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

OFFICE OF PREVENTION,
PESTICIDES, AND TOXIC SUBSTANCES

TXR No.: 0054488

MEMORANDUM

DATE: December 12, 2006

SUBJECT: FLUOPICOLIDE: Report of the Cancer Assessment Review Committee

PC Code: 027412

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

Jessica Kidwell

TO: Myron Ottley, Toxicologist (RAB3)
Nancy Dodd, Risk Assessor (RAB3)
Health Effects Division (7509P)

Janet Whitehurst
Fungicide Branch, Registration Division (7505P)

The Cancer Assessment Review Committee met on November 15, 2006 to evaluate the carcinogenic potential of FLUOPICOLIDE. Attached please find the Final Cancer Assessment Document.

cc: J. Pletcher
Y. Woo

CANCER ASSESSMENT DOCUMENT

EVALUATION OF THE CARCINOGENIC POTENTIAL OF ***FLUOPICOLIDE***

PC CODE: 027412

FINAL
December 12, 2006

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

DATA PRESENTATION

Myron Ottley, Toxicologist

DOCUMENT PREPARATION:

Jessica Kidwell, Executive Secretary

COMMITTEE MEMBERS IN ATTENDANCE:

(Signature indicates concurrence with the assessment unless otherwise stated).

William Burnam, Chair

Marion Copley

Nancy McCarroll

Esther Rinde — Minority Opinion Attached

Jess Rowland

Linda Taylor

NON-COMMITTEE MEMBERS IN ATTENDANCE

(Signature indicates concurrence with the pathology report)

John Pletcher, Consulting Pathologist — See Attached Sheet - next page

OTHER ATTENDEES: Paula Deschamp (HED/RAB3), Nancy Dodd (HED/RAB3)

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EXECUTIVE SUMMARY

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

Myron Ottley of Registration Action Branch 3 presented the chronic toxicity and carcinogenicity study in CD rats (MRID 46474139) and the carcinogenicity study in C57BL/6 mice (MRID 46474130). In the rat chronic toxicity/carcinogenicity study, AE C638206 (Fluopicolide, 95.9%, a.i.) 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 an 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. In the mouse carcinogenicity study, AE C638206 (Fluopicolide) (95.9% a.i.) 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.

The CARC concluded the following:

Carcinogenicity

Rat

- No treatment-related tumors were seen in male or female CD rats.

- *Adequacy of Dosing:* The CARC considered dosing at the high dose of 2500 ppm to be adequate to assess the carcinogenicity of fluopicolide in rats. This was based on decreased overall body weight gain in male (↓11%) and female (↓17%) rats at 2500 ppm, increased thyroid weight, and the non-neoplastic thyroid lesions observed at 2500 ppm in males. Effects were minimal in female rats. However, a reproductive study and a companion, supplemental study providing 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. These results, along with those of the main study, indicate that dosing in the chronic rat study at 2500 ppm was adequate for both sexes.

Mouse

- In male C57BL/6 mice, the incidences of liver tumors for the control, 50, 400, and 3200 ppm dose groups, respectively, were as follows:

Adenomas: 5/47 (11%), 0/49 (0%), 5/48 (10%), 11/49 (22%)
Carcinomas: 3/47 (6%), 1/49 (2%), 0/48 (0%), 2/49 (4%)
Combined: 7/47 (15%), 1/49 (2%), 5/48 (10%), 13/49 (27%)

There were 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. However, the incidences of adenomas (22%), carcinomas (4%) and combined (27%) at the high dose, exceeded the historical control data from the testing laboratory (6%, 10%, adenomas; 0%1%, carcinomas; 7%, 10%, combined). Therefore, the CARC concluded that the liver tumors (adenoma driven) noted at the highest dose tested (3200 ppm) in males were considered to be treatment-related. The CARC also noted that the liver tumor response seen in males was weaker than that seen in the females.

- In female C57BL/6 mice, the incidences of liver tumors for the control, 50, 400, and 3200 ppm dose groups, respectively, were as follows:

Adenomas: 1/58 (2%), 2/60 (3%), 1/60 (2%), 19/57 (33%)
Carcinomas: 0/58 (0%), 0/60 (0%), 2/60 (3%), 0/57 (0%)
Combined: 1/58 (2%), 2/60 (3%), 3/60 (5%), 19/57 (33%)

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 incidences of adenomas (33%) and combined (33%) at the high dose exceeded the historical control data (0%, 2%, adenomas; 0%, 3%, combined) for the testing laboratory. Therefore, the CARC concluded that the liver tumors (adenoma driven) noted at the highest dose tested (3200 ppm) in females were considered to be treatment-related.

- *Adequacy of Dosing:* The CARC considered dosing at 3200 ppm to be adequate, but not excessive, to assess the carcinogenic potential of fluopicolide in mice. This was based on significant decreases in body weight of both male and female mice at 3200 ppm. From weeks 13-52 of the study, body weight decreases ranged from 10-22% for males and 7-20% for females. At 78 weeks, the body weights of 3200 ppm males were 20% lower, females were 16% lower than of the control group. When evaluating effects on body weight in the mouse, the CARC considers body weight to be more relevant than body weight gain. No increased mortality was observed. Other effects seen at 3200 ppm included increased liver weights accompanied by an increased incidence of liver masses and nodules, and altered liver foci.

Mutagenicity

Fluopicolide has intrinsic mutagenic potential but this activity is not expressed *in vivo*. Consequently, there is no concern for mutagenicity at this time.

Structure Activity Relationship (SAR)

SAR was of limited use in the weight-of-evidence evaluation. Fluazinam is an appropriate analog for fluopicolide, in that it has a pyridine ring like fluopicolide. Fluazinam was classified a "Suggestive Evidence of carcinogenicity, but not sufficient to assess human carcinogenic potential" based on thyroid gland follicular cell tumors in male rats and liver tumors in male mice.

Mode of Action

The CARC concluded that the available data are sufficient to support a plausible non-linear, non-genotoxic mode of action for liver carcinogenicity. A series of steps involved in the mitogenic response have been identified that form the basis for the MOA for liver tumor induction in mice. The initial key event in the induction of liver tumors in mice treated with fluopicolide is enhancement of P450 microsomal enzymes, leading to cell proliferation, and ultimately resulting in liver tumors. This conclusion is based on the following:

- Data from genetic toxicology studies do not suggest a mutagenic concern;
- There is dose-concordance between liver tumors, cell proliferation and hepatic microsomal enzyme induction;
- A temporal relationship supporting the MOA was demonstrated. The mitogenic proliferative response was identified as early as 7 days after the onset of treatment, which declined after 28 days of treatment.

Classification and Quantification of Carcinogenic Potential

In accordance with the EPA's Final Guidelines for Carcinogen Risk Assessment (March, 2005), the CARC classified Fluopicolide as **"Not Likely to be Carcinogenic to Humans"** based on convincing evidence that a non-genotoxic, mitogenic mode of action for liver tumors was established in the mouse and that the carcinogenic effects were not likely at doses that do not cause perturbations of the liver. Quantification of carcinogenic potential is not required. The cRfD, which is based on the chronic toxicity/carcinogenicity rat study, is protective of both chronic and carcinogenic effects.

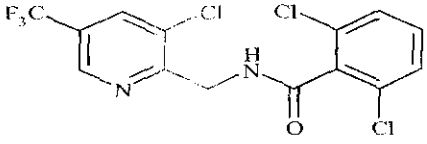
I. INTRODUCTION

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.

Figure 1. Structure of Fluopicolide



III. EVALUATION OF CARCINOGENICITY STUDIES

1. Combined Chronic Toxicity/Carcinogenicity Study with Fluopicolide in CD 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 up to 2500 ppm with AE C638206 for 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 and kidney 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 CD Rats Fed Fluopicolide

Organ/Lesion	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	7 (1.3)	10 (1.2)	9 (1.3)	20** (1.3)	20** (2.1)
Medulla granular casts	0	0	0	0	7** (1.6)
Males (104 weeks)					
THYROID	n = 60	n = 37	n = 37	n = 35	n = 60
cystic follicular hyperplasia	0	1	0	4	7**
KIDNEY	n = 60	n = 60	n = 60	n = 60	n = 60
Tubular casts	24 (1.6)	30 (1.5)	32 (1.4)	32 (1.5)	45** (2.0)
Cortical tubular dilatation	10 (1.8)	9 (2.0)	9 (2.0)	8 (2.0)	27** (2.0)
Cortical cysts	3 (2.0)	2 (2.0)	6 (2.3)	9 (2.1)	11* (2.5)
Papilla mineralization	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.
( ) Indicates severity of lesion 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

The CARC considered dosing at the high dose of 2500 ppm to be adequate to assess the carcinogenicity of fluopicolide in rats. This was based on decreased overall body weight gain in male (↓11%) and female (↓17%) rats at 2500 ppm, increased thyroid weight, and the non-neoplastic thyroid lesions observed at 2500 ppm in males. Effects were minimal in female rats. 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, along with those of the main study, indicate that dosing in the chronic rat study at 2500 ppm was adequate for both sexes.

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 Mortality and Tumor Data

Mortality

There were no statistically significant incremental changes in mortality with increasing doses of Fluopicolide in male or female mice (Tables 2 and 3) (Memo, L. Brunsman, 10/17/06, TXR No. 0054375).

Table 2. 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.

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.

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 4) (Memo, L. Brunsman, 10/17/06, TXR No. 0054375).

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 5) (Memo, L. Brunsman, 10/17/06, TXR No. 0054375).

Table 4. 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 5. 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 $p < 0.01$.

When compared to historical controls of the testing facility (Table 6), the incidence of hepatocellular adenomas and combined adenomas and/or carcinomas in the current study exceeded that of the historical control in both males and females. 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 6. Incidence of Tumors in Historical Controls C57B1/6 Mice		
	Males (%) (N = 149)	Females (%) (N = 150)
Adenoma	11 (3.7)	2 (0.7)
Carcinoma	24 (8.0)	32 (10.7)
Adenoma + Carcinoma	35 (11.7)	34 (11.4)

C. Non-Neoplastic Lesions

The non-neoplastic lesions observed in the liver of both sexes of mice are presented in Table 7. 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. The hepatocellular hypertrophy was correlated in both sexes with increased liver weight (at 400 and 3200 ppm) and liver enlargement (at 3200 ppm). However, the liver changes at 400 ppm were considered to be adaptive and were not considered to be adverse. The high-dose animals also had a significantly increased incidence of altered liver cell foci at 3200 ppm.

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

The CARC considered dosing at 3200 ppm to be adequate, but not excessive, to assess the carcinogenic potential of fluopicolide in mice. This was based on significant decreases in body weight of both male and female mice at 3200 ppm. From weeks 13-52 of the study, body weight decreases ranged from 10-22% for males and 7-20% for females. At 78 weeks, the body weights of 3200 ppm males were 20% lower, females were 16% lower than of the control group. When evaluating effects on body weight in the mouse, the CARC considers body weight to be more relevant than body weight gain. No increased mortality was observed. Other effects seen at 3200 ppm included increased liver weights accompanied by an increased incidence of liver masses and nodules, and altered liver foci.

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 (MRIDs 46474224–46474237). 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.

2. Mutagenicity

Fluopicolide was tested in 12 genetic toxicity assays, in an effort to characterize its mutagenic potential. One of five *Salmonella typhimurium* bacterial reverse mutation assays was positive in independently conducted plate incorporation and pre-incubation assays but only at precipitating concentrations and only in the presence of S9 activation. The remaining Ames assays (performed by different investigators and at different time intervals) were consistently negative when assayed using either the plate incorporation or pre-incubation techniques up to comparable and insoluble levels. The evidence of gene mutations in bacteria is, therefore, conflicting and was not supported by the negative findings of the mammalian cell gene mutation assay in cultured Chinese hamster lung (V79) cells In contrast, fluopicolide was clastogenic in the V79 cell line; the response was generally seen at cytotoxic concentrations (<50% decrease in the mitotic index, MI); however, activity was still detected (in the form of chromatid breaks, chromosome deletions, rings and dicentrics) at levels causing moderate to low cytotoxicity. This response was not seen in either human lymphocytes *in vitro* up to cytotoxic and precipitating levels or in three independently conducted mouse micronucleus assays using either the oral or intraperitoneal route of administration and levels up to the limit dose or an overtly toxic dose. Similarly, fluopicolide did not induce unscheduled DNA synthesis in rat hepatocytes harvested from male rats treated up to the limit dose. From the overall results, it was concluded that fluopicolide has intrinsic mutagenic potential but this activity is not expressed *in vivo*. Consequently, there is no concern for mutagenicity at this time.

All studies were classified as **acceptable/guideline** and satisfied the requirement for FIFRA Test Guideline 84-2 for mutagenicity data. It should be noted that fluopicolide as AE C638206 Technical, AE C638206 00 IC99 0005, AE C638206 00 IB99 0002, or AE C638206 00 IC99 0001 were tested in the mutagenicity assays as indicated. These designations are company codes and are all for fluopicolide technical. Each code provides the Chemical, Batch No, and Purity (%) in one expression (MRID 46474245). Summaries of the 12 assays are presented below:

(i) In three independent trials of the Ames assay, AE C638206 (fluopicolide, 97.8%), when tested in *Salmonella typhimurium* strains TA98, TA100, TA1535 and TA1537 at concentrations of 2000 to 5000 µg/plate +S9 , (fluopicolide) is considered mutagenic only at precipitating concentrations in strain TA 98 using both the plate incorporation and pre-incubation protocols. (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 0002 (fluopicolide, 95.6%),in the presence and

absence of metabolic activation at concentrations up to 5000 *u*g/plate, no increases in revertant colonies were found in either test series (both the plate incorporation and pre-incubation protocols) 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 (fluopicolide, 97.8%), 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 *u*g/plate. No increases in revertant colonies were found in either test series (both the plate incorporation and pre-incubation protocols) 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 **acceptable/guideline** and satisfies 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 *u*g/plate. No increases in revertant colonies were found in either test series (both the plate incorporation and pre-incubation protocols) at concentrations up to the limit dose, 5000 ug/plate. Therefore, AE C638206 00 IB99 0002 (fluopicolide, 99.3%), 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 (fluopicolide, 95.9%), 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 *u*g/plate. No increases in revertant colonies were found in either test series (both the plate incorporation and pre-incubation protocols) 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 (fluopicolide, **97.8%),** 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 (38.2 *u*g/mL –S9; 30 *u*g/mL +S9). Compound precipitation was noted at ≥30 *u*g/mL. 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 to Technical AE C638206 (fluopicolide, 97.8%), in the presence and absence of metabolic activation for 3 hours, at 7 concentrations ranging from 3.2 to 100 *u*g/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 *u*g/mL, and sampled at the end of treatment (EXPERIMENT II).

Dose-related severe cytotoxicity (<50% reduction in survival and mitotic indices relative to control values) was evident in both trials at ≥6.3 *u*g/mL –S9 and 100 *u*g/mL +S9. Macroscopically visible precipitation of the test article was observed at ≥ 250 *u*g/mL. Under non-activated and activated conditions of both trials, however, there were reproducible statistically significant ($p \leq 0.05$) increases in structural aberrations (manifested as chromatid breaks, chromosome deletions, rings and dicentrics). The incidence of numerical chromosome aberrations were unaffected by treatment. Therefore, AE C638206 is considered a clastogen in the *in vitro* Chinese hamster lung (V79) test 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 (fluopicolide, **95.9%)** was not clastogenic up to concentrations showing ≈ 50% cytotoxicity (156.25 or 19.53 *u*g/mL, 3 or 21 hour exposure -S9 or ≥312.5 *u*g/mL, 3 exposure +S9) in this *in vitro* mammalian ~~cell~~ test system (MRID 46474208).

(ix) In a mouse bone marrow micronucleus assay groups of NMRI mice (5M:5F/group) were administered AE C638206 00IC99 0005 (fluopicolide, **97.8%)** 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 (fluopicolide, **96.1%)** 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 slightly increased compared to the concurrent vehicle (negative) control (1.50 *vs.* 0.88 PCEs/1000 cells). The finding was considered anomalous; therefore, AE C638206 is classified as negative in this *in vivo* test system (MRID 46474212).

(xi) In a bone marrow micronucleus assay, male NMRI mice were administered two doses 24 hours apart of AE C638206 (fluopicolide, 99.4%) 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

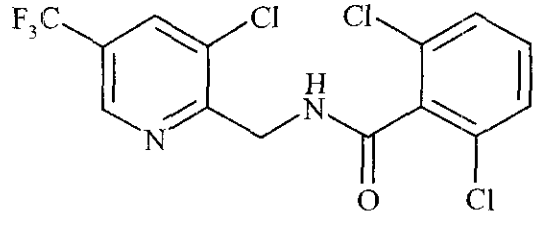
increase ($p < 0.05$) in the number of NCEs per PCEs at the highest dose tested (HDT). However, at no dose up to the HDT was a significantly increased number of mPCEs recorded, in the presence of a statistically increased ratio of PCEs to NCEs (evidence of interference with erythropoiesis), either when compared to vehicle controls, or to the laboratory's 8-year historical control database. 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 ip up to a clinically toxic level (MRID 46474214).

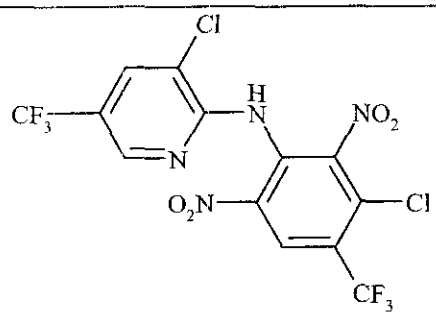
(xii) In an in vivo unscheduled DNA synthesis (UDS) assay, 2 groups of male rats (4/group) were administered single oral doses of AE C638206 00 IC99 0005 (fluopicolide, **97.7%**) at levels of 600 or 2000 mg/kg (the latter corresponds to the internationally recognized limit dose). There was no evidence (or dose-related positive response) that UDS, as determined by radioactive tracer procedures [nuclear silver grain counts], was induced at either timed sacrifice (MRID 46474216).

3. Structure-Activity Relationship

The CARC concluded that Fluazinam is an appropriate analog, in that it has a pyridine ring like fluopicolide. See structure below. It also targets the same organ—the liver. Although the chlorine in the benzene ring is expected to be activated by the two nitro groups to yield potential eletrophilic arylating intermediates, the finding of negative genotoxicity argues against a genotoxic mechanism.

Fluopicolide, however, is unlikely to be electrophilic, and hence not genotoxic. For fluopicolide, chlorine in the pyridine ring is in the inactive meta position, not the active ortho position. Chorine in the benzene ring will be also inactive for fluopicolide.

Chemical Name	**Structure**
Fluopicolide (AE C638206 or 2,6-dichloro-*N*-[[3-chloro-5-(trifluoromethyl)-2-pyridinyl]methyl]benzamide) Caswell#: None 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 **"Suggestive evidence of carcinogenicity, but not sufficient to assess human carcinogenic potential"** based on the following weight-of-the-evidence considerations: 1) There was some evidence in that fluazinam induced an increase in thyroid gland follicular cell tumors in male rats, but not in female rats. In one study in mice, there was clear evidence that an increased incidence of hepatocellular tumors observed in the male mice was treatment-related. In another study in mice, there was equivocal/some evidence that fluazinam may have induced an increase in hepatocellular tumors in the male mice. Increases in hepatocellular tumors observed in the female mice in the latter study were not statistically significant and some occurred at an excessively toxic dose level. The thyroid gland follicular cell tumors of concern were seen only in male rats and the hepatocellular tumors of concern were seen only in male mice. 2) Fluazinam was negative in mutagenicity assay

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 treatment-related effects were noted for 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. 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.

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

The registrant submitted mode of action studies (MRIDs 46474132, 46474133) in connection with the observed mouse liver tumors (MRID 46474130), which suggest Fluopicolide produces liver tumors by a mechanism similar to that of the drug Phenobarbital. The observed effects are increased hepatocyte proliferation and increased levels of hepatic microsomal enzymes, such as cytochrome P450. Executive Summaries of the two studies are listed below.

Fluopicolide Mechanistic Study: 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. Total Cytochrome P-450 (CYP450) was measured in liver microsomes following reduction by carbon monoxide. The activities of CYPIA1 and CYPIA2 were measured 1by 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 β-naphtho-flavone (CYPIA activities), phenobarbital (CYPII and CYP III activities), and clofibric acid (CYPIVA activity) were used in the assessment of each isoenzyme.

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), 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.

Summarized Key Events

Available data from the mode of action studies (MRIDs 46474132, 46474133) suggest that Fluopicolide induces P450 expression, leading to cell proliferation, and ultimately resulting in liver adenomas. The key events in the non-genotoxic mode of action are discussed below:

- *Induction of hepatic P450 microsomal enzymes.*

  The total cytochrome P450 content of liver microsomes was 2-fold greater in mice treated for 7 days with 3200 ppm fluopicolide than in control mice (2.19 vs 1.11 nmol/mg protein, $p<0.01$). Treatment for 7 days with 3200 ppm fluopicolide greatly induced the activities of microsomal enzymes benzoxyresorufin O-debenzylase (BROD; 19x controls) and pentoxyresorufin O-dependentylase (PROD; 12x controls), but had less impact on ethoxyresorufin O-deethylase (EROD; 1.8x controls) and lauric acid hydroxylase (0.33x of controls) (Table 8). These microsomal enzyme changes are similar to those resulting

from treatment with phenobarbital.

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 (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 62-68 of MRID 46474132. [1]Number in parentheses is the multiple of the control value, calculated by the reviewer. *Statistically different ($p \leq 0.05$) from the control.

In the phenobarbital mechanistic study, 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.

In addition, there are a number of characteristic morphological changes that are associated with CYP induction including an increase in liver weight and centrilobular hypertrophy, as follows:

- Increased Liver Weights: In the 28 day fluopicolide mechanistic study (MRID 46474132), mean absolute and relative (to body or brain) liver weight was increased at both the interim sacrifice (27-38%) on Day 8 and final sacrifice (48-59%) on Day 29 (Table 9). 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).

TABLE 9. 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. [1]Numbers in parentheses are the percent of control, calculated by the reviewer using data on pages 49-50 of MRID 46474132. ** Statistically different ($p < 0.01$) from the control.

In the phenobarbital mechanistic study (MRID 46474133), 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.

In the mouse cancer study (MRID 46474130), absolute and relative liver weights were significantly increased in 400 ppm males (15-30%), and in 3200 ppm males and females (35-99%), after 52 weeks. 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.

- Hepatocellular hypertrophy and altered cell foci: The effects on liver hypertrophy seen in the mouse cancer study were similar to the effects seen in the mouse mechanistic study, as were the microscopic changes. In the fluopicolide 28-day mechanistic study, 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) (Table 10).

TABLE 10. 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.

In the phenobarbital mechanistic study, 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.

In the mouse cancer study at 3200 ppm, altered liver cell foci were observed (most common type was acidophilic) after 78 weeks, and a non-significant increase after 52 weeks (≤2/10 for each lesion).

- *Hepatic cell proliferation.*

In the fluopicolide 28-day mechanistic study, liver samples from interim sacrifice animals (7 days) had a significant increase in the BrdU labeling index (6.5x of controls), consistent with increased cell proliferation (Table 11). At terminal sacrifice, however, the BrdU labeling index was not increased, indicating that AE C638206 induced a marked but transient increase in hepatocyte proliferation. This pattern of increased cell proliferation that is not sustained is indicative of a chemical with a mitogenic MOA.

TABLE 11 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. [1]The number in parentheses is the multiple of the control value, calculated by the reviewer. ** Statistically different ($p < 0.01$) from the control group.

• *MOA Limitations:* The CARC acknowledges several limitations with the current MOA data. The first is that only one dose was tested (albeit it was the dose at which tumors occurred) and, therefore, no dose response data were able to be evaluated for the key events. In addition, results for the concurrent positive control data used to validate the measurement of the P450 microsomal enzymes were not included with the MOA study, however, a positive control study with Phenobarbital performed a year earlier showed that the same CYPs (e.g. PROD and BROD) were induced by phenobarbital and fluopicolide.

Alternative Modes of Action

Fluopicolide does not act through a genotoxic MOA. Alternative modes of action were not presented by the registrant.

Reversibility

No studies were submitted to address the reversibility of the effects. Although these data are desirable, the lack of a reversibility study does not discount the MOA.

Relevance to Humans

The key events in this non-genotoxic mode of action are considered plausible to humans. It is possible that increased cell proliferation and tumor response can occur if the dose levels were high enough.

The MOA is applicable to all populations including children. Although metabolic enzyme systems in children do not reach adult levels of activity until six – 12 months of age, it can still be assumed that the MOA will hold, and the NOAEL will be protective.

MOA CONCLUSION: The CARC concluded that the available data are sufficient to support a plausible non-linear, non-genotoxic mode of action for liver carcinogenicity. The CARC agreed that a series of steps involved in the mitogenic response have been identified that form the basis for the MOA for liver tumor induction in mice. The initial key event in the induction of liver tumors in mice treated with fluopicolide is enhancement of P450 microsomal enzymes, leading to cell proliferation, and ultimately resulting in liver tumors.

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

The Committee's assessment of the weight-of-the-evidence is discussed below:

1. Carcinogenicity

Rat

- No treatment-related tumors were seen in male or female CD rats.

- *Adequacy of Dosing:* The CARC considered dosing at the high dose of 2500 ppm to be adequate to assess the carcinogenicity of fluopicolide in rats. This was based on decreased overall body weight gain in male (↓11%) and female (↓17%) rats at 2500 ppm, increased thyroid weight, and the non-neoplastic thyroid lesions observed at 2500 ppm in males. Effects were minimal in female rats. However, a reproductive study and a companion, supplemental study providing 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. These results, along with those of the main study, indicate that dosing in the chronic rat study at 2500 ppm was adequate for both sexes.

Mouse

- In male C57BL/6 mice, the incidences of liver tumors for the control, 50, 400, and 3200 ppm dose groups, respectively, were as follows:

  Adenomas: 5/47 (11%), 0/49 (0%), 5/48 (10%), 11/49 (22%)
  Carcinomas: 3/47 (6%), 1/49 (2%), 0/48 (0%), 2/49 (4%)
  Combined: 7/47 (15%), 1/49 (2%), 5/48 (10%), 13/49 (27%)

There were 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. However, the incidences of adenomas (22%), carcinomas (4%) and combined (27%) at the high dose, exceeded the historical control data from the testing laboratory (6%, 10%, adenomas; 0%1%, carcinomas; 7%, 10%, combined). Therefore, the CARC concluded that the liver tumors (adenoma driven) noted at the highest dose tested (3200 ppm) in males were considered to be treatment-related. The CARC also noted that the liver tumor response seen in males was weaker than that seen in the females.

- In female C57BL/6 mice, the incidences of liver tumors for the control, 50, 400, and 3200 ppm dose groups, respectively, were as follows:

Adenomas: 1/58 (2%), 2/60 (3%), 1/60 (2%), 19/57 (33%)
Carcinomas: 0/58 (0%), 0/60 (0%), 2/60 (3%), 0/57 (0%)
Combined: 1/58 (2%), 2/60 (3%), 3/60 (5%), 19/57 (33%)

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 incidences of adenomas (33%) and combined (33%) at the high dose exceeded the historical control data (0%, 2%, adenomas; 0%, 3%, combined) for the testing laboratory. Therefore, the CARC concluded that the liver tumors (adenoma driven) noted at the highest dose tested (3200 ppm) in females were considered to be treatment-related.

▪*Adequacy of Dosing:* The CARC considered dosing at 3200 ppm to be adequate, but not excessive, to assess the carcinogenic potential of fluopicolide in mice. This was based on significant decreases in body weight of both male and female mice at 3200 ppm. From weeks 13-52 of the study, body weight decreases ranged from 10-22% for males and 7-20% for females. At 78 weeks, the body weights of 3200 ppm males were 20% lower, females were 16% lower than of the control group. When evaluating effects on body weight in the mouse, the CARC considers body weight to be more relevant than body weight gain. No increased mortality was observed. Other effects seen at 3200 ppm included increased liver weights accompanied by an increased incidence of liver masses and nodules, and altered liver foci.

2. Mutagenicity

Fluopicolide has intrinsic mutagenic potential but this activity is not expressed *in vivo*. Consequently, there is no concern for mutagenicity at this time.

3. Structure Activity Relationship (SAR)

SAR was of limited use in the weight-of-evidence evaluation. Fluazinam is an appropriate analog for fluopicolide, in that it has a pyridine ring like fluopicolide. Fluazinam was classified a "Suggestive Evidence of carcinogenicity, but not sufficient to assess human carcinogenic potential" based on thyroid gland follicular cell tumors in male rats and liver tumors in male mice.

4. *Mode of Action*

The CARC concluded that the available data are sufficient to support a plausible non-linear, non-genotoxic mode of action for liver carcinogenicity. A series of steps involved in the mitogenic response have been identified that form the basis for the MOA for liver tumor induction in mice. The initial key event in the induction of liver tumors in mice treated with fluopicolide is enhancement of P450 microsomal enzymes, leading to cell

proliferation, and ultimately resulting in liver tumors. This conclusion is based on the following:

- Data from genetic toxicology studies do not suggest a mutagenic concern;
- There is dose-concordance between liver tumors, cell proliferation and hepatic microsomal enzyme induction;
- A temporal relationship supporting the MOA was demonstrated. The mitogenic proliferative response was identified as early as 7 days after the onset of treatment, which declined after 28 days of treatment.

VI. CLASSIFICATION OF CARCINOGENIC POTENTIAL

In accordance with the EPA's Final Guidelines for Carcinogen Risk Assessment (March 2005), the CARC classified Fluopicolide as **"Not Likely to be Carcinogenic to Humans"** based on convincing evidence that a non-genotoxic, mitogenic mode of action for liver tumors was established in the mouse and that the carcinogenic effects were not likely at doses that do not cause perturbations of the liver.

VII. QUANTIFICATION OF CARCINOGENIC POTENTIAL

Quantification of carcinogenic potential is not required. The cRfD, which is based on the chronic toxicity/carcinogenicity rat study, is protective of both chronic and carcinogenic effects.

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Attachment: Minority Opinion on Fluopicolide

DATE: December 12, 2006

FROM: Esther Rinde, Ph.D. Esther Rinde

TO: William Burnam, Chair
Cancer Assessment Review Committee (CARC)

Administration of Fluopicolide (a food use chemical) in the diet was associated with a treatment related increase in liver adenomas in both sexes of the mouse. The incidences at the highest dose exceeded that of the performing laboratory's historical control data in both sexes. There was a statistically significant trend ($p<0.01$) for these tumors in both sexes - and in female mice there was also pairwise statistical significance for adenomas ($p<0.01$) at the highest dose; in male mice there was a doubling in the incidence of adenomas at the highest dose. This highest dose tested in the mouse was deemed adequate and not excessive, but the spacing between the highest and middle dose was too wide: 0, 50, 400, 3200 ppm (which may be why tumors were only seen at 3200 ppm.).

Mode of Action

The registrant submitted only <u>one</u> mode of action study wth Fluopicolide, which was designed to show that Fluopicolide (like Phenobarbital) induces hepatocyte proliferation and increased levels of hepatic microsomal enzymes, such as Cytochrome P-450, in the mouse. However, unlike Phenobarbital, for which these effects have been well established, <u>only 1 dose level</u> of Fluopicolide was tested. No studies were submitted to address the reversibility of the effects. Also, no concurrent positive control data were provided. Alternative Modes of Action were not presented by the Registrant.

Our Guidelines (1-10) state:
"Elucidation of a mode of action for a particular cancer response in animals or humans is a **data-rich** *{emphasis added}* determination. Significant information should be developed to ensure that a scientifically justifiable mode of action underlies the process leading to cancer at a given site. In the absence of sufficiently, scientifically justifiable mode of action information, EPA generally takes public-health-protective default positions regarding the interpretation of the toxicologic and epidemiologic data.."

Our Guidelines (2-47) also state:
"Experimental challenge to the hypothesized mode of action, where interrupting the sequence of key events suppresses the tumor response or enhancement of key events increases the tumor response, creates very strong support for the mode of action."

The MOA evidence provided by the Registrant was not **data-rich** or sufficient to depart from the public-health-protective default position on the mouse liver tumors. Therefore, I disagree with the CARC conclusion that *".. the available data are sufficient to support a plausible non-linear...mode of action for liver carcinogenicity"*, because in the Registrant's submission of MOA data - <u>Only one dose was tested</u> and supporting information was not provided: thus, a more appropriate descriptor for the mouse liver tumors would have been "Suggestive Evidence."

13544

R134768

Chemical:	**Thiophanate-methyl**
	PC Code:
	102001
HED File Code:	**61400 SRRD DERs**
Memo Date:	**10/14/2006**
File ID:	**00000000**
Accession #:	**412-07-0027**

HED Records Reference Center
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