%)	1/58 (2)	2/60 (3)	3/60 (5)	19/57 (33)
p =	0.0000**	0.5128	0.3222	0.0000**

+Number of tumor bearing animals/Number of animals examined, excluding those that died before week 46.
[a]First adenoma observed at week 46, dose 3200 ppm.
[b]First carcinoma observed at week 79, dose 400 ppm.
Note: Significance of trend denoted at control.
Significance of pair-wise comparison with control denoted at dose level.
If *, then $p < 0.05$. If **, then $p < 0.01$.

Tables 6a and 6b show there were no significant dose-related changes in mortality.

Table 6a

Fluopicolide – C57BL/6 N Crl:BR SPF VAF Mouse Study (MRID 46474130)
Male Mortality Rates[+] and Cox or Generalized K/W Test Results

	Weeks				
Dose (ppm)	1-26	27-52	52[i]	53-80	Total
0	2/59[a]	0/57	10/57	6/47	8/49 (16)
50	0/60	1/60	10/59	5/49	6/50 (12)
400	1/60	1/59	10/58	3/48	5/50 (10)
3200	0/60	1/60	10/59	6/49	7/50 (14)

[+]Number of animals that died during interval/Number of animals alive at the beginning of the interval.
[i]Interim sacrifice at week 52.
[f]Final sacrifice at weeks 78-80.
[a]One accidental death at week 26, dose 0 ppm.
( )Percent.

Note: Time intervals were selected for display purposes only.
Significance of trend denoted at control.
Significance of pair-wise comparison with control denoted at dose level

Table 6b.

Fluopicolide – C57BL/6 N Crl:BR SPF VAF Mouse Study (MRID 46474130)
Female Mortality Rates[+] and Cox or Generalized K/W Test Results

	Weeks				
Dose (ppm)	1-26	27-52	52[i]	53-80	Total
0	0/60	1/60	10/59	4/49	5/50 (10)
50	0/60	0/60	10/60	9/50	9/50 (18)
400	0/60	0/60	10/60	2/48[a]	2/48 (4)
3200	0/60	5/60	10/55	4/45	9/50 (18)

[+]Number of animals that died during interval/Number of animals alive at the beginning of the interval.
[i]Interim sacrifice at week 52.
[f]Final sacrifice at weeks 78-80.
[a]Two accidental deaths at week 70, dose 400 ppm.
( )Percent.
Note: Time intervals were selected for display purposes only.
Significance of trend denoted at control.
Significance of pair-wise comparison with control denoted at dose level

C. Non-Neoplastic Lesions

The non-neoplastic lesions observed in the liver and stomachs of both sexes of mice are presented in Table 7.

TABLE 7: Incidence of microscopic non-neoplastic liver lesions in mice treated 52 weeks (satellite study) or 78 weeks (main study) with AE C638206.

Microscopic lesion	0 ppm	50 ppm	400 ppm	3200 ppm	0 ppm	50 ppm	400 ppm	3200 ppm
	Males				Females			
Hepatocellular hypertrophy								
52 weeks	0/10	0/10	5/10*	10/10**	0/10	0/10	6/10**	9/10**
78 weeks	0/50	0/50	20/50**	49/50**	0/50	0/50	41/50**	46/50**
Altered cell foci all types								
52 weeks	0/10	0/10	0/10	0/10	0/10	0/10	0/10	2/10
78 weeks	1/50	8/50*	5/50	18/50**	0/50 [1]	3/50	4/50	25/50**

Data obtained from pages 39, 148, 155, 160, and 161 of MRID 46474130.
*$p < 0.05$, **$p < 0.01$: Statistically different from the control group, determined by the reviewer using the Fisher exact test.
[1]The incidence was reported as 1/50 on p. 39 of MRID 46474130, which is inconsistent with the data on p. 148 and the individual animal data for the control group on pages 1293-1412 of MRID 46474130.

D. Adequacy of Dosing for Assessment of Carcinogenicity

There was a marked decrease in body weights and body weight gains at 3200 ppm. After 78 weeks, the body weights of 3200 ppm males were 20% lower, females were 16% lower than of the control group, and overall body weight gains were 45% lower for males and 35% lower for females.

This is an indication that 3200 ppm was an excessive test concentration. Although this could have impacted the study results, the lack of life shortening-suggests that the study and the conclusions drawn from it were valid. Therefore, dosing was considered adequate for assessment of carcinogenicity.

IV. TOXICOLOGY

1. Metabolism

Fluopicolide (radiolabeled in the benzene or pyridine position) was administered orally to male and female rats as a single 10 mg/kg or 100 mg/kg dose, or to male and female rats in 14 daily oral doses of 10 mg/kg. In single dosing experiments, the rats were sacrificed at intervals from 8-168 hours post-dosing and in repeat dose studies, six days after final dosing.

In these studies, regardless of dose, sex, or radiolabel position, the test material was rapidly and extensively absorbed. The radiolabeled test material was rapidly distributed to all tissues, and was found in the highest concentrations in the organs of elimination; intestine/intestinal contents, liver, and kidney. No sites of sequestration were found.

No significant differences in the whole blood maximum concentrations were found between sexes or radiolabel position. In addition, the time interval to maximum concentration was relatively rapid and similar for both radiolabel positions and sexes of rats of the low-dose group.

Elimination was moderately rapid for the males but slower for the females, being approximately twice as long.

For all test groups regardless of dose, radiolabel position, or sex, the feces were the primary route of elimination. For most studies, the urine accounted for ~10% of the radiolabel, which rose to ~22% in the 14-day repeat low-dose study. No radiolabel was detected in the expired air.

The test material, regardless of dose, radiolabel position, or sex, was rapidly and extensively metabolized. Up to 49 individual metabolites were detected in the urine of low-dose male and female rats and up to 42 metabolites in the urine of high-dose male and female rats within 48 hours of treatment. None of the radioactivity detected in the urine was associated with the parent compound and none of the metabolites accounted for more than 3% of the administered dose. Many of the metabolites were the products of glucuronide, sulphate, or glutathione conjugation.

The greatest amount of radioactivity recovered in the feces of male and female high-dose rats was attributed to the parent compound. In the feces of low-dose male and female rats, two metabolites that constituted >5% of the radiolabel recovered were the products of an initial conjugation with glutathione with subsequent degradation to the S-methyl group. All other metabolic products constituted <5% of the administered dose. The presence of glutathione derivatives strongly suggests formation of electrophilic species during the metabolic process. MRIDs 46474224 - 46474237

2. <u>Mutagenicity</u>:

Fluopicolide was tested in 12 genetic toxicity assays, in an effort to characterize its mutagenic potential. Two *in vitro* assays, i.e., one bacterial reverse mutation assay and one chromosomal aberration assay, gave positive results; however, genotoxicity was not demonstrated in the *in vivo* assays.

Unless otherwise noted, all studies were classified as **acceptable/guideline** and satisfied the requirement for FIFRA Test Guideline 84-2 for mutagenicity data.

(i) In an Ames assay, when tested in Salmonella typhimurium strains TA98, TA100, TA1535 and TA1537 at concentrations up to 5000 µg/plate, AE C638206 (fluopicolide) is considered mutagenic only at precipitating concentrations in the conventional battery of bacterial strains. (MRID 46474142)

(ii) In independent reverse mutation assays in bacteria, four histidine-requiring strains of *S. typhimurium* (TA98, TA100, TA1535, and 1537) and one tryptophan-requiring strain of *E. coli*, were exposed to AE C638206 00 IC99 0005 in the presence and absence of metabolic activation at concentrations up to 5000 *ug*/plate, no increases in revertant colonies were found in either test series at concentrations up to the limit dose, 5000 ug/plate. Therefore, AE C638206 Technical is considered nonmutagenic in the conventional battery of bacterial strains (MRID 46474144).

(iii) In independent reverse mutation assays in bacteria, four histidine-requiring strains of *S. typhimurium* (TA98, TA100, TA1535, and 1537) and one tryptophan-requiring strain

(auxotrophic *try*⁻) of *E. coli* (WP2 *uvrA*) were exposed to AE C638206 00 IC99 0005 in the presence and absence of metabolic activation. No evidence of cytotoxicity was observed at any concentration in either test series, but precipitation of the test article occurred at ≥ 1000 *ug*/plate. No increases in revertant colonies were found in either test series at concentrations up to the limit dose, 5000 ug/plate. Therefore, AE C638206 00 IC99 0005 is considered nonmutagenic in the conventional battery of bacterial strains. (MRID 46474146)

This study is classified as **unacceptable/guideline** (upgradeable) because purity information for this batch of the test material was not provided. Therefore, the study does not satisfy the requirement for FIFRA Test Guideline 84-2 for *in vitro* mutagenicity (bacterial reverse gene mutation) data.

(iv) In independent reverse mutation assays in bacteria, in the presence and absence of metabolic activation, no evidence of cytotoxicity was observed at any concentration in either test series, but precipitation of the test article occurred at ≥ 1000 *ug*/plate. No increases in revertant colonies were found in either test series at concentrations up to the limit dose, 5000 ug/plate. Therefore, AE C638206 00 IB99 0002 is considered nonmutagenic in the conventional battery of bacterial strains (MRID 46474148).

(v) In independent reverse mutation assays in bacteria, four histidine-requiring strains of *S. typhimurium* (TA98, TA100, TA1535, and 1537) and one tryptophan-requiring strain of *E. coli* were exposed to AE C638206 00 IC99 0001 in the presence and absence of metabolic activation. No evidence of cytotoxicity was observed at any concentration in either test series, but precipitation of the test article occurred at ≥ 1000 *ug*/plate. No increases in revertant colonies were found in either test series at concentrations up to the limit dose, 5000 ug/plate. Therefore, AE C638206 00 IC99 0001 is considered nonmutagenic in the conventional battery of bacterial strains (MRID 46474202).

(vi) In replicate mammalian cell gene mutation assays, cultures of Chinese hamster lung cells were exposed for four hours to AE C638206 in the presence or absence of metabolic activation. At no concentration was a biologically relevant, reproducible increase in mutant colonies found at concentrations up to the highest sub-cytotoxic levels. Therefore, AE C638206 is considered non-mutagenic in the Chinese hamster lung system (MRID 46474204).

(vii) In repeat *in vitro* chromosome assays, cultures of Chinese hamster lung (V79) cells were exposed Technical AE C638206, in the presence and absence of metabolic activation (S9-mix) for 3 hours, at 7 concentrations ranging from 3.2 to 100 *ug*/mL, and sampled 17 hours later (EXPERIMENT I); and for 20 hours in the absence of S9-mix at 7 concentrations ranging from 0.1 to 6.3 *ug*/mL, and sampled at the end of treatment (EXPERIMENT II).

Dose-related cytotoxicity (reduced survival and mitotic indices relative to control values) was evident in both Trials. Macroscopically visible precipitation of the test article was observed at ≥ 250 *ug*/mL. Of greater importance under non-activated and activated conditions of both trials, however, were reproducible statistically significant increases in structural aberrations (but not polyploidy or aneuploidy) at low to moderate levels of

cytotoxicity. Therefore, this batch-mixture of AE C638206 is considered a clastogen in the *in vitro* Chinese hamster lung (V79) cell system (MRID 46474206).

(viii) In independent, repeat assays for chromosome aberrations, human lymphocytes were exposed to the test article, AE C638206 in the presence and absence of metabolic activation. AE C638206 was clastogenic in both the presence and absence of metabolic activation in this *in vitro* mammalian cell test (MRID 46474208).

(ix) In a mouse bone marrow micronucleus assay groups of NMRI mice (5M:5F/group) were administered AE C638206 in two oral doses of 200, 600 or 2000 mg/kg/day, 24 hours apart, and bone marrow cells were harvested 24 hours after the second dose. No adverse clinical signs were observed during the main study. The ratio of polychromatic to normochromatic erythrocytes was unaffected by treatment. Additionally, at no dose level up to the limit dose (2000 mg/kg/day) were increased numbers of mPCEs induced by the test article, compared with the marked increases observed in CPA-treated cells. Therefore, AE C638206 was neither clastogenic (causing structural chromosome aberrations) nor aneugenic (causing numerical aberrations) in this mouse micronucleus assay (MRID 46474210).

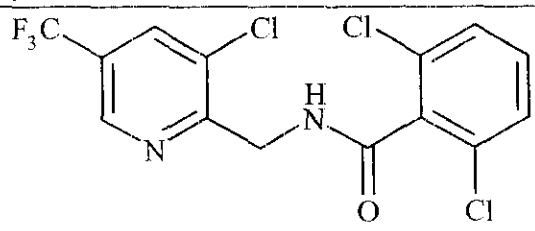
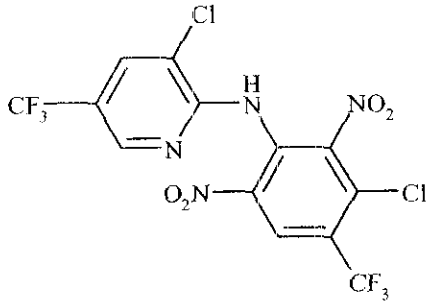
(x) In a cytogenetic (micronucleus) assay, technical AE C638206 was administered by oral gavage twice (24 hours apart) to 6 male CD-1 CrL: CD-1(ICR)BR mice at 2000 mg/kg/day (the limit dose for regulatory purposes). In the main assay, no adverse clinical toxicity was observed in any animal during the study. However, the group mean frequency of mPCE in the test group was found to be close to 2 times that observed in the concurrent vehicle (negative) control (1.50 *vs.* 0.88 PCEs/1000 cells), but was not statistically significant ($p > 0.05$). Therefore, the biological significance of these results is deemed questionable (MRID 46474212).

(xi) In a bone marrow micronucleus assay, male NMRI mice were administered two doses 24 hours apart of AE C638206 by intraperitoneal (ip) injection at 150, 300, and 600 mg/kg/day. Clinical toxicity (*e.g.* apathy, spasms, breathing difficulties) was observed in all test article-treated mice, but all animals survived until sacrifice. There was a significant increase ($p < 0.05$) in the number of NCEs per PCEs at the highest dose tested (HDT). However, at no dose up to the HDT was a significantly increased number of mPCEs recorded, in the presence of a statistically increased ratio of PCEs to NCEs (evidence of interference with erythropoiesis), either when compared to vehicle controls, or to the laboratory's 8-year historical control data base. The positive control registered a marked increase in mPCEs, in the absence of any alteration of erythropoietic effects. Therefore, Batch OP2350005 of Technical AE C638206 did not induce a clastogenic effect in male mice treated by ip up to a clinically toxic level (MRID 46474214).

(xii) In a mammalian unscheduled DNA synthesis (UDS) assay, 2 groups of male rats (4/group) were administered single oral doses of AE C638206 00 IC99 0005 at levels of 600 or 2000 mg/kg (the latter corresponds to the internationally recognized limit dose). There was no evidence (or dose-related positive response) that unscheduled DNA synthesis, as determined by radioactive tracer procedures [nuclear silver grain counts], was induced at either timed sacrifice (MRID 46474216).

2. **Structure-Activity Relationship**

One chemical was identified, Fluazinam, for which there is cancer assessment data.

Chemical Name	Structure
Fluopicolide (AE C638206 or 2,6-dichloro-*N*-[[3-chloro-5-(trifluoromethyl)-2-pyridinyl]methyl]benzamide) Caswell#: CAS Registry No.: 239110-15-7	
Fluazinam; IKF-1216 3-Chloro-*N*-[3-chloro-2,6-dinitro-4-(trifluoromethyl)phenyl]-5-(trifluoromethyl)-2-pyridinamine Caswell#: 959 CAS Registry No.: 79622-59-6	

Fluazinam** is classified as a **"suggestive evidence of carcinogenicity to humans."

1. There was some evidence that fluazinam was carcinogenic to male Sprague-Dawley rats because a) there were statistically significant positive trends for thyroid gland follicular cell adenocarcinomas and combined follicular cell adenomas/ adenocarcinomas. There was also a statistically significant increase by pair-wise comparison of 1000 ppm (40 mg/kg/day) or high dose group with the controls for combined follicular cell adenomas/adenocarcinomas. b) The incidences of thyroid gland adenomas at > or =100 ppm (> or =3.8 mg/kg/day) and adenocarcinomas at 1000 ppm were slightly outside their respective ranges for the historical controls (range: adenomas, 0%-13%; adenocarcinomas, 0%-5%). MRID 42248620. 1988.

2. There was clear evidence that fluazinam was carcinogenic to male CD-1 mice because: 1) There were statistically significant positive trends for hepatocellular adenomas, carcinomas and combined adenomas/carcinomas. There were also statistically significant increases by pair-wise comparison of the 1000 ppm (107 mg/kg/day) or high dose group with the controls for hepatocellular adenomas, carcinomas and for combined adenomas/carcinomas; and 2) The incidence of

hepatocellular adenomas and hepatocellular carcinomas at 1000 ppm exceeded the range of the historical controls for 1986-1988 (range: 8-23% and 5-13%, respectively). MRID 42208405. 1988.

3. In a more recent CD-1 mouse study, there was equivocal/some evidence that fluazinam was carcinogenic to male mice because: 1) There were no statistically significant positive trends for hepatocellular adenomas, carcinomas or combined adenomas/carcinomas for the male mice. However, there was a statistically significant increase by pair-wise comparison with the controls for hepatocellular adenomas at 3000 ppm (377 mg/kg/day) or mid dose, and combined adenomas/carcinomas at > or =3000 ppm; and 2) The incidence of hepatocellular adenomas at 3000 ppm was outside the historical control range for 1991-1993 (8-34%) and for 1987-1993 (0-31%), but the incidence at 7000 ppm (964 mg/kg/day) was within the range for the comparable historical control data. Similarly, the incidence of combined hepatocellular adenomas/carcinomas at 3000 ppm was outside the historical control range for 1987-1993 (4-42%), but the incidence at 7000 ppm was within the range for the comparable historical control data. The tumorigenic response did not occur in a dose-related manner. The highest dose level tested for the male mice in this study was considered to be adequate and not excessive. MRID 44807222. 1996.

Fluazinam was negative in mutagenicity assays.

4. Subchronic and Chronic Toxicity

a) Subchronic Toxicity

90-day Study – CD-1 Mouse

In a 92-day oral toxicity study (MRID 46474114) AE 638206 (Fluopicolide, 96.9% and 97.3% a.i., Batch Nos. AE C638206 00 1C99 0005) was administered to groups of 10 CD-1 mice/sex/dose in diet at dose levels of 0, 32, 320, 3200, or 6400 ppm (equivalent to 0, 4.7, 46, 461, and 944 mg/kg bw/day for males and 0, 6.2, 60, 629, and 1239 mg/kg bw/day for females).

No significant effects treatment-related were noted on body weight or body weight gain and no toxicologically relevant effects were noted in the hematology results. The activities of AST, ALT, and AP were slightly increased in male mice treated with ≥ 3200 ppm test material and the activity of ALT was slightly increased in female mice treated with ≥ 3200 ppm test material. These results, in conjunction with increased absolute and relative liver weight of mice in these groups, are consistent with liver hypertrophy. The incidence of microscopically observable hepatocellular hypertrophy was slightly increased in these groups.
Based on the study results, a LOAEL for AE C638206 was not identified. **The NOAEL for male and female CD-1 mice is greater than the maximum concentration administered, 6400 ppm (944 mg/kg/day for males and 1239 mg/kg/day for females).**

This 92-day oral toxicity study in the CD-1 mouse is **Acceptable/Guideline** and satisfies the guideline requirement for a 90-day oral toxicity study (OPPTS 870.3100; OECD 408) in the mouse.

90-day Study – C57BL/6JICO Mouse

In a 90-day oral toxicity study (MRID 46474116, summarized in MRID 46474115), AE C638206 (Fluopicolide, 95.9% a.i., Batch # OP2050046) was administered to 10 C57BL/6JICO mice/sex/dose in the diet at concentrations of 0, 50, 200, 800, or 3200 ppm (approximately 10.4, 37.8, 161, or 770 mg/kg/day for males and 12.6, 52.8, 207, or 965 mg/kg/day for females, respectively). Doses were selected based on previous results from a 90-day mouse dietary study with AE C638206 using Crl:CD1 (1 CR) Br mice (MRIDs 46474114 and 46474113).

There were nine deaths that appeared unrelated to treatment (no dose-response relationship). There were no adverse effects on clinical signs or neurological parameters noted for the surviving animals. Although body weight of males and females in the 3200 ppm group was lower by 7-10% early in the study, final mean body weights were comparable with the controls (both 97% of controls). The overall weight gain was slightly reduced in males in the 800 and 3200 ppm groups and in females in the 3200 ppm group (86-93% of control gain). There were some clinical chemistry variations such as slight decreases in the concentration of albumin and total cholesterol in animals treated with ≥800 ppm of AE C638206 and slightly increased alkaline phosphatase enzyme activity in males in the 3200 ppm group.

There was a slight dose-related increase in absolute (110 - 125% of control) and relative (114 - 130% of control) liver weight in animals treated with ≥800 ppm of AE C638206. These weight changes were associated with a diffused centrilobular hepatocellular liver hypertrophy. Microscopic examination revealed this lesion in 4/8 and 8/8 surviving male mice (control: 0/8) and in 8/9 and 10/10 surviving female mice (control: 0/8) at 800 and 3200 ppm of AE C638206, respectively. In addition, there was a dose-related increase in liver oval cell proliferation in females: 2/9, 2/9, 3/10, 4/9, and 8/10 in the control through the high dose groups, respectively. The toxicological significance of dark coloration of the liver in 4/8 males and 9/10 females treated with 3200 ppm was not determined.

Under the conditions of this study, the LOAEL for AE C638206 in male mice is not established; the LOAEL for female is 3200 ppm based on liver oval cell proliferation. The NOAEL for AE C638206 in male mice is ≥3200 ppm and for female mice is 800 ppm.

This 90-day oral toxicity study in the mouse is **Acceptable/Guideline**, and satisfies the guideline requirement for a 90-day oral toxicity study (OPPTS 870.3100; OECD 408).

90-day Study--Rat

In a 90-day oral toxicity study (MRID 46474112) Fluopicolide (Lot # AE C638206 00 1C99 0005; 97.2% a.i.) was administered to groups of 10 male and 10 female Sprague Dawley rats in a diet containing 0, 100, 1400 or 20,000 ppm (equivalent to 0, 7.4, 109 or 1668 mg/kg/day for males, and 8.4, 119 or 1673 mg/kg/day for females) for 13 weeks. Ten additional rats/sex from the control and high dose group were maintained on control diet for a further four weeks to determine the reversibility of any effects seen.

Two nontreatment-related mortalities were noted in the high dose group. Body weight gain over the course of the 20,000 ppm treatment was reduced by 41% in males and 29% in females, while the corresponding mean food consumption was reduced by 22% and 19% ($p<0.01$). Body weight gain was dramatically affected the first week of the study as evidenced by essentially no weight gain at the highest dose as compared to controls that gained an average of 58 g for males and 39 g for females. Reduced food consumption was also most dramatic during this week at about 50% for both sexes. Water consumption was 43% higher for females relative to the controls ($p<0.01$) during this same time frame and was somewhat higher for the remainder of the study. An increase in urinary volume and a slight decrease in specific gravity was observed in females only which corresponds to the increased water intake. No toxicologically relevant hematological or clinical chemistry findings were noted. Microscopic examination showed a minimal to slight hypertrophy of the zona glomerulosa in the adrenal of 17/20 of the rats at the highest dose level compared to one of each sex in the controls, and minimal changes were seen in 3/10 females at the 1400 ppm level. Minimum to slight trabecular hyperostosis of the bone joint was observed in 7/10 males and all females at the 20,000 ppm level compared to 0/10 males and 3/10 females in the control group. Decreased cellularity of the bone

marrow was observed for 7/10 males and 9/10 females at 20,000 ppm, and in 8/10 females at 1400 ppm compared to 0/10 males and 1/10 females in the control group. No treatment-related effects were observed at the 100 ppm dose level.

Following the four week off-dose period there was a complete or partial recovery of all treatment-related effects.

The *LOAEL is 20,000 ppm in the diet* (1668 mg/kg/day) for males based on hypertrophy of the zona glomerulosa in the adrenal, trabecular hyperostosis of the bone joint, and decreased cellularity of the bone marrow. The LOAEL for females is 1400 ppm in the diet (*119 mg/kg/day) based on* hypertrophy of the zona glomerulosa in the adrenal and decreased cellularity of the bone marrow. The NOAEL is 1400 ppm (109 mg/kg/day) for males and 100 ppm (7.9 mg/kg/day) for females.

This 90-day oral toxicity study in the rat is **Acceptable (Guideline)** and satisfies the guideline requirement for a 90-day oral toxicity study (OPPTS 870.3100; OECD 408) in the rat.

b) Chronic Toxicity

Mouse Cancer Study

In a carcinogenicity study (MRID 46474130) AE C638206 (Fluopicolide) (95.9% a.i., batch #OP2050046) was administered to 50 C57BL/6 mice/sex/dose in the diet at dietary levels of 0, 50, 400, or 3200 ppm (equivalent to 0, 7.9, 64.5, 551.0 mg/kg bw/day for males, and 0, 11.5, 91.9, and 772.3 mg/kg bw/day for females) for 18 months. Satellite groups of 10 C57BL/6 mice/sex/dose were similarly treated for 12 months. Historical control incidences of hepatocellular lesions were provided (MRID 46474135).

The incidence of mortality and clinical signs was similar in treated and control groups. Body weights and body weight gains of only the 3200 ppm animals were significantly decreased throughout the study. After 78 weeks, the body weights of 3200 ppm males were 20% lower, females were 16% lower than of the control group, and overall body weight gains were 45% lower for males and 35% lower for females. Food consumption was decreased in the 3200 ppm satellite and main group animals up to 18% throughout the study. The overall (week 1-78) food efficiency was decreased 40% for males and 30% for females at 3200 ppm. Hematology evaluations were not conducted, and there were no treatment-related changes in serum enzyme activities. After 52 weeks, absolute and relative liver weights were significantly increased in 400 ppm males (15-30%), and in 3200 ppm males and females (35-99%). After 78 weeks, liver weights were increased in both sexes at 400 ppm (15-33%) and 3200 (46-81%). At both 400 and 3200 ppm, the liver weight increases were correlated with a significant increase in the incidence of hepatocyte hypertrophy after 52 and 78 weeks, in males and females. The 3200 ppm animals had statistically significant increases in the incidence of enlarged liver and altered liver cell foci (most common type was acidophilic) after 78 weeks, and a non-significant increase after 52 weeks (≤2/10 for each lesion).

The LOAEL for AE C638206 in mice is 3200 ppm for both sexes (551.0 mg/kg/day for males, 772.3 mg/kg/day for females), based on severely decreased body weights and body weight gains and liver lesions in both sexes. The NOAEL is 400 ppm in both sexes (64.5 mg/kg/day for males, 91.9 mg/kg/day for females).

This carcinogenicity study is **Acceptable/Guideline** and satisfies guideline requirements for a carcinogenicity study [OPPTS 870.4200b; OECD 451] in mice.

Rat Chronic/Carcinogincity Study

In a combined chronic toxicity/carcinogenicity study (MRID 46474139), AE C638206 (Fluopicolide, 95.9%, a.i.; Batch No. OP2050046) was administered to 60 Crl:CD (SD) IGS BR rats/sex/dose in the diet at concentrations of 0 (controls), 50, 200, 750 or 2500 ppm (equivalent to 0, 2.1, 8.4, 31.5 or 109.4 mg/kg bw/day in males and 0, 2.8,

10.8, 41.0 or 142.2 mg/kg bw/day in females) for up to 104 weeks. An additional 20 animals/sex/dose were administered the same concentration and sacrificed after 52 weeks of treatment for a interim sacrifice. A third set of 10 animals/sex/dose were fed the treated diet at the same concentrations for 52 weeks followed by 13 weeks of being fed basal diet prior to sacrifice in a recovery study.

Clinical signs observed were in the females rats and consisted of yellow perigenital staining, brown staining of the pinna and brown staining of the dorsum.

There was no statistically significant difference in body weight in any of the treated groups. A statistically significant ($p< 0.05$ or $p<0.01$) decrease in mean body weight gain was observed in weeks 0-1 in both studies at the highest dose in males (33%) and females (28%), compared to controls. In the main study (104 week), a statistically significant ($p<0.01$) decrease was also seen in the females at 200 (20%) and 750(32%) ppm groups. The only significant decrease in body weight gain in weeks 1-2 of the main study was in males at 2500 ppm (11%) and females at 50 (15%) and 2500 (42%) ppm when compared to controls. Overall, body weight gain in the 2500 ppm groups of the main study was lower than controls by 11% (M) and 17% (F). In the animals dosed for 52 weeks, the similar effect of decreased body weight gain in the highest dosed males and females was observed with statistical significance in the first 2 weeks. Both male and female rats had comparable body weight gain by the end of the recovery period.

Statistical differences in hematology and clinical chemistry were not toxicologically significant.

Statistically significant increases ($p<0.01$ or 0.05) in relative and absolute kidney (122- 137%), thyroid (154-163%) and liver (122-134%) weights were observed in the males at 2500 ppm in the main study. These same increases in kidney (relative) and liver (relative and absolute) were observed in the males at 2500 ppm in the 52 week study. Females at 2500 ppm in the 52 week study had statistically significant increases in relative liver and kidney weights.

The lowest-observed-adverse effect level (LOAEL) for AE C638206 is 2500 ppm (109.4 (M), 142.2 (F) mg/kg/day) based on decreases in body weight gain (M/F) and an increase in thyroid organ weight with a corresponding increase in the incidence of thyroid lesions (M only). The no-observed-adverse effect level (NOAEL) for AE C638206 is 750 ppm (31.5 (M), 41.0 (F) mg/kg/day).

This chronic/carcinogenicity study in the rat is **ACCEPTABLE/GUIDELINE** and satisfies the guideline requirement for a chronic/ carcinogenicity study OPPTS 870.4300); OECD 453] in rats.

5. Mode of Action Studies

The registrant has submitted mode of action studies (MRIDs 46474132, 46474133) in connection with the observed mouse liver adenomas (MRID 46474130), which suggest Fluopicolide produces a transient effect on the liver, by a mechanism similar to that of the widely-prescribed drug Phenobarbital. The observed effects are increased hepatocyte proliferation, and increased levels of hepatic microsomal enzymes such at cytochrome P450. Again, these effects were observed to be transient in duration, with recovery occurring by the end of the 28-day study. Such a situation would mitigate against concern for prolonged effects on the liver, progressing to malignant tumors.

Fluopicolide Mechanistic Study: In a non-guideline mechanistic study (MRID 46474132, 2004) AE C638206 (Fluopicolide) (99.3% a.i., batch #R001737) was administered to groups of 15 C57BL/6 female mice in the diet at dietary levels of 0 or 3200 ppm (equivalent to 0 and 575 mg/kg bw/day) for 28 days. Satellite groups of 20 C57BL/6 females/dose were similarly treated with 0 or 3200 ppm (equivalent to 0 and 472 mg/kg bw/day) for 7 days. For all animals, necropsy was conducted and liver samples were examined histologically. Bromodeoxyuridine (BrdU) was administered in drinking water for 7 days prior to sacrifice to all animals, and liver sections were evaluated

immunologically for hepatocyte cell proliferation. Liver microsomal preparations from the satellite animals were assessed for the induction of several cytochrome P-450 isozymes.

No clinical signs of toxicity or mortality occurred during the study. The mean body weight of the 3200 ppm group was decreased (≤9%) throughout treatment, and the body weight gain was lower for days 1-7, 15-22, and 22-28. Food consumption was decreased for days 1-7 (25%) only. The mean absolute and relative (to body or brain) liver weight was increased at both the interim sacrifice (27-38%) and final sacrifice (48-59%). Gross pathology consisted of dark-appearing liver (9/20 interim and 11/15 terminal sacrifice vs. none in controls) and enlarged liver (1/20 interim and 3/15 terminal sacrifice vs. none in controls). Microscopic analysis showed diffuse, perilobular to panlobular hepatocellular hypertrophy in all treated interim (20/20 vs. 0/20 control) and terminal (15/15 vs 0/15 control) sacrifice animals, which was associated with a decreased incidence of diffuse, mainly centrilobular hepatocellular vacuolation (3/20 interim vs. 20/20 control; 3/15 terminal vs. 15/15 control).

Liver samples from interim sacrifice animals had a significant increase in the BrdU labeling index (6.5x of controls), consistent with increased cell proliferation. At terminal sacrifice, however, the BrdU labeling index was not increased, indicating that AE C638206 induced a marked but transient increase in hepatocyte proliferation. Liver microsomal preparations from interim sacrifice animals had 97% greater total cytochrome P-450 content than controls. Treatment greatly induced the activities of microsomal enzymes benzoxyresorufin O-debenzylase (BROD; 19x controls) and pentoxyresorufin O-depentylase (PROD; 12x controls), but had less impact on ethoxyresorufin O-deethylase (EROD; 1.8x controls) and lauric acid hydroxylase (0.33x of controls). These microsomal enzyme changes are similar to those resulting from treatment with phenobarbital.

This mechanistic study is **Acceptable (Non-Guideline)**. It provides credible supplemental information describing the physical and molecular changes in livers of female mice treated for 7 or 28 days with AE C638206.

Phenobarbital Mechanistic Study: In a 28-day oral toxicity study (MRID 46474133, 2002), 80 mg/kg phenobarbital was administered (>99% a.i., Batch No. 079H0561) to groups of 30 female mice by daily gavage at a concentration of 0 or 80 mg/kg/day. After seven days of treatment, half the mice in each group were sacrificed while the remaining mice were sacrificed after 28 days of treatment. The liver, brain, and duodenum were removed.

Treatment of female mice with 80 mg/kg/day phenobarbital did not increase mortality or clinical signs of toxicity. The average body weight was not affected by treatment, but the body weight gain for the first week of the study was decreased ~80%. At interim sacrifice, dark livers were found in 10/15 mice and in 6/15 treated mice after 28 days of treatment. The liver absolute and relative (to body or brain weight) of treated mice was significantly increased after 28 days of treatment. A slight to mild diffuse panlobular hypertrophy with a tendency towards the disappearance of diffuse centrilobular and panlobular microvacuolation was noted in treated mice at both the interim and terminal sacrifices. Hepatocellular proliferation was increased ~6.5 fold after one week of treatment with phenobarbital, but no significant increase was found at study termination. After seven days of treatment, the liver microsomal activities of total CYP450 and CYPIA were increased ~2-fold while that of CYPIIB and CYPIIIA were increased 11 and 20-fold respectively. Treatment with phenobarbital decreased CYPIVA activity. The results of this study agree with numerous similar studies conducted over the last several decades.

This 28-day oral toxicity study in the mouse is considered Acceptable/Nonguideline.

The labeling indices for duodenal tissue and the results of the positive control CYP microsomes were not included with the study report. However, these do not adversely influence interpretation of the study results.

V. COMMITTEE'S ASSESSMENT OF THE WEIGHT-OF-THE-EVIDENCE

TBA

VI. CLASSIFICATION OF CARCINOGENIC POTENTIAL

TBA

VII. QUANTIFICATION OF CARCINOGENIC POTENTIAL

TBA

VIII BIBLIOGRAPHY

MRID No.	CITATION

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

OFFICE OF
PREVENTION, PESTICIDES, AND
TOXIC SUBSTANCES

TXR No. 0054375

MEMORANDUM

DATE: October 17, 2006

SUBJECT: **Fluopicolide:** Qualitative Risk Assessment Based On C57BL/6 N Crl:BR SPF VAF Mouse Carcinogenicity Dietary Study

P.C. Code: 027412

TO: Myron S. Ottley, Pharmacologist
Registration Action Branch 3
Health Effects Division (7509P)

FROM: Lori L. Brunsman, Statistician
Science Information Management Branch
Health Effects Division (7509P)

THROUGH: Jessica Kidwell, EPS
Science Information Management Branch
Health Effects Division (7509P)

AND: Jess Rowland, Branch Chief
Science Information Management Branch
Health Effects Division (7509P)

BACKGROUND

A carcinogenicity study in C57BL/6 N Crl:BR SPF VAF mice was conducted by Centre International de Toxicologie, Evreux Cedex, France, for Bayer AG, Bayer CropScience, Monheim, Germany, and dated November 20, 2003 (Laboratory Project ID No. 21557 TCS, Bayer Report No. C038732, MRID No. 46474130).

The study design allocated groups of 50 mice per sex to dose levels of 0, 50, 400 or 3200 ppm (0, 7.9, 64.5 or 551.0 mg/kg/day for males; 0, 11.5, 91.9 or 772.3 mg/kg/day

for females) of Fluopicolide for 80 weeks. An additional 10 mice per sex per dose were designated for interim sacrifice at week 53.

ANALYSES

Survival Analyses

There were no statistically significant incremental changes in mortality with increasing doses of Fluopicolide in male or female mice (Tables 1 and 3).

Tumor Analyses

Male mice had statistically significant trends in liver adenomas, and adenomas and carcinomas combined, both at $p < 0.01$. There were no statistically significant pair-wise comparisons of the dosed groups with the controls. The statistical analyses of the tumors in male mice were based upon Fisher's Exact Tests for pair-wise comparisons and Exact Tests for trend since there were no statistically significant trends in mortality (Table 2).

Female mice had statistically significant trends, and statistically significant pair-wise comparisons of the 3200 ppm dose group with the controls, for liver adenomas, and adenomas and carcinomas combined, all at $p < 0.01$. The statistical analyses of the tumors in female mice were based upon Fisher's Exact Tests for pair-wise comparisons and Exact Tests for trend since there were no statistically significant trends in mortality (Table 4).

Table 1. Fluopicolide – C57BL/6 N Crl:BR SPF VAF Mouse Study (MRID 46474130)

Male Mortality Rates[+] and Cox or Generalized K/W Test Results

	Weeks				
Dose (ppm)	1-26	27-52	52[i]	53-80	Total
0	2/59[a]	0/57	10/57	6/47	8/49 (16)
50	0/60	1/60	10/59	5/49	6/50 (12)
400	1/60	1/59	10/58	3/48	5/50 (10)
3200	0/60	1/60	10/59	6/49	7/50 (14)

[+]Number of animals that died during interval/Number of animals alive at the beginning of the interval.

[i]Interim sacrifice at week 52.
[f]Final sacrifice at weeks 78-80.

[a]One accidental death at week 26, dose 0 ppm.

( )Percent.

Note: Time intervals were selected for display purposes only.
Significance of trend denoted at control.
Significance of pair-wise comparison with control denoted at dose level

Table 2. Fluopicolide – C57BL/6 N Crl:BR SPF VAF Mouse Study (MRID 46474130)

Male Liver Tumor Rates[+] and Fisher's Exact Test
and Exact Trend Test Results

	Dose (ppm)			
	0	50	400	3200
Adenomas (%)	5[a]/47 (11)	0/49 (0)	5/48 (10)	11/49 (22)
p =	0.0018**	1.0000[n]	0.6435	0.0999
Carcinomas (%)	3/47 (6)	1/49 (2)	0/48 (0)	2[b]/49 (4)
p =	0.4738	0.9463[n]	1.0000[n]	0.8319[n]
Combined (%)	7[c]/47 (15)	1/49 (2)	5/48 (10)	13/49 (27)
p =	0.0019**	0.9976[n]	0.8326[n]	0.1244

+Number of tumor bearing animals/Number of animals examined, excluding those that died or were sacrificed before week 53.

[a]First adenoma observed at week 66, dose 0 ppm.
[b]First carcinoma observed at week 71, dose 3200 ppm.

[c]One animal in the control group had both an adenoma and a carcinoma.

[n]Negative change from control.

Note: There were no tumors observed in interim sacrifice animals.
Significance of trend denoted at control.
Significance of pair-wise comparison with control denoted at dose level.
If *, then $p < 0.05$. If **, then $p < 0.01$.

Table 3. Fluopicolide – C57BL/6 N Crl:BR SPF VAF Mouse Study (MRID 46474130)

Female Mortality Rates[*] and Cox or Generalized K/W Test Results

	Weeks				
Dose (ppm)	1-26	27-52	52[i]	53-80	Total
0	0/60	1/60	10/59	4/49	5/50 (10)
50	0/60	0/60	10/60	9/50	9/50 (18)
400	0/60	0/60	10/60	2/48[a]	2/48 (4)
3200	0/60	5/60	10/55	4/45	9/50 (18)

[*]Number of animals that died during interval/Number of animals alive at the beginning of the interval.

[i]Interim sacrifice at week 52.
[f]Final sacrifice at weeks 78-80.

[a]Two accidental deaths at week 70, dose 400 ppm.

( )Percent.

Note: Time intervals were selected for display purposes only.
Significance of trend denoted at control.
Significance of pair-wise comparison with control denoted at dose level

Table 4. Fluopicolide – C57BL/6 N Crl:BR SPF VAF Mouse Study (MRID 46474130)

Female Liver Tumor Rates[+] and Fisher's Exact Test
and Exact Trend Test Results

	Dose (ppm)			
	0	50	400	3200
Adenomas (%)	1/58 (2)	2/60 (3)	1/60 (2)	19[a]/57 (33)
p =	0.0000**	0.5128	0.7605	0.0000**
Carcinomas (%)	0/58 (0)	0/60 (0)	2[b]/60 (3)	0/57 (0)
p =	0.5729	1.0000	0.2564	1.0000
Combined (%)	1/58 (2)	2/60 (3)	3/60 (5)	19/57 (33)
p =	0.0000**	0.5128	0.3222	0.0000**

+Number of tumor bearing animals/Number of animals examined, excluding those that died before week 46.

[a]First adenoma observed at week 46, dose 3200 ppm.
[b]First carcinoma observed at week 79, dose 400 ppm.

Note: Significance of trend denoted at control.
Significance of pair-wise comparison with control denoted at dose level.
If *, then $p < 0.05$. If **, then $p < 0.01$.

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DATA SUBMISSION VOLUME ____

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DATA REQUIREMENT:

200600003

870.4200

STUDY TITLE:

Historical Control Data for Long-Term Studies in C57bl/6 Mice

AUTHOR:

Jefferson Fowles

STUDY COMPLETED:

October 24, 2005

PERFORMING LABORATORY:

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LABORATORY PROJECT IDENTIFICATION:

JRF.24.10.05

STUDY VOLUME:

1 of 1

TOTAL PAGES:

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__ _ __ Identity of product impurities.

__ _ __ Description of the product manufacturing process.

__ _ __ Description of quality control procedures.

_____ Identity of the source of product ingredients.

_____ Sales or other commercial/financial information.

_____ A draft product label.

_____ The product confidential statement of formula.

_____ Information about a pending registration action.

__X__ FIFRA registration data.

_____ The document is a duplicate of page(s) _____.

_____ The document is not responsive to the request.

_____ Internal deliberative information.

_____ Attorney-Client work product.

_____ Claimed Confidential by submitter upon submission to the Agency.

The information not included is generally considered confidential by product registrants. If you have any questions, please contact the individual who prepared the response to your request.

DATA EVALUATION RECORD

FLUOPICOLIDE/ PC Code 027412
[Non-Guideline]

STUDY TYPE: MECHANISTIC STUDY – MOUSE
MRID 46474132

Prepared for

Health Effects Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
1801 Bell St.
Arlington, VA 22202

Prepared by

Toxicology and Hazard Assessment Group
Life Sciences Division
Oak Ridge National Laboratory
Oak Ridge, TN 37831
Task Order No. 114-2005

Primary Reviewer:
Sylvia Milanez, Ph.D., D.A.B.T. Signature: _____

Date: _____

Secondary Reviewers:
Kowetha Davidson, Ph.D., D.A.B.T. Signature: _____

Date: _____

Robert H. Ross, M.S., Group Leader Signature: _____

Date: _____

Quality Assurance:

Lee Ann Wilson, M.A. Signature: _____

Date: _____

Disclaimer

This review may have been altered subsequent to the contractor's signatures above.

Oak Ridge National Laboratory managed and operated by UT-Battelle, LLC., for the U.S. Department of Energy under Contract No. DE-AC05-00OR22725.

EPA Reviewer: Myron S. Ottley, Ph.D., Science Information Management Branch, Health Effects Division (7509C) **Signature:** _____ **Date**_____
EPA Work Assignment Manager: Ghazi Dannan, Ph.D. Registration Action Branch 3, Health Effects Division (7509C) **Signature**: _____ **Date**_____

Template version 11/01

TXR#: 053597

DATA EVALUATION RECORD

STUDY TYPE: Mechanistic study - mice, feeding [non-guideline]

PC CODE: 027412 **DP BARCODE:** D315502

SUBMISSION NO.: not given

TEST MATERIAL (PURITY): AE C638206 (Fluopicolide) (99.3% a.i.)

SYNONYMS: 2,6-dichloro-N-[3-chloro-5-(trifluoromethyl)-2-pyridylmethyl]benzamide; acylpicolide

CITATION: Langrand-lerche, C. (2004) AE C638206: 28-Day explanatory toxicity study in the C57BL/6 female mouse. Bayer CropScience, 355 rue Dostoïevski, BP 153, F-06903 Sophia-Antipolis Cédex, France. Laboratory project ID SA 03313, Bayer Report no. C040806, March 26, 2004. MRID 46474132. Unpublished.

SPONSOR: Bayer AG, Bayer CropScience, Alfred-Nobel-Strasse 50, 40789 Monheim, Germany.

EXECUTIVE SUMMARY: In a non-guideline mechanistic study (MRID 46474132) AE C638206 (Fluopicolide) (99.3% a.i., batch #R001737) was administered to groups of 15 C57BL/6 female mice in the diet at dietary levels of 0 or 3200 ppm (equivalent to 0 and 575 mg/kg bw/day) for 28 days. Satellite groups of 20 C57BL/6 females/dose were similarly treated with 0 or 3200 ppm (equivalent to 0 and 472 mg/kg bw/day) for 7 days. For all animals, necropsy was conducted and liver samples were examined histologically. Bromodeoxyuridine (BrdU) was administered in drinking water for 7 days prior to sacrifice to all animals, and liver sections were evaluated immunologically for hepatocyte cell proliferation. Liver microsomal preparations from the satellite animals were assessed for the induction of several cytochrome P-450 isozymes.

No clinical signs of toxicity or mortality occurred during the study. The mean body weight of the 3200 ppm group was decreased (≤9%) throughout treatment, and the body weight gain was lower for days 1-7, 15-22, and 22-28. Food consumption was decreased for days 1-7 (25%) only. The mean absolute and relative (to body or brain) liver weight was increased at both the interim sacrifice (27-38%) and final sacrifice (48-59%). Gross pathology consisted of dark-appearing liver (9/20 interim and 11/15 terminal sacrifice vs. none in controls) and enlarged liver (1/20 interim and 3/15 terminal sacrifice vs. none in controls). Microscopic analysis showed diffuse, perilobular to panlobular hepatocellular hypertrophy in all treated interim (20/20 vs. 0/20 control) and terminal (15/15 vs 0/15 control) sacrifice animals, which was associated with a decreased incidence of diffuse, mainly centrilobular hepatocellular vacuolation (3/20 interim vs. 20/20 control; 3/15 terminal vs. 15/15 control).

Liver samples from interim sacrifice animals had a significant increase in the BrdU labeling

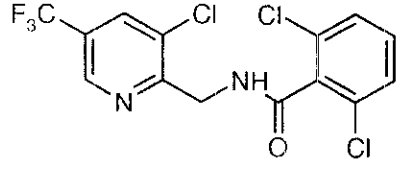
index (6.5x of controls), consistent with increased cell proliferation. At terminal sacrifice, however, the BrdU labeling index was not increased, indicating that AE C638206 induced a marked but transient increase in hepatocyte proliferation. Liver microsomal preparations from interim sacrifice animals had 97% greater total cytochrome P-450 content than controls. Treatment greatly induced the activities of microsomal enzymes benzoxyresorufin O-debenzylase (BROD; 19x controls) and pentoxyresorufin O-depentylase (PROD; 12x controls), but had less impact on ethoxyresorufin O-deethylase (EROD; 1.8x controls) and lauric acid hydroxylase (0.33x of controls). These microsomal enzyme changes are similar to those resulting from treatment with phenobarbital.

This mechanistic study is **Acceptable (Non-Guideline)**. It provides credible supplemental information describing the physical and molecular changes in livers of female mice treated for 7 or 28 days with AE C638206.

COMPLIANCE: Signed and dated GLP, Quality Assurance and Data Confidentiality statements were provided.

I. MATERIALS AND METHODS:

A. MATERIALS:

1. Test material:	AE C638206
Description:	technical, beige powder
Batch #:	R001737
Purity:	99.3 % a.i.
Compound Stability:	Sample stable at least until February 8, 2005 when stored in an air-tight, light resistant container at 5 ± 5°C
CAS # of TGAI:	239110-15-7
Structure:	

2. Vehicle and/or positive control: The dosing vehicle was diet. No positive control was used.

3. Test animals:	
Species:	Mouse
Strain:	C57BL/6 J
Age/weight at study initiation:	Approximately 10 weeks, 18.0 - 22.2 g
Source:	Charles River Laboratories, L'Arbresle, France
Housing:	Individually housed in suspended steel wire mesh cages
Diet:	A04C-10 P1 powdered rodent diet from Scientific Animal Food and Engineering (Epinay-sur-Orge, France), *ad libitum*
Water:	Filtered and softened tap water, *ad libitum*
Environmental conditions:	**Temperature:** 22 ± 2°C **Humidity:** 55 ± 15% **Air changes:** 15 cycles/hr target; not monitored **Photoperiod:** 12 hrs dark/ 12 hrs light
Acclimation period:	8 days

B. STUDY DESIGN:

1.**In life dates:** Main study: Start: November 6, 2003; End: December 4, 2003 (sacrifice)
Satellite study: Start: November 6, 2003; End: November 13, 2003 (sacrifice)

2.**Animal assignment/dose levels:** Animals were assigned to the test groups noted in Table 1 using a computerized stratification procedure so that all animals were within 20% of the group mean body weight on the day of randomization.

TABLE 1. Study design

Test Group	Conc. in Diet (ppm)	Main study: 28 days		Interim sacrifice: 7 days	
		No. Animals	Dose to animal (mg/kg/day)	No. Animals	Dose to animal (mg/kg/day)
Control	0	15	0	20	0
High dose	3200	15	575	20	472

Data from pages 16, 23, and 45 of MRID 46474132.

3.**Dose selection:** The selected dose level was based on the results of carcinogenicity study 21557 TCS (MRID 46474130), in which AE C638206 was administered to 50 C57BL/6 mice/sex/dose in the diet at 0, 50, 400, or 3200 ppm for 18 months, and to 10 C57BL/6 mice/sex/dose for 12 months. At 3200 ppm, both sexes had significantly decreased body weight gains throughout the study, increased liver weights after 52 and 78 weeks, hepatocyte hypertrophy after 52 and 78 weeks, and increased incidences of liver masses and nodules, enlarged liver, altered liver cell foci, and hepatocellular adenoma after 78 weeks. The study LOAEL was determined to be 3200 ppm for both sexes (551.0 mg/kg/day for males, 772.3 mg/kg/day for females), based on severely decreased body weight gains and liver lesions in both sexes, and the NOAEL was 400 ppm in both sexes (64.5 mg/kg/day for males, 91.9 mg/kg/day for females).

4.**Diet preparation and analysis:** The test diet was prepared once for the entire study, and was stored at approximately -18°C when not in use. The 3200 ppm diet was prepared by grinding the test substance to a fine powder and dry mixing it with the diet. Stability of the test substance in the diet was established in a previous study using 25 ppm and 10,000 ppm diets following 7 weeks of frozen storage and 1 week of room temperature storage (Study No. SA 00363; Report No. 605303; report pages 314-315). The 3200 ppm test diet homogeneity and concentration were evaluated at room temperature prior to the start of the study. Homogeneity was evaluated in two samples taken from the surface and bottom of the diet, and the mean of the four samples was used as the dietary AE C638206 concentration.

Results:

Homogeneity analysis: The mean of duplicate assays of samples from the top and bottom was 97-99% of the nominal concentration of 3200 ppm (mean = 98% or 3140.5 ppm).

Stability analysis: Established in a previous study using 25 ppm and 10,000 ppm diets (Study No. SA 00363; Report No. 605303; report pages 314-315).

Concentration analysis: The mean of the four samples evaluated for homogeneity, i.e. 3140.5 ppm (98% of nominal), was used as the dietary AE C638206 concentration.

The analytical data indicated that the mixing procedure was adequate and that the variance between nominal and actual dosage to the animals was acceptable.

5.**Statistics**: The following parameters were analyzed statistically: body weight, body weight gain/day, mean food and water consumption/day, total microsomal P-450 isoenzyme activities, 5-bromo-2'-deoxyuridine (BrdU) labeling indexes; and organ weights. The means and standard deviations were calculated for each group, and per time period for body weight gain/day and mean food consumption/day. The F test was used to compare the homogeneity of group variances. If the F test was not significant, the mean of the exposed group was compared to the mean of the control group using the 2-sided t-test, but if the F test was significant, the means of the exposed and control groups were compared using the modified 2-sided t-test. For body weight and mean food or water consumption/day, if the F test was significant, data were transformed using the log transformation. If the F test on log transformed data was not significant, the exposed and control group means were compared using the 2-sided t-test on log transformed data. If the F test was significant even after log transformation, the means of the exposed and control groups were compared using the modified 2-sided t-test. If one or more group variance(s) equaled 0, means were compared using the non-parametric Mann-Whitney test (2-sided). Statistical analyses were carried out using SAS programs for the P-450 content, and Path/Tox System V4.2.2. (Module Enhanced Statistics) for all other parameters.

Group means were compared at the 5% and 1% levels of significance. The reviewer considers the statistical analyses used to be appropriate.

C.METHODS:

1.**Observations:** Animals were inspected at least once daily for signs of toxicity and mortality. Clinical examinations were conducted at least once weekly during treatment.

2.**Body weight:** Main study group animals were weighed twice during the acclimation period, on the first day of treatment, weekly during treatment, and before necropsy. Interim sacrifice animals were weighed on treatment days 1 and 7, and before sacrifice.

3.**Food and water consumption and compound intake:** Food consumption for each group was determined as the difference in the weight of the supplied food and the food remaining after approximately each week of consumption. Water consumption was determined similarly. From the food consumption data, the mean daily diet consumption was calculated as g food/mouse/day. Compound intake (mg/kg bw/day) was calculated from the weekly consumption and body weight gain data.

4.**Sacrifice and pathology**: All animals were sacrificed on day 8 (interim) or 29 (final), after fasting overnight, by exsanguination under pentobarbital anesthesia. All were subjected to gross pathological examination of all major organs, tissues and body cavities. For all sacrificed animals, samples of the duodenum and liver were collected and fixed in 10% neutral buffered formalin, for evaluation of histology and immunohistochemistry. Histological sections were prepared from the left and median liver lobes and the duodenum, and stained with hematoxylin and eosin. The liver samples were examined histopathologically from all animals.

5.**Cell proliferation (BrdU) assay:**

a.**Preparation of BrdU (bromodeoxyuridine) solutions:** BrdU solutions were prepared twice

by dissolving BrdU (lot 023K1260) in drinking water at 0.8 g/L. The solutions were stored at ambient temperature. Three samples were taken from the surface of each solution and their concentration was determined spectrophotometrically with monitoring at 278 nm. The measured mean concentrations of the two BrdU preparations were 0.8364 g/L (105% of nominal) and 0.8338 g/L (104% of nominal). This variability from the nominal concentration is acceptable. The stability of the BrdU aqueous solution was determined over 14 days in a previous study (SA 01416).

b. **Administration of BrdU:** Prepared BrdU solutions (0.8 g/L in drinking water) was given in water bottles to animals for 7 days prior to sacrifice: days 1-8 for the interim sacrifice mice, and days 22-29 for the main study mice. The amount of BrdU intake was determined as the amount of water consumption, i.e., by measuring the difference in weight of the water bottles containing BrdU prior to administration and prior to the scheduled sacrifice.

c. **Cell proliferation assessment:** Hepatocyte cell cycling was assessed immunohistochemically by visualizing the incorporation of BrdU in two formalin-fixed liver samples, and in one duodenum sample that served as a positive staining control. The samples were incubated with a BrdU-specific monoclonal antibody, followed by signal amplification with a biotinylated secondary antibody and a streptavidin-horseradish peroxidase complex. The antibody complex was detected using diaminobenzidine and a Feulgen nuclear counterstain. The labeling index, or fraction of 1000 hepatocytes testing BrdU-positive, was evaluated separately in approximately 1000 centrilobular and 1000 periportal cells using an automated counter. The labeling index mean and standard deviation were determined for each animal, group, and liver zone.

7. **Induction of cytochrome P-450 at interim sacrifice:** The "hepatotoxic potential" of AE C638206 was determined by its ability to induce various cytochrome P-450 isozymes in hepatocyte microsome preparations. Liver samples were pooled from 5 mice/group, homogenized, microsomal preparations made (method not stated), and the total and specific cytochrome P-450 isozyme contents determined.

The total cytochrome P-450 content was measured spectrophotometrically using a reduced CO differential spectrum, once per sample. The enzyme activity levels of CYP IA, CYP II, and CYP III P-450 isozymes were evaluated by spectrofluorimetry using the substrates ethoxyresorufin (EROD), pentoxyresorufin (PROD), and benzoxyresorufin (BROD), respectively. The reactions were followed for 2, 5, or 7 minutes at 37°C. The level of CYP IV A was determined using lauric acid as substrate (10 minutes at 37°C), which produced 12-hydroxylauric acid, followed by derivatization with 4-bromomethyl-7-methoxycoumarin and measurement of the products by HPLC with fluorimetric detection. The level of 12-hydroxylauric acid was determined in the incubation mixes with method no. ANL/046-94E (details not provided). Positive control samples were run concurrently but their results were not reported: rat microsomes induced by β-naphthoflavone, phenobarbital, and clofibric acid.

II. RESULTS:

A. OBSERVATIONS:

1. **Clinical signs of toxicity:** None were observed.

2.**Mortality**: None occurred during the study.

B.BODY WEIGHT: Mean body weight of the 3200 ppm group was lower than of the control group (91-94% of controls, $p<0.01$) at all time points throughout treatment, as shown in Table 2. For days 1-7, treated animals lost weight (-0.35 g/day) whereas the weight of the control group was unchanged. The mean body weight gain of the main study animals was also lower than of the controls for days 15-22 and 22-28.

TABLE 2. Mean body weights and body weight gains/day of females treated for 7 or 28 days with AE C638206

Study day	Number of animals	0 ppm	3200 ppm
		Body Weight (g)	
1	n = 35 [7-day (n=20) + 28-day (n=15)]	20.1 ± 0.9	20.4 ± 0.8
7	n = 35 [7-day (n=20) + 28-day (n=15)]	20.1 ± 1.0	18.3** ± 0.8 (91)[1]
15	n = 15 [28-day]	20.5 ± 1.1	19.3** ± 0.9 (94)
22	n = 15 [28-day]	20.5 ± 1.2	19.2** ± 0.9 (94)
28	n = 15 [28-day]	21.0 ± 1.4	19.3** ± 0.9 (92)
Study days	Number of animals	Body Weight Gain /Day (g)	
1-7	n = 35 [7-day (n=20) + 28-day (n=15)]	0.00 ± 0.09	-0.35* ± 0.14
7-15	n = 15 [28-day]	0.05 ± 0.06	0.08 ± 0.07
15-22	n = 15 [28-day]	0.00 ± 0.08	-0.02 ± 0.07
22-28	n = 15 [28-day]	0.08 ± 0.08	0.03** ± 0.06

Data obtained from pages 39 and 41 of MRID 46474132.
* Statistically different ($p<0.05$) from the control.
** Statistically different ($p<0.01$) from the control.
[1]Numbers in parentheses are the percent of the control group, calculated by the reviewer.

C.FOOD CONSUMPTION AND COMPOUND INTAKE:

1.Food and water consumption: Food consumption of the 3200 ppm females was only 75% that of controls for days 1-7 ($p<0.01$), but was comparable to the control group thereafter. Results are summarized in Table 3. Water consumption was unaffected by treatment.

TABLE 3. Mean food consumption /mouse/ day (g) of females treated for 7 or 28 days with AE C638206

Study day(s)	Number of animals	Food consumption	
		0 ppm	3200 ppm
1-7	n = 35 [7-day (n=20) + 28-day (n=15)]	3.6 ± 0.3	2.7** ± 0.5 (75)[1]
7-15	n = 15 [28-day]	3.7 ± 0.3	3.5 ± 0.5 (95)
15-22	n = 15 [28-day]	3.8 ± 0.5	3.5 ± 0.4 (92)
22-28	n = 15 [28-day]	4.0 ± 0.6	4.0 ± 0.9 (100)

Data obtained from page 43 of MRID 46474132.
** Statistically different ($p<0.01$) from the control.
[1]Numbers in parentheses are the percent of the control group, calculated by the reviewer.

2.Compound consumption: The time-weighted-average doses for each group are presented in Table 1, for week1 and weeks 1-4.

D.SACRIFICE AND PATHOLOGY:

1.Organ weight: The 3200 ppm interim sacrifice animals had significantly greater absolute and

relative (to body or brain) liver weights than controls (27-38%, p<0.01), as shown in Table 4. These animals also had slightly lower terminal body weight (-7%; p<0.01) and a correspondingly greater brain-to-body weight ratio (+8%, p<0.01) than the controls that was not considered toxicologically relevant.

The final sacrifice 3200 ppm animals' mean body weight was similar to controls, but their absolute and relative liver weights were increased (+48-59%, p<0.01). The slightly lower absolute brain weight at 3200 ppm (-5%, p=0.01) was within normal biological variation.

TABLE 4. Organ weight (g) of female mice treated for 7 or 28 days with AE C638206

Parameter	Interim Sacrifice (Day 8, n=20)		Final Sacrifice (Day 29, n=15)	
	0 ppm	3200 ppm	0 ppm	3200 ppm
Terminal body weight (g)	16.6 ± 0.7	15.4** ± 0.8 (93)	17.4 ± 1.3	16.6 ± 0.7 (95)[1]
Absolute brain weight (g)	0.43 ± 0.02	0.43 ± 0.01 (100)	0.44 ± 0.02	0.42** ± 0.01 (95)
Absolute liver weight (g)	0.75 ± 0.13	0.95** ± 0.13 (127)	0.77 ± 0.07	1.14** ± 0.09 (148)
Brain : body weight ratio	0.026 ± 0.002	0.028** ± 0.001 (108)	0.025 ± 0.001	0.025 ± 0.008 (100)
Liver : body weight ratio	0.045 ± 0.008	0.062** ± 0.007 (138)	0.044 ± 0.002	0.069** ± 0.005 (157)
Liver : brain weight ratio	1.7 ± 0.3	2.22** ± 0.33 (131)	1.7 ± 0.1	2.7** ± 0.2 (159)

Data are from report pages 49-50 of MRID 46474132.
** Statistically different (p <0.01) from the control.
[1] Numbers in parentheses are the percent of control, calculated by the reviewer using data on pages 49-50 of MRID 46474132.

2. **Gross pathology:** At the interim sacrifice, 9/20 (p<0.01) of the 3200 ppm mice had a liver that appeared to be dark compared to controls, and 1/20 had an enlarged liver (each 0/20 in controls). In the main study group, 11/15 (p<0.01) had a dark liver and 3/15 had an enlarged liver at terminal sacrifice (each 0/20 in controls). Other macroscopic changes seen at the interim or terminal sacrifice were incidental to treatment. The gross pathology results are summarized in Table 5.

TABLE 5. Gross liver pathology incidence in female mice treated for 7 or 28 days with AE C638206

Liver lesions	Interim Sacrifice (Day 8, n=20)		Final Sacrifice (Day 29, n=15)	
	0 ppm	3200 ppm	0 ppm	3200 ppm
Obviously large	0/20	1/20	0/15	3/15
Dark	0/20	9/20**	0/15	11/15**

Data from pages 52-53 of MRID 46474132.
** Statistically different (p <0.01) from the control, determined by the reviewer using the Fisher exact test.

3. **Microscopic pathology:** At the interim and terminal sacrifice, all 3200 ppm animals had minimal to moderate diffuse, perilobular to panlobular hepatocellular hypertrophy, which was associated with a markedly decreased incidence of diffuse, mainly centrilobular hepatocellular vacuolation. The 3200 ppm interim and terminal sacrifice animals had a slightly increased incidence of mitotic cells and foci of single cell necrosis /apoptosis relative to controls, although this change appeared to be transient since it was statistically significant at only the interim sacrifice. Other microscopic changes were rare and considered incidental to treatment. The microscopic liver lesions are summarized in Table 6.

TABLE 6. Microscopic liver pathology incidence in female mice treated for 7 or 28 days with AE C638206

Liver lesions	Interim Sacrifice (Day 8, n=20)		Final Sacrifice (Day 29, n=15)	
	0 ppm	3200 ppm	0 ppm	3200 ppm
Hepatocellular hypertrophy, perilobular to panlobular - diffuse	0/20	20/20**	0/15	15/15**
Increased number of mitoses	0/20	5/20*	0/15	2/15
Single cell necrosis / apoptosis	0/20	5/20*	0/15	1/15
Hepatocellular vacuolization, mainly centrilobular, diffuse	20/20	3/20**	15/15	3/15**

Data from pages 55-56 of MRID 46474132.

*p<0.05; **p <0.01 Statistically different from the control, determined by the reviewer using the Fisher exact test.

4.Cell proliferation assessment: The mean centrilobular, perilobular, and total BrdU labeling indexes, indicators of cell proliferation, were 4.1-fold, 8.9-fold, and 6.5-fold higher ($p<0.01$ for each), respectively, in treated than control animals at the interim sacrifice. The values are shown in Table 7. The increases were statistically significant and considered treatment-related. At terminal sacrifice, however, the mean BrdU labeling index was comparable to (perilobular) or lower (centrilobular) in treated animals than controls, indicating a lack of cell proliferation.

TABLE 7.Mean 5-bromo-2'-deoxyuridine (BrdU) liver labeling index of female mice treated for 7 days with AE C638206

Region of liver	Interim Sacrifice (Day 8, n=20)		Final Sacrifice (Day 29, n=15)	
	0 ppm	3200 ppm	0 ppm	3200 ppm
Centrilobular	22.95 ± 19.00	93.55** ± 35.40 (4.1x)[1]	25.79 ± 17.94	7.23 ± 2.51 (0.3x)
Perilobular	24.16 ± 20.32	215.88 **± 57.91 (8.9x)	33.29 ± 18.01	28.53 ± 10.90 (0.9x)
Total	23.55 ± 18.97	152.95** ± 39.64 (6.5x)	29.62 ± 16.72	17.00 ± 5.27 (0.6x)

Data from page 58 of MRID 46474132.

** Statistically different ($p<0.01$) from the control group.

[1]The number in parentheses is the multiple of the control value, calculated by the reviewer.

5.Induction of cytochrome P-450 at interim sacrifice:

a.Total cytochome P-450 content: The total cytochrome P-450 content of liver microsomes was 2-fold greater in mice treated for 7 days than in control mice (2.19 vs. 1.11 nmol/mg protein, $p<0.01$).

b.Microsomal enzyme activities: As shown in Table 8, treatment with 3200 ppm AE C638206 markedly induced BROD and PROD activities (19x and 12x of controls, respectively, $p<0.05$). The effect of treatment on EROD activity (1.8x of controls, $p<0.05$) and lauric acid hydroxylation (0.33x of controls, $p<0.05$) was less pronounced.

TABLE 8. Mean activity (pmol/min/mg protein) of liver microsomal enzymes of female mice treated for 7 days with AE C638206

Liver microsome enzyme activity	No. pooled samples analyzed (5 animals/sample)	Mean activity[1] (pmol/min/mg prot)

		0 ppm	3200 ppm
Benzoxyresorufin o-debenzylation (BROD)	4	57.3 ± 10.5	1079.8 ± 27.3 (19x)[1]
Ethoxyresorufin o-deethylation (EROD)	4	71.2 ± 8.1	127.2* ± 10.0 (1.8x)
Pentoxyresorufin o-depentylation (PROD)	4	18.3 ± 2.0	227.4* ± 9.8 (12x)
Lauric acid hydroxylation	4	15.37 ± 0.64	5.09* ± 0.06 (0.33x)

Data from pages 52-68 of MRID 46474132.
*Statistically different ($p \leq 0.05$) from the control.
[1]Number in parentheses is the multiple of the control value, calculated by the reviewer.

III. DISCUSSION AND CONCLUSIONS:

A. INVESTIGATOR'S CONCLUSIONS: The investigator concluded that the test compound adversely affected body weight throughout the study, body weight gain for days 1-7, 15-22, and 22-28, and food consumption for days 1-7. Both the interim and terminal sacrifice animals had increased absolute and relative liver weights, and some had grossly visible dark and enlarged livers. All treated animals (7 or 28 days) had microscopic diffuse, perilobular to panlobular hepatocellular hypertrophy, generally accompanied by a decreased incidence of hepatocellular vacuolation. An increased number of mitotic cells and some foci of single cell necrosis/apoptosis were seen after 7 and 28 days of treatment, but the increase was greater after 7 days. Interim sacrifice animals, but not terminal sacrifice animals, had an elevated BrdU labeling index (6.5-fold higher than controls), indicative of a marked but transient hepatocellular proliferation. Liver microsomes prepared from treated interim sacrifice animals had increased total cytochrome P-450 content (+97%) and in BROD (+1785%) and PROD (+1143%) activities, with a phenobarbital-like profile.

B. REVIEWER COMMENTS: The reviewer concurs with the investigator's findings. Treatment for 7 or 28 days with 3200 ppm AE C638206 caused no clinical signs of toxicity or mortality. Treatment did cause lowered body weights and body weight gains throughout treatment, as well as increased absolute and relative liver weights, grossly visible dark-appearing liver, enlarged liver, and diffuse perilobular to panlobular hepatocellular hypertrophy after 7 or 28 days of treatment. Liver from interim sacrifice animals had a significantly increased BrdU labeling index, consistent with increased cell proliferation, but this was not evident at terminal sacrifice. The interim sacrifice animals had increased liver microsomal total cytochrome P-450 content and activities of microsomal benzoxyresorufin O-debenzylase and pentoxyresorufin O-dependentylase, but little change in ethoxyresorufin O-deethylase and lauric acid hydroxylase activities. These microsomal enzyme changes are similar to those resulting from treatment with phenobarbital.

This mechanistic study is **Acceptable (Non-Guideline)** and provides credible information regarding the effects of AE C638206 treatment on female mice.

C. STUDY DEFICIENCIES: No deficiencies that would invalidate the study results were identified. It would have been helpful if (1) results were presented for the positive control samples used to validate the measurement of the P-450 microsomal enzyme activities, and (2) the statistical significance of the gross and microscopic lesions had been determined by the study author.

DATA EVALUATION RECORD

FLUOPICOLIDE (AE C638206)/027412

STUDY TYPE: LIVER CELL PROLIFERATION
MRID 46474133

Prepared for

Health Effects Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
1801 Bell Street
Arlington, VA 22202

Prepared by

Toxicology and Hazard Assessment Group
Life Sciences Division
Oak Ridge National Laboratory
Oak Ridge, TN 37830
Work Assignment No. 114-2005

Primary Reviewer:
H. T. Borges, Ph.D., D.A.B.T. Signature:_____
Date: _____

Secondary Reviewers:
R.A. Young, Ph.D., D.A.B.T. Signature:_____
Date: _____

Robert H. Ross, M.S. Group Leader Signature:_____
Date: _____

Quality Assurance:
Lee Ann Wilson, M.A. Signature:_____
Date: _____

Disclaimer

This review may have been altered subsequent to the contractor=s signatures above.

Oak Ridge National Laboratory, managed and operated by UT-Battelle, LLC., for the U.S. Dept. of Energy under contract DE-AC05-00OR22725.

EPA Reviewer: M.S. Ottley, Ph.D. **Signature:** _____
Science Information Mgmt. Branch, Health Effects Division (7509C) **Date:** _____
EPA Secondary Reviewer: G. Dannan, Ph.D **Signature:** _____
Registration Action Branch 3, Health Effects Division (7509C) **Date:** _____

TXR#: 053597

DATA EVALUATION RECORD

STUDY TYPE: 28-day Liver Proliferation Study – Mice; Non-guideline

PC CODE: 027412 **DP BARCODE:** D315502

TEST MATERIAL (PURITY): Phenobarbital (>99% a.i.)

SYNONYMS: 5-ethyl-5-phenyl-2,4,6-(1H,3H,5H)pyrimidinetrione; 5-Ethyl-5-phenylbarbituric acid

CITATION: Langrand-Lerche, C. (2002) Phenobarbital: 28-day hepato-toxicity study in the C57BL/6 mouse. Bayer CropScience, 355, rue Dostoïevski, BP 153, F—6903 Sophia-Antipolis Cédex, France. Laboratory Study No. SA 02013. September 9, 2002. MRID 46474133.

SPONSOR: Bayer AG, Bayer CropScience, Alfed-Nobel-Strasse 50, 40789 Monheim, Germany.

EXECUTIVE SUMMARY: In a 28-day oral toxicity study (MRID 46474133), 80 mg/kg phenobarbital was administered (>99% a.i., Batch No. 079H0561) to groups of 30 female mice by daily gavage at a concentration of 0 or 80 mg/kg/day. After seven days of treatment, half the mice in each group were sacrificed while the remaining mice were sacrificed after 28 days of treatment. The liver, brain, and duodenum were removed.

Treatment of female mice with 80 mg/kg/day phenobarbital did not increase mortality or clinical signs of toxicity. The average body weight was not affected by treatment, but the body weight gain for the first week of the study was decreased ~80%. At interim sacrifice, dark livers were found in 10/15 mice and in 6/15 treated mice after 28 days of treatment. The liver absolute and relative (to body or brain weight) of treated mice was significantly increased after 28 days of treatment. A slight to mild diffuse panlobular hypertrophy with a tendency towards the disappearance of diffuse centrilobular and panlobular microvacuolation was noted in treated mice at both the interim and terminal sacrifices. Hepatocellular proliferation was increased ~6.5 fold after one week of treatment with phenobarbital, but no significant increase was found at study termination. After seven days of treatment, the liver microsomal activities of total CYP450 and CYPIA were increased ~2-fold while that of CYPIIB and CYPIIIA were increased 11 and 20-fold respectively. Treatment with phenobarbital decreased CYPIVA activity. The results of this study agree with numerous similar studies conducted over the last several decades.

This 28-day oral toxicity study in the mouse is considered Acceptable/Nonguideline.

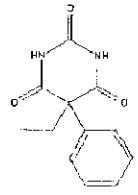
The labeling indices for duodenal tissue and the results of the positive control CYP microsomes were not included with the study report. However, these do not adversely influence interpretation of the study results.

COMPLIANCE: Signed and dated GLP, Quality Assurance, and Data Confidentiality statements were provided

I. MATERIALS AND METHODS:

A. MATERIALS:

1. **Test material:** Phenobarbital

Description:	White powder
Lot/batch #:	079H0561
Purity:	>99% a.i.
Compound stability:	Not reported
CAS # of TGAI:	50-06-6
Structure:	

2. **Vehicle and/or positive control:** 0.5% aqueous methylcellulose

3. **Test animals:**

Species:	Mice
Strain:	C57BL/6 J@Ico
Age/weight at study initiation:	Female, 9 weeks; 16.4-19.8 g
Source:	Iffa-Credo, L'Arbresle, France
Housing:	Individually in suspended stainless steel mesh cages
Diet:	A04C-10 P1, Usine d'Alimentation Rationnelle, Villemoisson-sur-Orge, France, *ad libitum*
Water:	Tap water, *ad libitum*
Environmental conditions:	**Temperature:** 20-24EC **Humidity:** 40-70% **Air changes:** 15/hr **Photoperiod:** 12 hours light/dark
Acclimation period:	16 days

B. STUDY DESIGN:

1. **In life dates:** Start: February 13, 2002; End: March 29, 2002

2. **Animal assignment:** Animals were randomly assigned based on body weight to the test groups noted in Table 1.

TABLE 1: Study design

Test group	Dose to animal (mg/kg/day)	# Females (Interim Sacrifice)	# Female (Final Sacrifice)
Control	0	15	15
Test	80	15	15

3. **Dose selection rationale:** The doses were chosen based on liver effects in a subacute toxicity study in the CD-1 mouse (SA 96420).

4. **Dose preparation:** The appropriate amount of phenobarbital was added to 0.5% aqueous methylcellulose. Each mouse in the test group received a daily gavage dose of phenobarbital to provide 80 mg/kg/day at a dosing volume of 10 mL/kg body weight. Seven days before scheduled sacrifice, the mice were given drinking water containing 0.8 g/L bromodeoxyuridine (BrdU). Water consumption during this 7-day interval was measured. The concentration of phenobarbital in methylcellulose was 101% of nominal while the concentration of BrdU in the drinking water was 96-100% of nominal.

5. **Statistics:** For water consumption data, group differences were compared by Bartlett's test. If the data were homogenous, group means were compared by Dunnett's test. If the data were heterogeneous, a modified t-test was used. Body weight, food consumption, organ weight, and cytochrome P-450 content group variances were compared by the F-test. Group differences were determined using a t-test or modified t-test, dependent on data homogeneity. Cytochrome P-450 isoenzyme activities and centrilobular and perilobular BrdU labeling indices were compared using the Mann-Whitney test.

C. METHODS:

1. **Observations:**

1a. Cageside observations: Animals were inspected daily for signs of toxicity and mortality.

1b. Clinical examinations: Clinical examinations were conducted weekly.

1c. Neurological evaluations: Neurological evaluations were not part of the study design.

2. **Body weight:** All mice were weighed on study days 1, 7, 15, 22, 28, and at scheduled necropsy.

3. **Food consumption and compound intake:** Food consumption for each animal was determined weekly and mean daily diet consumption was calculated as g food/kg body weight/day. .

4. **Ophthalmoscopic examination:** Ophthalmoscopic observations were not part of the study design.

5. **Necropsy:** On study days 8 and 29, the interim and final sacrifice animals were necropsied. The animals were fasted overnight before sacrifice. At necropsy, all major organs, tissues, and body cavities were examined. Abnormalities were noted, but not collected. The duodenum, brain and liver were sampled, the liver and brain weighed, and the liver and duodenum fixed in 10% neutral formalin for histological (hematoxylin and eosin) and immunohistological examination. All liver tissue was histologically examined.

6. **Cell Proliferation:** Immunohistochemical staining was used to visualize BrdU incorporation into hepatocellular DNA. Duodenal tissue was used as a positive control. The zonal labeling index, expressed as the number of BrdU-positive cells / 1000 cells was measured on random

fields comprised of 1000 centrilobular or 1000 periportal hepatocytes. The average labeling index for each zone was calculated.

7. **Total and Isoenzymatic Cytochrome P-450 activities:** Total Cytochrome P-450 (CYP450) was measured in liver microsomes following reduction by carbon monoxide. The activities of CYPIA1 and CYPIA2 were measured by the deethylation of 7-ethoxyresorufin (EROD) to resorufin. CYPII isoenzyme (CYPIIB1, CYPIIB2, and CYPIIE) activities were measured by O-deethylation of pentoxyresorufin (PROD), while CYPIIIA1 and A2 activities were monitored by the O-dealkylation of benzoxyresorufin (BROD). CYPIVA activity was monitored fluorimetrically by derivatization of 4-(bromomethyl)-7-methoxycoumarin of 12-hydroxy-lauric acid. Positive controls, consisting of microsomes induced by •-naphtho-flavone (CYPIA activities), phenobarbital (CYPII and CYP III activities), and clofibric acid (CYPIVA activity) were used in the assessment of each isoenzyme.

II. RESULTS:

A. OBSERVATIONS:

1. **Clinical signs of toxicity:** No treatment-related clinical signs of toxicity were noted.

2. **Mortality:** Two mice died during the study; one control animal on day 6, and one test animal on day 10. Both deaths were unrelated to the test material.

B. BODY WEIGHT AND WEIGHT GAIN:

No treatment-related effects were found on body weight; however, the body weight gain of treated mice was statistically but not biologically decreased by ~80% on day 7.

C. FOOD CONSUMPTION AND COMPOUND INTAKE:

1. **Food consumption:** The mean food consumption was statistically but not biologically significantly increased in the treated group during study days 8-15, 15-22, and 22-28 by 12%, 21%, and 12%, respectively.

2. **Compound consumption:** Compound consumption is in Table 1, above.

D. OPHTHALMOSCOPIC EXAMINATION:

Ophthalmoscopic observations were not part of the study design.

E. NECROPSY:

At interim sacrifice, the liver of 10/15 treated female mice was darkly colored, while the liver of 6/14 treated mice was darkly colored at terminal sacrifice. No other treatment-related effects were noted.

1. **Organ weight:** Shown in Table 2 are the body weight, the brain absolute and relative to body weights, and the liver absolute and relative to body weight and brain weights of control and treated female mice. At the interim sacrifice, no treatment-related effect was noted on the absolute body, liver, or brain weight of treated mice. The liver relative to body weight of treated female mice was statistically increased ~7% relative to control mice. After 28 days of treatment, the liver absolute (14%) and relative to brain (11%) or body weight (10%) were significantly increased relative to control animals

TABLE 2. Absolute and relative brain and liver weights of female mice treated daily with 80 mg/kg phenobarbital

Group	Tissue	Interim sacrifice (7 days)	Terminal sacrifice (28 days)
Control	Body Wt (g)	15.29 ± 0.656	15.84 ± 0.999
	Liver (g)	0.68 ± 0.056	0.71 ± 0.062
	Liver (%) relative to body wt.	4.44 ± 0.282	4.56 ± 0.290
	Liver (%) relative to brain wt.	161.77 ± 16.393	169.09 ± 11.830
	Brain (g)	0.42 ± 0.014	0.42 ± 0.014
	Brain (%) relative to body wt.	2.76 ± 0.168	2.64 ± 0.137
Test	Body Wt (g)	14.79 ± 0.829	16.11 ± 0.898
	Liver (g)	0.71 ± 0.096	0.81** ± 0.062
	Liver (%) relative to body wt.	4.77* ± 0.505	5.01** ± 0.359
	Liver (%) relative to brain wt.	167.87 ± 20.697	187.17** ± 14.808
	Brain (g)	0.42 ± 0.016	0.43* ± 0.014
	Brain (%) relative to body wt.	2.85 ± 0.149	2.68 ± 0.112

Data from Tables 6-8, pages 40-47, MRID 46474133
* = $p \leq 0.05$; ** = $p \leq 0.01$

2. **Microscopic pathology:** A slight to mild diffuse panlobular hepatocellular hypertrophy was observed in 13/15 treated female mice at the interim sacrifice and in all female mice after 28 days of treatment. The change was associated with the disappearance of centrilobular and panlobular microvacuolation. No other treatment-related effects were noted.

3. **Hepatocellular proliferation:** After 7 days of treatment, the BrdU labeling index was increased 6½ times the control index (17.88 ± 15.85 control vs 117.6** ± 44.63 ($p \leq 0.01$) treated). After 28 days of treatment with phenobarbital, the labeling was approximately that of control mice (20.7 ± 21.22 control vs 27.9 ± 16.61 treated). The labeling indices of the duodenal cells (positive control) for untreated and treated mice at both sacrifice periods could not be found in the report. However, the reported results of the liver indices are consistent with numerous results reported in the literature.

4. **Total and Isoenzymatic Cytochrome P-450 Activities:** Shown in Table 3 are the average activities of total CYP450 and the isoenzymes CYPIA, CYPIIB, CYPIIIA, and CYPIVA. As expected, treatment with phenobarbital for seven days slightly increased the liver microsomal protein and doubled the activities of total CYP450 and CYPIA while dramatically increasing the activities of CYPIIA and CYPIIIA. CYPIVA activity was decreased with phenobarbital treatment.

TABLE 3. Total and isoenzymatic CYP450 activity in liver microsomes of female mice treated with Phenobarbital for 7 days.						
Group	**Protein (mg/mL)**	**CYP450 (nmol/mg protein)**	**CYPIA (pmol/min/ mg protein)**	**CYPIIB (pmol/min/ mg protein)**	**CYPIIIA (pmol/min/ mg protein)**	**CYPIVA (nmol/min/ mg protein)**
Control	5.17	1.35	45.13	18.69	63.1	12.91
Treated	8.25	2.81**	85.96*	200.94	1264.4	4.33
Fold Difference	1.6	2.1	1.9	10.8	20.0	0.3

Data extracted from Appendix L, pages 166-167 of MRID 46474133
* = $p \leq 0.01$; ** = $p \leq 0.01$

III. DISCUSSION AND CONCLUSIONS:

A. INVESTIGATORS= CONCLUSIONS:

The study author concluded that treatment of female mice with phenobarbital for one week induced a transient hepatocellular proliferation that returned to control levels by 28 days of treatment. Phenobarbital was shown to be a potent inducer of CYPIIA and CYPIIIA activities.

B. REVIEWER COMMENTS:

Treatment of female mice with 80 mg/kg/day phenobarbital did not increase mortality or clinical signs of toxicity. The average body weight was not affected by treatment, but the body weight gain for the first week of the study was decreased ~80%. This decrease was not considered biologically relevant. At interim sacrifice, dark livers were found in 10/15 female mice after 7 days of treatment and in 6/15 treated female mice after 28 days of treatment. The liver absolute and relative (to body or brain weight) of treated mice was significantly increased after 28 days of treatment. A slight to mild diffuse panlobular hypertrophy and a tendency towards the disappearance of diffuse centrilobular and panlobular microvacuolation was noted in treated mice at both the interim and terminal sacrifices. Hepatocellular proliferation was increased ~6.5 fold after one week of treatment with phenobarbital, but no significant increase was found at study termination. After seven days of treatment, the liver microsomal activity of total CYP450 and CYPIA were increased ~2-fold while that of CYPIIB and CYPIIIA was increased 11 and 20-fold respectively. Treatment with phenobarbital decreased CYPIVA activity. The results of this study agree with numerous similar studies conducted over the last several decades.

C. STUDY DEFICIENCIES:

The labeling indices for duodenal tissue and the results of the positive control CYP microsomes were not included with the study report. However, these do not adversely influence interpretation of the study results.

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Registrant's Discussion

of the

Cancer Potential of Fluopicolide

and

Mode of Action Data

From: Fluopicolide. Toxicology Data Summary and Endpoint Selection Justification, pages 17 – 19. (MRID 46708548)

mineralization of the papillary/pelvic epithelium (high dose level only). No evidence of neoplastic lesions was observed.

Therefore, the NOAEL for toxicity was 200 ppm in both males and females, (equivalent to 8.4 mg/kg/day in males and 10.8 mg/kg/day in males females). Furthermore, there was no evidence of carcinogenicity with Fluopicolide up to and including the dose level of 2500 ppm (equivalent to 109.4 mg/kg/day in males and 142.2 mg/kg/day and females).

Oncogenicity in the Mouse

Fluopicolide was administered to C57/BL6 mice in the diet at concentrations up to 3200 ppm for 78 weeks. After a 52-week treatment period, 10 animals/sex/group were killed for assessment of chronic toxicity. After a 78-week treatment period, the carcinogenicity was evaluated from all oncogenicity phase animals (50 animals/sex/group).

Fluopicolide administered daily for 78 weeks produced severe reduction of the body weight gain (-45% in males and -35% in females) at 3200 ppm indicating that the Maximal Tolerated Dose (MTD) was reached. The target organ identified was the liver. Higher liver weights, enlarged liver, masses and nodules in the liver were observed at 400 and 3200 ppm at 52 and 78 weeks. These changes were associated with hepatocellular hypertrophy at 52 and 78 weeks and a high incidence of altered cell foci at 3200 ppm at 78 weeks. A high incidence of hepatocellular adenoma was observed at 3200 ppm at 78 weeks in both males and females, and to a lesser extent, at 52 weeks in females. These findings in the liver could be attributed in part to the fact that 3200 ppm clearly exceeded the MTD. The incidence of hepatocellular carcinomas was not affected.

Therefore, the NOAEL was:

a. 50 ppm for toxicity (equivalent to 7.9 mg/kg/day in males and 11.5 mg/kg/day in females), and
b. 400 ppm for carcinogenicity (equivalent to 64.5 mg/kg/day in males and 91.9 mg/kg/day in females).

These benign liver tumors occurred at the highest dose reaching the MTD (severe body weight gain reduction in high dose animals) and without any dose-effect relationship, thereby suggesting a threshold mechanism. Moreover, no tumors were observed in other mouse tissues, and these tumors did not progress into malignant neoplasia during the lifespan of these animals. No hepatocellular carcinoma was observed in any groups after a 78-week treatment period. Altogether, these findings clearly indicate that the slight increased incidence of hepatocellular adenoma in mice is a weak carcinogenic response which is considered of no relevance to man.

Based on these findings, a 28-day explanatory toxicity study was performed with Fluopicolide in C57Bl/6 female mice to investigate liver cell proliferation and cytochrome P450 induction (study No. C040806, MRID 46474132). These latter parameters were compared to those obtained in a 28-day hepato-toxicity study (study No. C026075 (MRID 46474133) and study No. C042531 (MRID 46474134)) performed with phenobarbital in female mice. Phenobarbital is a reference product, known to induce cytochrome P450 enzyme activities as well as liver cell proliferation followed by the development of hepatocellular adenoma after a chronic

exposure in rodents (Cunningham, 1996, MRID 46474136). These findings are clearly specific to rodents and are of no relevance to humans (Ito, 1992, MRID 46474137).

These explanatory toxicity studies showed that both Fluopicolide and phenobarbital induced a marked transient hepatocellular proliferation in C57BL/6 female mice which returned to control levels after 28 days of treatment. This is a very well known mechanism of hepatocellular proliferation in rodents induced by liver tumor promoters such as phenobarbital (Cunningham, 1996; Ito, 1992). Moreover, Fluopicolide showed clear induction of total cytochrome P-450 and related enzyme activities indicating a clear phenobarbital-like profile.

Altogether, these findings clearly indicate that the increased incidence in benign hepatocellular adenoma observed in high dose mice after a chronic exposure to Fluopicolide is the consequence of a threshold mechanism involving liver cell proliferation with a phenobarbital-like profile, a well known mechanism of tumor development in rodents of no relevance in humans. In conclusion, given the weak carcinogenic response in a single organ and a single rodent species only at high dose levels, together with the rodent specific mechanism of action, the absence of genotoxicity in all the *in vivo* studies, Fluopicolide is not considered to present a carcinogenic risk to humans.

DATA EVALUATION RECORD

FLUOPICOLIDE/ 027412
[OPPTS 870.4300 (§ 83-5)]

STUDY TYPE: COMBINED CHRONIC TOXICITY/CARCINOGENICITY- RAT
MRID 46474139/46474138

Prepared for

Health Effects Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
1801Bell Street
Arlington, VA 22202

Prepared by

Toxicology and Hazard Assessment Group
Life Sciences Division
Oak Ridge National Laboratory
Oak Ridge, TN 37831
Task Order No. 114-2005

Primary Reviewer: Dana F. Glass, D.V.M.	Signature: _____ Date: _____
Secondary Reviewer: K.A. Davidson, PhD., D.A.B.T.	Signature: _____ Date: _____
Robert H. Ross, M.S., Group Leader	Signature: _____ Date: _____
Quality Assurance: Lee Ann Wilson, M.A.	Signature: _____ Date: _____

Disclaimer

This review may have been altered subsequent to the contractor's signatures above.

Oak Ridge National Laboratory managed and operated by UT-Batelle, LLC., for the U.S. Department of Energy under Contract No. DE-AC05-00OR22725.

EPA Reviewer: Myron S. Ottley, Ph.D. **Signature:**
Registration Action Branch 3, Health Effects Division (7509C) **Date**
EPA Secondary Reviewer: Ghazi Dannan, Ph. D. **Signature:**
Registration Action Branch 3, Health Effects Division (7509C) **Date**

Template version 08/05

TXR#: 053597

DATA EVALUATION RECORD

STUDY TYPE: Combined chronic toxicity/carcinogenicity- diet- rat; OPPTS 870.4300 [§83-5]; OECD 453.

PC CODE: 027412 **DP BARCODE:** D315502

TEST MATERIAL (PURITY): AE C638206 (Fluopicolide), 95.9%, a.i.

SYNONYMS: 2, 6-dichloro-N-5-trifluromethylpyridine-2-methyl)benzcumide

CITATION: Cooper, S. 2003. AE C638206: Combined carcinogenicity and toxicity study by dietary administration to CD rats for 104 weeks. Huntingdon Life Sciences, Ltd., England. Laboratory Project ID No. AES 024/032124 and Project No. AES/024. November 18, 2003. MRID 46474139. Unpublished.

U. S. EPA. 2004. EPA Study Profile- Fluopicolide (AE C638206). Health Effects Division- Office of Pesticide Programs. Report Nos. C038733 and AES 024/032124; Study No. AES 024. December 18, 2004. EPA Volume No. 81. MRID 46474138.

SPONSOR: Bayer CropScience AG, Monheim, Germany

EXECUTIVE SUMMARY: In a combined chronic toxicity/carcinogenicity study (MRID 46474139), AE C638206 (Fluopicolide, 95.9%, a.i.; Batch No. OP2050046) was administered to 60 Crl:CD (SD) IGS BR rats/sex/dose in the diet at concentrations of 0 (controls), 50, 200, 750 or 2500 ppm (equivalent to 0, 2.1, 8.4, 31.5 or 109.4 mg/kg bw/day in males and 0, 2.8, 10.8, 41.0 or 142.2 mg/kg bw/day in females) for up to 104 weeks. An additional 20 animals/sex/dose were administered the same concentration and sacrificed after 52 weeks of treatment for a interim sacrifice. A third set of 10 animals/sex/dose were fed the treated diet at the same concentrations for 52 weeks followed by 13 weeks of being fed basal diet prior to sacrifice in a recovery study. A report, MRID 46474138, which consisted of a summary of the study profile was provided as an additional source of information.

Clinical signs observed were in the females rats and consisted of yellow perigenital staining, brown staining of the pinna and brown staining of the dorsum.

There was no statistically significant difference in body weight in any of the treated groups. A statistically significant ($p< 0.05$ or $p<0.01$) decrease in mean body weight gain was observed in weeks 0-1 in both studies at the highest dose in males (33%) and females (28%), compared to controls. In the main study (104 week), a statistically significant ($p<0.01$) decrease was also seen in the females at 200 (20%) and 750(32%) ppm groups. The only significant decrease in body weight gain in weeks 1-2 of the main study was in males at 2500 ppm (11%) and females at 50 (15%) and 2500 (42%) ppm when compared to controls. Overall, body weight gain in the 2500 ppm groups of the main study was lower than controls by 11% (M) and 17% (F). In the animals dosed for 52 weeks, the similar effect of decreased body weight gain

in the highest dosed males and females was observed with statistical significance in the first 2 weeks. Both male and female rats had comparable body weight gain by the end of the recovery period.

Statistical differences in hematology and clinical chemistry were not toxicologically significant.

Statistically significant increases ($p<0.01$ or 0.05) in relative and absolute kidney (122- 137%), thyroid (154-163%) and liver (122-134%) weights were observed in the males at 2500 ppm in the main study. These same increases in kidney (relative) and liver (relative and absolute) were observed in the males at 2500 ppm in the 52 week study. Females at 2500 ppm in the 52 week study had statistically significant increases in relative liver and kidney weights.

Males had a statistically significant increase in the incidence and severity of non-neoplastic microscopic lesions in the thyroid, kidney and liver in the main study. A corresponding increase ($p< 0.05$) in the incidence of enlarged kidneys and thyroids were present in the males at 2500 ppm compared to controls on gross observation. Histopathological examination showed an increased incidence of thyroid cystic follicular hyperplasia in the males. This was observed in 0/60, 1/37, 0/37, 4/35 and 7/60 ($p < 0.05$) of the dosing regimen (control, 50, 200, 750 or 2500 ppm). During the recovery period, all lesions present were reversed except for a slight increase in the severity of the renal cortical tubular basophilia in the males. Females had no statistically significant differences in lesions in any of the dose groups in either the toxicity or the main study. Also, there was not a treatment related increase in neoplastic tumor incidence of any type in animals dosed to 2500 ppm AE C638206 for up to 104 weeks.

The lowest-observed-adverse effect level (LOAEL) for AE C638206 is 2500 ppm (109.4 (M), 142.2 (F) mg/kg/day) based on decreases in body weight gain (M/F) and an increase in thyroid organ weight with a corresponding increase in the incidence of thyroid lesions (M only). The no-observed-adverse effect level (NOAEL) for AE C638206 is 750 ppm (31.5 (M), 41.0 (F) mg/kg/day).

Dosing was considered adequate based on the decreased body weight gain in the male and female rats at 2500 ppm, and the non-neoplastic lesions observed at 2500 ppm in males. While effects were minimal in the female, a reproductive study, MRID 46474124 (main study) and 46474125 (supplemental study - histopathological evaluation of liver and kidneys) indicated kidney toxicity (microscopic lesions) in male and female rats in both parental generations and decreased body weight gain in the F_0 females treated with AC638206 for 16 weeks at 2000 ppm indicating adequate dosing in this study at 2500 ppm.

This chronic/carcinogenicity study in the rat is **ACCEPTABLE/GUIDELINE** and satisfies the guideline requirement for a chronic/ carcinogenicity study OPPTS 870.4300); OECD 453] in rats.

COMPLIANCE: Signed and dated GLP, Quality Assurance and Data Confidentiality statements were provided.

I. MATERIALS AND METHODS:

A. MATERIALS:

1. Test material: AE C638206, fluopicolide

Description:	technical grade; fine powder, stored at <30°C in the dark
Lot/Batch #:	Batch No. OP2050046
Purity:	95.9 % a.i.
Compound Stability:	stable, expiration date was Jan. 4, 2003

CAS # for TGAI: 239110-15-7

2. **Vehicle and/or positive control:** The vehicle used was the diet, Rat and Mouse No. 1 Maintenance diet. No positive control was used.

3. **Test animals:**

Species:	rat	
Strain:	Crl:CD (SD) IGS BR	
Age/weight at study initiation:	Males: 40-44 days old; 159-234 grams Females: 41-45 days old; 137- 196 grams	
Source:	Charles River (UK) Ltd., Margate, Kent, England	
Housing:	4/sex/dose/cage (main study) or 3-4/sex/dose/cage (recovery period)	
Diet:	Rat and mouse diet No. 1 maintenance diet, *ad libitum,* supplied by Special Diets Ltd., Witham, Essex, England	
Water:	Tap water, *ad libitum*	
Environmental conditions:	**Temperature:**	19 to 25°C
	Humidity:	40-70%
	Air changes:	Not reported
	Photoperiod:	12 hrs dark/ 12 hrs light
Acclimation period:	14 days for males and 15 days for females	

B. STUDY DESIGN:

1. **In life dates:**

Start: September 6, 2000 (males) and September 7, 2000 (females)
End: September 10, 2002 (males) and September 11, 2002 (females)

2. **Animal assignment/dose levels:** Animals were assigned randomly to the test groups noted in Table 1. The criteria for placement into random groups was not included in the study report.

TABLE 1: Study design in rats treated with AE C68206 in diet

Test group	Conc. in diet (ppm)	Dose to animal (mg/kg bw/day)		Main Study (104 weeks)		Toxicity study Interim Sac. (52 weeks)		Toxicity study (52 weeks with recovery)[a]	
		M	F	M	F	M	F	M	F
1	0 (control)	0	0	60	60	20	20	10	10
2	50	2.1	2.8	60	60	20	20	10	10
3	200	8.4	10.8	60	60	20	20	10	10
4	750	31.5	41.0	60	60	20	20	10	10
5	2500	109.4	142.2	60	60	20	20	10	10

Data from MRID 46474139, p. 11, 17, 18 and 38

[a] Rats were administered treated or control diet for 52 weeks followed by basal diet for 13 weeks

3. **Dose selection:** The dose levels were selected based on the results from a previous 13-week toxicity study in rats (MRID 46474111) performed by the Sponsor (Aventis CropScience Report No. TOX/00/283-4) in which rats were treated with AE C638206 in the diet at concentrations of 100, 1400 or 20,000 ppm. In this study, 100 ppm was the NOAEL and 20,000 ppm was considered too high for use in the carcinogenicity study. Several changes were identified in the liver and kidney at 1400 ppm.

4. **Diet preparation and analysis:** Diet was prepared weekly by mixing appropriate amounts of test substance with SDS Rat and Mouse No. 1 maintenance diet and was stored at room temperature in sealed metal containers. A pre-mix of the correct dietary concentration was prepared by adding an approximately equal quantity of plain diet to the required weight of AE C638206 before mixing with a spoon. Additional amounts of plain diet were added that equaled this mixture and also mixed with a spoon. This doubling-up was followed until a homogenous pre-mix was achieved and the pre-mix was blended in a Turbula Mixer. A second pre-mix was then made from this first pre-mix using this doubling-up procedure and also blended in the Turbula Mixer. The 2500 and 750 ppm formulations were made by direct dilution of the first pre-mix with additional untreated diet and the 200 and 50 ppm made by direct dilution of the second pre-mix with additional untreated diet. All blending was done with the Turbula Mixer set at 100 cycles.

   Homogeneity and stability were tested using the 50 and 2500 ppm pre-mix. Each diet formulation was randomly sampled in duplicate from the top, center and bottom of the turbula mixer drum. Each homogeneity sample was sub-sampled for stability and the remainder retained for homogeneity determination. The sub-samples were sub-divided to produce 5 diet samples with one sample retained frozen and the others kept at room temperature (21°C). The stability determinations were made from the 21°C formulation on Days 0, 8 and 22. Samples of each formulation administered to the rats during Weeks 1, 13, 26, 39, 52, 65, 79, 91 and 103 were analyzed in duplicate for concentration from the Turbula mixer drum.

Results

Homogeneity analysis: ± 3.92 %; Homogeneity analysis of the 50 ppm premix yielded results ranging from 47.2 to 53.2 ppm with a mean of 50.8 and a coefficient of variation of 3.92%. The 2500 ppm premix yielded results ranging from 2360 to 2580 ppm with a mean of 2470 ppm and a coefficient of variation of 3.10%.

Stability analysis: ± 6.4 %; Stability analysis of the 50 ppm premix yielded results ranging from 50.6 to 53.2 ppm with a relative mean error of +1.4 to +6.4%. Stability analysis of the 2500 ppm premix yielded results ranging from 2470 to 2600 ppm with a relative mean error of -1.2 to +2.8%.

Concentration analysis: ± 6.8 %; The relative mean error (% deviation from nominal) for mean concentration ranged from -6.8 to +4.4

The mean concentration of AE C638206 analyzed confirmed accurate formulation and the homogeneity and stability also were confirmed by analytical methods. Stability was confirmed for 22 days at ambient temperature. The analytical data indicated that the mixing procedure was adequate and that the variance between nominal and actual dosage to the animals was acceptable.

5. **Statistics:** All statistical analysis was carried out separately for males and females. The analysis utilized the individual animal as the basic experimental unit. The following data were analyzed at each time point separately: body weight, blood chemistry, hematology, urinalysis, organ weights (both absolute and adjusted for terminal body weight), and pathological findings. Fisher's Exact test was used for categorical data using the treated groups versus the controls. Bartlett's test was used for continuous data to first test the homogeneity of variance between groups. In analysis of body weight

gain and organ weights, when Bartlett's test found statistically significant findings, a Behrns-Fisher test was used to do pair-wise comparisons; otherwise a Dunnett's test was used.

A sequence of statistical tests was used for clinical pathology data. If 75% of the data were the same value, then a frequency analysis was applied. Treatment groups were compared using a Mantel test for a trend in proportions and also pair-wise Fisher's Exact test for dose group against the control. If Bartlett's test for variance homogeneity was not significant at the 1% level, then parametric analysis was used. Then depending on whether the F1 test for monotonicity of dose-response was or was not significant at the 1% level, either Dunnett's test or William's test was performed, respectively. If Bartlett's test was significant at the 1% level, the logarithmic and square-root transformations were applied. If Barlett's test was still significant, then non-parametric tests were applied. Log-rank methods were used to analyze the number of animals with tumors across treatment groups with males and females calculated separately. Shirley's test for monotonic trend was used where the H1 test was not significant at 1% and Steel's test was used where the H1 test was significant at the 1% level. Peto's test was used for mortality and tumor incidence data comparisons. Significance is indicated by $p<0.05$ or $p<0.01$. The reviewer finds the statistical methods employed adequate for the study.

C. **METHODS:**

1. **Observations:**

   1a. **Cageside observations**: Animals were inspected at least twice daily for signs of toxicity and mortality.

   1b. **Clinical examinations**: Clinical examinations which included palpation for masses were conducted weekly.

   1c. **Neurological evaluations**: Neurological evaluations were not conducted. A previous subchronic feeding study in the rat (Report No. C019700) and a 90-day neurotoxicity rat study (Report No. C019700)are available for data on neurotoxicity evaluations of rats treated with technical grade AE C638206.

2. **Body weight:** Animals were weighed on the first day of treatment (week 0), once each week for the first 16 weeks of treatment, once every 4 weeks thereafter and prior to necropsy.

3. **Food consumption and compound intake:** Total food consumption values were calculated from the weekly group mean values and were calculated as g food/animal/week. Weekly group mean food consumption and standard deviations were derived from unrounded cage values. Group mean food efficiency was calculated for the first 16 weeks of treatment and was derived from cage values. The formula used was:

$$\text{Food conversion efficiency (\%)} = \frac{\text{body weight gain (g)}}{\text{total food consumption (g/rat)}} \times 100$$

The group mean achieved dosage for each sex was expressed as mg/kg/day and calculated by the following formula:

$$\text{Achieved food intake (mg/kg/day)} = \frac{\text{food consumed (g/rat) x ppm test substance}}{\text{midweek body weight (g) x 7}}$$

4. **Ophthalmoscopic examination:** Eyes were examined prior to treatment, during week 51 for all controls and 2500 ppm animals and during weeks 78 and 104 for 20 males and 20 females in the control and 2500 ppm groups.

5. **Hematology & clinical chemistry:** After an overnight fast from food only, blood for hematology and clinical chemistry was taken from the retro-orbital sinus under isoflurane anesthesia. For the hematology samples, all animals in the 52 week study were bled at weeks 13, 26 and 52, and in the main study, 20 males and 20 females were bled at weeks 78 and 104. Blood smears were collected from all main study animals not used for hematology during weeks 52, 78 and 104. During Week 13 of recovery, all surviving animals were evaluated. Blood samples for clinical chemistry were obtained in ten males and ten females at weeks 13, 26 and 52 in the toxicity study and in ten males and ten females in the main study at weeks 78 and 104. The CHECKED (X) parameters were examined.

a. **Hematology**

X	Hematocrit (HCT)*	X	Leukocyte differential count*
X	Hemoglobin (HGB)*	X	Mean corpuscular HGB (MCH)*
X	Leukocyte count (WBC)*	X	Mean corpusc. HGB conc.(MCHC)*
X	Erythrocyte count (RBC)*	X	Mean corpusc. volume (MCV)*
X	Platelet count*		Reticulocyte count
	Blood clotting measurements*		
X	(Thromboplastin time)		
	(Clotting time)		
X	(Prothrombin time)		

* Recommended for combined chronic/carcinogenicity studies based on Guideline 870.4300.

b. **Clinical chemistry**

	ELECTROLYTES		OTHER
X	Calcium	X	Albumin*
X	Chloride	X	Creatinine*
	Magnesium	X	Urea nitrogen*
X	Phosphorus	X	Total Cholesterol*
X	Potassium*		Globulins
X	Sodium*	X	Glucose*
	ENZYMES (more than 2 hepatic enzymes eg., *)	X	Total bilirubin
X	Alkaline phosphatase (ALK)*	X	Total protein (TP)*
	Cholinesterase (ChE)	X	Triglycerides
X	Creatine phosphokinase		Serum protein electrophoresis
	Lactic acid dehydrogenase (LDH)		
X	Alanine aminotransferase (ALT/ SGPT)*		
X	Aspartate aminotransferase (AST/ SGOT)*		
X	Gamma glutamyl transferase (GGT)*		
	Sorbitol dehydrogenase*		
	Glutamate dehydrogenase*		

* Recommended for combined chronic and carcinogenicity studies based on Guideline 870.4300.

6. **Urinalysis:** To collect urine, animals were placed into metabolism cages after being fasted overnight from food and water. Urine was collected from 10 males and 10 females at weeks 12, 25, 51 (interim sacrifice group), 77 and 103 (main study group). All surviving animals were assessed at Week 13 of

recovery. During Week 13 of recovery, all surviving animals were evaluated. The CHECKED (X) parameters were examined.

X	Appearance*	X	Glucose*
X	Volume*	X	Ketones
X	Specific gravity / osmolality*	X	Bilirubin
X	pH*	X	Blood/ red blood cells*
X	Sediment (microscopic)		Nitrate
X	Protein*		Urobilinogen

* Recommended for combined chronic and carcinogenicity studies based on Guideline 870.4300.

7. **Sacrifice and pathology**: All animals that died and those killed in extremis or sacrificed on schedule were subjected to gross pathological examination. Animals were killed by carbon dioxide asphyxiation. The CHECKED (X) tissues were collected and preserved in 10% neutral buffered formalin except for the testes and epididymides which were fixed in Bouin's fluid and eyes which were fixed in Davidson's fluid. Samples of any gross lesions or masses were also processed for examination. Tissue samples were dried, embedded in paraffin wax, sectioned and stained with hematoxylin and eosin except for the testes which were stained with the periodic acid/Schiff (PAS) method. Microscopic examination was performed on all tissues and gross lesions from animals killed or dying during the study and all animals from the controls and 2500 ppm group on completion of the 52 week and main study. In the 50, 200 or 750 ppm groups, the kidneys, liver and lungs were examined at the completion of the main study, 52 week study and at the end of the recovery period. The (XX) organs, in addition, were weighed.

	DIGESTIVE SYSTEM		CARDIOVASC./HEMAT.		NEUROLOGIC
	Tongue	X	Aorta, thoracic*	XX	Brain (multiple sections)*+
X	Salivary glands*	XX	Heart*+	X	Periph.nerve*
X	Esophagus*	X	Bone marrow*	X	Spinal cord (3 levels)*
X	Stomach*	X	Lymph nodes*	X	Pituitary*
X	Duodenum*	XX	Spleen*+	X	Eyes (retina, optic nerve)*
X	Jejunum*	XX	Thymus		**GLANDULAR**
X	Ileum*			XX	Adrenal gland*+
X	Cecum*		**UROGENITAL**	X	Lacrimal gland
X	Colon*	XX	Kidneys*+	XX	Parathyroids*
X	Rectum*	X	Urinary bladder*	XX	Thyroids*
XX	Liver*+	XX	Testes*+		**OTHER**
	Gall bladder* (not rat)	XX	Epididymides*+	X	Bone (sternum and/or femur)
	Bile duct (rat)	X	Prostate*	X	Skeletal muscle
X	Pancreas*	X	Seminal vesicle*	X	Skin*
	RESPIRATORY	XX	Ovaries*+	X	All gross lesions and masses*
X	Trachea*	XX	Uterus*+		
X	Lung*++	X	Mammary gland*		
X	Nose*				
X	Pharynx*				
X	Larynx*				

* Required for combined chronic/carcinogenicity studies based on Guideline 870.4300.
+Organ weight required in combined chronic/carcinogenicity studies.
++Organ weight required if inhalation route.

II. RESULTS:

A. OBSERVATIONS:

1. **Clinical signs of toxicity:** The only clinical signs observed were in females rats. Statistical significance was not performed for the incidence of these clinical signs. Yellow perigenital staining was observed in females at 750 and 2500 ppm in both the 52 week and main studies. In the main study, staining was first observed at week 13 and continued throughout the study but was minimized by week 66. The yellow perigenital staining was most evident at week 47 and was seen in 0/60 of controls, 1/58 at 50 ppm, 3/60 at 200 ppm, 11/58 at 750 ppm and in 16/59 at 2500 ppm. Similar observations were seen in the 52 week study.

   Brown staining of the pinna was also observed in the females beginning at approximately week 13. It was most evident in week 53 when it was seen in 1/60 controls, 2/56 at 50 ppm, 0/58 at 200 ppm , 6/58 at 750 ppm and in 18/59 at 2500 ppm. This effect was only observed in about 3% of the females at 2500 ppm by week 72. Again, similar results were observed in the 52 week study.

   The final clinical sign observed in females was a brown staining of the dorsum. This effect was most prevalent at week 21 when it was observed in 12/60 (20%) controls and 29/60 (48%) at 2500 ppm. Other dose groups showed lower incidences which were not strictly dose related.

   These clinical signs are not likely to have toxicological significance due to no effects observed in the urinalysis or histopathology, and they were transient with most effects diminishing around week 66.

2. **Mortality:** There were no treatment-related increases in mortality. Survival at the end of the 104 weeks in each group ranged from 25 to 52% thus allowing a sufficient number of animals to be examined (Table 2).

TABLE 2:Cumulative mortality in main (104 weeks) study

	0 ppm	50 ppm	200 ppm	750 ppm	2500 ppm
Males	37/60	32/60	32/60	29/60	31/60
Females	39/60	45/60	37/60	36/60	35/60

3. **Neurological evaluations**: Because neurological deficits were not observed in earlier studies with AE C638206 performed by the same laboratory, Functional Observation Battery examinations were not conducted in this study.

B. BODY WEIGHT: Data on body weight and body weight gain are provided in Tables 3 and 4. There were no statistically significant differences observed in body weight. A statistically significant ($p< 0.05$ or $p<0.01$) decrease in mean body weight gain during week 0-1 in the 52 week and main studies (104 week) was observed in the 2500 ppm males and females. In the main study, a statistically significant ($p<0.01$) decrease was also seen in the females at 200 and 750 ppm during week 0-1. In the highest dosed males in week 0-1, body weight gain was 33% lower than that of controls. In females, mean body weight gain was 20, 32 and 28% lower than controls in the 200, 750 and 2500 ppm dose groups during this time, respectively. Significant decreases in body weight gain in weeks 1-2 of the main study was observed in the males (11%) at 2500 ppm and the females at 50 and 2500 ppm (15%, 42%) compared to controls. The overall body weight gain of the 2500 ppm groups (M/F) was decreased by 11% (M) and 17% (F) when compared to controls in the main study.

In the 52 week study, body weight gain was statistically significantly ($p< 0.01$) decreased in the males at 2500 ppm and in the females at 200, 750 and 2500 ppm during week 0-1. Body weight gain in the males at 2500 ppm was 39% lower than controls in week 0-1. The females' body weight gain in week 0-1 was 35, 39 and 54% lower than controls in the 200, 750 and 2500 ppm groups, respectively. Both male and female rats had comparable body weight gain by the end of the recovery period.

TABLE 3: Mean body weights (BW ± SD) and body weight gains (BWG ± SD) in main (104 weeks) study					
g + SD	0 ppm	50 ppm	200 ppm	750 ppm	2500 ppm
MALES Initial BW	198 ± 15.1	197 ± 14.0	198 ± 12.3	197 ± 10.5	194 ± 11.1
BW wk 1	250 ± 20.0	250 ± 18.8	254 ± 17.0	246 ± 14.2	228 ± 12.9
BW wk 13	553 ± 56.3	559 ± 59.6	566 ± 62.2	548 ± 47.2	509 ± 43.2
BW wk 28	667 ± 73.6	671 ± 80.0	681 ± 80.7	651 ± 60.2	624 ± 61.5
BW wk 52	778 ± 99.5	788 ± 105.6	787 ± 105.2	754 ± 83.0	729 ± 81.7
BW wk 76	834 ± 109.7	857 ± 138.0	847 ± 136.0	829 ± 101.9	772 ± 89.8
BW wk 104	817 ± 132.5	825 ± 134	861 ± 136.8	795 ± 108.2	746 ± 121.3 (91)[a]
BWG 0-1	52 ± 12.7	53 ± 7.4	56 ± 7.7* (108)	49 ± 8.5	35 ± 7.7** (67)
BWG 1-2	54 ± 10.1	52 ± 9.4	51 ± 8.3	51 ± 9.1	48 ± 8.5** (89)
BWG 0-13[b]	355	362	368	351	315 (89)
BWG 13-28[b]	114	112	115	103	115
BWG 28-52[b]	111	117	106	103	105
BWG 52-104[b]	39	37	74	41	17 (44)
BWG 0-52[b]	580	591	589	557	535
BWG 0-104	623 ± 129.6	629 ± 135.0	665 ± 134.4	602 ± 106.7	555 ± 120.3 (89)
FEMALES initial BW	165 ± 11.4	166 ± 11.4	163 ± 9.3	164 ± 12.6	167 ± 11.5
BW wk 1	191 ± 14.4	189 ± 14.7	183 ± 12.3	182 ± 15.0	186 ± 13.1
BW wk 2	217 ± 16.1	211 ± 17.3	209 ± 16.5	206 ± 18.6	201 ± 15.2
BW wk 13	318 ± 26.9	312 ± 31.7	309 ± 24.7	310 ± 33.5	296 ± 25.1
BW wk 28	373 ± 41.9	358 ± 40.3	360 ± 37.9	362 ± 47.0	338 ± 40.4
BW wk 52	461 ± 72.0	443 ± 72.1	440 ± 61.8	451 ± 80.2	411 ± 62.7
BW wk 76	538 ± 98.8	502 ± 83.5	517 ± 84.2	527 ± 107.9	481 ± 81.0
BW wk 104	554 ± 98	508 ± 113.1	542 ± 127.7	558 ± 140.5	486 ± 97.2 (88)
BWG 0-1	25 ± 6.1	23 ± 6.8	20 ± 6.8 (80)**	17 ± 7.9** (68)	18 ± 9.0** (72)
BWG 1-2	26 ± 5.4	22 ± 6.0** (85)	27 ± 7.4	25 ± 6.5	15 ± 7.8** (58)
BWG 0-13[b]	153	146	146	146	129 (84)
BWG 13-28[b]	55	46	51	52	42 (76)
BWG 28-52[b]	88	85	80	89	73 (83)
BWG 52-104[b]	93	65	102	107	75 (81)
BWG 0-52[b]	296	277	277	287	244 (82)
BWG 0-104	390 ± 97.0	346 ± 107.4	378 ± 122.2	396 ± 136.8	322 ± 94.8 (83)

Data obtained from Tables 4C, pages 94-103 in MRID 46474139.

[a] Number in parentheses is percent of control, provided for in report or calculated by reviewer

[b] Body weight gain was calculated by reviewer.

* Statistically different ($p < 0.05$) from the control.

** Statistically different ($p < 0.01$) from the control.

TABLE 4: Mean body weights (BW ± SD) and body weight gains (BWG ± SD) in toxicity (52 weeks) study					
g + SD	0 ppm	50 ppm	200 ppm	750 ppm	2500 ppm
MALES Initial BW	196 ± 15.0	195 ± 12.3	196 ± 14.2	199 ± 12.2	197 ± 13.0
BW wk 52	751 ± 92.9	754 ± 87.0	756 ± 91.8	752 ± 85.1	723 ± 67.5 (96)[a]
BWG 0-1	57 ± 7.2	56 ± 5.8	53 ± 6.0	50 ± 6.0** (88)	35 ± 6.5** (61)
BWG 1-2	50 ± 7.5	53 ± 10.1	51 ± 7.2	51 ± 6.5	49 ± 9.6
BWG 2-13[b]	234	234	233	231	222
BWG 0-52	556 ± 81.8	559 ± 86.5	559 ± 85.8	554 ± 78.9	527 ± 67.2
BWG Recovery 0-12	35 ± 11.1	44 ± 42.3	40 ± 20.3	50 ± 17.4* (143)	44 ± 31.5
FEMALES Initial BW	169 ± 9.5	164 ± 10.1	165 ± 11.2	164 ± 10.3	165 ± 10.3
BW wk 52	465 ± 76.0	438 ± 62.8	438 ± 59.0	421 ± 67.3	387 ± 64.1 (83)
BWG 0-1	28 ± 6.3	32 ± 8.4 (114)*	18 ± 4.6**(64)	17 ± 6.1** (61)	13 ± 5.5** (46)
BWG 1-2	18 ± 6.3	17 ± 7.1	26 ± 5.0** (144)	24 ± 4.9** (133)	20 ± 4.8 (111)
BWG 2-13[b]	99	99	99	93	83 (84)
BWG 0-52	297 ± 72.2	273 ± 60.3	273 ± 50.6	257 ± 61.3	222 ± 60.9 (75)**
BWG Recovery 0-12	29 ± 25.3	22 ± 15.0	31 ± 9.9	28 ± 23.4	49 ± 25.4

Data obtained from Tables 4A and 4B, pages 84-93 in MRID 46474139.
[a] Number in parentheses is percent of control, provided in report and calculated by reviewer
[b] BWG calculated by reviewer
* Statistically different ($p < 0.05$) from the control.
** Statistically different ($p < 0.01$) from the control.

C. FOOD CONSUMPTION AND COMPOUND INTAKE

1. **Food consumption**: No treatment-related signs of toxicity were observed.

2. **Compound consumption**: Compound consumption in the males high dose group had an 8% reduction by the treated rats at 52 weeks in Table 1.

3. **Food efficiency**: No treatment-related signs of toxicity were observed. Food conversion efficiency was decreased in males and females in the 2500 ppm groups compared to controls in both the 52 week and main study (104 week), but only in week one. By week 2-3, all groups were comparable to controls.

D. OPHTHALMOSCOPIC EXAMINATION: No treatment-related findings were identified on any of the animals during ophthalmoscopic examinations in either the 52 week or main study.

E. BLOOD ANALYSES:

1. **Hematology:** Statistically significant differences for hematology in several red blood cell parameters were observed primarily in the 2500 ppm group animals, especially males. Although differences were

statistically significant, they were minor (< 8% of controls), sporadic and did not follow a dose-related trend and were not considered an adverse effect of treatment.

2. **Clinical chemistry**: Statistically significant differences in clinical chemistry parameters were mostly observed in the males. While some of the parameters followed a dose-related trend, the differences were usually ≤15% of the control values and not considered of biological significance.

F. **URINALYSIS:** No treatment-related signs of toxicity were observed.

G. **SACRIFICE AND PATHOLOGY:**

1. **Organ weight**: Data for organ weights are included in Table 5. In the 52 week study, male rats in the 750 and 2500 ppm groups had statistically significant ($p<0.05$ and $p<0.01$) increases in relative liver and kidney weight, and absolute liver weight in the 2500 ppm group when compared to controls. Relative kidney and liver weight were both 9% greater than controls in the 750 ppm males, and 13 and 20%, respectively, in the males at 2500 ppm. Absolute liver weight was 15% greater than controls in males at 2500 ppm. The only statistically significant increase ($p<0.01$) in organ weight in treated female rats was in relative kidney and liver weight at 2500 ppm in females in the 52 week study; however, this was not toxicologically significant due to a significant corresponding decrease in body weight.

In the main study (104 week), male rats were observed to have statistically significant ($p<0.05$ or $p<0.01$) increases in organ weight compared to controls at 750 and 2500 ppm. These increases were toxicologically significant only in the 2500 ppm group with absolute and relative kidney weight being 22% and 37%, respectively, more than controls. The absolute and relative liver weight was 22 and 34%, respectively, more than controls in the 2500 ppm group. Statistically significant ($p<0.05$) increases in relative and absolute thyroid (including parathyroids) weight were also observed in the highest dosed males in the main study. There were no effects in females on organ weights at 104 weeks.

Rats in the recovery study had terminal organ weights comparable to controls in all the dose groups.

Based on corresponding histopathological findings, the only toxicologically significant findings were increased thyroid weight in the highest dosed male rats.

TABLE 5. Group mean values of absolute (g ± SD) and relative (% of body weight) organ weights					
Organ	Dietary concentration (ppm)				
	0	50	200	750	2500
Males (52 weeks)					
Terminal body weight	740.2 ± 98.5	735.3 ± 77.4	738.9 ± 104.0	727.7 ± 87.5	711.6 ± 73.4
Kidney, absolute	3.87 ± 0.49	4.00 ± 0.39	3.98 ± 0.60	4.13 ± 0.46	4.20 ± 0.43
Kidney, relative	0.526 ± 0.053	0.546 ± 0.040	0.541 ± 0.051	0.571 ± 0.067* (109)[a]	0.592 ± 0.051** (113)
Liver, absolute	21.51 ± 2.92	22.17 ± 3.10	22.86 ± 5.03	23.17 ± 3.75 (108)	24.81 ± 3.31* (115)
Liver, relative	2.917 ± 0.279	3.015 ± 0.287	3.075 ± 0.371	3.183 ± 0.329* (109)	3.491 ± 0.367** (120)
Males (104 weeks)					
Terminal body weight	810.7 ± 132.0	820.6 ± 132.6	856.6 ± 137.4	791.8 ± 108.6	744.9 ± 127.0
Kidney, absolute	4.67 ± 0.43	4.83 ± 1.05	5.10 ± 0.73*	5.08 ± 0.85* (109)	5.69 ± 1.87** (122)
Kidney, relative	0.586 ± 0.082	0.592 ± 0.093	0.603 ± 0.089	0.655 ± 0.160* (112)	0.800 ± 0.396** (137)
Liver, absolute	22.49 ± 4.34	23.03 ± 3.53	24.18 ± 3.57	24.21 ± 4.13	27.42 ± 4.72** (122)
Liver, relative	2.803 ± 0.538	2.843 ± 0.417	2.850 ± 0.361	3.100 ± 0.680 (111)	3.757 ± 0.908** (134)
Thyroid – parathyroid, absolute	0.039 ± 0.011	0.041 ± 0.022	0.047 ± 0.031	0.041 ± 0.011	0.060 ± 0.049* (154)
Thyroid+ parathyroid, relative	0.0049 ± 0.0015	0.0050 ± 0.0021	0.0055 ± 0.0038	0.0053 ± 0.0016	0.0080 ± 0.0058* (163)
Females (52 weeks)					
Terminal body weight	464.1 ± 85.3	427.9 ± 64.1	438.8 ± 64.5	413.9 ± 60.3	386.0 ± 49.6** (83)
Kidney, absolute	2.78 ± 0.51	2.60 ± 0.29	2.72 ± 0.39	2.64 ± 0.38	2.67 ± 0.34
Kidney, relative	0.604 ± 0.065	0.617 ± 0.083	0.622 ± 0.055	0.643 ± 0.082	0.694 ± 0.074** (115)
Liver, absolute	14.47 ± 2.55	14.07 ± 2.17	14.51 ± 2.27	13.94 ± 1.76	14.31 ± 1.97
Liver, relative	3.130 ± 0.215	3.307 ± 0.366	3.319 ± 0.312	3.384 ± 0.240	3.719 ± 0.351** (119)

Data from MRID 46474139, Tables: 11A, 11B, 11E and 11F, p. 201-212 and 225-256.
[a] Number in parentheses is percent of control, as reported in study
significant when compared to controls, * $p<0.05$ and ** $p<0.01$

2. **Gross pathology:** At 104 weeks, males in the 2500 ppm group had a statistically significant increase in the incidence of enlarged kidneys and thyroids compared to controls. The enlarged kidneys were observed in 1/60 of the controls and 8/60 of the 2500 males ($p<0.05$). The enlarged thyroids were observed in 0/60 of the controls and 12/60 of the 2500 males ($p<0.05$). The incidence of scabs observed on the tails of the males at 2500 ppm was decreased compared to controls in the main study. This was observed in 28/60 of the controls and 12/60 of the 2500 group ($p<0.01$). The female group at 2500 ppm had a lower incidence of adrenal masses (11/60 controls, 1/60 at 2500 ppm; $p<0.01$) when compared to controls. No treatment-related findings were reported among the interim sacrifice or recovery animals.

3. **Microscopic pathology**:

   a. **Non-neoplastic**: Table 6 provides the data on microscopic pathology. In the main (104 week) and 52 week study, statistical increases in the incidence and severity of non-neoplastic microscopic lesions were observed in the males. Females did not have any toxicologically significant lesions observed, e.g. eosinophilic foci, an increase (nonsignificant) in kidneys). In the main study, an increased incidence of thyroid cystic follicular hyperplasia was present in the males. This was observed in 0/60 of controls, 4/35 (11%) at 750 ppm and in 7/60 (12%, $p<0.05$) at 2500 ppm. Degenerative changes in the kidney were also observed in the highest dosed males. Renal tubular casts were observed in 24/60 of the controls and 45/60 ($p<0.01$) of the 2500 ppm males. Other statistically significant increases in renal lesions in the males at 2500 ppm included hyaline droplet formation, cortical tubular dilatation, cortical cysts and papilla mineralization. In the main study, centrilobular hepatocyte hypertrophy was observed; however, this was not toxicologically significant and was considered an adaptive change to treatment.

   In the 52 week study, statistically significant increases ($p<0.01$) in the incidence and severity of renal lesions were observed in the males at 2500 ppm. These lesions included cortical tubular basophilia, and medulla granular casts. The only lesion in the liver was a increase in hepatic centrilobular hypertrophy. Neither the kidney nor liver lesions were considered toxicologically significant.

   During the recovery period, all lesions present were reversed except for a slight increase in the severity of the cortical tubular basophilia in the male rat's kidneys.

TABLE 6. Treatment-related microscopic lesions and severity in rats						
Organ/Lesion	Severity	Dietary concentration (ppm)				
		0	50	200	750	2500
Males (52 weeks)						
KIDNEY		n = 20	n = 20	n = 15	n = 20	n = 20
Cortical tubular basophilia	total (avg. severity)[a]	7 (1.3)	10 (1.2)	9 (1.3)	20** (1.3)	20** (2.1)
Medulla granular casts	total (avg. severity)	0	0	0	0	7** (1.6)
Males (104 weeks)						
THYROID		n = 60	n = 37	n = 37	n = 35	n = 60
cystic follicular hyperplasia	total	0	1	0	4	7**
KIDNEY		n = 60	n = 60	n = 60	n = 60	n = 60
Tubular casts	total (avg. severity)	24 (1.6)	30 (1.5)	32 (1.4)	32 (1.5)	45** (2.0)
Cortical tubular dilatation	total (avg. severity)	10 (1.8)	9 (2.0)	9 (2.0)	8 (2.0)	27** (2.0)
Cortical cysts	total (avg. severity)	3 (2.0)	2 (2.0)	6 (2.3)	9 (2.1)	11* (2.5)
Papilla mineralization	total (avg. severity)	0	1 (1.0)	2 (2.0)	2 (1.0)	12** (1.4)

Data obtained from MRID 46474139, Text Tables 5, 6, 9, and 10, pp. 41-45 and Table 13H, p. 446-466.
^ Severity is as follows: (1) = minimal, (2) = slight, (3) = moderate and (4) = marked
* statistically different from controls, $p < 0.05$
** statistically different from controls, $p < 0.01$

b. **Neoplastic:** The incidence of neoplastic lesions did not show any statistically significant differences between the control and treated animals in the main study. No neoplastic lesions were found related to treatment with AE C638206. Statistical analysis was performed separately for males and females for benign, malignant and benign/malignant combined tumors. Log-rank analysis showed the total observed and expected number of tumors common to each sex was not increased.

An increase in the incidence of benign skin tumors was observed in male rats (Table 7). Although not statistically significant, there was an increasing trend over the testing regimen.
The incidence was stated to exceed the usual background incidence for the testing laboratory. This is probably not treatment-related based on the typical result of skin injury.

TABLE 7. Treatment-related microscopic lesions and severity in rats					
Tumor	Dietary concentration (ppm)				
	0	50	200	750	2500
Males (104 weeks)					
	n = 24	n = 28	n = 25	n = 26	n = 34
Keratoacanthoma	3	3	5	3	8
Squamous cell papilloma	2	0	1	2	5
Total tumor count [a]	5 (20)	3 (11)	6 (24)	5 (19)	13 (38)

[a] Number in parentheses is percent of group affected , calculated by reviewer

III. DISCUSSION AND CONCLUSIONS:

A. **INVESTIGATORS' CONCLUSIONS:** The investigator stated that the low body weight gain and food conversion efficiency during the first week of treatment in those treated with 2500 ppm was a combination of non-specific toxicity and an initial low palatability of the treated food. The study investigator also stated that the clinical signs observed in the females did not appear to have a specific cause and there was no corresponding treatment-related effect on the urinalysis that would have caused the perigenital staining. The decrease in palpable masses in the females corresponded to the lower body weight gain observed in the treated rats.

The investigator identified the liver and the kidney as the target organs in this study based on the increased liver and kidney weights observed mostly in the 2500 ppm animals. The investigator also thought the centrilobular hepatocytic hypertrophy, while commonly an adaptive response to treatment, might be more significant due to the accompanying degenerative changes and foci of alteration observed in the livers of the highest dosed. The changes in the thyroid were secondary to the effects in the liver according to the investigator. The kidney findings were related to the common findings in aged male rats that are associated with alpha-2-U- globulin. The investigator also stated that some age-related kidney findings were increased in occurrence and severity. Only a slight increase in the severity of the cortical tubular basophilia in the kidneys of the 2500 ppm males was present after the recovery period.

Statistical analysis of the number and type of neoplastic lesions identified did not correlate any type of neoplasia with treatment. The number and type of tumors found was either that commonly found in aged rats or within the range of historical data accumulated by the laboratory.

Therefore, the investigator concluded that the administration of AE C638206 to rats at concentrations to 2500 ppm for up to 104 weeks did not provide any evidence of oncogenic potential. The liver and kidney were identified as the target organs. The investigator considered 200 ppm as the no-observed-adverse-effect level (NOAEL) in males and females since the few findings at this dose were limited to adaptive changes in the liver. The no-observed-effect level (NOEL) was established as 50 ppm. The investigator did not include a lowest-observed-adverse-effect level.

B. **REVIEWER COMMENTS:** Although statistical analysis was not included, the reviewer finds no toxicological significance in the clinical signs observed in the female rats treated with AE C638206. The signs observed were a transient increased incidence of yellow perigenital staining, brown staining of the pinna, and brown staining of the dorsum. Urinalysis, clinical chemistry and histopathological lesions did not support these findings. The reviewer agrees with the investigator that a true cause of the signs was not clear. Weight gain in the 2500 ppm males and females decreased significantly in the first 2 weeks of dosing and had corresponding decreased food consumption and food conversion efficiency. Both food consumption and loss of body weight were also observed in the 200 and 750 ppm females in the main study. Although not statistically significant, the reviewer also noted that the overall body weight was consistently less than controls in the 2500 ppm dosed animals. Although body weight gain data for time points past week 2 were not provided in the study, when the reviewer calculated these, a decrease in body weight gain continued throughout most of the study in the 2500 ppm females.

The reviewer found no direct toxicologically significant effect on the hematology and clinical chemistry parameters measured. While differences were present and statistically significant,

corresponding histopathological lesions were not identified or the differences were minor, sporadic and not consistent. While palpable masses were observed and monitored, there was not a treatment-related increase in the incidence of these masses.

Statistically significant increases in the liver, kidney, and thyroid organ weights were seen primarily in the males dosed with 2500 ppm. The reviewer does not consider the increased liver weight to be toxicologically significant because the histopathological lesions identified were those most commonly associated with an adaptive response of the liver to treatment. The liver effect, centrilobular hepatocyte hypertrophy, observed both at 52 and 104 weeks was considered to be an adaptive change due to treatment. Lesions in the kidney were those associated with the alpha -2μ- globulin accumulation normally present in male aged rats and are not considered adverse. The increase in thyroid weight was statistically significant in the 2500 ppm males and was accompanied by corresponding increases in the incidence of thyroid cell hyperplasia thus making it toxicologically significant. Although thyroid effects may be secondary to liver changes, there is insufficient evidence to support this conclusion (no UDPGT or TH assays).

The lowest-observed-adverse effect level (LOAEL) for AE C638206 is 2500 ppm (109.4 (M), 142.2 (F) mg/kg/day) based on decreases in body weight gain (M/F) and an increase in thyroid organ weight with a corresponding increase in the incidence of thyroid lesions (M only). The no-observed-adverse effect level (NOAEL) for AE C638206 is 750 ppm (31.5 (M), 41.0 (F) mg/kg/day).

The reviewer agrees that dosing of male and female rats with up to 2500 ppm AE C638206 did not provide any evidence of oncogenic potential in any animals at any dose level.

While overall the clinical signs, observations and lesions identified were not seen consistently in both males and females, the reviewer finds enough evidence to state dosing was adequate in this study and that some toxicological effects were observed based on slightly decreased body weight gain at 2500 ppm (M/F).

C. <u>STUDY DEFICIENCIES:</u>

The study report did not include statistical significance on the incidence of clinical signs, food consumption, or food conversion efficiency. Weekly body weight gain was only provided for the first two weeks of treatment. The deficiencies do not affect the study but would add useful data for establishing the NOAEL and LOAEL values.

DATA FOR ENTRY INTO ISIS

Chronic/Carcinogenicity Study - rodents (870.4300)

PC code	MRID	Study	Species	Duration	Route	Admin	Dose range (mg/kg/day)	Doses (ppm)	NOAEL mg/kg/day	LOAEL mg/kg/day	Target organs	Comments
027412	46474139 46474138	chronic/ carcinogenicity	rat	104 days	oral	diet	2.1- 142.2	0, 50, 200, 750 or 2500	NOAEL- 750 ppm [31.5 (m) and 41.0 (f) mg/kg/day]	LOAEL- 2500 ppm [109.4 (m) and 142.2 (f) mg/kg/day]	thyroid	

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DATA EVALUATION RECORD

FLUOPICOLIDE/ PC Code 027412
[OPPTS § 870.4200b]

STUDY TYPE: CARCINOGENICITY – MOUSE
MRID 46474130 and 46474135

Prepared for

Health Effects Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
1801 Bell St.
Arlington, VA 22202

Prepared by

Toxicology and Hazard Assessment Group
Life Sciences Division
Oak Ridge National Laboratory
Oak Ridge, TN 37831
Task Order No. 114-2005

Primary Reviewer:	
Sylvia Milanez, Ph.D., D.A.B.T.	Signature: _____
	Date: _____
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Kowetha Davidson, Ph.D., D.A.B.T.	Signature: _____
	Date: _____
Robert H. Ross, M.S., Group Leader	Signature: _____
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Lee Ann Wilson, M.A.	Signature: _____
	Date: _____

Disclaimer

This review may have been altered subsequent to the contractor's signatures above.

Oak Ridge National Laboratory managed and operated by UT-Battelle, LLC., for the U.S. Department of Energy under Contract No. DE-AC05-00OR22725.

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EPA Reviewer: Myron S. Ottley, Ph.D., Science Information Management Branch, Health Effects Division (7509C)
EPA Work Assignment Manager: Ghazi Dannan, Ph.D.
Registration Action Branch 3, Health Effects Division (7509C)

Signature:
Date
Signature:
Date
Template version 11/01

TXR#: 053597

DATA EVALUATION RECORD

STUDY TYPE: Carcinogenicity - mice, feeding [OPPTS 870.4200b (§83-2b)] OECD 451.

PC CODE: 027412

DP BARCODE: D315502
SUBMISSION NO.: not given

TEST MATERIAL (PURITY): AE C638206 (Fluopicolide) (95.9% a.i.)

SYNONYMS: 2,6-dichloro-N-[3-chloro-5-(trifluoromethyl)-2-pyridylmethyl]benzamide; acylpicolide

CITATION: Chevalier, G. (2003) AE C638206: Carcinogenicity study by oral route (Dietary admixture) in C57BL/6 mice. Centre International de Toxicologie, Evreux Cedex, France. Laboratory project ID 21557 TCS; Bayer Report no. C038732, November 20, 2003. MRID 46474130. Unpublished.

Frith, C.H., B. Highman, G. Burger, and W.D. Sheldon. 1983. Spontaneous lesions in virgin and retired breeder BALB/c and C57BL/6 mice. Laboratory Animal Sciences 33: 273-286. MRID 46474135. Published.

SPONSOR: Bayer AG, Bayer CropScience, Alfred-Nobel-Strasse 50, 40789 Monheim, Germany.

EXECUTIVE SUMMARY: In a carcinogenicity study (MRID 46474130) AE C638206 (Fluopicolide) (95.9% a.i., batch #OP2050046) was administered to 50 C57BL/6 mice/sex/dose in the diet at dietary levels of 0, 50, 400, or 3200 ppm (equivalent to 0, 7.9, 64.5, 551.0 mg/kg bw/day for males, and 0, 11.5, 91.9, and 772.3 mg/kg bw/day for females) for 18 months. Satellite groups of 10 C57BL/6 mice/sex/dose were similarly treated for 12 months. Historical control incidences of hepatocellular lesions were provided (MRID 46474135).

The incidence of mortality and clinical signs was similar in treated and control groups. Body weights and body weight gains of only the 3200 ppm animals were significantly decreased throughout the study. After 78 weeks, the body weights of 3200 ppm males were 20% lower, females were 16% lower than of the control group, and overall body weight gains were 45% lower for males and 35% lower for females. Food consumption was decreased in the 3200 ppm satellite and main group animals up to 18% throughout the study. The overall (week 1-78) food efficiency was decreased 40% for males and 30% for females at 3200 ppm. Hematology evaluations were not conducted, and there were no treatment-related changes in serum enzyme activities. After 52 weeks, absolute and relative liver weights were significantly increased in 400 ppm males (15-30%), and in 3200 ppm males and females (35-99%). After 78 weeks, liver

weights were increased in both sexes at 400 ppm (15-33%) and 3200 (46-81%). At both 400 and 3200 ppm, the liver weight increases were correlated with a significant increase in the incidence of hepatocyte hypertrophy after 52 and 78 weeks, in males and females. The 3200 ppm animals had statistically significant increases in the incidence of enlarged liver and altered liver cell foci (most common type was acidophilic) after 78 weeks, and a non-significant increase after 52 weeks (≤2/10 for each lesion).

There was a treatment related increase in the incidence of hepatocellular adenoma when compared to controls. The 3200 ppm animals had statistically significant increases in hepatocellular adenoma in both sexes after 78 weeks, and a small increase after 52 weeks. The adenoma incidence after 78 weeks of the dosing regimen (0, 50, 400, 3200 ppm) was 5/50, 0/50, 5/50, and 11/50 in males, respectively, and 1/50, 2/50, 0/50, and 16/50 in females, respectively. After 52 weeks, hepatocellular adenoma was found in 3/10 high-dose females but no males. The adenomas were correlated with an increased incidence of liver masses and nodules at necropsy.

The LOAEL for AE C638206 in mice is 3200 ppm for both sexes (551.0 mg/kg/day for males, 772.3 mg/kg/day for females), based on severely decreased body weights and body weight gains and liver lesions in both sexes. The NOAEL is 400 ppm in both sexes (64.5 mg/kg/day for males, 91.9 mg/kg/day for females).

This carcinogenicity study is **Acceptable/Guideline** and satisfies guideline requirements for a carcinogenicity study [OPPTS 870.4200b; OECD 451] in mice.

COMPLIANCE: Signed and dated GLP, Quality Assurance and Data Confidentiality statements were provided.

I. MATERIALS AND METHODS:

A. MATERIALS:

1. **Test material:** AE C638206

Description:	technical, beige powder
Batch #	OP2050046
Purity:	95.9 % a.i.
Compound Stability:	Sample stable through May 3, 2003 when stored at room temperature, protected from light
CAS # of TGAI:	239110-15-7
Structure:	

2. **Vehicle and/or positive control:** The dosing vehicle was diet. No positive control was used.

3. **Test animals:**

Species	Mouse
Strain:	C57BL/6 N Crl:BR, SPF, VAF
Age/weight at study initiation:	Approximately 7 weeks only on the first day of treatment

	Males: 21.5 to 26.2 g, mean 23.3 g; Females: 17.7 to 22.3 g, mean 19.5 g	
Source:	Charles River, Saint-Aubin lès Elbeuf, France	
Housing:	Individually housed in polycarbonate cages containing autoclaved sawdust	
Diet:	M20 EXTRALABO (supplier SDS, Vigny, France) *ad libitum*	
Water:	Filtered tap water *ad libitum*	
Environmental conditions:	**Temperature:**	22 ± 2°C
	Humidity:	50 ± 20%
	Air changes:	12 cycles/hr
	Photoperiod:	12 hrs dark/ 12 hrs light
Acclimation period:	18 days	

B. STUDY DESIGN:

1. **In life dates:** Main study: Start: May 7, 2001; End: November 4-15, 2002 (necropsy);Satellite study: Start: May 7, 2001; End: May 6-7, 2002 (necropsy)

2. **Animal assignment/dose levels:** Animals were assigned to the test groups noted in Table 1 using a computerized stratification procedure so that the average body weight in each group was similar.

TABLE 1. Study design

Test Group	Conc. in Diet (ppm)	Dose to animal (mg/kg/day)		Main Study: 18 months (546-557 days)		Interim sacrifice: 12 months (364-365 days)	
		Male	Female	Male	Female	Male	Female
Control	0	0	0	50	50	10	10
Low (LDT)	50	7.9	11.5	50	50	10	10
Mid (MDT)	400	64.5	91.9	50	50	10	10
High (HDT)	3200	551.0	772.3	50	50	10	10

Data from pp. 21 and 29 of MRID 46474130.

3. **Dose selection:** The dose levels were selected based on the results from a 90-day study (SA 00363) in which C57BL/6 mice received dietary levels of 50-3200 ppm. Reduced body weight gain of approximately 10% occurred in the 3200 ppm dose group, and increased liver weight associated with hepatocellular hypertrophy was seen in almost all the animals dosed at 800 ppm and 3200 ppm. The NOEL in this range-finding study was 50 ppm.

4. **Diet preparation and analysis:** Diet was prepared at least every 10 days by mixing appropriate amounts of test substance with M20 EXRALABO diet (SDS, Vigny, France) to achieve dietary concentrations of 50, 400, and 3200 ppm. The diets were stored at room temperature protected from light prior to use. Homogeneity was tested at room temperature in diets containing 50 ppm and 3200 ppm before treatment and after 1, 13, 39 and 65 weeks of storage. Duplicate samples were taken from the top, middle, and bottom of each dietary preparation for analysis. Stability was evaluated in 50 and 3200 ppm diets when stored in open feeders under animal room conditions (on days 0, 10 and 16), and at room temperature in closed bags (days 0, 5, 10, 13, 16, 23 and 35 at 50 ppm; days 0, 10 and 35 at 3200 ppm).

All diets (50, 400, and 3200 ppm) prepared for use in weeks 1, 2, 3, 4, 13, 26, 39, 52, 65, and 78 were analyzed for concentration.

Results:

Homogeneity analysis: The mean of duplicate assays of samples from the top, middle, and bottom was within 8% of the nominal at both 50 and 3200 ppm. Values ranged from 43.6-57.3 ppm and 3040-3250 ppm, respectively.

Stability analysis: In the open feeders, compared to day zero, levels were 3% greater on day 10 and 22% lower on day 16 at 50 ppm; levels were 1% greater on day 10 and 3% lower on day 16 at 3200 ppm. In closed bags, at room temperature, mean AE C638206 levels were within 8% of day zero for days 5, 10, and 13 at 50 ppm, and days 10 and 35 at 3200 ppm. Stability in closed bags was notably lower (29-40%) at days 16, 23, and 35 in the 50 ppm diets.

Concentration analysis: Dietary concentrations of AE C638206 ranged from -8% to +10% of nominal at all tested weeks throughout the study, for all three test concentrations.

The analytical data indicated that the mixing procedure was adequate and that the variance between nominal and actual dosage to the animals was acceptable.

5. **Statistics**: The main group animals and satellite animals were evaluated separately. Body weight, food consumption, hematology, blood chemistry and organ weight data were analyzed first by the Kolmogorov-Lilliefors test for normality of data distribution. If the data distribution was not normal, it was transformed logarithmically (except organ weight) and re-tested for homogeneity. If still not homogenous, the untransformed data were then compared for treated and control groups. For data that were distributed normally, the homogeneity of variance was determined using the Bartlett test (for ≥3 groups) or the Fischer test (for ≤3 groups). If the data variance was non-homogenous, then treated and control groups were compared with the Dunn test (following Bartlett test), or the Mann-Whitney / Wilcoxon test (following Fisher test). If the variance between the groups was homogenous, treated and control groups were compared with Student's test (for two groups) or the Dunnett test (for ≥3 groups). Survival rates were compared using the Chi-squared test. The number of neoplasms (per group and per organ) were compared by Peto's test. The reviewer considers the analyses used to be appropriate.

C. **METHODS**:

1. **Observations:** Animals were inspected at least once daily for signs of toxicity and mortality. Clinical examinations were conducted at least once weekly. All mice were palpated for masses every 4 weeks until week 52, after which they were palpated every 2 weeks.

2. **Body weight**: Animals were weighed before allocation to dose groups, on the first day of treatment, once weekly during the first 13 weeks of treatment, once every 4 weeks from week 13 to week 31, and once every two weeks from week 32 to the end of the study.

3. **Food consumption, food efficiency, and compound intake:** Food consumption for each animal was determined for every 7-day period during the first 13 weeks, then every 4 weeks until week 30, and once every two weeks from week 32 to the end of the study. The mean daily diet consumption was calculated as g food/animal/day. Compound intake (mg/kgbw/day) values were calculated as time-weighted averages from the consumption and body weight gain data. Food efficiency was not reported in MRID 46474130, although the reviewer did calculate the values for the overall study as [(total 78-week body weight gain in g/total 78-week food consumption in g) X 100]. The overall food consumption was calculated as the mean daily food consumption/animal (from page 33 of MRID 46474130) multiplied by 546 days.

4. **Ophthalmoscopic examination:** Examination of eyes was not conducted and is not required.

5. **Hematology and clinical chemistry:** For each surviving animal in the principal group, and when possible in animals killed prematurely, blood was collected from a tail vein during week 52 and at the terminal sacrifice (week 78). Blood smears were prepared but the differential blood counts were not determined. The main study animals were not fasted and not anesthetized at the time of blood collection. For animals in the satellite group, blood was collected from the orbital sinus of all surviving fasted animals under light isoflurane anesthesia to assess serum enzyme activities. The CHECKED (X) parameters were examined in blood from satellite animals. Bone (femoral) marrow smears were prepared from all mice killed at week 53 and at terminal sacrifice. The bone marrow differential cell count was not evaluated, however.

   a. **Hematology:** not conducted

   b. **Clinical chemistry*:** Although these data were not required based upon OPPTS Guideline 870.4200, limited clinical chemistry evaluations were conducted.

	ELECTROLYTES		**OTHER**
	Calcium		Albumin
	Chloride		Creatinine
	Magnesium		Urea nitrogen
	Phosphorus		Total Cholesterol
	Potassium		Globulins
	Sodium		Glucose
	ENZYMES		Total bilirubin
x	Alkaline phosphatase (ALK)		Total protein (TP)
	Cholinesterase (ChE)		Triglycerides
	Creatine phosphokinase		Serum protein electrophoresis
	Lactic acid dehydrogenase (LDH)		
x	Alanine aminotransferase (ALT/also SGPT)		
x	Aspartate aminotransferase (AST/also SGOT)		
	Gamma glutamyl transferase (GGT)		
	Glutamate dehydrogenase		

* Not required for carcinogenicity studies based on Guideline 870.4200.

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6. **Urinalysis**: Not conducted or required per the carcinogenicity guidelines (OPPTS 870.4200).
7. **Sacrifice and pathology**: All animals that died and those sacrificed on schedule were subjected to gross pathological examination. The CHECKED (X) tissues were collected and examined histologically in all animals in the main group. For all satellite group animals, the macroscopic lesions and liver were examined microscopically. The (XX) organs, in addition, were weighed in all animals.

	DIGESTIVE SYSTEM		CARDIOVASC./HEMAT.		NEUROLOGIC
X	Tongue	X	Aorta, thoracic*	XX	Brain (multiple sections)*+
X	Salivary glands*	XX	Heart*+	X	Periph. nerve*
X	Esophagus*	X	Bone marrow*	X	Spinal cord (3 levels)*
X	Stomach*	X	Lymph nodes*	X	Pituitary*
X	Duodenum*	XX	Spleen*+	X	Eyes (retina, optic nerve)*
X	Jejunum*	X	Thymus		GLANDULAR
X	Ileum*			XX	Adrenal gland*+
X	Cecum*		UROGENITAL		Lacrimal gland
X	Colon*	XX	Kidneys*+	X	Parathyroids*
X	Rectum*	X	Urinary bladder*	X	Thyroids*
XX	Liver*+	XX	Testes*+		OTHER
X	Gall bladder* (not rat)	XX	Epididymides*+	X	Bone (sternum and/or femur)
	Bile duct* (rat)	X	Prostate*	X	Skeletal muscle
X	Pancreas*	X	Seminal vesicle*	X	Skin*
	RESPIRATORY	XX	Ovaries*+	X	All gross lesions and masses*
X	Trachea*	XX	Uterus*+		
X	Lung*+	X	Mammary gland*		
X	Nose*	X	Vagina		
X	Pharynx*				
X	Larynx*				

* Required for carcinogenicity studies based on Guideline 870.4200.
+Organ weight required in carcinogenicity studies.

II. RESULTS:

A. OBSERVATIONS:

1. **Clinical signs of toxicity**: The nature, incidence and onset of clinical signs were similar in animals of both sexes from the control and treated groups. The weekly detailed examinations revealed no signs of neurotoxic effects, and the incidence of palpable masses was similar in treated and control animals.

2. **Mortality**: The incidence of mortality was similar in treated and control groups of both sexes. The survival rates at 0, 50, 400, and 3200 ppm were 82%, 88%, 90%, and 88%, respectively, for males and 90%, 82%, 92%, and 82%, respectively, for females.

B. BODY WEIGHT: Body weights and weight gains of the 50 and 400 ppm groups were comparable to the controls, for both sexes. At 3200 ppm, the body weights of both males and females were significantly lower than of controls throughout the study, starting at week 2. The final body weight of males was 20% lower and of females was 16% lower, than the

corresponding controls. The high-dose groups also had significantly lower body weight gains throughout most of the study, and the overall body weight gain was decreased 45% in males and 35% in females, relative to controls. The results are summarized in Table 2.

TABLE 2:Mean body weights (BW) and body weight gains (BWG) of main group mice treated up to 78 weeks with AE C638206

Parameter	0	50 ppm	400 ppm	3200 ppm
Males [Mean (g) ± Standard Deviation]				
Initial BW (week 1, day 1)	23.1 ± 0.76	23.0 ± 0.81 (100)[1]	23.2 ± 0.79 (100)	23.3 ± 0.74 (101)
Week 13	29.8 ± 1.73	29.9 ± 1.66 (100)	29.0* ± 1.48 (97)	26.9* ± 0.99 (90)
Week 26	34.9 ± 2.54	34.8 ± 2.99 (100)	33.8 ± 2.32 (97)	29.1** ± 1.05 (83)
Week 52	40.8 ± 4.34	41.3 ± 4.50 (101)	39.0 ± 4.17 (96)	31.9** ± 2.09 (78)
Final BW (week 78)	41.4 ± 5.45	43.5 ± 5.71 (105)	42.0 ± 5.02 (101)	33.3** ± 1.94 (80)
BWG Wk 2-13	5.9	6.0 (102)	5.0** (85)	3.9** (66)
BWG Wk 13-26	5.1	4.9 (96)	4.8 (94)	2.2** (43)
BWG Wk 26-52	5.8	6.4 (110)	5.3 (91)	2.8** (48)
BWG Wk 52-78	0.6	1.9 (317)	3.0** (500)	1.1 (183)
Overall BWG Wk 1-78	18.3	20.5 (112)	18.8 (103)	10.0 ** (55)
Females [Mean (g) ± Standard Deviation]				
Initial BW (week 1, day 1)	19.4 ± 0.69	19.3 ± 0.76 (99)	19.4 ± 0.70 (100)	19.3 ± 0.62 (99)
Week 13	24.5 ± 1.13	24.5 ± 1.29 (100)	24.8 ± 1.16 (101)	22.8** ± 0.92 (93)
Week 26	28.1 ± 2.53	28.8 ± 3.31 (102)	28.7 ± 2.77 (102)	24.5** ± 1.13 (87)
Week 52	33.3 ± 4.16	34.5 ± 5.44 (104)	34.1 ± 4.44 (102)	26.7** ± 1.88 (80)
Final BW (week 78)	34.7 ± 4.92	36.1 ± 6.35 (104)	36.3 ± 5.37 (105)	29.2** ± 2.71 (84)
BWG Wk 2-13	4.2	4.1 (98)	4.5 (107)	3.6* (86)
BWG Wk 13-26	3.6	4.3 (119)	3.9 (108)	1.7** (47)
BWG Wk 26-52	5.2	5.7 (110)	5.4 (104)	2.2** (42)
BWG Wk 52-75	1.4	1.6 (114)	2.2 (157)	2.5 (179)
Overall BWG Wk -1-75	15.3	16.8 (110)	16.9 (110)	9.9** (65)

Data obtained from pp. 34, 81-85, 89-93, and 113-114 of MRID 46474130.
* Statistically different ($p < 0.05$) from the control.
** Statistically different ($p < 0.01$) from the control.
[1]Number in parentheses is the percent of the control group, calculated by the reviewer.

C. FOOD CONSUMPTION AND COMPOUND INTAKE:

1. **Food consumption:** Throughout the study, food consumption was similar in the control, 50 ppm, and 400 ppm groups, but was significantly lower at 3200 ppm (up to 16% in main

study; 18% in satellite study, $p<0.01$). Food consumption for selected time intervals is shown in Table 3.

TABLE 3. Mean food consumption (g/animal/day) and food efficiency for main group mice treated up to 78 weeks with AE C638206

Parameter	0 ppm	50 ppm	400 ppm	3200 ppm
Males				
Food consumption				
Weeks 1-12	5.8	5.7 (98)[2]	5.7 (98)	5.6** (97)
Weeks 13-16	5.4	5.6 (104)	5.5 (102)	4.9** (91)
Weeks 29-52	5.4	5.5 (102)	5.4 (100)	4.8** (89)
Weeks 53-77	5.0	5.1 (102)	5.0 (100)	4.6** (92)
Weeks 1-78 (overall)	5.4	5.5 (102)	5.4 (100)	5.0** (93)
Food efficiency, weeks 1-78 [1]	0.62	0.69 (110)	0.64 (103)	0.37 (60)
Females				
Food consumption				
Weeks 1-12	7.8	7.7 (99)	7.9 (101)	6.7** (86)
Weeks 13-16	6.7	6.9 (103)	6.9 (103)	6.1** (91)
Weeks 29-52	6.0	6.1 (102)	6.0 (100)	5.9** (98)
Weeks 53-77	5.6	5.7 (102)	5.5 (98)	5.1** (91)
Weeks 1-78 (overall)	6.4	6.5 (102)	6.5 (102)	5.9** (92)
Food efficiency, weeks 1-78 [1]	0.44	0.47 (107)	0.48 (109)	0.31 (70)

Data obtained from pp. 33 of MRID 46474130.
** Statistically different ($p<0.01$) from the control mean.
[1]Calculated by reviewer as [(total 78-week body weight gain in g/total 78-week food consumption in g) X 100].
[2]Number in parentheses is the percent of the control mean, calculated by the reviewer for food efficiency.

2. **Compound consumption:** The time-weighted-average doses for each group are presented in Table 1.

3. **Food efficiency:** As shown in Table 3, overall food efficiency (for weeks 1-78) of low- and mid-dose males and females was comparable to or greater than of the controls. For the high-dose groups, food efficiency was decreased relative to controls for both the males (40% lower) and females (30% lower).

D. OPHTHALMOSCOPIC EXAMINATION: Neither required nor performed.

E. BLOOD ANALYSES:

1. **Hematology:** Hematology evaluations were not conducted.

2. **Clinical chemistry:** There were no treatment-related changes in the activities of the examined enzymes. Alkaline phosphatase activity was statistically increased in the 3200 ppm females (564 ± 895 vs. 180 ± 22 for controls, $p<0.01$) due to two outliers.

F. URINALYSIS: Urinalysis was neither required nor performed.

G. SACRIFICE AND PATHOLOGY:

1. **Organ weight:** The liver was the only organ with statistically significant and dose-related differences in weight from the controls. Liver weights are shown in Table 4. Males had significantly increased absolute and relative liver weights after both 52 and 78 weeks at 400 ppm (15-30%, p<0.01) and 3200 ppm (35-79%, p<0.01). The 400 ppm females had significantly increased absolute liver weights only after 78 weeks (33%, p<0.01), whereas the 3200 ppm females had increased absolute and relative liver weights after both 52 weeks (50-99%, p<0.01) and 78 weeks (56-81%, p<0.01).

TABLE 4:Absolute and relative-to-body liver weight of mice treated 52 weeks (satellite study) or 78 weeks (main study) with AE C638206.

Exposure time	0	50 ppm	400 ppm	3200 ppm
Males [Mean (g) ± Standard Deviation]				
Terminal body weight				
52 weeks	37.66 ± 5.21	37.90 ± 5.29 (101)	42.56 ± 3.93 (113)	31.25 ± 1.79** (83)
78 weeks	38.58 ± 5.28	40.62 ± 5.92 (105)	39.09 ± 4.71 (101)	31.15 ± 2.18** (81)
52 weeks, absolute weight	1.59 ± 0.326	1.71 ± 0.274 (108)[1]	2.06** ± 0.261 (130)	2.15** ± 0.243 (135)
52 weeks, relative weight	4.21 ± 0.512	4.53 ± 0.425 (108)	4.84** ± 0.340 (115)	6.85** ± 0.489 (163)
78 weeks, absolute weight	1.62 ± 0.258	1.85 ± 0.491 (114)	1.91** ± 0.310 (118)	2.37** ± 0.344 (146)
78 weeks, relative weight	4.26 ± 0.743	4.65 ± 1.75 (109)	4.90** ± 0.746 (115)	7.62** ± 1.17 (179)
Females [Mean (g) ± Standard Deviation]				
Terminal body weight				
52 weeks	36.23 ± 4.75	34.14 ± 6.03 (94)	34.03 ± 5.06 (94)	26.58 ± 2.45** (73)
78 weeks	32.61 ± 4.71	33.77 ± 6.15 (104)	33.73 ± 4.62 (103)	27.17 ± 3.10** (83)
52 weeks, absolute weight	1.51 ± 0.128	1.44 ± 0.290 (95)	1.57 ± 0.241 (104)	2.26** ± 0.637 (150)
52 weeks, relative weight	4.21 ± 0.398	4.20 ± 0.447 (100)	4.61 ± 0.331 (110)	8.39** ± 1.53 (199)
78 weeks, absolute weight	1.66 ± 0.396	1.64 ± 0.312 (99)	2.20** ± 1.65 (133)	2.59** ± 1.36 (156)
78 weeks, relative weight	5.18 ± 1.43	4.94 ± 1.01 (95)	6.62 ± 4.87 (128)	9.37** ± 3.57 (181)

Data obtained from pages 36 and 118-125 of MRID 46474130.
** Statistically different (p <0.01) from the control mean.
[1]Number in parentheses is the percent of the control group, calculated by the reviewer.

2. **Gross pathology:** In the satellite (52-week) study animals, statistical differences from the control group were not found for any lesion in males or females. However, several findings at 3200 ppm may have been treatment-related. These consist of a slightly increased incidence of enlarged liver (2/10 males) and of liver masses + nodules (2/10 females) after 78 weeks, which were correlated with hepatocellular hypertrophy (males) or adenoma (females).

   The main study significant findings are summarized in Table 5. The 3200 ppm males and females had an increased incidence of liver enlargement and of masses and nodules (all types combined), which were statistically significant (p<0.01) except for masses and nodules in males. The masses and nodules were primarily correlated with hepatocellular adenoma, and enlarged liver with hepatocellular hypertrophy. [The reviewer notes that on p. 37 of MRID

46474130 it states that the incidence of masses + nodules in control males was 3/50, but this contradicts the 5/50 reported on p. 137, which is confirmed by the individual animal data on pages 211, 219, 224, and 225. Additionally, the incidence of masses + nodules in the 400 ppm and 3200 ppm was given as 6/50 and 10/50, respectively, on p. 37 of MRID 46474130. Per the individual animal data, the number of affected animals (i.e., incidence) was 5/50 at 400 ppm (see p. 295) and 9/50 at 3200 ppm (see p. 318), since two lesions were found in one animal at each dose.]

TABLE 5:Incidence of gross liver lesions in mice treated for up to 52 weeks (satellite study) or 78 weeks (main study) with AE C638206.

Gross lesion	0	50 ppm	400 ppm	3200 ppm	0	50 ppm	400 ppm	3200 ppm
	Males				Females			
Liver enlargement:								
52 weeks	0/10	0/10	1/10	2/10	0/10	1/10	0/10	0/10
78 weeks	2/50	6/50[1]	6/50	28/50**	8/50	3/50	5/50	29/50**
Masses + nodules:								
52 weeks	0/10	0/10	0/10	0/10	0/10	0/10	0/10	2/10
78 weeks[2]	5/50	2/50	5/50	9/50	1/50	2/50	3/50	9/50**

Data obtained from pages 37, 126, 127, 137, 142, and 196-351 of MRID 46474130.
** Statistically different ($p < 0.01$) from the control, determined by the reviewer using the Fisher exact test.
[1]Reported erroneously as 7/50 on page 37; 6/50 is reported on page 137 and is consistent with the individual animal data.
[2]The incidence was reported as 3/50, 2/50, 6/50, and 10/50, respectively on p. 37 of MRID 46474130. The reviewer considers these values to be erroneous, as explained in the text above (section II. G. 2.).

3. Microscopic pathology:

a) Non-neoplastic: The liver was the main target organ in both sexes. The incidence of hepatocellular hypertrophy was increased significantly at 400 and 3200 ppm, following either 52 or 78 weeks of treatment (Table 6). The hepatocellular hypertrophy was correlated in both sexes with increased liver weight (at 400 and 3200 ppm), and liver enlargement (at 3200 ppm). The high-dose animals also had a significantly increased incidence of altered liver cell foci. The incidence of several other lesions was increased statistically in the main study high-dose animals, including degeneration of seminiferous tubules in testes of males (18/50 vs. 9/50 controls, $p<0.05$), fibro-osseus proliferation in the femur of females (11/49 vs. 2/50 controls, $p<0.01$), and lymphoid cell infiltration of the ovaries of females (10/46 vs. 3/49 controls, $p<0.05$). The toxicological significance and relationship to treatment of these findings is unclear.

TABLE 6:Incidence of microscopic non-neoplastic liver lesions in mice treated 52 weeks (satellite study) or 78 weeks (main study) with AE C638206.

Microscopic lesion	0 ppm	50 ppm	400 ppm	3200 ppm	0 ppm	50 ppm	400 ppm	3200 ppm
	Males				Females			
Hepatocellular hypertrophy								
52 weeks	0/10	0/10	5/10*	10/10**	0/10	0/10	6/10**	9/10**
78 weeks	0/50	0/50	20/50**	49/50**	0/50	0/50	41/50**	46/50**

Altered cell foci, all types								
52 weeks	0/10	0/10	0/10	0/10	0/10	0/10	0/10	2/10
78 weeks	1/50	8/50*	5/50	18/50**	0/50 [1]	3/50	4/50	25/50**

Data obtained from pages 39, 148, 155, 160, and 161 of MRID 46474130.
*p <0.05, **p <0.01: Statistically different from the control group, determined by the reviewer using the Fisher exact test.
[1]The incidence was reported as 1/50 on p. 39 of MRID 46474130, which is inconsistent with the data on p. 148 and the individual animal data for the control group on pages 1293-1412 of MRID 46474130.

b) Neoplastic: At the interim sacrifice, 1/10 of the 400 ppm females, and 3/10 of the 3200 ppm females had hepatocellular adenoma (Table 7). Historical control C57BL/6 mouse incidences of hepatocellular neoplasms reported by Frith et al. (1983) are 1/232 (0.4%) for males, and 0/233 for females. The increased incidence of adenoma at 3200 ppm is likely treatment-related, but the finding in 1/10 females at 400 ppm did not appear to be treatment-related since the incidence was 0/50 after 78 weeks at 400 ppm. None of the animals had hepatocellular carcinoma.

At terminal sacrifice, the incidence of hepatocellular adenoma was statistically increased at 3200 ppm in both sexes. The adenoma was correlated with an increased incidence of liver masses and nodules in both sexes. A few animals had hepatocellular carcinoma, but their incidence did not differ from the control groups, as shown in Table 7. The combined incidence of hepatocellular adenoma and carcinoma was statistically significant ($p<0.05$ or 0.01) only for the 3200 ppm females. There were no significant differences between the control and treated groups for the number of animals with neoplasms, with >1 primary neoplasm, or with metastases. The number of animals with benign tumors was increased slightly, but not statistically significantly, in the 3200 ppm males (13/50 vs. 8/50 controls) and females (18/50 vs. 11/50 controls), due to the increased incidence of hepatocellular adenoma. The incidence of hepatocellular neoplasms found in the control groups in this study after 78 weeks (16% for males and 2% for females) is in line with the historical control incidence reported by Frith et al. (1983) for female C57BL/6 mice (5/216 or 2.3%) but was much greater than the historical male incidence of 10/215 (4.7%).

TABLE 7:Incidence of neoplastic liver lesions in mice treated 52 weeks (satellite study) or 78 weeks (main study) with AE C638206.

	0 ppm	50 ppm	400 ppm	3200 ppm	0 ppm	50 ppm	400 ppm	3200 ppm
Hepatocellular lesion	**Males**				**Females**			
Adenoma:								
52 weeks	0/10	0/10	0/10	0/10	0/10	0/10	1/10	3/10†
78 weeks	5/50	0/50	5/50	11/50†	1/49	2/50	0/50	16/50**‡
Carcinoma:								
52 weeks	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10
78 weeks	3/50	1/50	0/50	2/50	0/49	0/50	2/50	0/50
Adenoma - carcinoma								
52 weeks	0/10	0/10	0/10	0/10	0/10	0/10	1/10	3/10
78 weeks	8/50	1/50	5/50	13/50	1/49	2/50	2/50	16/50**‡

Data obtained from pages 40, 162, 164, and 167 of MRID 46474130.
*p <0.05, **p <0.01: Statistically different from the control group, determined by the reviewer using the Fisher exact test.
†p <0.05, ‡p <0.01: Statistically different from the control group, determined by the study author.

III. DISCUSSION AND CONCLUSIONS:

A. **INVESTIGATOR'S CONCLUSIONS:** The investigator concluded that the test compound caused increased liver weight. This was complemented by increased incidences of enlarged liver, liver masses and nodules, and of hepatocellular hypertrophy at both 400 and 3200 ppm. Treatment-induced changes seen only at 3200 ppm included severely reduced body weight gain and food consumption, increased incidences of altered liver cell foci, and hepatocellular adenoma in both sexes. The time-to-tumor was decreased in the high-dose females. The severe reduction in body weight gain at 3200 ppm indicated, per the study author, that "the Maximal Tolerated Dose" was reached, which may at least in part be responsible for the occurrence of hepatocellular adenoma at 3200 ppm. The investigator identified the study NOAEL as 50 ppm for toxicity, and 400 ppm for carcinogenicity.

B. **REVIEWER COMMENTS:** The reviewer concurs with the reviewer's findings that there were no treatment-related effects on clinical observations or mortality, but that treatment caused decreased food consumption, body weight, body weight gain, and liver lesions at 3200 ppm. The reviewer disagrees with the investigator's conclusion that the liver changes at 400 ppm (increased liver weight, hepatocellular hypertrophy) were a toxic effect of treatment, but views these changes as a non-specific adaptive response to a xenobiotic agent and is not considered adverse. Also, the reviewer does not consider the incidence of enlarged liver and of liver masses and nodules at 400 ppm to be significantly different from the control groups. Treatment with 3200 ppm elicited effects that are consistent with an adaptive response to a xenobiotic agent (increased liver weights, increased incidences of enlarged liver, hepatocellular hypertrophy), but also caused other changes indicative of compound toxicity: severely reduced body weights and body weight gains, decreased food efficiency, an increased incidence of liver masses and nodules and altered liver cell foci.

A treatment-related toxic effect caused a significant increase in hepatocellular adenoma in both sexes at 3200 ppm after 78 weeks, and possibly after 52 weeks in females (3/10 vs. 0/10 controls). The reviewer did not conclude that the time-to-tumor was decreased significantly for the 3200 ppm females (one tumor at week 46, one at 78, and all 14 others at week 79). It is unknown why the control incidence of hepatocellular neoplasms in males after 78 weeks (16%) was so much greater than reported by Frith et al. (4.7%; 1983). The marked decrease in weekly body weights (16-20% at week 78) and body weight gains (35-45% at week 78) seen at 3200 ppm throughout the study in both sexes indicates that 3200 ppm was an excessive test concentration.

The LOAEL for AE C638206 in mice is 3200 ppm for both sexes (551.0 mg/kg/day for males, 772.3 mg/kg/day for females), based on severely decreased body weights and body weight gains and liver lesions in both sexes. The NOAEL is 400 ppm in both sexes (64.5 mg/kg/day for males, 91.9 mg/kg/day for females).

C. **STUDY DEFICIENCIES:** The marked decrease in body weights and body weight gains indicated that 3200 ppm was an excessive test concentration. Although this could have impacted the study results, the lack of life shortening suggests that the study and the conclusions drawn from it were valid.

Minor deficiencies include the lack of statistical analysis of the incidences of gross pathology and of non-neoplastic microscopic pathology, several typographic errors, and failure to determine differential blood counts from the blood smears, which is required for rodent carcinogenicity studies [OPPTS 870.4200b (§83-2b)]. The study author states that this was not done because the study sponsor "considered that no specific signs of toxicity evidenced the need to determine the differential white cell count." For similar reasoning, the bone marrow differential cell count was not determined in prepared bone marrow smears. The reviewer concurs that there was no indication that the hematopoietic system was a target for compound toxicity, and this omission probably did not impact the study results. Typographic errors were present in several places in MRID 46474130, which did not impact the interpretation of the study results.

DATA FOR ENTRY INTO ISIS

Carcinogenicity Study - mice (870.4200b)

PC code	MRID	Study	Species	Duration	Route	Admin	Dose range mg/kg/day	Doses mg/kg/day	NOAEL mg/kg/day	LOAEL mg/kg/day	Target organ	Comments
027412	46474130	Carcinogenicity	mice	18 months	oral	diet	7.9-772.3	M: 7.9, 64.5, 551.0 F: 11.5, 91.9, 772.3	M: 64.5 F: 91.9	M: 551.0 F: 772.3	Body weight gain decrease, liver lesions and adenoma	Toxicity, Neoplasia

DATA EVALUATION RECORD

FLUOPICOLIDE/PC 027412
(AE C638206)

STUDY TYPE: SUBCHRONIC (90 DAY) ORAL TOXICITY STUDY [(RODENTS OPPTS (8703100)]
MRID 46474112

Prepared for

Health Effects Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
1801 Bell St.
Arlington, VA 22202

Prepared by

Toxicology and Hazard Assessment Group
Life Sciences Division
Oak Ridge National Laboratory
Oak Ridge, TN 37831
Task Order No. 05-114

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Date: _____

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Date: _____

Robert H. Ross, M.S., Group Leader Signature: _____
Date: _____

Quality Assurance:
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Date: _____

Disclaimer

This review may have been altered subsequent to the contractor=s signatures above.

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EPA Reviewer: Myron S. Ottley, PhD **Signature:**
Registration Action Branch 3, Health Effects Division (7509C) **Date**
EPA Work Assignment Manager: Ghazi Dannan, PhD **Signature:**
Registration Action Branch 3, Health Effects Division (7509C) **Date**

Template version 11/01

TXR#: 053597

DATA EVALUATION RECORD

STUDY TYPE: Subchronic Oral Toxicity: Feeding; OPPTS 870.3100 ['82-1a] (Rat); OECD 408.

PC CODE: 027412 **DP BARCODE: D315502**
SUBMISSION NO.:

TEST MATERIAL (PURITY): AEC638206 (96.9% and 97.5%)

SYNONYMS: Fluopicolide

CITATION: Mallyon, B.A. (2000) AE C638206: Rat 90-day dietary toxicity study with 4 week off-dose period. Aventis CropScience UK Ltd, Chesterford Park, Saffron Walden Essex, England. Report No. TOX/00/283-4, December 1, 2000. MRID 46474112. Unpublished

SPONSOR: Bayer CropScience AG, Monheim, Germany

EXECUTIVE SUMMARY: In a 90-day oral toxicity study (MRID 46474112) Fluopicolide (Lot # AE C638206 00 1C99 0005; 97.2% a.i.) was administered to groups of 10 male and 10 female Sprague Dawley rats in a diet containing 0, 100, 1400 or 20,000 ppm (equivalent to 0, 7.4, 109 or 1668 mg/kg/day for males, and 8.4, 119 or 1673 mg/kg/day for females) for 13 weeks. Ten additional rats/sex from the control and high dose group were maintained on control diet for a further four weeks to determine the reversibility of any effects seen.

Two nontreatment-related mortalities were noted in the high dose group. Body weight gain over the course of the 20,000 ppm treatment was reduced by 41% in males and 29% in females, while the corresponding mean food consumption was reduced by 22% and 19% ($p<0.01$) Where do these percentages come from? Not cited in any of the tables. Body weight gain was dramatically affected the first week of the study as evidenced by essentially no weight gain at the highest dose as compared to controls that gained an average of 58 g for males and 39 g for females. Reduced food consumption was also most dramatic during this week at about 50% for both sexes. Water consumption was 43% higher for females relative to the controls ($p<0.01$) during this same time frame and was somewhat higher for the remainder of the study. An increase in urinary volume and a slight decrease in specific gravity was observed in females only which corresponds to the

increased water intake. No toxicologically relevant hematological or clinical chemistry findings were noted. Microscopic examination showed a minimal to slight hypertrophy of the zona glomerulosa in the adrenal of 17/20 of the rats at the highest dose level compared to one of each sex in the controls, and minimal changes were seen in 3/10 females at the 1400 ppm level. Minimum to slight trabecular hyperostosis of the bone joint was observed in 7/10 males and all females at the 20,000 ppm level compared to 0/10 males and 3/10 females in the control group. Decreased cellularity of the bone marrow was observed for 7/10 males and 9/10 females at 20,000 ppm, and in 8/10 females at 1400 ppm compared to 0/10 males and 1/10 females in the control group. No treatment-related effects were observed at the 100 ppm dose level.

Following the four week off-dose period there was a complete or partial recovery of all treatment-related effects.

The LOAEL is 20,000 ppm in the diet (1668 mg/kg/day) for males based on hypertrophy of the zona glomerulosa in the adrenal, trabecular hyperostosis of the bone joint, and decreased cellularity of the bone marrow. The LOAEL for females is 1400 ppm in the diet (119 mg/kg/day) based on hypertrophy of the zona glomerulosa in the adrenal and decreased cellularity of the bone marrow. The NOAEL is 1400 ppm (109 mg/kg/day) for males and 100 ppm (7.9 mg/kg/day) for females.

This 90-day oral toxicity study in the rat is **Acceptable (Guideline)** and satisfies the guideline requirement for a 90-day oral toxicity study (OPPTS 870.3100; OECD 408) in the rat.

COMPLIANCE: Signed and dated GLP, Quality Assurance, and Data Confidentiality statements were provided.

I. MATERIALS AND METHODS:

A. MATERIALS:

1. **Test material:** AE C638206

Description:	Technical grade AE C638206; a fine light brown solid
Lot/Batch #:	AE C638206 00 1C99 0005
Purity	97.2% a.i.
Compound Stability:	Two test substance stability analyses were conducted. The first analysis was conducted on June 10, 1999 and supported use of the test substance through September 10, 1999. The second analysis was conducted on August 16, 1999 and supported use through November 16, 1999.
CAS No. of TGAI:	239110-15-7
Structure:	Not available

2. **Vehicle and/or positive control:** Powdered laboratory rodent diet; no positive control.

3. Test animals:

Species:	Rat	
Strain:	Sprague-Dawley (Crl: CD IGS BR)	
Age/weight at study initiation:	Approximately 28-35 days old Males: 136 grams. Females: 124 grams	
Source:	Charles River UK Ltd., Margate, Kent UK	
Housing:	5/sex/dose/cage	
Diet:	Powdered laboratory rodent diet (Modified SQC Expanded Ground Rat and Mouse Maintenance Diet No. 1) *ad libitum*	
Water:	Tap water *ad libitum*	
Environmental conditions:	**Temperature:**	19-23EC
	Humidity:	45-65%
	Air changes:	Not reported
	Photoperiod:	12 hrs dark/ 12 hrs light
Acclimation period:	7 days	

B. STUDY DESIGN:

1. In life dates:

Start: July 13, 1999; End: October 12, 1999

2. Animal assignment: Animals were assigned to the test groups noted in Table 1 based upon body weight so that each group had similar initial mean body weight and weight distributions. Ten rats/group/sex were fed the test material for 13 weeks, while an additional ten rats/group/sex of the controls and the highest dosed were observed for 4 weeks after completion of the exposure period to determine the potential for the reversal of any adverse effects.

TABLE 1: Study design

Test group	Conc. in diet (ppm)	Dose (mg/kg bw/day)		# Male[a]	# Female[a]
		Males	Females		
Control	0	0	0	20	20
Low	100	7.4	8.4	10	10
Mid	1400	109	119	10	10
High	20,000	1668	1673	20	20

Data from p. 24, MRID46474112

[a]Ten rats/group/sex of the controls and the highest dosed were observed for 4 weeks after completion of the exposure period to determine the potential for the reversal of any adverse effects

3. Dose selection rationale: The dose levels were selected based on the results from a 28-day study (MRID#) in which dietary-administration of up to 20,000 ppm resulted in no mortalities and no clinical signs of toxicity.

4. **Diet preparation and analysis:** Diet was prepared weekly by mixing appropriate amounts of test substance with the rodent feed and was stored at room temperature. Homogeneity and stability were tested before dosing was initiated. During the study, samples of treated food (100, 1400 and 20,000 ppm) were analyzed during weeks 1, 5, 10, and 14 for concentration.

Results:

Homogeneity analysis: Overall, the analyses ranged from 97.0 to 103.0% of nominal (means of 4 replicates). Samples taken from the top, middle, and bottom of 100 - 20,000-ppm diet preparations were within less than 5% of each other.

Stability analysis: Stability of the diet was satisfactory over the 8-day time frames for each preparation used. The analyses ranged from 94.3 to 103.2% of nominal (means of 4 replicates) and the percent decline from nominal had a maximum of 7% over 15 days at room temperature.

Concentration analysis: The variance of actual to nominal ranged from a low of 95.7% to a high of 105.9% (means of 4 replicates from a total of 48 samples analyzed).

The analytical data indicated that the mixing procedure and stability of the resulting preparations were adequate, and that the variance between nominal and actual dosage to the animals was acceptable.

5. **Statistics**: Body weight, food consumption, water consumption, organ weight, motor activity and assessments from the functional observational battery were analyzed from homogeneity of group variances using Bartlett=s test. If not significant at the 5% level, the data were then analyzed by one way analysis of variance to establish the significance of variability among the groups. If significant, pair-wise comparisons of each treated group, in turn with the controls. were made using Dunnett=s test of significance. Where group variances were heterogeneous using Bartlett=s test, pair-wise comparisons were made using a modified t-test. For pair-wise comparisons, significance was tested at the 5% and 1% levels ($p < 0.05$ and $p < 0.01$).

C. METHODS:

1. Observations:

1a. Cageside observations: Animals were inspected at least once each day for signs of toxicity and mortality.

1b. Clinical examinations: Detailed clinical observations were conducted weekly.

1c. Neurological evaluations: The following evaluations and measurements were performed:

1. Functional Observational Battery (FOB) (Week 11)

X	**HOME CAGE OBSERVATIONS**	X	**HANDLING OBSERVATIONS**	X	**OPEN FIELD OBSERVATIONS**
	Posture*	x	Reactivity*/ease of removal	x	Mobility
	Biting	x	Lacrimation* / chromodacryorrhea	x	Rearing+
x	Convulsions*	x	Salivation*	x	Arousal/ general activity level*
x	Tremors*		Piloerection*	x	Convulsions*
	Abnormal Movements*		Fur appearance	x	Tremors*
x	Palpebral closure*	x	Palpebral closure* (open field)		Abnormal movements*
	Feces consistency		Respiratory rate+ (open field)	x	Urination / defecation*
	Spontaneous vocalization		Red/crusty deposits*		Grooming
	SENSORY OBSERVATIONS		Mucous membranes /eye /skin color	x	Gait abnormalities / posture*
x	Approach response+	x	Eye prominence*		Gait score*
	Touch response+		Muscle tone*		Bizarre / stereotypic behaviour*
x	Startle response*	x	Vocalization	x	Posture
	Pain response*				
x	Pupil response*				
	Eyeblink response		**PHYSIOLOGICAL OBSERVATIONS**		**NEUROMUSCULAR OBSERVATIONS**
	Forelimb extension	x	Body weight*		Hindlimb extensor strength
x	Hindlimb extension	x	Body temperature+	x	Forelimb grip strength*
x	Righting reflex+			x	Hindlimb grip strength*
	Olfactory orientation			x	Landing foot splay*
			OTHER OBSERVATIONS		Rotarod performance
		x	Tail pinch		

*Required parameters; +Recommended parameters

1d. Motor activity (Week 12): The motor activity of all animals scheduled for termination after 13 weeks of treatment was measured during Week 12. Activity was monitored using a commercially available tracking, motion analysis and behavior recognition system. The experimental apparatus consisted of a video camera, video recorder, and color monitor. In each session, the animals from each group were monitored in numerical order. The video recorder was activated to record animals over a 60 minute period for each session. The analogue video images from each tape were translated into digital information and analyzed to derive the total distance moved (cm) by each rat during the whole 60 minute period.

2. **Body weight**: Animals were weighed weekly.

3. **Food and water consumption and compound intake**: Food consumption for each animal was determined and mean daily diet consumption was calculated as g food/kg body weight/day. Food efficiency [(body weight gain in kg/food consumption in kg per unit time) X 100] and compound intake (mg/kg bw/day) values were calculated as time-weighted averages from the consumption and body weight gain data. Water consumption was measured over a 4-day period each during Weeks 4, 8, 11, and 17.

4. **Ophthalmoscopic examination:** Eyes were examined before the start of treatment and during Week 13 for all animals in the control and highest dose groups.

5. **Hematology and clinical chemistry:** Blood was collected from the reto-orbital sinus of each animal during Week 13 and Week 17 for hematology and clinical chemistry on all surviving animals. The CHECKED (X) parameters were examined.

a. Hematology:

X	Hematocrit (HCT)*	X	Leukocyte differential count*
X	Hemoglobin (HGB)*	X	Mean corpuscular HGB (MCH)*
X	Leukocyte count (WBC)*	X	Mean corpusc. HGB conc.(MCHC)*
X	Erythrocyte count (RBC)*	X	Mean corpusc. volume (MCV)*
X	Platelet count*	X	Reticulocyte count
X	Blood clotting measurements*		
X	(Thromboplastin time)		
	(Clotting time)		
X	(Prothrombin time)		

* Recommended for 90-day oral rodent studies based on Guideline 870.3100

b. Clinical chemistry:

	ELECTROLYTES		OTHER
X	Calcium	X	Albumin*
X	Chloride	X	Creatinine*
	Magnesium	X	Urea nitrogen*
X	Phosphorus	X	Total Cholesterol*
X	Potassium*	X	Globulins
X	Sodium*	X	Glucose*
	ENZYMES (more than 2 hepatic enzymes)*	X	Total bilirubin
X	Alkaline phosphatase (ALK)*	X	Total protein (TP)*
	Cholinesterase (ChE)		Triglycerides
X	Creatine phosphokinase		Serum protein electrophoresis
	Lactic acid dehydrogenase (LDH)	X	A/G ratio
X	Alanine aminotransferase (ALT/also SGPT)*		
X	Aspartate aminotransferase (AST/also SGOT)*		
	Sorbitol dehydrogenase*		
X	Gamma glutamyl transferase (GGT)*		
	Glutamate dehydrogenase		

* Recommended for 90-day oral rodent studies based on Guideline 870.3100

6. **Urinalysis*** :Urine was collected from fasted animals during Weeks 12 and 17. The CHECKED (X) parameters were examined.

X	Appearance*	X	Glucose
X	Volume*	X	Ketones
X	Specific gravity/osmolality*	X	Bilirubin
X	pH*	X	Blood/blood cells*
X	Sediment (microscopic)		Nitrate
X	Protein*	X	Urobilinogen

*Optional for 90-day oral rodent studies

7. **Sacrifice and pathology:** All animals that died and those sacrificed on schedule were subjected to gross pathological examination and the CHECKED (X) tissues were collected for histological examination. The (XX) organs, in addition, were weighed.

	DIGESTIVE SYSTEM		CARDIOVASC./HEMAT.		NEUROLOGIC
X	Tongue	X	Aorta*	XX	Brain*+
X	Salivary glands*	XX	Heart*+	X	Peripheral nerve*
X	Esophagus*	X	Bone marrow*	X	Spinal cord (3 levels)*
X	Stomach*	X	Lymph nodes*	X	Pituitary*
X	Duodenum*	XX	Spleen*+	X	Eyes (optic nerve)*
X	Jejunum*	XX	Thymus*+		GLANDULAR
X	Ileum*			XX	Adrenal gland*+
X	Cecum*		UROGENITAL	X	Lacrimal gland
X	Colon*	XX	Kidneys*+	X	Parathyroid*
X	Rectum*	X	Urinary bladder*	X	Thyroid*
XX	Liver*+	XX	Testes*+		OTHER
	Gall bladder (not rat)*	XX	Epididymides*+	X	Bone (sternum and/or femur)
	Bile duct (rat)	X	Prostate*	X	Skeletal muscle
X	Pancreas*	X	Seminal vesicles*	X	Skin*
	RESPIRATORY	XX	Ovaries*+	X	All gross lesions and masses*
X	Trachea*	XX	Uterus*+	X	Haderian gland
X	Lung*	X	Mammary gland*	X	Coagulating gland
X	Nose*	X	Vagina	X	Diaphram
X	Pharynx*				
X	Larynx*				

* Recommended for 90-day oral rodent studies based on Guideline 870.3100
+ Organ weights required for rodent studies.

II. RESULTS

A. OBSERVATIONS:

1. **Clinical signs of toxicity**: Clinical signs were observed in the 20,000 ppm dose group as shown in Table 2.

TABLE 2. Clinical signs of toxicity observed at 20,000 ppm dose level				
Clinical Sign over 90 days (20 animals/sex observed)	Males (Number/ mean number days)	Females (Number/ mean number days)	Persisted During Recovery Period (10 animals/sex observed Days 99-121)	
			Males (Number/ mean number days)	Females (Number/ mean number days)
Hair loss	17/65.5	20/83.7	10/18.5	10/20.7
Body soiling: loss of coat condition	11/30.3	6/13.3	No	No
Soiled urogenital region	1/8.0	6/13.3	1/8.0	2/11.5

Data taken from p. 38-42 MRID 46474112

There were no treatment-related clinical signs observed at the lower dose levels nor in controls.

2. **Mortality**: Two mortalities were observed in the high dose group, neither of which is believed to be treatment related.

3. **Neurological evaluations**: There were no treatment related findings regarding the Functional Observational Battery, or the motor activity testing.

B. BODY WEIGHT AND WEIGHT GAIN:

Statistically significantly lower body weights compared to controls were observed in high-dose males and females beginning on Day 8 ($p<0.01$; Table 3) which persisted throughout the remaining study and recovery periods (Table 3). The impact was most marked in the first week when the average weight gain for males and females was only about 2g, while controls gained 40 to 60 g. Food efficiency estimates (discussed in Section C and shown in Table 5) indicate that this weight loss may be due to both decreased food consumption and food efficiency, because the food efficiency values were 20% lower when compared to controls. However, no statistical results were reported on food efficiency. No substance-related effects on body weight were seen in the lower dose groups.

During the recovery period, the body weight gain in the 20,000 ppm dose group, relative to controls, was considerably greater in males ($p<0.01$) and slightly greater in females ($p<0.05$). There were no effects on body weight gain at the lower dose levels.

TABLE 3. Average body weight and body weight gain during 90 days of treatment[b] followed by a 30-day recovery period.									
Diet conc. (ppm)	Average body weight (g ∀ SD)							Average total weight gain by week 13	
	Day 1	Day 8	Week 4	Week 7	Week 10	Week 13	Week 17[B]	g	% Change from control

Male									
0	134 ∀ 10.2	192 ∀ 16.0	346 ∀ 24.8	443 ∀ 36.8	501 ∀ 46.7	543 ∀ 52.9	583 ∀ 56.7	409	B
100	137 ∀ 9.4	194 ∀ 14.3	346 ∀ 20.3	436 ∀ 31.0	500 ∀ 42.2	533 ∀ 48.7	B	396	-3.2
1400	136 ∀ 10.5	183 ∀ 13.9	323 ∀ 23.4* (-7)	419 ∀ 32.4	475 ∀ 40.6	512 ∀ 49.9	B	376	-8.1
20000	135 ∀ 8.5	136 ∀ 9.3* (-29)*	235 ∀ 27.5** (-32)	306 ∀ 34.5** (-31)	342 ∀ 39.6** (-32)	378 ∀ 41.2** (-30)	446 ∀ 52.5** (-23)	243	-40.6
Female									
0	125 ∀ 7.6	164 ∀ 13.4	229 ∀ 19.2	270 ∀ 24.7	293 ∀ 28.5	306 ∀ 32.0	323 ∀ 36.1	181	B
100	125 ∀ 8.9	162 ∀ 14.2	230 ∀ 23.1	270 ∀ 27.8	292 ∀ 29.5	304 ∀ 29.5	B	179	-1.1
1400	124 ∀ 10.1	160 ∀ 14.4	228 ∀ 20.5	267 ∀ 25.7	288 ∀ 32.6	300 ∀ 31.6	B	176	-2.8
20000	123 ∀ 8.3	125 ∀ 81** (-24)	193 ∀ 11.7** (-16)	227 ∀ 14.2** (-16)	240 ∀ 15.0** (-18)	251 ∀ 16.0** (-18)	266 ∀ 23.0** (-18)	128	-29.3

b. Data obtained from pages 55 to 61, .MRID 46474112.
R Recovery Period
* Statistically different ($p < 0.05$) from the control.
** Statistically different ($p < 0.01$) from the control.
Numbers in ( ) are % difference from controls calculated by reviewer

C. FOOD AND WATER CONSUMPTION AND COMPOUND INTAKE

1. **Food and water consumption:** At 20000 ppm, mean food consumption over the treatment period was reduced by 22% in males and 19% (where do these percentages come from? From the table, it should be 17 and 15% for males and females)in females (Table 4) when compared to controls ($p<0.01$). This effect was more severe during the first week of treatment when food consumption was reduced by 54% in males and by 48% in females relative to controls. There were no effects on food consumption at the lower dose levels.

During the recovery period, on week 17, the mean food intake for both sexes was similar to the controls

Water consumption in high dose females was noticeably elevated throughout the treatment period, but was statistically significant ($p<0.01$) only during the first measurement interval (Week 4). The increase at that point was 43% relative to controls and was 20% and 15% at Weeks 8 and 11, respectively.

TABLE 4. Food consumption during 90 days of treatment followed by a 30-day recovery period.				
Diet Conc. (ppm)	Average food consumption (g/animal/day ∀ SD)[a]			
	Week 1	Week 7	Week 13	Week 17[b]
Male				

0	26.1 ∀ 1.2	30 ∀ 0.6	29 ∀ 1.3	24 ∀ 1.4
100	26 ∀ 0.0	28 ∀ 2.1	27 ∀ 1.4	--
1400	24 ∀ 0.7*	28 ∀ 0.0	28 ∀ 0.7	--
20000	12 ∀ 1.0** (-54)	24 ∀ 2.2** (-20)	24 ∀ 2.4** (-17)	23 ∀ 1.4 (-4)
Female				
0	21 ∀ 1.2	21 ∀ 0.6	20 ∀ 1.0	17 ∀ 0.0
100	21 ∀ 0.0	21 ∀ 1.4	21 ∀ 1.4	--
1400	21 ∀ 1.4	21 ∀ 0.0	20 ∀ 1.4	--
20000	11 ∀ 0.8** (-48)	18 ∀ 1.0** (-14)	17 ∀ 0.6** (-15)	17 ∀ 0.7

[a]Data obtained from pages 211 to 216, MRID 46474112.
[b]Recovery period
* Statistically different ($p < 0.05$) from the control.
** Statistically different ($p < 0.01$) from the control.
Numbers in ( ) are % difference from controls calculated by reviewer

2. **Compound consumption:** The time-weighted average Fluopicolide intakes are summarized in the Table 1.

3. **Food efficiency:** At 20,000 ppm, mean food efficiencies (Table 5) over the course of the study were reduced by an average of about 29% in males and 10% (show these percentages if possible in the table) in females when compared to controls. Statistical significance was not reported. Statistically significant body weight effects were limited to the 20,000 ppm dose group (Table 3.).

TABLE 5. Mean food efficiency by dose level.

Week	Males				Females			
	0 ppm	100 ppm	1400 ppm	20,000 ppm	0 ppm	100 ppm	1400 ppm	20,000 ppm
1	32	31.4	29.1	0.6	26.3	25.4	24.4	2.6
2	29.5	29.8	27.9	26.1	18.1	18.6	18.5	21.6
3	25.9	26.1	26.7	22.1	15.4	16.3	16.5	17.6
4	18.8	18.3	14.8	13.2	12.4	11.9	11.4	12.9
5	17.8	17.2	19.6	16.9	12.2	10.8	12.7	10.9
6	16.4	17	16	13.4	9.1	8.2	7.8	9.6
7	12.4	11.6	11.5	9.8	6.2	7	4.9	5.2
8	8.6	13.6	12.4	11.2	7.7	8.9	9.3	6.9
9	10.9	9	7.8	5.6	3.7	3.5	2.1	1.3

10	8.7	10.4	8.4	5.2	4.6	4.1	3.9	3.3
11	9.8	10.2	9.1	10	4.4	5.4	6.5	5.3
12	3.7	2	1.3	5	2.2	0.3	-0.7	0.6
13	7.5	4.9	9	6.7	2.4	2.7	2.1	3

Data are from report pages 73-74, MRID 46474112.

D. OPHTHALMOSCOPIC EXAMINATION:

There were no treatment-related effects.

E. BLOOD ANALYSES:

1. **Hematology:** Hematology data for the highest dose relative to controls are summarized in Table 6. At 20,000 ppm, hemoglobin concentration ($p<0.01$), hematocrit ($p<0.05$), mean cell hemoglobin ($p<0.01$) and mean cell hemoglobin concentration ($p<0.001$) were slightly decreased in both sexes relative to controls. Activated partial thromboplastin time was slightly increased in males only compared to controls ($p<0.001$).

   Other statistically significant differences relative to controls were not considered toxicologically significant because they were not dose-related or, as the report stated, the values were within normal ranges of historical controls (these data were not provided).

   At the end of the 4-week recovery period, all hematological parameters in both sexes were comparable to those in the control group.

TABLE 6. Selected hematology data

Parameter[a]	Males (Week 13)		Females (Week 13)	
	0 ppm	20,000 ppm	0 ppm	20,000 ppm
Hemoglobin concentration (g/L)	152 ∀ 6.5	143 ∀ 6.4**	151 ∀ 4.9	137 ∀ 3.7***
Hematocrit (L/L)	0.45 ∀ 0.022	0.43 ∀ 0.021**	0.43 ∀ 0.015	0.41 ∀ 0.015**
Mean cell hemoglobin concentration (MCHC) (g/L)	342 ∀ 4.0	332 ∀ 10.3**	349 ∀ 2.5	340 ∀ 2.9**
Activated partial thromboplastin time (APTT) (seconds)	19.9 ∀ 1.66	26.5 ∀ 2.17**	19.8 ∀ 1.37	19.0 ∀ 3.26

a. Data obtained from pages 83-88, MRID 46474112.
* p # 0.05
** p # 0.01
*** p # 0.001

2. **Clinical chemistry:** Selected clinical chemistry data are summarized in Table 7. There was a dose-dependent increase in cholesterol in males ($p<0.05$, $p<0.001$, $p<0.001$, respectively at 100, 1400, and 20,000 ppm), but in females only the highest dose group showed an increase ($p<0.001$). At 20,000 ppm, there was a slight increase in total protein, total globulin, and creatinine in males and females.

   Other statistically significant differences relative to controls were not considered toxicologically significant because they were not dose-related or, as the report stated, the values were within normal ranges of historical controls (these data were not provided).

   At the end of the 4-week recovery period, all clinical chemistry parameters in both sexes were comparable to those in the control group.

TABLE 7. Selected clinical chemistry data

Mean Measurement[a]	Males (Week 13)				Females (Week 13)			
	0 ppm	100 ppm	1400 ppm	20,000 ppm	0 ppm	100 ppm	1400 ppm	20,000 ppm
Cholesterol concentration (mmol/L)	1.63 ∀ 0.259	1.94 ∀ 0.304*	2.23 ∀ 0.349***	3.63 ∀ 0.826***	2.11 ∀ 0.322	2.11 ∀ 0.388	2.26 ∀ 0.354	3.51 ∀ 0.553***
Total protein (g/L)	64.3 ∀ 1.49	65.7 ∀ 2.10	66.0 ∀ 2.17	70.5 ∀ 4.44**	68.4 ∀ 3.10	67.0 ∀ 3.43	69.5 ∀ 3.30	73.5 ∀ 2.82***
Total globulin (g/L)	33.2 ∀ 1.79	33.9 ∀ 1.66	34.0 ∀ 1.37	36.5 ∀ 2.99**	33.7 ∀ 2.83	32.4 ∀ 2.17	34.5 ∀ 1.50	37.2 ∀ 1.61***
Creatinine (umol/L)	41 ∀ 2.8	43 ∀ 3.3	46 ∀ 4.9	51 ∀ 5.6***	54 ∀ 3.4	50 ∀ 2.6**	52 ∀ 2.9	57 ∀ 3.5*

a. Data obtained from pages 91-96, MRID 46474112.
* p # 0.05
** p # 0.01
*** p # 0.001

F. URINALYSIS:

Selected urinalysis data are summarized in Table 8.

At both 1400 and 20,000 ppm, there were statistically significant (but only females show * statistical significance on the table) changes consisting of a slight increase in the number of epithelial cells in the urine sediment of males only (where is this on the table?), a slight increase in urinary volume of females only, and a slight decrease in specific gravity of females only. The effects observed in females are consistent with the increased water consumption noted above.

There were no treatment-related effects at 100 ppm in either sex.

At the end of the 4-week recovery period, all urinalysis parameters in both sexes were comparable to those in the control group.

TABLE 8. Selected urinalysis data

Mean measurement[a]	Males (Week 12)			Females (Week 12)		
	0 ppm	1400 ppm	20,000 ppm	0 ppm	1400 ppm	20,000 ppm
Urinary volume (mL)	16 ∀ 5.5	15 ∀ 7.4	19 ∀ 8.6	16 ∀ 6.8	22 ∀ 5.9*	25 ∀ 6.0**
Specific gravity	1.022 ∀ 0.0064	1.024 ∀ 0.0090	1.017 ∀ 0.0054	1.019 ∀ 0.107	1.011 ∀ 0.0046**	1.009 ∀ 0.0036**

a. Data obtained from page 99 and 105, MRID46474112.
* p #0.05
** p #0.01

G. SACRIFICE AND PATHOLOGY:

1. **Organ weight**: Selected organ weight data are summarized in Table 9. (These percentage changes do not match up with what was presented in the tables. I also would change all the "body weight ratios" to "relative organ weight changes". Its less confusing. See strikethrough text.)

   At 20,000 ppm, when compared to controls, absolute liver weight was increased 22% in females only and absolute spleen weight was decreased by 45% in males and by 40% in females. Also, body weight ratios increased in males and females for liver (50% and 49%, respectively), and brain (38% and 15%); ~~body weight ratios~~ relative spleen weight decreased in males and females ~~for spleen~~ (24% and 29%); body weight ratios increased in males only for kidney (15%), testes (38%), and epididymides (31%).

   At 1400 ppm, when compared to controls absolute spleen weight was decreased by 10% in males and by 16% in females. Also, body weight ratios increased in males only for liver (15%) and kidney (11%); spleen:body weight ratios decreased by 19% in females only.

   There were no effects on organ weight in either sex at 100 ppm.
   Other statistically significant differences were considered to be of no toxicological significance because they were either not dose-related or were a consequence of reduced terminal body weight.

   At the end of the 4-week recovery period, there was a trend of reversal of the above effects.

TABLE 9. Selected organ weight data

Organ	Mean organ weight[a] (g)

	0 ppm		1400 ppm		20,000 ppm	
	Males	Females	Males	Females	Males	Females
Liver	16.44 ∀ 1.614	9.68 ∀ 1.423	17.99 ∀ 2.262	10.03 ∀ 1.390	16.75 ∀ 2.079	11.79 ∀ 0.929** (22)
Spleen	0.87 ∀ 0.166	0.62 ∀ 0.087	0.78 ∀ 0.166 (-10)	0.52 ∀ 0.066* (-16)	0.48 ∀ 0.081** (-45)	0.37 ∀ 0.032** (-40)

[a] Data obtained from pages 111 and 113, .MRID 46474112
* p #0.05
** p #0.01
*** p #0.001
Numbers in ( ) are % difference from controls calculated by reviewer

Organ	Mean organ: body weight ratio[a]					
	0 ppm		1400 ppm		20,000 ppm	
	Males	Females	Males	Females	Males	Females
Liver	3.13 ∀ 0.319	3.21 ∀ 0.304	3.59 ∀ 0.310** (15)	3.37 ∀ 0.277	4.71 ∀ 0.350** (50)	4.77 ∀ 0.335** (49)
Spleen	0.17 ∀ 0.031	0.21 ∀ 0.030	0.16 ∀ 0.020	0.17 ∀ 0.016** (-19)	0.13 ∀ 0.015* (-24)	0.15 ∀ 0.017** (-29)
Kidney	0.54 ∀ 0.053	0.62 ∀ 0.063	0.60 ∀ 0.063* (11)	0.63 ∀ 0.103	0.62 ∀ 0.049** (15)	0.61 ∀ 0.102
Testes	0.71 ∀ 0.092	NA	0.67 ∀ 0.095	NA	0.98 ∀ 0.086** (38)	NA
Epidydimides	0.245 ∀ 0.0219	NA	0.245 ∀ 0.0383	NA	0.322 ∀ 0.0219** (31)	NA
Brain	0.40 ∀ 0.037	0.67 ∀ 0.071	0.42 ∀ 0.039	0.66 ∀ 0.056	0.55 ∀ 0.050* (38)	0.77 ∀ 0.057* (15)

[a] Data obtained from pages 115-118, .MRID 46474112
* p #0.05
** p # 0.01
*** p #0.001
Numbers in ( ) are % difference from controls calculated by reviewer

2. **Gross pathology**: At 20,000 ppm a speckled appearance of both kidneys was observed in 4/10 male rats sacrificed after 13 weeks. Both kidneys had a speckled appearance in 3/10 male rats and one additional male rat had one kidney with this appearance after the 4-week recovery period.

   At 1400 ppm both kidneys had a speckled appearance in 3/10 male rats and an additional three male rats had one kidney with this appearance after the 13-week treatment period.

   There were no treatment-related macroscopic abnormalities at 100 ppm.

3. **Microscopic pathology:** Histological changes were observed in the adrenals, bone joint, bone marrow, liver and kidney.

   a. **Adrenals (Table 10):** At 20,000 ppm, there was an increase in severity and incidence of hypertrophy of the zona glomerulosa of the adrenal cortex in both sexes sacrificed after 13 weeks exposure. In animals sacrificed after the 4-week recovery period, the severity of the hypertrophy had decreased in both sexes.

   There were no treatment-related histopathological changes in either sex at 100 or 1400 ppm.

TABLE 10. Selected histopathology data on adrenals

Time/severity [a]	Hypertrophy of the Zona Glomerulosa (incidence)							
	Males				Females			
	0 ppm	100 ppm	1400 ppm	20,000 ppm	0 ppm	100 ppm	1400 ppm	20,000 ppm
After 13 weeks:								
Minimal	1/10	0	0	0	1/10	1/10	3/10	3/10
Slight	0	0	0	7/10	0	0	0	7/10
After 4 weeks recovery:								
Minimal	0			8/10	1/10			4/10
Slight	0			0	0			1/10

[a] Severity scale: minimal, slight, moderate, severe. Data obtained from pages 136-161, .MRID 46474112

   b. **Liver:** Minimal to moderate hypertrophy of centrilobular hepatocytes was observed in treated rats at 20,000 and 1400 ppm, but this finding was not seen as significant toxicologically. At the end of the 4-week recovery period, a reversal of all liver effects was seen.

   c. **Bone joint (Table 11):** At 20,000 ppm, slight to moderate trabecular hyperostosis of the bone joint was observed in 7/10 males and 9/10 females.

   In animals sacrificed after the 4-week recovery period, there was a decrease in the incidence and severity of the bone joint effects.

TABLE 11. Selected histopathology data on bone joint

Time/severity [a]	Trabecular hyperostosis of the bone							
	Males				Females			
	0 ppm	100 ppm	1400 ppm	20,000 ppm	0 ppm	100 ppm	1400 ppm	20,000 ppm
After 13 weeks:	0/10	0/10	0/10	7/10(2.0)	3/10(1.0)	1/10(1.0)	8/10(2.1)	9/10(2.3)
After 4-weeks recovery:	0/10			0/10	1/10(2.0)			7/10(1.7)

[a] Severity scale: minimal(1), slight(2), moderate(3), severe(4). Data obtained from pages 136-149 and 159-161, MRID 46474112.

d. **Bone marrow (Table 12)**: At 20,000 ppm, decreased cellularity in the bone marrow relative to controls was observed in 7/10 males and 9/10 females.

In animals sacrificed after the 4-week recovery period, there was a partial recovery from the above effects.

TABLE 12. Selected histopathology data on bone

Time/severity [a]	Decreased cellularity in the bone marrow							
	Males				Females			
	0 ppm	100 ppm	1400 ppm	20,000 ppm	0 ppm	100 ppm	1400 ppm	20,000 ppm
After 13 weeks:	0/10	1/10(1.0)	0/10	7/10(1.6)	3/10(1.0)	1/10(1.0)	8/10(2.1)	9/10(2.3)
After 4-weeks recovery:	0/10			3/10(1.0)	4/10(1.0)			5/10(1.6)

[a] Severity scale: minimal(1), slight(2), moderate(3), severe(4). Data obtained from pages 136-161, MRID 46474112.

e. **Kidneys**: In females, there were no effects on kidneys at any dose. At 20,000 and 1400 ppm in males only, there were a number of kidney effects (e.g., increased incidence and severity of accumulation of hyaline droplets, single cell death in the proximal tubule epithelium, foci of basophilic tubules and granual casts) consistent with the formation of hyaline droplets and were not considered toxicologically significant. In animals sacrificed after the 4-week recovery period, there was a partial recovery of these kidney effects.

There were no treatment-related histopathological kidney changes in males treated at 100 ppm.

III. DISCUSSION AND CONCLUSIONS:

A. INVESTIGATORS= CONCLUSIONS:

The no observed adverse effect level (NOAEL) for both sexes was 100 ppm, equivalent to a daily intake for the combined sexes of 7.9 mg/kg/bw/day.

The target organs for toxicity were the liver, kidneys, adrenals, bone joint and bone marrow. Following a 4-week off-dose period, there was a full or partial recovery of all treatment-related changes.

B. REVIEWER COMMENTS:

The LOAEL is 20,000 ppm in the diet (1668 mg/kg/day) for males based on hypertrophy of the zona glomerulosa in the adrenal, trabecular hyperostosis of the bone joint, and decreased cellularity of the bone marrow. The LOAEL for females is 1400 ppm in the diet (119 mg/kg/day) based on hypertrophy of the zona glomerulosa in the adrenal and trabecular hyperstosis in the bone joint. The NOAEL is 1400 ppm (109 mg/kg/day) for males and 100 ppm (7.9 mg/kg/day) for females. The discrepancy with the investigator=s conclusions regarding the LOAEL and NOAEL is based on considering toxicologically insignificant both the hypertrophy of centrilobular hepatocytes and the kidney effects consistent with hyaline droplet formation in treated rats at 20,000 and 1400 ppm.

The body weight ratios for brain in both males and females at 20,000 ppm were significantly increased and this elevation persisted in males that were off-dose for 4 weeks after the 13-week treatment period. For males in the 20,000 ppm group, the epididymides and testes had elevated body weight ratios and this elevation persisted in the 4-week off-dose group. No clinical chemistry or treatment-related histopathology was noted for these organs. Therefore, their toxicological significance is questionable.

C. STUDY DEFICIENCIES:

No significant study deficiencies were identified.

DATA FOR ENTRY INTO ISIS

Subchronic Oral Study - rodents (870.3100)

PC code	MRID #	Study type	Species	Duration	Route	Dosing method	Dose range mg/kg/day	Doses tested mg/kg/day	NOAEL mg/kg/day	LOAEL mg/kg/day	Target organ(s)	Comments
027412	46474112	subchronic	rat	13 weeks	oral	diet	7.4-1673	0, 7.9, 114, 1671	Male: 109 Female: 7.9	Male: 1668 Female: 119	Adrenal, bone	None

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DATA EVALUATION RECORD

FLUOPICOLIDE (AE C638206)/027412

STUDY TYPE: Subchronic Oral Toxicity - Mouse [OPPTS §870.3100 (OECD 408)] MRID 46474113 and 46474114

Prepared for

Health Effects Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
1801 Bell Street
Arlington, VA 22202

Prepared by

Toxicology and Hazard Assessment Group
Life Sciences Division
Oak Ridge National Laboratory
Oak Ridge, TN 37830
Work Assignment No. 114-2005

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Date:
Quality Assurance:
Lee Ann Wilson, M.A. Signature:
Date:

Disclaimer

This review may have been altered subsequent to the contractor=s signatures above.

Oak Ridge National Laboratory, managed and operated by UT-Battelle, LLC., for the U.S. Dept. of Energy under contract DE-AC05-00OR22725.

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EPA Reviewer: M.S. Ottley, Ph.D. **Signature:** _____
Science Information Mgmt. Branch, Health Effects Division (7509C) **Date:** _____
EPA Work Assignment Manager: G. Dannan, Ph.D. **Signature:** _____
Registration Action Branch 3, Health Effects Division (7509C) **Date:** _____

Template version 02/06

TXR#: 053597

DATA EVALUATION RECORD

STUDY TYPE: 90-Day Oral Toxicity, Feeding Study, Mouse OPPTS 870.3100 ['82-1a]; OECD 408.

PC CODE: 027412 **DP BARCODE:** D315502

TEST MATERIAL (PURITY): AE C638206 (Fluopicolide, 96.9% and 97.3% a.i.)

SYNONYMS: 2,6-dichloro-*N*-[3-chloro-5-(trifluoromethyl)-2-pyridylmethyl]benzamide; 2,6-dichloro-*N*-[[3-chloro-5-(trifluoromethyl)-2-pyridinyl]methyl]benzamide

CITATION: Mallyon, B.A. (2000) AE 638206: Mouse 90-day dietary toxicity study. Aventis CropScience UK Limited, Toxicology, Chesterford Park, Saffron Walden, Essex, CB10 1XL, England. Laboratory Report No. TOX/00/283-5. September 6, 2000. MRID 46474114. Unpublished.

Author not reported. (2004). AE 638206: Mouse 90-day dietary toxicity study. Aventis CropScience UK Limited, Toxicology, Chesterford Park, Saffron Walden, Essex, CB10 1XL, England. Laboratory Report No. C008604. December 17, 2004. MRID 46474113. Unpublished.

SPONSOR: Bayer CropScience AG, Monheim, Germany.

EXECUTIVE SUMMARY:

In a 92-day oral toxicity study (MRID 46474114) AE 638206 (Fluopicolide, 96.9% and 97.3% a.i., Batch Nos. AE C638206 00 1C99 0005) was administered to groups of 10 CD-1 mice/sex/dose in diet at dose levels of 0, 32, 320, 3200, or 6400 ppm (equivalent to 0, 4.7, 46, 461, and 944 mg/kg bw/day for males and 0, 6.2, 60, 629, and 1239 mg/kg bw/day for females).

No significant effects treatment-related were noted on body weight or body weight gain and no toxicologically relevant effects were noted in the hematology results. The activities of AST, ALT, and AP were slightly increased in male mice treated with ≥ 3200 ppm test material and the activity of ALT was slightly increased in female mice treated with ≥ 3200 ppm test material. These results, in conjunction with increased absolute and relative liver weight of mice in these groups, are consistent with liver hypertrophy. The incidence of microscopically observable hepatocellular hypertrophy was slightly increased in these groups.

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Based on the study results, a LOAEL for AE C638206 was not identified. **The NOAEL for male and female CD-1 mice is greater than the maximum concentration administered, 6400 ppm (944 mg/kg/day for males and 1239 mg/kg/day for females).**

This 90-day oral toxicity study in the CD-1 mouse is **Acceptable/Guideline** and satisfies the guideline requirement for a 90-day oral toxicity study (OPPTS 870.3100; OECD 408) in the mouse.

COMPLIANCE: Signed and dated GLP, Quality Assurance, and Data Confidentiality statements were provided.

I. MATERIALS AND METHODS:

A. MATERIALS:

1. Test material: AE C638206

Description:	Technical; fine, light brown solid; stored below 30/C in the dark
Lot/batch #:	AE C638206 00 1C99 0005
Purity:	96.9 and 97.3 % a.i..
Compound stability:	Sample expiration dates September 10, 1999 and November 16, 1999
CAS # of TGAI:	239110-15-7
Structure:	Not available

2. Vehicle: Diet

3. Test animals:

Species:	Mouse
Strain:	Sprague-Dawley CD-1 (outbread, albino)
Age/weight at study initiation:	Young adult; Males: 19.3 to 25.3 grams; Females: 18.6 to 25.3 grams
Source:	Charles River UK Ltd, Margate, Kent, UK
Housing:	5/sex/dose/cage
Diet:	Powdered laboratory rodent diet (Modified SQC Expanded Ground Rat and Mouse Maintenance Diet No. 1, supplied by Special Diet Services Ltd., Stepfiled, Witham Esses, UK), *ad libitum*
Water:	Tap water, *ad libitum*
Environmental conditions:	Temperature: 19-23°C Humidity: 45-65% Air changes: Not reported (and not required by the 1998 EPA guidelines) Photoperiod: 12 hrs dark/ 12 hrs light
Acclimation period:	6 days (July 28, 1999 to August 2, 1999)

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B. STUDY DESIGN:

1. **In life dates:** Start: August 3, 1999; End: November 2, 1999.

2. **Animal assignment:** Animals were assigned to the test groups noted in Table 1 based upon body weight so that each group had similar initial mean body weight and body weight distribution.

TABLE 1: Study design

Test group	Conc. in diet (ppm)	Dose to animal -M/F mg/kg/day)	# Male	# Female
Control	0	0/0	10	10
Low	32	4.7/6.2	10	10
Mid - 1	320	46/60	10	10
Mid - 2	3200	461/629	10	10
High	6400	944/1239	10	10

Data from page 19, MRID 46474114

3. **Dose selection rationale:** The doses were selected based on the results of a 28-day range-finding study.

4. **Diet preparation and analysis:** Diet was prepared weekly by mixing appropriate amounts of test substance with Modified SQC Expanded Ground Rat and Mouse Maintenance Diet No. 1 and was stored at room temperature. Homogeneity was not tested due to the small size of the samples (<5 kg/dose). Stability of technical grade AE C638206 in rodent diet was determined to be 29 days when stored at room temperature

Results:

Homogeneity analysis: Not tested as part of this study due to the small sample size. However, homogeneity was done in other studies for the test material and was found acceptable.

Stability analysis: In a previous study, all diets were within 10% of nominal for 29days.

Concentration analysis: 93.5% to 107.0% of nominal, with the exception of the 32 ppm diet fed during week 9 which was determined to be 111.5% of nominal.

The analytical data indicated that the variance between nominal and actual was acceptable.

4. **Statistics:** Body weight, food consumption and organ weight were analyzed for homogeneity using Bartlett's test. If not significant at the 5% level, the data were then analyzed by ANOVA. If significant, the pair-wise comparisons with controls were made using Dunnett's test. Where group variances were heterogeneous , pair-wise comparisons were made using Cochran and Cox's modified test. Hematology and clinical chemistry results were evaluated by the Bartlett's test. If the data were homogenous, ANOVA and the Student's T-test were done to determine differences from control. If the data were heterogeneous, the Kruskal-Wallis Rank Sum Test was done. Incidence data were not statistically analyzed.

C. METHODS:

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1. **Observations:**

   1a. Cageside observations: Animals were inspected at least twice daily during business days for signs of toxicity and mortality.

   1b. Clinical examinations: Clinical examinations were conducted weekly.

   1c. Neurological evaluations: No neurological evaluations beyond those routinely included in subchronic feeding studies were conducted as part of this study.

2. **Body weight:** Animals were weighed weekly.

3. **Food consumption and compound intake:** Food consumption for each animal was determined and mean daily diet consumption was calculated as g food/kg body weight/day. Food efficiency (food conversion) and compound intake (mg/kg bw/day) values were calculated as time-weighted averages from the consumption and body weight gain data.

4. **Ophthalmoscopic examination:** The eyes were not examined during the study.

5. **Hematology and clinical chemistry:** Blood was collected during week 13 for hematology and clinical chemistry from all surviving animals. The animals were not fasted before blood collection. The CHECKED (X) parameters were examined.

   a. **Hematology:**

X	Hematocrit (HCT)*	X	Leukocyte differential count*
X	Hemoglobin (HGB)*	X	Mean corpuscular HGB (MCH)*
X	Leukocyte count (WBC)*	X	Mean corpusc. HGB conc.(MCHC)*
X	Erythrocyte count (RBC)*	X	Mean corpusc. volume (MCV)*
X	Platelet count*	X	Reticulocyte count
	Blood clotting measurements*		
	(Thromboplastin time)		
	(Clotting time)		
	(Prothrombin time)		

* Recommended for 90-day oral rodent studies based on Guideline 870.3100

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b. Clinical chemistry:

	ELECTROLYTES		OTHER
X	Calcium	X	Albumin*
X	Chloride	X	Creatinine*
	Magnesium	X	Urea nitrogen*
X	Phosphorus	X	Total Cholesterol*
X	Potassium*	X	Globulins
X	Sodium*	X	Glucose*
	ENZYMES (more than 2 hepatic enzymes eg., *)	X	Total bilirubin
X	Alkaline phosphatase (ALK)*	X	Total protein (TP)*
	Cholinesterase (ChE)		Triglycerides
	Creatine phosphokinase		Serum protein electrophoresis
	Lactic acid dehydrogenase (LDH)		Albumin/globulin (A/G) ratio
X	Alanine aminotransferase (ALT/also SGPT)*		
X	Aspartate aminotransferase (AST/also SGOT)*		
	Sorbitol dehydrogenase*		
X	Gamma glutamyl transferase (GGT)*		
	Glutamate dehydrogenase		

* Recommended for 90-day oral rodent studies based on Guideline 870.3100

6. **Urinalysis***: Urinanalyses was not done

7. **Sacrifice and pathology**: All animals that died and those sacrificed on schedule were subjected to gross pathological examination and the CHECKED (X) tissues were collected for histological examination. The (XX) organs, in addition, were weighed. All tissues and organs were examined.

	DIGESTIVE SYSTEM		CARDIOVASC./HEMAT.		NEUROLOGIC
X	Tongue	X	Aorta*	XX	Brain*+
X	Salivary glands*	XX	Heart*+	X	Peripheral nerve*
X	Esophagus*	X	Bone marrow*	X	Spinal cord (3 levels)*
X	Stomach*	X	Lymph nodes*	X	Pituitary*
X	Duodenum*	XX	Spleen*+	X	Eyes (optic nerve)*
X	Jejunum*	XX	Thymus*+		**GLANDULAR**
X	Ileum*			XX	Adrenal gland*+
X	Cecum*		**UROGENITAL**	X	Lacrimal gland
X	Colon*	XX	Kidneys*+	X	Parathyroid*
X	Rectum*	X	Urinary bladder*	X	Thyroid*
X	Liver*+	XX	Testes*+		**OTHER**
X	Gall bladder (not rat)*	XX	Epididymides*+	X	Bone (sternum and/or femur)
	Bile duct (rat)	X	Prostate*	X	Skeletal muscle
X	Pancreas*	X	Seminal vesicles*	X	Skin*
	RESPIRATORY	XX	Ovaries*+	X	All gross lesions and masses*
X	Trachea*	XX	Uterus*+	X	Haderian gland
X	Lung*	X	Mammary gland*		
X	Nose*		Oviduct		
X	Pharynx*		Preputial gland		
X	Larynx*		Vagina		

* Recommended for 90-day oral rodent studies based on Guideline 870.3100

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+ Organ weights required for rodent studies.

II. RESULTS:

A. OBSERVATIONS:

1. **Clinical signs of toxicity:** No treatment-related clinical signs of toxicity were observed.

2. **Mortality:** None of the mice died during the study.

3. **Neurological evaluations:** Neurological signs were not evaluated.

B. BODY WEIGHT AND WEIGHT GAIN: The overall body weight gain of mice in the 6400 ppm group was decreased 20% in males and 31% in females. At 3200 ppm, the overall body weight gain was reduced 22% in females. These effects were not statistically significant.

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TABLE 2. Average body weights and body weight gains during 92 days of treatment[a]

Dose (ppm)	Body weights (g~SD)				Total weight gain[b]	
	Day 1	Day 8	Week 7	Week 13	g	% of control
			Male			
0	24.5 ± 2.53	292.9 ± 1.45	37.8 ± 2.39	41.1 ± 3.36	16.6	--
32	25.9 ± 1.81	29.9 ± 1.48	38.9 ± 1.84	41.9 ± 2.60	16.0	96.4
320	26.3 ± 2.28	29.2 ± 2.97	39.0 ± 2.76	43.6 ± 3.78	17.3	104
3200	26.0 ± 1.98	29.2 ± 1.44	37.7 ± 2.21	40.6 ± 2.89	14.6	88
6400	26.4 ± 1.40	27.8 ± 2.03	36.0 ± 2.77	39.7 ± 3.07	13.3	80.1
			Female			
0	23.9 ± 1.74	25.0 ± 1.76	31.3 ± 4.49	34.2 ± 4.93	10.3	--
32	23.0 ± 1.70	24.7 ± 1.99	31.3 ± 4.22	33.7 ± 6.48	10.7	104
320	23.7 ± 2.09	24.6 ± 1.56	30.0 ± 2.17	32.2 ± 2.46	8.5	82.5
3200	22.7 ± 1.34	23.8 ± 1.08	28.7 ± 2.11	30.7 ± 2.71	8.0	77.7
6400	23.8 ± 1.68	24.2 ± 2.06	29.6 ± 1.97	30.8 ± 1.86	7.0	68.0

[a] Data obtained from pages 27-30 of MRID 46474114

[b] Calculated by reviewer

C. FOOD CONSUMPTION AND COMPOUND INTAKE:

1. **Food consumption:** No treatment-related effects on food consumption were found.

2. **Compound consumption:** Time-weighted compound consumption is in Table 1 above.

3. **Food efficiency:** No treatment-related effects on food efficiency were found.

D. OPHTHALMOSCOPIC EXAMINATION: No ophthalmoscopic examinations were done.

E. BLOOD ANALYSES:

1. **Hematology:** Although statistically significant differences were found, none were of clinical or toxicological relevance.

2. **Clinical chemistry:** The only significant treatment-related effects were slight increases of enzyme activities indicative of liver function of male and female mice in the 3200 ppm and 6400 ppm groups (Table 3). While other statistically-significant effects were noted, none were of toxicological or clinical relevance.

TABLE 3. Enzyme activities of male and female mice associated with liver function at 14 weeks.

Enzyme	Males			Females		
	0 ppm	3200 ppm	6400 ppm	0 ppm	3200 ppm	6400 ppm
ALT (U/L)	42 ± 11.6	83** ± 63.6	75** ± 20.2	32 ± 9.7	69*** ± 51.9	79*** ± 35.2
AST (U/L)	61 ± 11.4	85* ± 40.3	103*** ± 23.5	78 ± 43.3	99 ± 49.2	86 ± 21.5
AP (U/L)	45 ± 24.0	71 ± 57.6	93** ± 79.8	59 ± 16.8	53 ± 9.8	67 ± 33.7
GGT (U/L)	<3	<3	<3	<3	<3	<3

Data from pages 48-50 of MRID 46474114

*p<0.05; **p<0.01;***p<0.001

F. URINALYSIS: Urinalysis was not done.

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G. SACRIFICE AND PATHOLOGY:

1. **Organ weight:** The absolute and relative liver weights were increased in the 3200 ppm and 6400 ppm male and female mice (Table 4). No other clinically significant treatment-related effects were found.

TABLE 4. Absolute (g) and relative (%) liver weight of male and female mice fed AE C638206 for 92 days

Parameter	0 ppm		3200 ppm		6400 ppm	
	Males	Females	Males	Females	Males	Females
Absolute wt.	1.83 ± 0.251	1.60 ± 0.289	2.44** ± 0.209 (33%)	2.30** ± 0.353 (44%)	2.60** ± 0.222 (42%)	2.56** ± 0.24 (60%)
Relative wt.	4.57 ± 0.484	4.81 ± 0.473	6.22** ± 0.308 (36%)	7.65** ± 0.863 (59%)	6.86** ± 0.303 (50%)	8.56** ± 0.87 (78%)

Data from page 51, 53, 55, and 57 of MRID 46474114
Results in parentheses are percent increase over control calculated by reviewer
**p≤0.01

2. **Gross pathology:** The only remarkable findings noted at necropsy were a slight increase in abnormal areas in the liver and liver enlargement of male and female mice. Abnormal areas were noted in the liver of three males and three females treated with 3200 ppm and in the liver of two males and three females treated with 6400 ppm test material. Enlarged livers were found in one male treated with 6400 ppm test material and in one female in each of the 3200 ppm and 6400 ppm groups.

3. **Microscopic pathology:** The only notable microscopic result was an increase of moderate to severe liver hypertrophy. Moderate liver hypertrophy was found in 4/10 male and 1/9 female mice treated with 3200 ppm test material and in 5/10 male and 3/10 female mice treated with 6400 ppm test material. The liver hypertrophy was classified as severe in 3/10 male mice and 0/10 female treated with 6400 ppm test material.

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III. DISCUSSION AND CONCLUSIONS:

A. **INVESTIGATORS= CONCLUSIONS:** Based on the results, the study author stated the no observed effect level (NOEL) was 32 ppm, equivalent to a daily intake of 5.5 mg/kg/day for the combined sexes. The no observed adverse effect level (NOAEL), based on histopathological changes in the liver, was 320 ppm, equivalent to a daily intake of 53 mg/kg/day for the combined sexes.

B. **REVIEWER COMMENTS:** In this study, no statistically significant effects were noted on body weight or body weight gain and no toxicologically relevant effects were noted in the hematology results. The activities of AST, ALT, and AP were slightly increased in male mice treated with ≥ 3200 ppm test material and the activity of ALT was slightly increased in female mice treated with ≥ 3200 ppm test material. These results, in conjunction with increased absolute and relative liver weights of mice in these groups, are consistent with hepatocellular hypertrophy. The incidence of microscopically observable hepatocellular hypertrophy was also slightly increased in these groups.

Based on the study results, a LOAEL for AE C638206 was not identified. **The NOAEL for male and female CD-1 mice is greater than the maximum concentration administered, 6400 ppm (944 mg/kg/day for males and 1239 mg/kg/day for females).**

C. **STUDY DEFICIENCIES:**

None that would affect interpretation of the study results.

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DATA EVALUATION RECORD

FLUOPICOLIDE/027412
(AE C638206)
STUDY TYPE: 90-Day Oral Toxicity in Rodents -Mouse
(OPPTS 870.3100/OECD 408)
MRIDs 46474115 and 46474116

Prepared for

Health Effects Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
1801 Bell Street
Arlington, VA 22202

Prepared by

Toxicology and Hazard Assessment Group
Life Sciences Division
Oak Ridge National Laboratory
Oak Ridge, TN 37831
Task Order No. 114-2005

Primary Reviewer:
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Date: _____

Secondary Reviewers:
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Date: _____

Robert H. Ross, M.S., Group Leader Signature: _____
Date: _____

Quality Assurance:
Lee Ann Wilson, M.A. Signature: _____
Date: _____

Disclaimer

This review may have been altered subsequent to the contractor=s signatures above.

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EPA Reviewer: M. S. Ottley Ph.D. **Signature:**
Science Information Management Branch, Health Effects Division (7509C) **Date**
EPA Work Assignment Manager: G. Dannan Ph.D. **Signature:**
Registration Action Branch 3, Health Effects Division (7509C) **Date**
Template version 11/01

TXR # 053597

DATA EVALUATION RECORD

STUDY TYPE: 90-Day Oral Toxicity (feeding)-mouse [OPPTS 870.3100 ('82-1a)] (rodent) OECD 408.

PC CODE: 027412 **DP BARCODE**:D315502
SUBMISSION NO.: N/A

TEST MATERIAL (PURITY): AE C638206 (95.9 %)

SYNONYMS: 2,6-Dichloro-N-{[3-chloro-5-(trichloromethyl)-2-pyridinyl]-methyl}benzamide

CITATION: Wason S. (2001) AE C638206: 90-Day Toxicity Study in the Mouse by Dietary Administration. Aventis CropScience, 355, rue Dostoïeveski, BP 153, F-06903 Sophia Antipolis Cedex, France. Study No. SA 00363 and DocMap No. 605303. July 20, 2001. MRID 46474116. Unpublished.

Anonymous (2001) AE C638206: 90-Day Toxicity Study in the Mouse by Dietary Administration. Bayer CropScience AG, Monheim, Germany. Report Nos.: C018138 and 605303 and Study No. SA 00363. July 20, 2001. MRID 46474115. Unpublished.

SPONSOR: Bayer CropScience AG, Monheim, Germany.

EXECUTIVE SUMMARY: In a 90-day oral toxicity study (MRID 46474116, summarized in MRID 46474115), AE C638206 (Fluopicolide, 95.9% a.i., Batch # OP2050046) was administered to 10 C57BL/6JICO mice/sex/dose in the diet at concentrations of 0, 50, 200, 800, or 3200 ppm (approximately 10.4, 37.8, 161, or 770 mg/kg/day for males and 12.6, 52.8, 207, or 965 mg/kg/day for females, respectively). Doses were selected based on previous results from a 90-day mouse dietary study with AE C638206 using Crl:CD1 (1 CR) Br mice (MRIDs 46474114 and 46474113).

There were nine deaths that appeared unrelated to treatment (no dose-response relationship). There were no adverse effects on clinical signs or neurological parameters noted for the surviving animals. Although body weight of males and females in the 3200 ppm group was lower by 7-10% early in the study, final mean body weights were comparable with the controls (both 97% of controls). The overall weight gain was slightly reduced in males in the 800 and 3200 ppm groups and in females in the 3200 ppm group (86-93% of control gain). There were some clinical

chemistry variations such as slight decreases in the concentration of albumin and total cholesterol in animals treated with ∃800 ppm of AE C638206 and slightly increased alkaline phosphatase enzyme activity in males in the 3200 ppm group.

There was a slight dose-related increase in absolute (110 - 125% of control) and relative (114 - 130% of control) liver weight in animals treated with ∃800 ppm of AE C638206. These weight changes appear to be associated with a diffused centrilobular hepatocellular liver hypertrophy. Microscopic examinations revealed this lesion in 4/8 and 8/8 surviving male mice (control: 0/8) and in 8/9 and 10/10 surviving female mice (control: 0/9) at 800 and 3200 ppm of AE C638206, respectively. Hepatocellular hypertrophy may be considered an adaptive response to chemical treatment. However, in consideration of the increased severity of centrilobular hepatocellular hypertrophy in the high dose groups, along with a dose-related increase of liver oval cell proliferation in females: 2/9, 2/9, 3/10, 4/9, and 8/10 in control and through the high dose groups, respectively, these effects do not preclude a treatment related adverse effect on the liver. The toxicological significance of dark coloration of the liver in 4/8 males and 9/10 females treated with 3200 ppm was not determined, but adds to the weight of evidence consideration for liver toxicity.

Under the conditions of this study, the LOAEL for AE C638206 is established at 3200 ppm based on liver toxicity exemplified by the increase in absolute liver weight, increased severity of centrilobular hepatocellular hypertrophy, consequent liver necrosis in the males and hepatocellular proliferation in the females. The NOAEL for AE C638206 is established at 800 ppm.

Consistent with the conclusions of this C57BL/6JIO 90-day oral mouse study is that of the Sprague-Dawley CD-1 90-day oral mouse study (MRID 46474114), which established a LOAEL at ≥3200 ppm based on liver histopathological findings and a NOAEL of 320 ppm. In addition to the alternate mice strain, the liver histopathological findings raise concerns that are evident in the chronic mouse study (MRID#?) that reported hepatocellular adenoma at ≥3200 ppm. Using the weight of evidence approach, the liver effects reported in this C57BL/6JIO 90-day oral mouse study and that of the Sprague-Dawley CD-1 90-day oral mouse study (MRID 46474114) are of toxicological significance and support a LOAEL of 3200 ppm.

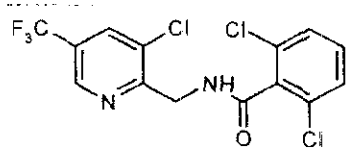
This 90-day oral toxicity study in the mouse is **Acceptable/Guideline,** and satisfies the guideline requirement for a 90-day oral toxicity study (OPPTS 870.3100; OECD 408).

COMPLIANCE: Signed and dated GLP, Quality Assurance, Data Confidentiality, and Flagging statements were provided.

I. MATERIALS AND METHODS:

A. MATERIALS:

1. **Test material:** AE C638206

Description:	fine beige powder
Lot/Batch #:	OP2050046
Purity:	95.9 % a.i.
Compound Stability:	Stable in diet for at least 56 days at ambient temperature or frozen for 7 weeks at below -15EC and ambient temperature for one week.
CAS No. of TGAI:	239110-15-7
Structure:	

2. **Vehicle and/or positive control:** Diet. A positive control was not used.

3. **Test animals:**

Species:	Mouse	
Strain:	*C57BL/6JICO*	
Age/weight at study initiation:	Approximately 5 weeks old, males 12.7 to 19.5 g; females 11.6 to 16.6 g.	
Source:	Iffa-Credo, St Germain-sur-L=Arbresle, France	
Housing:	Housed individually in suspended stainless steel wire mesh cages	
Diet:	M 20 contrôlé (Pietrement, Previns, France), *ad libitum.*	
Water:	*Filtered and softened municipal water, ad libitum*	
Environmental conditions:	**Temperature:**	20-24EC
	Humidity:	40-70%
	Air changes:	10-15 air changes/hr
	Photoperiod:	12 hrs dark/ 12 hrs light from 7 a.m. to 7 p.m.
Acclimation period:	7 days	

B. STUDY DESIGN:

1. **In life dates:** Toxicity Study--Start: September 6, 2000; End: December 7, 2000.

2. **Animal assignment:** Animals were weighed and then grouped into two weight classes. They were then assigned on a random basis to the test groups (Table 1).

TABLE 1: Study design

Test group	Concentration in diet (ppm)	Dose to animals (mg/kg/day)			Number of animals/sex
		Males	Females	Combined***	
1	0	0	0	0	10
2	50	10.4	12.6	8.4	10
3	200	37.8	52.8	33.3	10
4	800	161	207	133	10
5	3200	770*	965**	533	10

Data taken from page 15 and page 20, MRID 46474116.
* No data recorded for Weeks 5 and 7 due to spillage

** No data recorded for Weeks 5,6,7, and 9 due to spillage
*** Theoretical intake of test materials based on food intake data for Weeks 1-13.

3. **Dose selection rationale:** The dose levels were selected based on the results from a 90-day mouse dietary study with AE C638206 using Crl:CD1 (1 CR) Br mice (MRID 46474114, Report No. Tox/00/283-5).

4. **Diet preparation and analysis:** Diets were prepared twice by mixing appropriate amounts of AE C638206 with diet (M 20 contrôlé). Diets were stored frozen at below -15EC. Stability of diets containing 25 and 10,000 ppm was tested, following storage at ambient temperature for up to 56 days or for 7 weeks at below -15EC. Homogeneity was verified for the 50 ppm and 3200 ppm concentrations before the dosing and during the study. For homogeneity, samples were taken from the surface, middle, and bottom levels. At each level, a single sample was taken and analyzed. Stability samples were taken as follows: 1) after 22 days, 4, 6, or 8 weeks at ambient temperature; 2) after 7 days at below -15EC and 15 days at ambient temperature; and 3) after 3, 5, or 7 weeks at below -15EC followed by 7 days at ambient temperature. During the study, samples of treated diet were analyzed twice at each dose level for concentration.

Results:

Homogeneity analysis: On the two sampling dates, top and bottom concentrations ranged from 93-95% and 96-101% of the 50 ppm nominal concentration and from 94-98% and 95-97% of the 3200 ppm nominal concentration.

Stability analysis: The average concentrations of samples stored at ambient temperature for 22 to 56 days or at below -15EC for 7 to 49 days and at ambient temperature for 5 to 15 days were 20.75 ppm (83%) and 22.7 ppm (91%) for the nominal concentration of 25 ppm. The samples from the 10,000 ppm diet stored under the identical conditions had average concentrations of 10,012 ppm (101%) and 9743 ppm (97%) for the nominal concentration of 10,000 ppm.

Concentration analysis: The mean concentration of samples taken two different dates during the study period were 48.2 ppm (97%); 199 ppm (99.5%); 762.5 ppm (95%); and 3076.4 ppm (96.1%) for the nominal concentrations of 50, 200, 800, and 3200 ppm, respectively.

The analytical data indicated that the mixing procedure was adequate. The variance between nominal concentration and actual dosage to the animals was acceptable.

5. **Statistics:** For the parameters of body weight, food consumption, clinical chemistry, organ weight, organ/body and organ/brain weight ratios, and standard deviation were calculated for each sex and dose group for each time period. Statistical analyses of clinical pathology data and organ weight were conducted using Bartlett=s test for homogeneity of variance between the groups. If the data were homogeneous, ANOVA followed by Dunnett=s was used. If the data were heterogeneous, a modified t-test was used. For body weight and food intake data, Dunnett=s test was used. Statistical significance was set at p # 0.05 and 0.01. Xybion PathTox System (version 4.2.2.) was used in all calculations and statistical analyses.

C. METHODS:

1. Observations:

1a. Cageside observations: Animals were inspected twice daily and once daily on weekends and holidays for signs of toxicity and mortality. Any deviations from normal were recorded. Cages were inspected daily for evidence of ill-health, such as blood or loose feces.

1b. Clinical examinations: Clinical examinations, which were performed on each animal once a week, included body surface and orifices, posture, general behavior, respiration and excretory products.

1c. Neurological evaluations: Neurological evaluations were not performed; however, daily cageside observations and weekly clinical examinations were made.

2. **Body weight**: Animals were weighed during the acclimation period, on the day treatment commenced, at weekly intervals throughout the treatment period, and before necropsy.

3. **Food consumption and compound intake**: Food consumption for each animal was determined weekly by weighing the amount of food utilized (difference between food supplied and food not consumed). Food efficiency data were not provided. Compound intake (mg/kg bw/day) values were calculated by the study author as time-weighted averages from the food consumption and body weight data (Table 1).

4. **Ophthalmoscopic examination**: Eye examinations were not performed.

5. **Hematology and clinical chemistry**: Hematology assays were not conducted. For clinical chemistry assays, blood was collected from all surviving fasted animals from the retro-orbital sinus under anesthesia with isoflurane. All blood samples were collected on Days 91, 92, 93, or 94 from equal numbers randomly distributed from each group. Blood (0.6 ml) was collected in lithium heparin for plasma chemistry determination. The CHECKED (X) parameters were examined.

Clinical chemistry:

	ELECTROLYTES		OTHER
	Calcium	x	Albumin*
	Chloride		Creatinine*
	Magnesium	x	Urea nitrogen*
	Phosphorus	x	Total Cholesterol*
	Potassium*		Globulins
	Sodium*		Glucose*
	ENZYMES (more than 2 hepatic enzymes)*	x	Total bilirubin
x	Alkaline phosphatase (ALK)*	x	Total protein (TP)*
	Cholinesterase (ChE)		Triglycerides

	Creatine phosphokinase		Serum protein electrophoresis
	Lactic acid dehydrogenase (LDH)		A/G ratio
x	Alanine aminotransferase (ALT)*		
x	Aspartate aminotransferase (AST)*		
	Sorbitol dehydrogenase*		
	Gamma glutamyl transferase (GGT)*		
	Glutamate dehydrogenase		

* Recommended for 90-day oral rodent studies based on Guideline 870.3100.

6. **Urinalysis:** Urinalysis was not performed.

7. **Sacrifice and pathology:** All animals that died and those sacrificed on schedule (fasted overnight) under deep anaesthesia (ip injection of pentobarbital, 60 mg/kg bw) were subjected to gross pathological examination. The CHECKED (X) tissues were collected for histological examination. The (XX) organs, in addition, were weighed before fixation. All tissues were fixed in neutral buffered 10% formalin with the exception of eye, optic nerve, Harderian gland, epididymis and testis that were fixed in Davidson=s fixative. Tissues except larynx were embedded in paraffin, sectioned and stained with hematoxylin and eosin. Pathological examinations were performed on all tissues from all animals in the control and high dose groups and all un-scheduled deaths. Macroscopic lesions were also examined in all intermediate dose groups.

	DIGESTIVE SYSTEM		CARDIOVASC./HEMAT.		NEUROLOGIC
x	Tongue	x	Aorta*	xx	Brain*+
x	Salivary glands*	xx	Heart*+	x	Peripheral nerve*
x	Esophagus*	x	Bone marrow*	x	Spinal cord (3 levels)*
x	Stomach*	x	Lymph nodes*	x	Pituitary*
x	Duodenum*	xx	Spleen*+	x	Eyes (optic nerve)*
x	Jejunum*	xx	Thymus*+		GLANDULAR
x	Ileum*			xx	Adrenal gland*+
x	Cecum*		UROGENITAL	x	Lacrimal gland
x	Colon*	xx	Kidneys*+	x	Parathyroid*
x	Rectum*	x	Urinary bladder*	x	Thyroid*
				x	Harderian gland
xx	Liver*+	xx	Testes*+		OTHER
x	Gall bladder (not rat)*	x	Epididymides*+	x	Bone (sternum and/or femur)
	Bile duct (rat)	x	Prostate*	x	Skeletal muscle
x	Pancreas*	x	Seminal vesicles*	x	Skin*
	RESPIRATORY	xx	Ovaries*+		All gross lesions and masses*
x	Trachea*	x	Uterus*+	x	Tatooed ears
x	Lung*	x	Mammary gland*	x	Zymbal gland
	Nose*	x	Vagina, cervix		
	Pharynx*	x	Urethra, ureters		
x	Larynx*				

* Recommended for 90-day oral rodent studies based on Guideline 870.3100.
\+ Organ weights required for rodent studies.

II. RESULTS:

A. OBSERVATIONS:

1. **Clinical signs of toxicity:** No clinical signs of toxicity related to treatment with AE C638206 were observed.

2. **Mortality:** One male from each of the control, 200, and 3200 ppm dose groups and one female from the 800 ppm group were found dead or moribund during Day 6 and 73. No cause of death could be determined, except one male control mouse may have had suppurative encephalitis. One male of the control and two at 800 ppm and one male at 3200 ppm died as the result of accidental trauma between Day 21 and 85. In addition, one female from each of the control and 50 ppm groups died during the anesthesia period prior to blood sampling on Day 94. There were no mortalities directly related to AE C638206 treatment.

3. **Neurological evaluations:** Specific neurotoxicity tests were not conducted, and no clinical signs of neurotoxicity were seen during daily observations.

B. BODY WEIGHT AND WEIGHT GAIN: Mean body weight and body weight gain are shown in Table 2. Treated animals of both sexes in all dose groups had final mean body weight comparable to the controls. However, early in the study, the males treated with 3200 ppm had lower weight which initially ranged from 10% ($p<0.05$) in Week 1 to 7% ($p<0.05$) lower in Week 6. Similarly, the high dose females had lower body weight (6-8%, $p<0.05$) from the beginning of the study to Week 7. The overall weight gain in the male 800 and 3200 ppm groups was slightly reduced to 86 and 93% of the control, respectively, and in females in the 3200 ppm group to 86% of the control weight gain.

TABLE 2: Average body weight (g) and body weight gain (g) during 90 days of treatment

Dose [ppm]	Body weight (Mean∀S. D.), g					Total weight gain
	Week 0	Week 1	Week 6	Week 7	Week 13	g
Males						
0	17.0∀2.0 8.252.062.02.02 02.02.0	19.4∀ 1.5	22.9∀1.1	24.1∀1.1	26.1∀0.7	9.0
50	17.1∀1.9	18.9∀1.3	22.9∀1.0	24.2∀0.8	26.0∀0.9(99)	8.9(98)
200	17.3∀1.1	19.6∀ 1.0	23.1∀ 1.1	24.5∀1.1	25.9∀ 1.3(99)	8.5(94)
800	16.9∀1.7	19.4∀1.1	22.1∀ 1.3	23.5∀1.2	25.2∀0.9(96)	7.7(86)
3200	17.3∀1.7	17.4∀1.0(90)*	21.4∀0.7(93)*	23.2∀0.6(96)	25.3∀0.6(97)	8.4(93)
Females						
0	14.6∀1.4	16.1∀0.9	19.4∀ 0.6	20.5∀0.8	21.2∀0.7	6.6
50	15.0∀0.9	16.5∀0.7	19.9∀0.6	21.0∀0.6	21.7∀0.5(102)	6.7(102)
200	15.0∀0.8	16.5∀0.9	19.3∀0.7	20.6∀0.7	21.7∀0.6(102)	6.7(102)
800	14.4∀1.4	16.3∀0.5	19.0∀1.1	20.3∀0.6	21.0∀0.6(99)	6.7(102)
3200	14.9∀1.1	15.0∀0.7(93)*	17.9∀1.3(92)*	19.3∀0.8(94)*	20.5∀0.9(97)	5.7(86)

Data obtained from pages 34-35 and page 38, MRID 46474116.
Data in parenthesis is percent of control calculated by reviewer.
* Significantly different ($p<0.05$) from the control.

C. FOOD CONSUMPTION AND COMPOUND INTAKE:

1. **Food consumption:** Food consumption data were provided on a weekly basis. Due to excessive food spillage during some weeks, food consumption data for those weeks were not provided. Food consumption appeared to be slightly lower than the control for males and females in the 3200 ppm groups only during the first week of the study.

2 **Compound consumption:** Time-weighted average compound consumption was calculated by the study author and is included in Table 1.

3. **Food efficiency:** Food efficiency was not reported by the study author. Due to excessive food spillage during the study, it was difficult to make an accurate assessment of food efficiency.

D. OPHTHALMOSCOPIC EXAMINATION: There were no ophthalmoscopic examinations conducted.

E. BLOOD ANALYSES:

5. Hematology: No hematology examinations were conducted.

2. **Clinical chemistry:** Very slight decreases (87-89% of control) in albumin levels were seen in male and female treated animals at dose levels of ∃800 ppm. There were decreases in total cholesterol in males (51-81% of control) and in females (77-84% of control) of all treated groups (Table 3). In contrast, there was a very slight increase in alkaline phosphatase activity at the highest dose level in both sexes. In addition, there were sporadic changes in bilirubin, alanine aminotransferase and urea levels. These variations were within the normal range of biological variation at the end of 13 weeks of study.

TABLE 3: Clinical chemistry- electrolytes after 90 days of treatment with AE C638206

Dose (ppm)	Mean ∀ S. D.		
	Albumin (g/l)	Alkaline phosphatase (IU/l)	Total cholesterol (mm/l)
Males			
0	38∀2	69∀6	1.82∀0.17
50	37∀2	71∀8	1.47∀0.19(81)**
200	36∀2	69∀6	1.36∀0.30(75)**
800	33∀2(87)**	72∀7(104)	0.95∀0.19(52)**
3200	33∀2(87)**	89∀8(129)**	0.91∀0.20(50)**
Females			
0	38∀2	99∀12	1.51.0∀0.15
50	37∀2	102∀10(103)	1.43∀0.17
200	35∀3	104∀9(105)	1.19∀0.14(79)**
800	34∀3(89)**	109∀5(110)	1.16∀0.14(77)**
3200	33∀2(87)**	106∀12(107)	1.27∀0.14(84)**

Data obtained from pages 44-51, MRID 46474116.
Data in parenthesis is percent of control calculated by reviewer.
** Significantly different ($p < 0.01$) from the control.

F. URINALYSIS: No urinalysis was conducted.

1. SACRIFICE AND PATHOLOGY:

2. **Organ weight:** There was a slight dose-related increase in absolute liver weight, 110% and 120% ($p<0.01$) of the control in males and 113 and 125% ($p<0.01$) of the control level in females treated with 800 and 32,00 ppm AE C638206, respectively (Table 4). There was also a slight increase in relative liver weight, 114 and 130% ($p<0.01$) of the control level in males and 116 and 134% of the control level ($p<0.01$) in females at 800 and 3200 ppm. These variations are not toxicological significant. Slight decreases in mean absolute and relative heart weight were seen in males and females in the 3200 ppm group.

TABLE 4: Absolute and relative(to body) organ weight in mice treated with AE C638206 orally for 13 weeks

Parameter	Dietary concentration (ppm)				
	0	50	200	800	3200
Males (mean ∀SD)					
Body weight, g	22.3∀0.5	22.3∀0.8	22.2∀1.1	21.4∀0.7	21.2∀0.7
Heart					
Absolute, g	0.13∀0.01	0.13∀0.01	0.13∀0.01	0.12∀0.01(92)	0.11∀0.01(85)**
Relative, g/100g	0.56∀0.05	0.56∀0.05	0.57∀0.03	0.54∀0.05(96)	0.51∀0.06(91)
Liver					
Absolute, g	1.0∀0.1	1.0∀0.1	1.0∀0.1	1.1∀0.1(110)	1.2∀0.0(120)**
Relative, g/100g	4.3∀0.3	4.3∀0.4	4.5∀0.2(105)	4.9∀0.2(114)**	5.6∀0.1(130)**
Females (mean ∀SD)					
	0	50	200	800	3200
Body weight, g	17.7∀0.8	18.0∀0.4	17.7∀0.8	17.7∀0.8	17.4∀0.8
Heart					
Absolute, g	0.11∀0.01	0.11∀0.02	0.11∀0.01	0.10∀0.01(91)	0.09∀0.01(82)**
Relative, g/100g	0.61∀0.04	0.61∀0.08	0.61∀0.06	0.57∀0.05(93)	0.54∀0.039)*
Liver					
Absolute, g	0.8∀0.1	0.8∀0.0	0.8∀0.1	0.9∀0.1(113)*	1.0∀0.1(125)**
Relative, g/100g	4.4∀0.3	4.3∀0.2	4.7∀0.4(107)	5.1∀0.4(116)**	5.9∀0.4(134)**

Data obtained from pages 52-70, MRID 46474116.
Data in parenthesis is percent of control calculated by reviewer.
* Significantly different ($p<0.05$) from the control.
** Significantly different ($p<0.01$) from the control.

3. **Gross pathology:** Examination of animals that were sacrificed moribund or that died from unscheduled deaths, accidental trauma, or during anesthesia were considered incidental to treatment. However, dark liver coloration was noted at the terminal sacrifice in 4/8 males and 9/10 females treated with 3200 ppm. There were no additional findings in the treated animals.

4. **Microscopic pathology:** In the surviving males, there was a dose-related increase in liver centrilobular cell hypertrophy in 4/8 and 8/8 mice (control: 0/8) treated with 800 and 3200 ppm AE C638206, respectively (Table 5). An increase was also detected in liver single cell necrosis in 4/8 males at the highest dose (control: 0/8). In females, there was also a dose-related increase in liver centrilobular cell hypertrophy in 8/9 and 10/10 (control: 0/9) mice treated with 800 and 3200 ppm of AE C638206, respectively. In addition, in females there

was a dose-related increase in liver oval cell proliferation in 2/9, 2/9, 3/10, 4/9, and 8/10 in the control through high-dose groups, respectively. This response was not seen in males. In deceased males, one from each of the 800 and 3200 ppm groups showed a diffused centrilobular hepatocellular hypertrophy.

TABLE 5: Histopathological findings in mice treated with AE C638206 orally for 13 weeks

Parameter	Dietary concentration (ppm)				
	0	50	200	800	3200
Males					
Liver:					
B Hypertrophy, hepatocellular, centrilobular, *diffuse*	0/8	0/10	0/9	4/8	8/8
B Single cell, necrosis, multifocal					
B Proliferation, oval cell, *focal to multifocal*,	0/8	0/10	0/9	0/8	4/8
	0/8	1/10	0/9	0/8	0/8
Females					
	0	**50**	**200**	**800**	**3200**
Liver:					
B Hypertrophy, hepatocellular, centrilobular, *diffuse*	0/9	0/9	0/10	8/9	10/10
- Single cell, necrosis, multifocal					
- Proliferation, oval cell, *focal to multifocal*,	0/9	0/9	1/10	0/9	0/10
	2/9	2/9	3/10	4/9	8/10**

Data obtained from pages 91-96, MRID 46474116.
a Statistical analysis -Fisher Exact test performed by reviewer.
* Significantly different ($p<0.05$) from the control.

III. DISCUSSION AND CONCLUSIONS:

A. INVESTIGATORS= CONCLUSIONS: No treatment related effects were seen in clinical signs or neurological symptoms. Four mice (one male each from the control, 200, and 3200 ppm dose groups and one female from the 800 ppm group) were found dead or moribund during Day 6 and 73. One male in the control and two in the 800 ppm group, and one female in the 3200 ppm group died of accidental trauma between Day 21 and 85. Finally, one female from each of the control and the 50 ppm groups died during anesthesia. These mortalities were considered incidental and not treatment related.

Terminal body weight was slightly reduced (3%) in males and females treated with 3200 ppm. Overall body weight gain was slightly reduced in males, to 86 and 93% of control in the 800 and 3200 ppm groups, respectively and in females at 3200 ppm (86% of control). There was no clear treatment-related effect on food consumption. Total cholesterol concentration showed a slight decrease (50 to 84% of control) in both sexes at dose levels ∃200 ppm (and in males at 50 ppm, $p<0.01$). Albumin level was decreased (87 to 89% of control) at dose levels ∃200 ppm. Absolute and relative liver weight were increased 110 and114% and 120 and 130% of the control weight in males and 113 and 116% and 125 and 134% of the control weight in females at 800 and 3200 ppm, respectively. In contrast, the absolute and relatively heart

weight were decreased slightly, 85 and 91% and 82 and 89% of the control weight in male and female mice at 3200 ppm.

Gross pathology examination of deceased animals showed no treatment-related changes. At the terminal sacrifice dark liver coloration was noted in 4/8 males and 9/10 females at 3200 ppm. Histopathological examination revealed diffuse centrilobular hepatocellular hypertrophy in one deceased male each in the 800 and 3200 ppm groups. The same finding was also observed in all surviving mice in the 3200 ppm groups and in 4/8 males and in 8/9 females in the 800 ppm groups.

The No Observed Effect Level for AE C638206 in mice in both sexes was 50 ppm.

B. REVIEWER COMMENTS: There was a high member of mortalities (a total of nine) during the study. These mortalities were present in all groups and could not attributed to treatment.

There was no assessment of hematology, urinalysis, or food and water consumption. There were no adverse effects on clinical signs or neurological parameters noted in the surviving animals. Except for Weeks 1 to 6 or 7, treated animals had mean body weight comparable with the controls. However, overall body weight gain was slightly reduced in males, 86 and 93% of control in the 800 and 3200 ppm groups, respectively and in females at 3200 ppm (86% of control). There were some clinical chemistry variations with statistical significance such as slight decreases in the concentration of albumin and total cholesterol in animals treated with ∃800 ppm of AE C638206 and slightly increased alkaline phosphatase enzyme activity in males in the 3200 ppm group. These changes were considered to be of no toxicological significance.

There was a slight dose-related increase in absolute (110 - 125% of control) and relative (114 - 130% of control) liver weight in animals treated with ∃800 ppm of AE C638206. These weight changes appear to be associated with a diffused centrilobular hepatocellular liver hypertrophy. Microscopic examinations revealed this lesion in 4/8 and 8/8 surviving male mice (control: 0/8) and in 8/9 and 10/10 surviving female mice (control: 0/9) at 800 and 3200 ppm of AE C638206, respectively. Hepatocellular hypertrophy may be considered an adaptive response to chemical treatment. However, in consideration of the increased severity of centrilobular hepatocellular hypertrophy in the high dose groups, along with a dose-related increase of liver oval cell proliferation in females: 2/9, 2/9, 3/10, 4/9, and 8/10 in control and through the high dose groups, respectively, these effects do not preclude a treatement related adverse effect on the liver. The toxicological significance of dark coloration of the liver in 4/8 males and 9/10 females treated with 3200 ppm was not determined, but adds to the weight of evidence consideration for liver toxicity.

Under the conditions of this study, the LOAEL for AE C638206 is established at 3200 ppm based on liver toxicity exemplified by the increase in absolute liver weight, increased severity of centrilobular hepatocellular hypertrophy, consequent liver necrosis in the males and hepatocellular proliferation in the females. The NOAEL for AE C638206 is established at 800 ppm.

Consistent with the conclusions of this C57BL/6JIO 90-day oral mouse study is that of the Sprague-Dawley CD-1 90-day oral mouse study (MRID 46474114), which established a LOAEL at ≥3200 ppm based on liver histopathological findings and a NOAEL of 320 ppm. In addition to the alternate mice strain, the liver histopathological findings raise concerns that are evident in the chronic mouse study (MRID#?) that reported hepatocellular adenoma at ≥3200 ppm. Using the weight of evidence approach, the liver effects reported in this C57BL/6JIO 90-day oral mouse study and that of the Sprague-Dawley CD-1 90-day oral mouse study (MRID 46474114) are of toxicological significance and support a LOAEL of 3200 ppm.

C. **STUDY DEFICIENCIES:** Food spillage and a high number of non-treatment related deaths were problems during the study; lack of hematological analysis and opthalmoscopic exam, lack of gross pathological examination of the nose, pharynx and all gross lesions and masses and organ weights of the epididymides and uterus are noted deficiencies of this study. These deficiencies, however, are not considered serious deficiencies that would invalidate the study results.

DATA FOR ENTRY INTO ISIS

Subchronic (90 day) Oral Study - rodents (870.3100)

PC code	MRID	Study	Species	Duration	Route	Admin	Dose range ppm	Doses ppm	NOAEL mg/kg/day	LOAEL mg/kg/day	Target organ	Comments
027412	46474115 46474116	subchronic	mice	13 wks.	oral	dietary	0-3200	0, 50, 200, 800, 3200	Males: 3200 ppm Females: 800 ppm	Males: Not established Females: 3200 ppm	Liver	oval cell proliferation

HED Cancer Assessment Review Committee Meeting

FLUOPICOLIDE
November 15, 2006

CARC Members	Signature
Lori Brunsman, Statistician	
William Burnam, Chair	
Marion Copley	
Vicki Dellarco	
Kit Farwell	
Abdallah Khasawinah	
Jessica Kidwell, Executive Secretary	
Nancy McCarroll	
Tim McMahon	
John Pletcher, Consulting Pathologist	
Esther Rinde	
Jess Rowland	
Linda Taylor	
Yin-Tak Woo	
Other Attendees	**Signature**
Myron Ottley (presenter)	
Paula A Descamps	
Nancy Dodd	

11/15/06 CARC MTG - FLUDPICOLIDE

TUMORS

RAT - No T-R increase in tumors - both sexes F344-rats (Doses: 0, 50, 200, 750, 2500 ppm)

ADEQUACY OF DOSING: ADEQUATE, NOT EXCESSIVE
- Non neoplastic lesions - males only / Repro study - histopath both sexes, 2000 ppm
- ↓BWG (overall) 11.6 ♂, 17.1 ♀ (based on toxicities observed up to 1 yr mainly)
- Kidney non-neoplastic lesions males & females

Mouse: Liver Tumors (C57BL/6N Crl:BR SPF VAF Mouse)

- Males:

Dose	0	50	400	3200 PPM	H.C. (testing lab)
Aden.	5/47**	0/49	5/48	11/49	6%, 10%
%	11	0	10	22	
CARCIN.	3/47	1/49	0/48	2/49	0%, 1%
%	6	2	0	4	
COMBO	7/47**	1/49	5/48	13/49	10%, 7%
%	15	2	10	27	

- Females:

Dose	0	50	400	3200 PPM	H.C (testing lab)
Aden	1/58**	2/60	1/60	19/57**	0%, 2%
%	2	3	2	33	
CARCIN	0/58	0/60	2/60	0/57	0%, 1%
%	0	0	3	0	
COMBO	1/58**	2/60	3/60	19/57**	0%, 3%
%	2	3	5	33	

♂ Treatment-related? Yes
- Trend only, adenoma-combo
- Adenoma-driven, doubling of adenomas at ½ next dose
- > HC
- weaker response than female

♀ Treatment-related? Yes
- Trend, PW HD - Aden & combo
- > H.C., Driven by aden.

Adeq. of Dosing: Adequate, Not excessive
- Based on BW (not BWG)
- no mortality
- toxicity present

11/15/06 CARC MTG - FLUOPICOLIDE

MOA: Mitogenesis associated with P450 Induction (PB - positive control separate study)

Key Events	Doses	0	50	400	3200	Time
1. Induction of P450 Enzymes						
- Total cyt P450					↑2x	7 days
- BROD (CYP1A/2B)					↑19x	
- EROD (CYP1A)					↑1.8x	Consistent w/ PB in separate study. Request results of Concurrent Pos control
- PROD (CYP2B)					↑12x	
- Lauric acid hydrox.					↑35x	
2. Increased cell prolif						
BrdU					X (↑6.5x)	↑ at D8. NOT SUSTAINED AT D29
3. Organ Toxicity						
Cell hypertrophy					X hypertrophy	Day 8, Day 29
Altered cell foci						
Increased liver weight					X ↑ liver wt	Day 8, Day 29
4 Inhibition of Apoptosis					↑ apoptosis/necrosis	D8, D29

Problems: Only used Ideal (linear dose) - no D-R
No oxidative stress markers
No concurrent positive control data included

Plausible MOA 7/8

11/15/06 CARC HTG - FLUOPICOLIDE

Mutagenicity: (12 studies) - WOE does not suggest mutagenic concern bec/ don't see it in vivo

- Neg Ames (2004)
- 1 weak positive Ames (2004) — Marginal evidence of genotoxicity in vitro, but not expressed in vivo

- Pos. chrm. aber in vitro (2004) - same product as weakly pos. Ames
- Neg gene mut - mammal cells
- Neg in vitro chrm aber in human lymphocytes
- Series of 4 in two assays - negative (Bone marrow micronuclei)
- Neg. UDS

Myron Get corrections from Nancy

SAR: See Hentel's write up

Fluazinam..

11/15/06 CARC MTG - FLUOPICOLIDE

CLASSIFICATION: "Not Likely at ..."

① Suggestive: 1
or
② "Not Likely" at doses that don't cause perturbation of liver: 7

WOE

- T-R benign tumor (liver) (mice) species, both sexes
- No tumors in rat
- Accept that they have plausible MOA that has been supported by data (threshold)
- No concerns for mutagenicity
- No SAR concerns

Quantification: Quant not required

Since tumors appear at HDT

NOAEL of 31.5 mg/kg/d adequately addresses concerns for chronic bird & mammal dose that cause tumor

No MOE for tumor

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R139259

Chemical: Fluopicolide

PC Code:
027412

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