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

OFFICE OF
PREVENTION, PESTICIDES, AND

MEMORANDUM

DATE: March 22, 2007

SUBJECT: Cancer Assessment Review Committee Meeting on Pyrasulfatole

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

TO: Addressees

Attached for your review is a package on Pyrasulfatole prepared by Rob Mitkus.

A meeting to review the carcinogenicity classification of this chemical is scheduled for Wednesday, 4/04/07 at 10 am in Room S-10100, PY1.

Addressees:

G. Akerman
L. Austin
K. Bailey
L. Brunsman
W. Burnam
M. Copley
V. Dellarco
K. Farwell
R. Kent
J. Kidwell
M. Manibusan
R. Mitkus
N. McCarroll
J. Pletcher
E. Rinde
J. Rowland
P.V. Shah
Yin-Tak Woo

CANCER ASSESSMENT DOCUMENT

FOR COMMITTEE DELIBERATION

EVALUATION OF THE CARCINOGENIC POTENTIAL OF
PYRASULFOTOLE

PC CODE 000692

April 4, 2007
Submitted by: Robert J. Mitkus, PhD

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

CONTENTS

4.

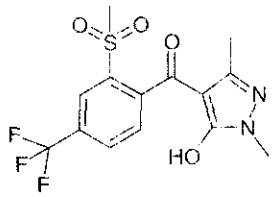
I. INTRODUCTION

On April 4, 2007, the Cancer Assessment Review Committee of the Health Effects Division of the Office of Pesticide Programs met for the first time to evaluate the carcinogenic potential of pyrasulfotole. The toxicological database is being jointly reviewed by Australian (APVMA), Canadian (PMRA), and American (EPA) regulators. Data reviews ("DERs") are currently in the process of being finalized.

II. BACKGROUND INFORMATION

Pyrasulfotole is a post-emergence benzylpyrazole herbicide that is proposed to be used on several broadleaf species of weeds in various cereal crops.

Chemical Name:	Pyrasulfotole
IUPAC Name:	(5-hydroxy-1,3-dimethyl-1H-pyrazol-4-yl)[2-(methylsulfonyl)-4-(trifluoromethyl)phenyl]methanone
Chemical Formula:	$C_{14}H_{13}F_3N_2O_4S$
Chemical Structure:	



CAS Registry #:	365400-11-9
PC CODE:	000692

Pyrasulfotole is an inhibitor of the enzyme 4-hydroxyphenylpyruvate dioxygenase (HPPD). In mammals, HPPD catalyzes the reversible conversion of 4-hydroxyphenylpyruvate (HPP) to homogentisate, primarily in the liver (Figure 1). Blockage of tyrosine catabolism at this point leads to a reconversion of HPP to tyrosine and a consequent increase in blood tyrosine concentrations (tyrosinemia). Blockage of HPPD in plants leads to inhibition of photosynthesis; this phytotoxicity is the basis of the herbicidal action of HPPD inhibitors. Based on selective modification of the model HPPD inhibitor NTBC, tight binding of HPPD inhibitors is accomplished between the enol tautomer of triketones and a ferric iron (Fe^{2+}) bound to HPPD (Wu et al. 2002).

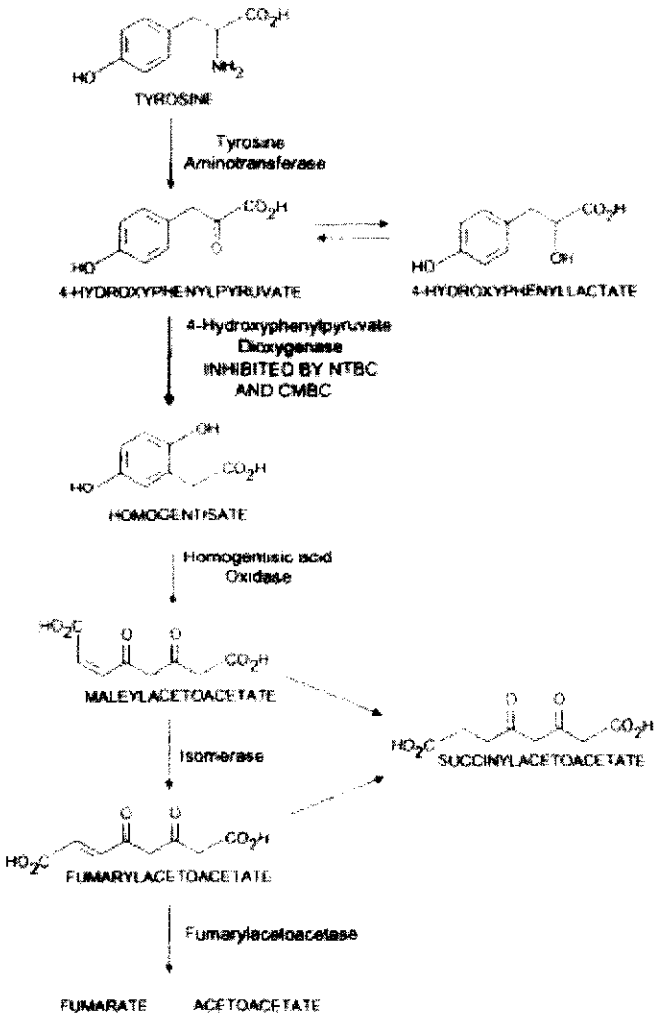


Figure 1. The pathway of mammalian tyrosine catabolism (Reproduced from Ellis et al 1995). Note: the conversion of tyrosine to 4-hydroxyphenylpyruvate by tyrosine aminotransferase is reversible.

III. EVALUATION OF CARCINOGENICITY STUDIES

1. Combined Chronic Toxicity/Carcinogenicity Study (Rats)

Reference: Wason S. (2006). 6-Month toxicity, chronic toxicity and carcinogenicity study of AE 0317309 in the Wistar rat by dietary administration. Bayer CropScience, Sophia Antipolis Cedex, France. Laboratory Study No.: SA 02453, February 28, 2006. MRID 46801910.

A. Experimental Design

Pyrasulfotole (95.7% a.i.) was administered in the diet to 75 six-week-old Wistar Rj:WI (IOPS HAN) rats/sex/group at dose levels of 0, 25, 250, 1000, or 2500 ppm (equivalent to 0/0, 1.0/1.4, 10/14, 41/57, or 104/140 mg/kg bw/day in males/females) for 24 months. Animals were sacrificed at 6 (10/dose group), 12 (10/dose group), and 24 months (55/dose group).

B. Discussion of Tumor Data

Mortality

Mortality was statistically significantly increased to 72.7% in high-dose males at 24 months. While treatment-related, the increase in mortality did not compromise the validity of the study, since it was less than 75% (OPPTS 870.4300).

Table 1. Mortalities in male rats treated with pyrasulfotole

Month	Dose (ppm)									
	Males					Females				
	0	25	250	1000	2500	0	25	250	1000	2500
Mortality (N=55)										
6	1	1	0	1	4	0	0	0	0	0
12	2	6	2	2	8	2	1	0	1	0
24	30	38	25	29	40*	28	19	25	22	26
Mortality (%)										
6	1.3	1.3	0.0	1.3	5.3	0.0	0.0	0.0	0.0	0.0
12	3.1	9.2	3.1	3.1	12.3	3.1	1.5	0.0	1.5	0.0
24	54.5	69.1	45.5	52.7	72.7	50.9	34.5	45.5	40.0	47.3

*p< 0.05

Corneal Tumors

An increased incidence of corneal tumors was observed in high-dose males (2500 ppm; 104 mg/kg/day). The incidences of these rare tumors observed in two males were not analyzed statistically, since they were so low individually (1/55 each) and when combined (2/110). However, the incidence did exceed the historical control incidence of these tumors (0/403 in males) in 7 studies conducted from 2000-05 at Bayer CropScience Centre de Recherche Sophia Antipolis under the current head of pathology. The registrant considered these tumors to be treatment-related and suggested that they resulted from corneal inflammation and regenerative hyperplasia due to tyrosinemia. Photos of the tumors are attached in the Appendix. Squamous cell corneal tumors were observed in male rats treated with the HPPD inhibitor tembotrione at 200 ppm (4/60) and 800 ppm (2/60); however, this study has not been reviewed by CARC. Ocular tumors were not seen when the HPPD inhibitors mesotrione, topramezone, and isoxaflutole were each tested in rats for two years at doses up to 190, 381.5, and 500 mg/kg/day, respectively.

Table 2. Male rats: Incidence of corneal tumors

Concentration (ppm):	25	250	1000	2500
Dose (mg/kg/day):	1	10	41	104
Tumor Type				
Squamous cell papilloma	0/55	0/55	0/55	1/55
Squamous cell carcinoma	0/55	0/55	0/55	1/55
Combined	0/55	0/55	0/55	2/110

No treatment-related tumors were observed in female rats.

C. Non-Neoplastic Histopathological Findings

Treatment-related, non-neoplastic microscopic findings were observed in the eyes, liver, pancreas, thyroid gland, and kidneys of both sexes at >25 ppm (Tables 3-7).

Eyes

In the eyes, the incidence of corneal inflammation was significantly increased at all time points in males at dietary concentrations of 250 ppm and above, and in females at all time points at 1000 and 2500 ppm. At 24 months, there was a slight increase (NSS) in corneal inflammation in males at 25 ppm (5%). The historical control range of unilateral and bilateral inflammation of the cornea is 0/60-3/59 (0-5%) in males and 0/60-2/60 (0-3%) in females in 7 studies conducted from 2000-05 at Bayer CropScience Centre de Recherche Sophia Antipolis under the current head of pathology. The incidences of corneal inflammation at ≥250 ppm were therefore outside the historical control range. Regenerative hyperplasia of the cornea was increased in males at all time points at 250 ppm and above, and in females at all time points at 1000 and 2500 ppm. Neovascularization of the cornea was increased in males at 6, 12, and 24 months at 250 ppm and above, in females at 6 and 12 months at 1000 and 2500 ppm, and in females at 24 months at 250 ppm and above. At 24 months, there was an increase in males in the incidence of mucous metaplasia of the cornea at 250 ppm and above. Also at 24 months, corneal atrophy was

increased in males at 250 ppm and above and in females at 1000 and 2500 ppm, and peripheral retinal atrophy was statistically significantly increased in both males and females at 250 ppm and above. The increase in corneal inflammation in males is likely a precursor event to the regenerative hyperplasia observed at this same dose. If treatment-related, the observed corneal tumors may be considered a consequence of the regenerative hyperplasia.

Table 3. Incidence of non-neoplastic microscopic findings in the eyes

Finding	Dose (ppm)									
	Males					Females				
	0	25	250	1000	2500	0	25	250	1000	2500
6 months										
N examined	9	10	10	10	10	10	10	10	10	10
Inflammation, cornea, unilateral or bilateral	0	0	6	8	8	0	0	0	6	7
Hyperplasia, cornea, regenerative, unilateral or bilateral	0	0	5	8	7	0	0	0	5	6
Neovascularization, cornea, unilateral or bilateral	0	0	5	7	6	0	0	0	3	6
12 months										
N examined	10	10	9	10	8	9	10	10	9	10
Inflammation, cornea, unilateral or bilateral	0	0	6	9	8	0	1	0	5	7
Hyperplasia, cornea, regenerative, unilateral or bilateral	0	0	6	9	8	0	1	0	4	6
Neovascularization, corneal, unilateral or bilateral	0	1	5	8	8	0	1	0	4	7
24 months										
N examined	55	55	55	55	55	55	55	54	55	55
Inflammation, cornea, unilateral or bilateral	1	3	39**	49**	44**	0	1	2	28**	32**
Hyperplasia, cornea, regenerative, unilateral or bilateral	0	0	31**	38**	32**	1	0	2	24**	25**
Neovascularization, cornea, unilateral or bilateral	2	3	46**	49**	53**	0	0	13**	41**	42**
Vacuolation, cornea, focal	0	0	2	0	2	1	0	0	5	5
Metaplasia, mucous, cornea	0	0	2	9**	11**	0	0	0	0	0
Atrophy, cornea	0	0	11**	25**	24**	0	0	0	5*	6*
Atrophy, retina, peripheral, unilateral or bilateral	2	3	17**	15**	17**	1	4	8*	20**	13**

*p<0.05; ** p< 0.01

Liver

At 6 months, centrilobular hepatocellular hypertrophy was increased in males at all doses and in females from 250 ppm onwards. However, at 12 and 24 months in males this finding was reported only from 250 ppm onwards. In females, there was no centrilobular hepatocellular hypertrophy at 12 months, and at 24 months it was only reported in one animal at 2500 ppm. Centrilobular hepatocellular vacuolation was increased in incidence in males at 6 and 12 months from 250 ppm, but was not observed in females at these time points or in either sex at 24 months. The most consistent finding was centrilobular hepatocellular hypertrophy, which was observed at all time points in males at 250 ppm and above. Plasma cholesterol was statistically significantly increased in both sexes at 250 ppm and above at 7 months (≥20%) and in males at 12 months (≥52%), and was statistically significantly increased in males at 1000 and 2500 ppm at 18 and 24 months (≥33%), and at 2500 ppm in females at 24 months (63%). Triglyceride levels were also increased (NSS) by 124% and 135% in males and females, respectively, at 2500 ppm. The combination of hepatocellular hypertrophy and the low magnitude of increased plasma cholesterol/triglycerides provide evidence of compound bioavailability, rather than hepatotoxicity.

Table 4. Incidence of non-neoplastic microscopic findings in the liver of rats

Finding	Dose of AE 0317309, dietary concentration in ppm									
	Males					Females				
	0	25	250	1000	2500	0	25	250	1000	2500
6 months										
N examined	9	10	10	10	10	10	10	10	10	10
Hypertrophy, hepatocellular, centrilobular	0	8	10	10	10	0	0	3	4	5
Vacuolation, hepatocellular, centrilobular	1	1	5	3	4	0	0	0	0	0
12 months										
N examined	10	10	9	10	8	9	10	10	9	10
Hypertrophy, hepatocellular, centrilobular	0	0	6	10	6	0	0	0	0	0
Vacuolation, hepatocellular, centrilobular	0	0	2	5	5	0	0	0	0	0
24 months										
N examined	55	55	55	55	55	55	55	54	55	55
Hypertrophy, hepatocellular, centrilobular	0	0	17**	11**	13**	0	0	0	0	1

*p < 0.05.; ** p < 0.01

Pancreas

In the pancreas, diffuse acinar degeneration/atrophy was reported at all time points in treated

groups; at 6 months, the incidence of this finding was clearly increased in males and females at 2500 ppm. Only single male animals were recorded with this finding at 12 months at 1000 and 2500 ppm, but the incidence was clearly increased in females at 2500 ppm. At 24 months, there were indications of a dose-response relationship in the incidence of diffuse acinar degeneration/atrophy in both sexes, with statistical significance in males at 1000 and 2500 ppm. Also at 24 months, the incidence of focal acinar degeneration/atrophy was increased in females at 2500 ppm. Other findings included fibrosis, inflammation, and interstitial oedema; however these were generally of a low or sporadic incidence (see table below). It is unlikely that pancreatic histopathology is directly relevant to the hypothesized corneal tumor formation; however, these results have been presented here as support for the adequacy of dosing.

Table 5. Non-neoplastic microscopic findings in the pancreas (number of animals affected)

Findings	Dose of AE 0317309, dietary concentration in ppm									
	Males					Females				
	0	25	250	1000	2500	0	25	250	1000	2500
6 months										
N examined	9	10	10	10	10	10	10	10	9	10
Degeneration / atrophy, acinar, diffuse	0	0	0	0	7	0	0	0	1	8
12 months										
N examined	10	10	9	10	8	9	10	10	9	10
Degeneration / atrophy, acinar, diffuse	0	0	0	1	1	0	0	0	0	7
Fibrosis, interstitial, diffuse	0	0	0	0	5	0	0	0	0	3
Inflammation, acute	0	0	0	1	0	0	0	0	0	5
24 months										
N examined	55	55	54	54	54	55	55	54	55	55
Degeneration / atrophy, acinar, diffuse	0	0	1	8**	9**	0	1	2	4	4
Degeneration / atrophy, acinar, focal	34	34	34	30	30	18	20	25	26	34**
Oedema, interstitial	1	1	0	6	1	0	1	4	6*	2

*p <0.05; ** p < 0.01.

Thyroid

In the thyroid, the incidence of altered colloid was consistently increased in males in all treatment groups. The incidence of increased follicular diameter was increased at 6 and 12 months in males from 250 ppm, but this finding was not observed in males at 24 months or in females at any time point. Pigment deposition in the follicular cells was increased in males at 6 and 12 months from 250 ppm, and at 24 months was statistically significantly increased in both sexes in all treated groups with some suggestion of a dose-response relationship. Focal follicular cell hyperplasia was observed in males at 12 months at 2500 ppm, and at 24 months the incidence was increased above that in control males from 250 ppm. The incidence of diffuse follicular cell hypertrophy was consistently increased in both sexes at 12 and 24 months from 250 ppm.

Table 6. Non-neoplastic microscopic findings in the thyroid (number of animals affected)

Findings	Dose of AE 0317309, dietary concentration in ppm									
	Males					Females				
	0	25	250	1000	2500	0	25	250	1000	2500
6 months										
N examined	9	10	10	10	10	10	10	10	10	10
Altered colloid, basophilic deposits	1	4	8	6	8	0	0	0	0	0
Increased follicular diameter	0	0	1	1	5	0	0	0	0	0
Pigment, brown, follicular cells	0	0	1	1	4	0	0	0	0	0
12 months										
N examined	10	10	9	10	8	9	10	10	9	10
Hyperplasia, follicular cell, focal	0	0	1	0	4	0	0	0	0	0
Hypertrophy, follicular cell, diffuse	0	0	4	5	7	0	0	1	2	1
Altered colloid, basophilic deposits	2	4	8	10	8	0	0	1	1	1
Increased follicular diameter	0	0	5	5	8	0	0	0	0	0
Pigment, brown, follicular cells	1	1	6	8	8	0	0	1	1	0
24 months										
N examined	55	55	54	55	55	55	55	54	55	55
Altered colloid, basophilic deposits	21	24	36**	30	33*	4	3	9	13*	8
Pigment, brown, follicular cells	3	14**	39**	33**	38**	0	7**	14**	14**	18**
Hypertrophy, follicular cell, diffuse	2	2	5	8*	4	0	0	3	7**	2
Hyperplasia, follicular cell, focal	3	2	9	12*	8	0	1	0	1	1

*p<0.05; ** p<0.01

Bayer CropScience sponsored Experimental Pathology Laboratories, Inc. to establish a scientific advisory group consisting of independent consultant pathologists to review several issues involving the thyroid arising from toxicology studies with AE 0317309. The pathology expert group noted that the colloid alterations were present in all groups including controls and that the morphology was similar between control and treated groups; the primary difference being an increase in the number of follicles affected in treated groups. Additionally, colloid changes were seen in the absence of follicular cell hypertrophy, and were not considered to indicate a persistent alteration in thyroid function in this study. Similarly, the brown pigment observed in the

follicular cells was considered to be similar in morphology between control and treated animals. This pigment was evaluated to be suggestive of lipofuscin, which is a normal pigment often associated with aging and seen in a number of organs under untreated conditions. It was the opinion of the pathology expert group that the colloid alteration and pigment deposition observed in rats administered AE 0317309 for two years were representative of normal age-related physiologic changes specific to the rat, and that these findings were not adverse.

Kidney

The incidence of chronic progressive nephropathy (CPN) was slightly increased in males at 6 months at 1000 and 2500 ppm and was clearly increased at 12 months from 250 ppm. At 24 months, chronic progressive nephropathy was common in controls, but the incidence was greater in all treated groups of both sexes, achieving statistical significance in the males. The historical control range for CPN was 27/60-41/60 (45-68%) in males and 16/50-43/60 (32-72%) in females. The incidence of hyperplasia of the collecting ducts was increased in males at 24 months in all treated groups, reaching statistical significance at 1000 and 2500 ppm (see table below). The historical control range for collecting duct hyperplasia was 0/60-9/50 (0-18%) in males and 0/60-6/50 (0-12%) in females.

Table 7. Non-neoplastic microscopic findings in the kidneys (number of animals affected)

Findings	Dose of AE 0317309, dietary concentration in ppm									
	Males					Females				
	0	25	250	1000	2500	0	25	250	1000	2500
6 months										
N examined	9	10	10	10	10	10	10	10	10	10
Nephropathy, progressive, chronic	2	2	2	3	5	0	0	0	0	0
12 months										
N examined	10	10	9	10	8	9	10	10	9	10
Nephropathy, progressive, chronic	3	5	9	10	8	0	0	1	1	0
24 months										
N examined	55	55	55	55	55	55	55	54	55	55
Nephropathy, progressive, chronic	44	51*	54**	52*	51*	37	42	45	45	42
Hyperplasia, collecting ducts	5	11	11	19**	19**	7	9	8	7	5

*p< 0.05; ** p< 0.01

D. Adequacy of the Dosing for Assessment of Carcinogenicity

Dosing may be considered excessive at the high dose in males, since mortality was increased in this group to 72.7% (P<0.05) at 24 months. Dosing was considered adequate, but not excessive in females and all other male treatment groups. Generally, body weight in males was decreased during the study at 1000 (≤10%) and 2500 ppm (≤12%), often reaching statistical significance. Body weights in the males at 250 ppm also tended to be somewhat reduced (≤7%), reaching

statistical significance at day 540. Body weight gains in males generally reflected this pattern, except late in the study (days 540-708) when body weights declined in all groups including controls. There were also indications of depressed final body weights (6%; NSS) and body weight gains (9%) in the female rats at and above 250 ppm. Increases in histopathology of the eyes, pancreas, thyroid gland, and kidneys of both sexes were observed at >25 ppm (Section 1.C). These finding support the adequacy of dosing in this study. Increases in centrilobular hepatocelullar hypertrophy along with minor increases in cholesterol/triglyceride levels were considered markers of compound bioavailability, rather than hepatotoxicity.

2. Carcinogenicity Study (Mice)

Reference: Steiblen, G. (2006). Carcinogenicity study of AE 0317309 in the C57BL/6 Mouse by dietary administration. Bayer CropScience, Sophia Antipolis Cedex, France. Laboratory Study No.: SA 03172, February 17, 2006. MRID 46801909. Unpublished.

A. Experimental Design

Pyrasulftole (95.7% w/w a.i.) was administered in the diet to C57BL/6J mice (50/dose) at doses of 0, 100, 1000, or 4000 ppm for males. Groups of 50 females/group received 0, 100, 1000, or 6000 ppm for the first 10 weeks. The high dose in females was reduced to 4000 ppm from week 11 onwards, because it was considered excessive due to increased mortality. The concentrations resulted in doses of 0/0, 13.6/16.7, 137/168, or 560/713 mg/kg/day (M/F) for up to 78 weeks. Additionally, an interim group of 10 mice/sex/dose were treated similarly for up to 52 weeks and then sacrificed.

B. Discussion of Tumor Data

Mortality

Both male and female mice showed statistically significant increasing trends for mortality with increasing doses of pyrasulfotole, as well as a significant pair-wise comparison of the 4000 ppm dose group with the controls, all at $p < 0.01$ (Tables 8 and 9).

Table 8. Male Mouse Mortality Rates[+] and Cox or Generalized K/W Test Results

	Weeks				
Concentration (ppm)	**1-26**	**27-52**	**53[i]**	**53-81[f]**	**Total**
0	2/60	1/58	9/57	5/48	8/51 (16)**
100	1/60	3/59	9/56	5/47	9/51 (18)
1000	2/59[a]	3/57	10/54	2/44	7/49 (14)
4000	4/60	10/56	9/46	13/37	27/51 (53)**

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

[i]Interim sacrifice at week 53.
[f]Final sacrifice at weeks 79-81.

[a]One accidental death at week 20, dose 1000 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.
If *, then $p < 0.05$. If **, then $p < 0.01$.

Table 9. Female Mouse Mortality Rates[+] and Cox or Generalized K/W Test Results

	Weeks				
Concentration (ppm)	**1-26**	**27-52**	**53[i]**	**53-81[f]**	**Total**
0	3/60	3/57	10/54	9/44	15/50 (30)**
100	0/60	4/60	9/56	5/47	9/51 (18)
1000	2/60	4/58	9/54	3/45	9/51 (18)
4000	14/60	7/46	7/39	12/32	33/53 (62)**

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

[i]Interim sacrifice at week 53.
[f]Final sacrifice at weeks 79-81.

( )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.
If *, then $p < 0.05$. If **, then $p < 0.01$.

Urinary Bladder Tumors

Male mice had statistically significant trends, and significant pair-wise comparisons of the 4000 ppm dose group with the controls, for urinary bladder transitional cell carcinomas, and papillomas and carcinomas combined, all at $p < 0.01$. There was a statistically significant trend for urinary bladder transitional cell papillomas at $p < 0.05$. In addition, although there was only one urethral transitional cell carcinoma at the high dose, there was a statistically significant trend at $p < 0.05$ due to increased mortality at the high dose. In conversations by Lori Brunsman with Dr. John Pletcher, EPA's consulting pathologist, this urethral transitional cell carcinoma should be considered the same tumor type as the transitional cell carcinomas in the urinary bladder. The statistical analyses of the tumors in male mice were based upon Peto's Prevalence Test since there were statistically significant survival disparities among the dose groups (Tables 10 and 11). The historical control incidence for bladder tumors in males was 0/394 across 5 studies (2000-05) performed at Bayer CropScience Centre de Recherche Sophia Antipolis (CRSA) since the appointment of the current head of pathology.

Table 10. Male Mouse Urinary Bladder Transitional Cell Tumor Rates[+] and Peto's Prevalence Test Results

	Concentration (ppm)			
	0	100	1000	4000
Papillomas (%)	0/46 (0)	0/44 (0)	0/43 (0)	3[a]/33 (9)
p =	0.02155*	-	-	0.06781
Carcinomas (%)	0/47 (0)	0/44 (0)	0/43 (0)	8[b]/34 (24)
p =	0.00000**	-	-	0.00011**
Combined (%)	0/47 (0)	0/44 (0)	0/43 (0)	11/34 (32)
p =	0.00000**	-	-	0.00002**

+Number of tumor bearing animals/Number of animals examined, excluding those that died before observation of the first tumor.

[a]First papilloma observed at week 71, dose 4000 ppm.
[b]First carcinoma observed at week 69, dose 4000 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 $p < 0.01$.

Table 11. Male Urethral Transitional Cell Tumor Rates[+] and Peto's Prevalence Test Results

	Concentration (ppm)			
	0	100	1000	4000
Carcinomas# (%)	0/43 (0)	0/42 (0)	0/42 (0)	1[a]/24 (4)
p =	0.01400*	-	-	0.09036

+Number of tumor bearing animals/Number of animals examined, excluding those that died before observation of the first tumor.

#No adenomas observed.

[a]First carcinoma observed at the final sacrifice at week 79, dose 4000 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 $p < 0.01$.

Female mice had statistically significant trends for urinary bladder transitional cell papillomas, carcinomas, and papillomas and carcinomas combined, all at $p < 0.01$. There were significant pair-wise comparisons of the 4000 ppm dose group with the controls for urinary bladder transitional cell papillomas and carcinomas, both at $p < 0.05$, and for urinary bladder transitional cell papillomas and carcinomas combined at $p < 0.01$. The statistical analyses of the tumors in female mice were based upon Peto's Prevalence Test since there were statistically significant survival disparities among the dose groups (Table 12). The historical control incidence for bladder tumors in females was 0/380 across 5 studies performed at CRSA.

Table 12. Female Urinary Bladder Transitional Cell Tumor Rates[+] and Peto's Prevalence Test Results

	Concentration (ppm)			
	0	100	1000	4000
Papillomas (%)	0/35 (0)	0/40 (0)	0/42 (0)	2[a]/19 (11)
p =	0.00042**	-	-	0.02632*
Carcinomas (%)	0/35 (0)	0/40 (0)	0/42 (0)	2[b]/19 (11)
p =	0.00042**	-	-	0.02632*
Combined (%)	0/35 (0)	0/40 (0)	0/42 (0)	4/19 (21)
p =	0.00000**	-	-	0.00260**

+Number of tumor bearing animals/Number of animals examined, excluding those that died before observation of the first tumor.

[a]First papilloma observed at the final sacrifice at week 79, dose 4000 ppm.
[b]First carcinoma observed at the final sacrifice at week 79, dose 4000 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 $p < 0.01$.

C. Non-Neoplastic Histopathological Findings

At 18 months, histopathologic findings included a dose-related increase in the incidence of minor to moderate centrilobular hepatocellular hypertrophy in males and females at 1000 and 4000 ppm, which was statistically significant in males at 1000 and 4000 ppm and females at 4000 ppm. Other findings were observed in the urinary system (kidney, urinary bladder, and ureters) at the high dose in males and females, as well as in the prostate in males, and were associated with stones and concretions observed in the urinary system at the same dose (Table 13). Gallstones were observed at an increased incidence in all treatment groups in both sexes. The historical control incidence of gallstones ranged from 0/60-4/60 (0-7%) in males and from 0/60-1/60 (0-2%) in females.

Table 13. Non-neoplastic microscopic findings in all mice at 18 months

Finding	Dose (ppm)							
	Males				Females			
	0	100	1000	4000	0	100	1000	4000
Liver								
N (includes unscheduled and scheduled deaths)	50	50	50	50	50	50	50	50
Centrilobular hepatocellular hypertrophy	0	1	14**	25**	0	0	3	7**
Hepatocellular vacuolation: diffuse	30	26	20	15	37	39	39	23
Hepatocellular vacuolation: mainly periportal: diffuse	0	0	0	7	0	0	0	6
Interstitial mixed cell infiltrate: focal / multifocal	14	19	18	26**	19	20	25	17
Gall bladder								
Gallstones	4	19**	22**	19**	0	5	14**	5**
Epithelial hyperplasia: focal / multifocal	1	2	6	3	0	4	1	3
Kidney								
Pelvic stones: unilateral	0	0	0	10**	0	0	0	21**
Pelvic stones: bilateral	0	0	0	3	0	0	0	3
Collecting ducts hyperplasia: unilateral	0	0	0	7**	2	0	2	13**
Collecting ducts hyperplasia: bilateral	0	0	0	2	0	1	1	0
Pelvic epithelium hyperplasia: unilateral: focal / multifocal	0	0	1	10**	0	0	1	11**
Pelvic epithelium hyperplasia: bilateral: focal / multifocal	0	0	0	1	0	0	1	4**
Papillary fibrosis / atrophy: unilateral	0	0	2	13**	0	0	1	19**
Papillary fibrosis / atrophy: bilateral	0	0	0	12**	0	0	0	4**
Atrophy / fibrosis / scar: cortex / medulla: unilateral	0	0	1	18**	7	10	20**	26**
Atrophy / fibrosis / scar: cortex / medulla: bilateral	0	1	0	21**	1	0	1	9**
Suburothelial mixed cell infiltrate: focal / multifocal	0	0	0	8*	0	0	0	5**
Interstitial hemorrhage(s): focal / multifocal	0	1	0	8**	0	0	0	7
Glomerular chamber dilatation: focal / multifocal	0	0	1	8**	2	3	4	17**
Tubular dilatation: cortex: diffuse	1	3	3	20**	10	4	8	26**
Pelvic dilatation: unilateral	2	1	2	6	2	0	1	12**
Pelvic dilatation: bilateral	0	1	1	33**	1	0	0	15**
Papillary necrosis: unilateral: focal / multifocal	1	0	1	5	0	1	2	4
Papillary necrosis: bilateral: focal / multifocal	0	0	0	2	0	0	0	0

Papillary necrosis: unilateral / bilateral: focal / multifocal	1	0	1	7*	0	1	2	4
Cortical basophilic tubules: unilateral	8	27**	15	14	18	20	13	23*
Collecting duct concretions: unilateral / bilateral	0	0	0	22**	1	3	3	15**
Cyst(s)	5	3	5	7	1	4	3	15**
Medullary tubular mineralization: focal / multifocal	2	1	1	0	0	1	0	6
Arteritis / periarteritis: focal / multifocal	0	0	0	6*	0	0	0	6**
Urinary bladder								
Stones: intraluminal	0	0	0	17**	0	0	0	8**
Stones: intraglandular	0	0	0	2	0	0	0	0
Urothelial hyperplasia: simple: multifocal / diffuse	0	0	1	20**	0	0	0	31**
Urothelial hyperplasia: nodular / glandular: multifocal / diffuse	0	0	0	40**	0	0	0	13**
Urothelial hyperplasia: squamous: multifocal / diffuse	0	0	0	28**	0	0	0	9**
Urothelial hyperplasia: atypical: focal / multifocal	0	0	0	1	0	0	0	2
Distention	11	7	5	37**	4	4	5	19**
Muscular hemorrhage(s) / necrosis: focal / multifocal	0	0	0	5	0	0	0	8
Vascular congestion: focal / multifocal	0	0	0	8*	0	0	0	10*
Interstitial edema: diffuse	0	0	0	42**	0	0	0	27**
Adenomyosis: focal / multifocal	0	0	0	6*	0	0	0	0
Intramuscular inflammatory cell infiltrate: focal / multifocal	3	0	0	27**	1	1	1	20**
Suburothelial mixed cell infiltrate: focal / multifocal	1	0	1	11*	0	0	1	4
Interstitial mixed cell infiltrate: focal / multifocal	0	0	0	17**	0	0	0	8**
Serosal mixed cell infiltrate: focal / multifocal	0	1	1	11**	0	0	0	12**
Prostate								
Intra-urethral stones	0	0	0	3				
Urethral urothelial hyperplasia: simple focal / multifocal	0	0	0	8**				
Adenomyosis: focal / multifocal	0	0	0	3				
Ureters								
Stones	0	0	0	2	0	0	0	0
Urothelial hyperplasia: simple: multifocal / diffuse	0	0	0	3	0	0	0	1

Urothelial hyperplasia: nodular / glandular: multifocal / diffuse	0	0	0	2	0	0	0	2
Dilatation	0	0	1	4	0	0	0	1
Adenomyosis: focal / multifocal	0	0	0	2	0	0	0	0

Data obtained from Tables 10a , b, c, pages 47 -57, and 186 – 247, in the study report.
* $p < 0.05$; ** $p < 0.01$.

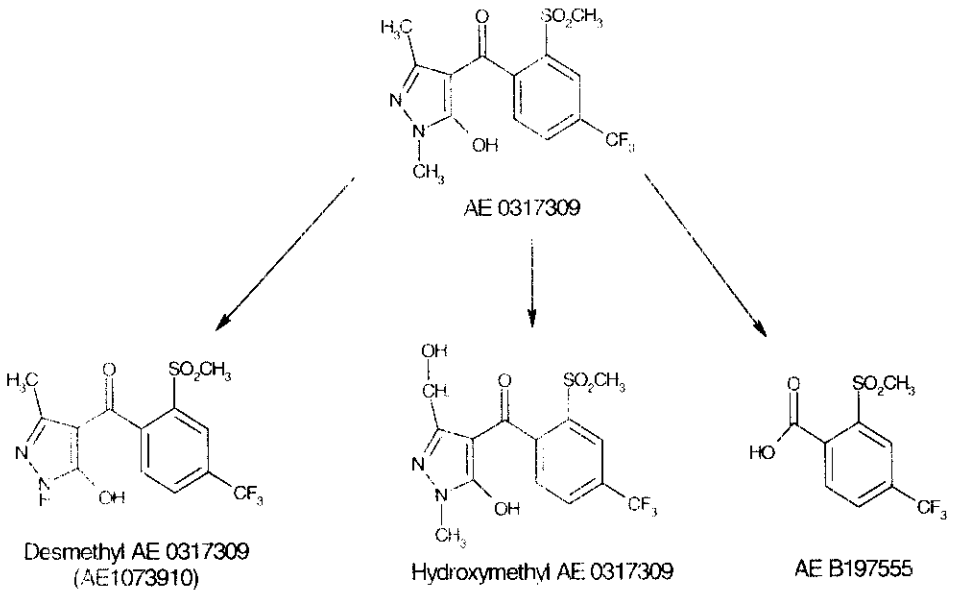
D. Adequacy of Dosing for Assessment of Carcinogenicity

Dosing was considered adequate, but not excessive, in both sexes based on an increase in the incidence of histopathology of the kidney, urinary bladder, gall bladder, and prostate (males only). Body weight gain was decreased by 27% in males and by 14% in females at 4000 ppm. Body weight was decreased by 3-8% in males throughout the study and by 5% in females at the end of the study at 4000 ppm. Body weight was unaffected at 1000 ppm in males. Body weight gain was statistically significantly decreased in this group over the first year of the study; however, overall body weight gain was only 3% for the study. Mean body weight and body weight gain in females were not affected at 1000 ppm.

IV. TOXICOLOGY

1. Metabolism

In a disposition study in male rats (MRID 46801918) in which single doses of pyrasulfotole (10 mg/kg bw) were administered orally, 100% of the dose was recovered in 52 hours, with 70% of the dose being excreted in urine and 30% in feces. Less than 2% of the administered dose remained in residual carcass and tissues after 52 hours. Hydroxymethyl AE 0317309 (2%), desmethyl AE 0317309 (<9%), and AE B197555 (benzoic acid; <2%) were observed as metabolites in urine & feces; further metabolism of pyrasulfotole is unknown. Metabolic transformations are depicted below:



2. Mutagenicity

An assessment of the mutagenicity of the **parent compound**, pyrasulfotole, was based on the following four acceptable/guideline genetic toxicology studies:

1. In an in vitro reverse gene mutation test with *Salmonella typhimurium* strains TA1535, TA100, TA1537, TA98 and TA102 at concentrations up to 5000 µg/plate (limit concentration), pyrasulfotole was not mutagenic with or without metabolic activation (MRID 46801911).

2. In an in vitro mammalian cell gene mutation assay in Chinese hamster V79 cells at the HGPRT locus at concentrations up to 960 µg/mL (pH of culture medium changed above 312.5 µg/mL in prelim. cytotox. test), pyrasulfotole was not mutagenic with or without metabolic activation (MRID 46801912).

3. In an in vitro mammalian cell cytogenetics assay with Chinese hamster V79 cells at concentrations up to 2500 µg/ml (cytotoxic) in the presence and absence of metabolic activation, there was no evidence of clastogenicity induced above background at non-cytotoxic concentrations (MRID 46801913).

4. In an in vivo micronucelus assay performed in NMRI mice, no increase in micronuclei was seen following i.p. dosing up to and including 500/1000 mg/kg bw (M/F) (MRID 46801914). Mortality was observed in a pilot study above 500 mg/kg bw; 8/10 females died at 1000 mg/kg bw in the main study.

An assessment of the mutagenicity of the **benzoic acid metabolite** (AE B197555; RPA 203328) of pyrasulfotole was also conducted using the following four

acceptable/guideline genetic toxicology studies:

1. In an in vitro reverse gene mutation test with *Salmonella typhimurium* strains TA 98, TA 100, TA 1535, and TA 1537 at concentrations up to 5000 µg/plate (limit concentration), pyrasulfotole was not mutagenic with or without metabolic activation (MRID 43904814).

2. In an in vitro mammalian cell gene mutation assay in Chinese hamster ovary cells at the HGPRT locus at concentrations up to 2700 µg/mL (cytotoxic), pyrasulfotole was not mutagenic with or without metabolic activation (MRID 44545303).

3. In an in vitro mammalian cell cytogenetics assay with Chinese hamster ovary cells at concentrations up to 2710 µg/ml (~10 mM) in the presence and absence of metabolic activation, there was no evidence of clastogenicity induced above background (MRID 44545301).

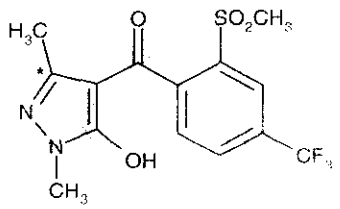
4. In an in vivo micronucelus assay performed in Crl:CD-1®(ICR)BR mice, no increase in micronuclei was seen following oral dosing up to and including 2000 mg/kg bw (limit dose) (MRID 44545302).

Based on the overall findings, the parent compound, pyrasulftole or its benzoic acid metabolite, AE B197555, does not pose a mutagenic concern.

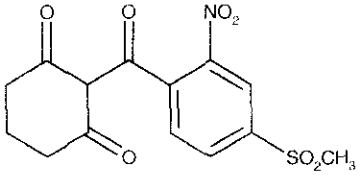
3. Structure-Activity Relationships

The compounds mesotrione, topramezone, isoxaflutole, and pyrasulfotole are herbicides that inhibit HPPD. All four compounds are similar in that they contain 2 rings joined by a carbonyl group. The structures of these compounds follow here:

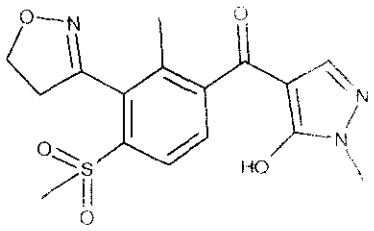
Pyrasulfotole



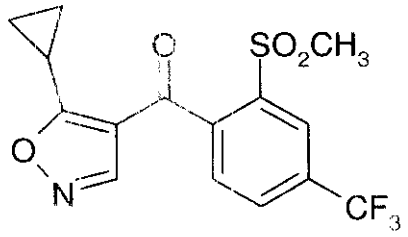
Mesotrione



Topramezone (BAS 670 H)



Isoxaflutole



Mesotrione was classified by HIARC in 2001 as "not likely to be carcinogenic to humans" by all routes of exposure based upon lack of evidence of carcinogenicity in rats and mice. Topramezone was classified by CARC in 2005 as "Not likely to be carcinogenic to humans at doses that do not alter rat thyroid hormone homeostasis" based on treatment-related increases in thyroid follicular cell tumors in male and females rats. There was no concern for mutagenicity for either mesotrione or topramezone. In 1996, isoxaflutole was classified as "likely to be a human carcinogen" based on increases in liver tumors in both sexes of mice and rats and increases in thyroid follicular cell tumor in male rats. There was no concern for mutagenicity for isoxaflutole.

4. Chronic and Subchronic Toxicity

a) Chronic Toxicity

Combined Chronic Toxicity/Carcinogenicity – Rat

Reference: Wason S. (2006). 6-Month toxicity, chronic toxicity and carcinogenicity study of AE 0317309 in the Wistar rat by dietary administration. Bayer CropScience, Sophia Antipolis Cedex, France. Laboratory Study No.: SA 02453, February 28, 2006. MRID 46801910. Unpublished.

In a combined chronic toxicity/carcinogenicity study (MRID 46801910), AE 0317309 (95.7% a.i., batch Op. 1-4) was administered in the diet to 75 six-week-old Wistar Rj:WI (IOPS HAN) rats/sex/group at dose levels of 0, 25, 250, 1000, or 2500 ppm (equivalent to 0/0, 1.0/1.4, 10/14, 41/57, or 104/140 mg/kg bw/day in males/females) for 24 months. Animals were sacrificed at 6 (10/dose group), 12 (10/dose group), and 24 months (55/dose group).

There were no treatment-related effects on food consumption and hematology. Mortality was 73% ($P<0.05$) in high-dose males at 24 months; however, survival was >25% after 24 months in control and all treated groups and therefore was considered acceptable for evaluating the carcinogenic potential of pyrasulfotole. Increased incidences of white area on the eye and soiled fur in one or more areas were observed clinically in males and females at all time points at ≥250

ppm. Body weight in males was decreased (≥10%) throughout the study at ≥1000 ppm. A consistent treatment-related effect on body weight or body weight gain was not observed in treated females. During ophthalmoscopic examination, corneal opacity, neovascularization of the cornea, edema of the cornea, and "snow flake" corneal opacities were observed in males at ≥250 ppm after 6, 12, and 24 months of treatment. At 24 months, increased incidences of corneal opacity and neovascularization of the cornea were observed in males at ≥25 ppm. Similar effects were observed in females during treatment at 250 ppm. Plasma cholesterol was increased in males and females at 250 ppm at 7 months (≥20%) and in males at 12 months (≥52%). Plasma cholesterol was also increased in males at ≥1000 ppm at 18 and 24 months (≥33%) and at 2500 ppm in females at 24 months (63%). Increases in cholesterol at ≥250 ppm were considered toxicologically significant in light of the increased incidence of hepatocellular hypertrophy and/or vacuolation and increased liver weight throughout the study. Triglyceride levels were also increased (NSS) by 124% and 135% in males and females, respectively, at 2500 ppm. Increased levels of ketones were observed in the urine of males and females at ≥1000 ppm at all collection periods and in males at 250 ppm at months 19 and 24 only. Urinary pH was decreased at ≥250 ppm in males at all time points and in females at 3 months only. Urinary protein was increased in males in all treatment groups from 6 months onwards. Decreases in urinary pH and increased urinary protein in males were considered toxicologically significant in conjunction with treatment-related increases in chronic progressive nephropathy and collecting duct hyperplasia. The increased level of ketones may have been due to unmetabolized test substance.

Absolute and relative liver and kidney weights were elevated in males at ≥250 ppm from 6 months onwards (liver: 20%; kidney: >15%). There were no significant effects on organ weights in females. At macroscopic examination, the incidence of eye opacities was increased in males at ≥250 ppm and in females at ≥25 ppm. Enlarged liver was observed in males at 6 months in all treated groups and at 12 months at ≥250 ppm. At 24 months, there was an increased incidence among males of pale kidneys and irregular surface of the kidney at ≥1000 ppm. Non-neoplastic histopathology is summarized above.

Carcinogenicity – Mouse

Reference: Steiblen, G. (2006) Carcinogenicity study of AE 0317309 in the C57BL/6 Mouse by dietary administration. Bayer CropScience, Sophia Antipolis Cedex, France. Laboratory Study No.: SA 03172, February 17, 2006. MRID 46801909. Unpublished.

In a carcinogenicity study (MRID 46801909), AE 0317309 (95.7% w/w a.i.) (95.7% w/w a.i., Batch No. OP. 1-4) was administered in the diet to C57BL/6J mice (50/dose) at doses of 0, 100, 1000, or 4000 ppm for males. Groups of 50 females/group received 0, 100, 1000, or 6000 ppm for the first 10 weeks, then reduced the high-dose to 4000 ppm from week 11 onwards. The high-dose in females was considered excessive because of increased mortality. The concentrations resulted in doses of 0/0, 13.6/16.7, 137/168 and 560/713 mg/kg/day in males and females for up

to 78 weeks. Additionally, 10 mice/sex/dose were treated similarly for up to 52 weeks and then scheduled for interim sacrifice.

At 4000 ppm. survival rate at 18 months was 50 and 40%, respectively in males and females. The survival rate is above the guideline recommended level and is acceptable. Survival rates at 100 and 1000 ppm were comparable to controls. At 4000 ppm clinical signs suggested of compound administration include hardness in the urinary bladder area, soiled fur, reduced motor activity, labored or rapid respiration and red urine. The study authors presumed red urine color was due to compound excretion, however, no urine analysis was performed to confirm. At 4000 ppm mean body weight significantly in both males and females. Body weight was unaffected 1000 ppm in males, however, body weight gain was significantly decreased; the incidence was dose-related. At this dose, in females, body weight gains were significantly decreased during two time periods (days 92-176 and 344-450). Red blood cells, Hb, Hct, and MCHC were decreased in females at 4000 ppm at 18 months (showing indications of dose-response relationships). MCV was slightly increased in females at 4000 ppm at 18 months. In males at 4000 ppm, similar hematological effects were generally observed at 18 months. The perturbations seen at 4000 ppm were considered to be treatment-related. Hematological changes at 100 ppm were comparable to controls.

The majority of statistically significant organ weight changes were restricted to 4000 ppm mice sacrificed at 18 months. Absolute and relative kidney weights increased in males and males at 4000 ppm dose group at the terminal sacrifice. Absolute brain weight was reduced at 4000 ppm at 18 months in males, although it was actually significantly increased as a percentage of body weight. Relative liver weights were increased in both males and females at 18 months, reaching statistical significance in all treated males, with no clear dose-response associated effects on absolute liver weights. Absolute and relative spleen weights were increased in males and relative spleen weights in females at 18 months in 4000 ppm group.

In all treatment groups (males and females) at 12 months, incidences (n= 7 - 10) of the following lesions were increased in the kidneys: (i) large kidneys (2-7 treated vs 0 control); (ii) small kidneys (1-4 treated vs 0 controls); stones (1-4 vs 0 control); pelvic dilation (3-6 vs 0 control); pale (1-3 vs 0 control) cysts (1-2 vs 0 control) gritty content in the bladder (6-8 vs 0 control) and distended bladder (4-8 vs 0 control). At 18 months, the majority of those which died unscheduled at 4000 ppm were found to have died due to acute or chronic renal failure, due to urinary tract blockage or chronic kidney and/or urinary bladder inflammation, respectively. Stones were found in the kidney and/or urinary bladder of these animals; other findings at necropsy of unscheduled deaths were enlarged or small kidneys, renal pelvic dilation, pale kidneys, renal cyst(s), distention of the urinary bladder, and gallbladder stones or concretions. Similar findings were observed in animals sacrificed at 18 months. The incidence of above findings at 4000 ppm were higher than the incidence at 12 months of sacrifice.

Increased incidence of gallstones was a relatively common macroscopic observation in all treated groups at scheduled sacrifice, although there was no dose-response relationship.

b) Subchronic Toxicity

90-day oral (rat)

Reference: Langrand-Lerche, C. (2003). AE0317309 90-day toxicity study in the rat by dietary administration. Bayer CropScience 355, rue Dostoievski, BP 153, F-06903 Sophia Antipolis Cedex , Laboratory Study No.: SA02017, July 30, 2005. MRID 46801842. Unpublished.

In a 90-day oral toxicity study (MRID 46801842), AE 0317309 (97.4% w/w a.i., batch H2235) was administered in the diet to groups of 10 Rj:WI(IOPS HAN) Wistar rats rats/sex/dose at dose levels of 0, 2, 30, 1000, 7000 and 12000 ppm (equivalent to 0.0, 0.13, 1.96, 66, 454, and 830 mg/kg bw/day for males and 0.0, 0.15, 2.32, 77, 537 and 956 mg/kg bw/day in females) for a period of 90 days.

No abnormalities were detected during the neurotoxicity assessment and there were no treatment related effects on hematological parameters. At 12000 ppm, six male and one female were either sacrificed for humane reasons or were found dead during the treatment period. The group was terminated at week 11 due to excessive toxicity. At 7000 ppm, two males were found dead or were sacrificed for humane reasons during the treatment period. Treatment related clinical signs were observed in a large number of rats at 7000 and 12000 ppm and consisted of intensely yellow colored urine associated on a few occasions with soiled anogenital area, soiled fur, piloerection, general pallor and wasted appearance. Other clinical signs noted included: few or no feces, cold to touch, reduced motor activity, labored respiration, hunched posture, increased salivation and soiling around the mouth. White areas on eyes were noted in two males at 7000 ppm and in one male and four females at 12000 ppm. At 1000 ppm, yellow coloured urine was noted in all males on a few days and one female presented a white area on the eyes.

At 12000 ppm, a reduction in mean body weight gain of 70% was recorded in males during the first week of exposure. At this dose level, the depressions in body weight gain ranged from 11.5 to 56% over days 22 to 70. In females at 7000 ppm and 12000 ppm, the depressions in mean body weight gain were 12.5 and 15.6%, respectively, relative to controls at the end of the 90 day period. At 12000 ppm food consumption in males was lower than control values throughout the study. In females at 12000, the mean food consumption was lower than control value on the first week of treatment only (reduction of 28%) without reaching statistical significance. At 7000 ppm, a reduction of food consumption was noted during the first week in males (28%) and females (15%), the difference with controls reaching statistical significance in males only. Very slight reductions thereafter were also observed in both sexes but were not statistically significant.

Neovascularization of the cornea and characteristic "snowflake" corneal opacities were noted at 7000 and 12000 ppm in males, and at 1000, 7000, and 12000 ppm in females. In males, bilirubin, AST and ALT, urea, and creatinine were increased (but not statistically

significantly) at 7000 ppm. Cholesterol was statistically significantly ($p \leq 0.01$) increased in males at 1000 ppm (45%) and 7000 ppm (51%). Triglycerides were statistically significantly ($p \leq 0.01$) increased in males at 1000 ppm (112%) and 7000 ppm (68%). Ketone levels were increased from 1000 ppm in both males and females. This is likely due to detection of the diketone structure of the test substance itself, as the vast majority of the parent molecule is excreted in the urine unchanged. There was an increased incidence of occult blood, erythrocytes, leukocytes, and epithelial cells in the urine in both males and females at 7000 ppm and in females at 12000 ppm (males in the 12000 ppm group did not survive until the end of the study and urine was therefore not collected).

At 1000 ppm and 7000 ppm, the relative liver to body weight in males was statistically significantly increased by 22 and 26% respectively. At 1000 ppm and 7000 ppm, relative kidney to body weight in males was increased 3.5 and 38.6%, respectively, the latter increase being statistically significant. For females relative liver weight was increased 8.7, 13 and 8.7% at 1000, 7000 and 12000 ppm and relative kidney weight was increased 8, 25.4 and 30%, respectively.

At 7000 ppm and 12000 ppm, abnormal shape of the kidneys, mottled kidneys, dilation of and gritty content in the renal pelvis, gritty content, distension of the urinary bladder, and gritty content of the ureters, and enlarged livers were observed in males and females. Livers were enlarged in 3/10 males at 1000 ppm. Prominent lobulation was noted in 1/7 male at 7000 ppm and in 2/10 males at 1000 ppm. The thyroid gland was enlarged in one male at 1000 ppm.

The 12000 ppm group was terminated early at 11 weeks and tissues from animals in this group were not microscopically examined. **Histological changes associated with the presence of calculi (urolithiasis) were found in the kidneys/urinary bladder/ureters in 4/8 males and 6/10 females at 7000ppm. Associated histological changes included: pelvic dilatation (unilateral or bilateral), urinary epithelial hyperplasia (pelvis, urinary bladder and ureters), interstitial fibrosis of the urinary tract, cystitis and ureteritis.** Slight to moderate diffuse centrilobular hepatocellular hypertrophy was observed in 6/7 males at 7000 ppm, in 9/10 males at 1000 ppm and in 1/10 female at 7000 ppm. In females, a periportal vacuolation was found in 8/10 animals at 7000 ppm and 3/10 animals at 1000 ppm.

28-day oral (mouse)

Reference: McElligott, A. (2002). AE 0317309: Preliminary 28-day toxicity study in the mouse by dietary administration. Bayer CropScience, Sophia Antipolis Cedex, France. Laboratory Study No.: SA 02080, September 17, 2002. MRID 46801843. Unpublished.

In a 28-day oral toxicity study (MRID 46801843), Pyrasulfotole (97.4% w/w a.i., batch H2235) was administered to 10 C57BL/6J mice/sex/dose in the diet at dose levels of 0, 200, 1000, or

5000 ppm (equal to 0/0, 35.8/45.0, 192/233, or 961/1082 mg/kg bw/day in males/females). There were no compound-related effects on mortality, clinical signs, body weight, food consumption, clinical chemistry, or organ weights. **Gritty content was found in the urinary bladder of 2/10 males at 5000 ppm. This finding was considered to be treatment-related, since analyses of gritty urinary tract content or urinary tract stones found in other studies (90-day rat toxicity study, mouse carcinogenicity study) have shown that the urinary tract material contains a high concentration of test substance.** Pale livers were noted in 5/10 females at 5000 ppm. Spleens with a black focus were observed in 4/10 females at 5000 ppm (vs. 1/10, 2/10, and 2/10 females at 0, 200, and 1000 ppm, respectively). In the absence of histopathology in the spleen, this effect was not considered toxicologically significant. **In 3/10 males at 5000 ppm, examination of the urinary bladder revealed diffuse urothelial hyperplasia, diffuse submucosal granulation tissue, and diffuse suburothelial mixed-cell infiltrate. Urinary calculi were also observed in one of these 3 males at 5000 ppm.**

Multifocal, centrilobular hepatocytic microvacuolation was observed in the livers of 8/10 and 9/10 males at 1000 and 5000 ppm, respectively (vs. 5/10 and 6/10 males at 0 and 10 ppm, respectively). Multifocal, centrilobular hepatocytic microvacuolation was also observed in 5/10 females at 5000 ppm (vs. 3/10, 4/10, and 4/10 females at 0, 200, and 1000 ppm, respectively). Hepatocytic microvacuolation may have been an adaptive response of the liver; however, since the study authors did not specify the contents of the vacuoles, the increased incidences at ≥1000 ppm (males) and 5000 ppm (females) were considered toxicologically significant. Focal/multifocal subcapsular hyperplasia of the adrenal glands was observed in 6/10 females at 5000 ppm (vs. 3/10, 0/10, and 0/10 females at 0, 200, and 1000 ppm, respectively). Because linear dose response was lacking for this observation and statistical analysis was not performed, the finding was considered a high-dose effect.

90-day oral (mouse)

Reference: Steiblen, G. (2003). AE 0317309: 90-day toxicity study in the mouse by dietary administration. Bayer CropScience, Sophia Antipolis Cedex, France. Laboratory Study No.: SA 03015, November 21, 2003. MRID 46801844. Unpublished.

In a 90-day oral toxicity study (MRID 46801844), pyrasulfotole (95.7% w/w a.i., batch Op. 1-4) was administered to 10 C57BL/6 J@ Ico mice/sex/dose in the diet at dose levels of 0, 100, 1500, or 3000 ppm (equal to 0/0, 16.5/19.7, 124/152, 259/326, or 500/617 mg/kg bw/day for males/females). There were no compound-related effects on mortality, clinical signs, ophthalmology, body weight, food consumption, clinical chemistry, organ weights, or gross and histologic pathology. **Urinary pH was slightly increased at 3000 ppm in females (6.3, $p<0.05$ vs. 6.0 in controls).** Due to the small number or volume of samples obtained, urinary pH was not measured in 3000 ppm males. Examination of the individual animal data revealed that urinary pH for the other male dose groups was similar to controls (~6.0).

5. Mode of Action

Corneal tumors (rat): In the study reports for the cancer studies in rats and mice, it was stated that corneal tumors were the result of a nongenotoxic mode of action that was secondary to corneal inflammation (cornea) and regenerative hyperplasia caused by tyrosine; however, a mode of action analysis was not submitted to support this conclusion. A search of the National Center for Biotechnology Information (NCBI) biomedical literature database, PubMed, did not yield any information supporting an association between tyrosinemia and ocular tumors in rodents or humans.

Bladder tumors (mouse): These tumors were considered by the registrant to be the result of a non-genotoxic proliferative mechanism due to the concurrent presence of secondary inflammation and hyperplastic findings in the urinary bladder, induced by the urinary stones; however, a mode of action analysis was not submitted to support this conclusion. A search of the National Center for Biotechnology Information (NCBI) biomedical literature database, PubMed, did not yield information that links tyrosinemia and bladder tumors in rodents or humans. It is noted that in the current carcinogenicity study in mice, bladder tumors were observed only in the presence of urinary bladder stones or concretions

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

TO BE ADDED AFTER THE CARC MEETING.

VI. CLASSIFICATION OF CARCINOGENIC POTENTIAL

TO BE ADDED AFTER THE CARC MEETING.

VII. QUANTIFICATION OF CARCINOGENIC POTENTIAL

TO BE ADDED AFTER THE CARC MEETING.

VIII. BIBLIOGRAPHY

MRID REFERENCE

To be added

LITERATURE REFERENCES

To be added

IX. Appendix: Photomicrographs of corneal tumors[1]

Figure 1. Microphotograph (25x magnification) of the corneal squamous cell carcinoma observed at 2500 ppm in the rat study conducted with AE 0317309.

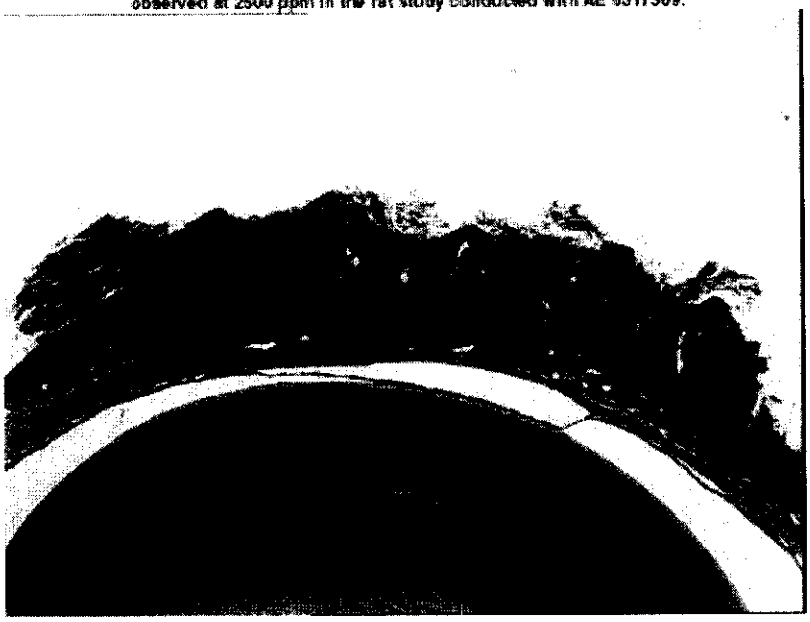
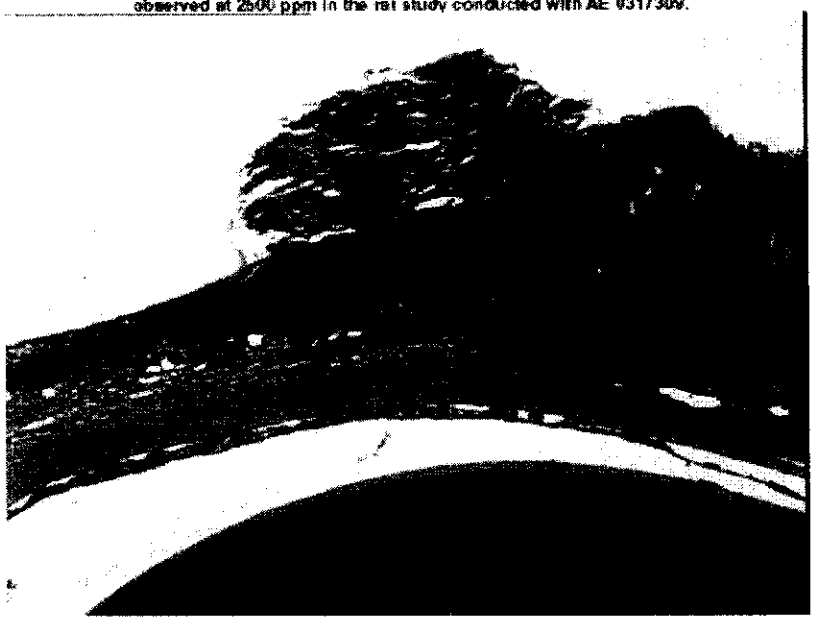


Figure 2. Microphotograph (50x magnification) of the corneal squamous cell carcinoma observed at 2500 ppm in the rat study conducted with AE 0317309.



1 Extracted from Bayer CropScience document "Historical control data for selected findings from the rat chronic/oncogenicity study SA 02453"; no MRID

Figure 3. Microphotograph (100x magnification) of the corneal squamous cell carcinoma observed at 2500 ppm in the rat study conducted with AE 0317309.

Figure 4. Microphotograph (50x magnification) of the corneal squamous cell papilloma observed at 2500 ppm in the rat study conducted with AE 0317309.

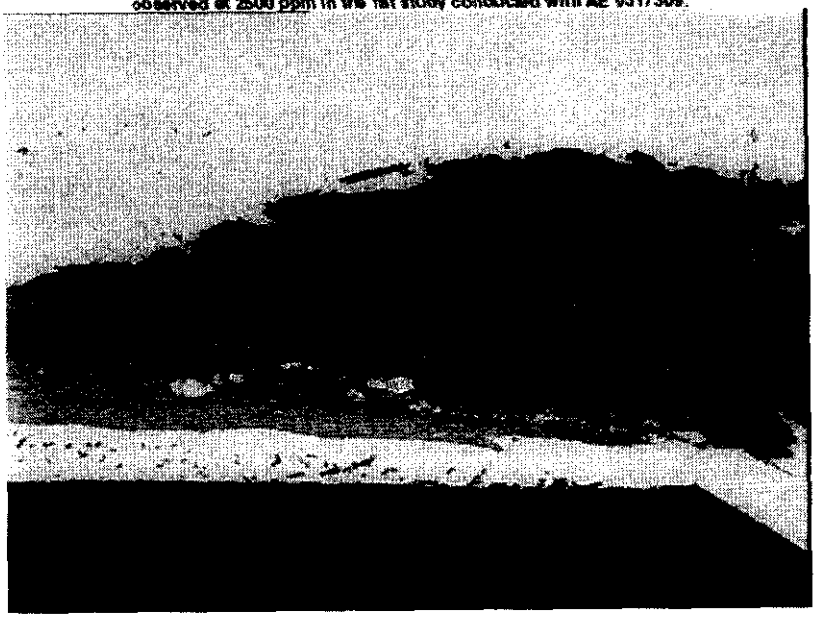
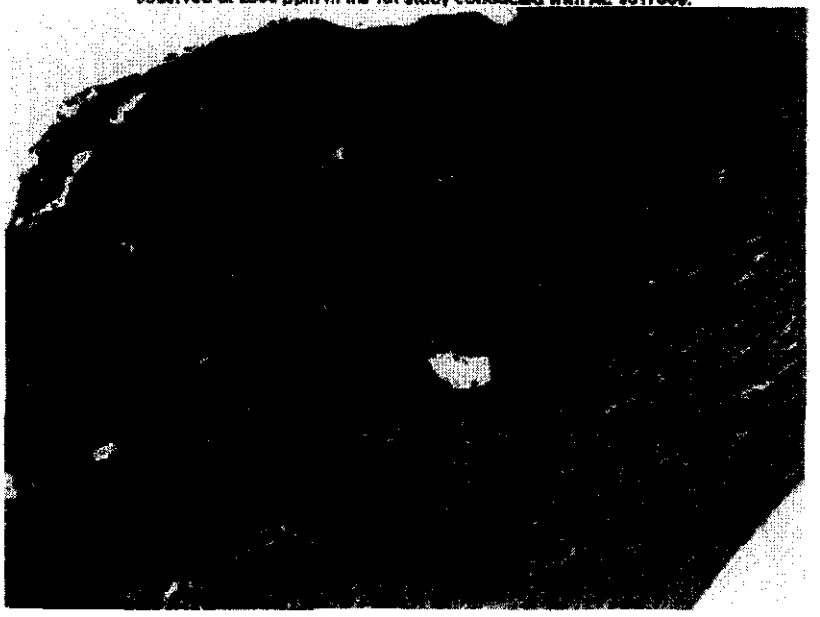


Figure 5. **Microphotograph (100x magnification) of the corneal squamous cell papilloma observed at 2500 ppm in the rat study conducted with AE 0317309.**



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

OFFICE OF
PREVENTION, PESTICIDES, AND
TOXIC SUBSTANCES

TXR No. 0054535

MEMORANDUM

DATE: March 14, 2007

SUBJECT: **Pyrasulfotole:** Qualitative Risk Assessment Based On C57BL/6J Mouse Carcinogenicity Dietary Study

***P.C. Code*: 000692**

TO: **Robert Mitkus, Toxicologist**
Registration Branch 1
Health Effects Division (7509P)

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

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

BACKGROUND

A carcinogenicity study in C57BL/6J mice was conducted by Bayer CropScience, Sophia Antipolis Cedex, France, for the Bayer AG, Bayer CropScience, Monheim, Germany, and completed February 17, 2006 (Study No. SA 03172, MRID No. 46801909).

The study design allocated groups of 60 mice per sex to dose levels of 0, 100, 1000 and 4000 ppm of Pyrasulfotole for 78 weeks. The high dose for the females was 6000 ppm for the first 10 weeks of the study, but was reduced to 4000 ppm from week 11 onward due to excessive mortality. Doses were equivalent to 0, 13.6, 137, and 560 mg/kg/day for males and 0, 16.7, 168, and 713 mg/kg/day for females. Ten mice per sex

per were designated for interim sacrifice at week 53. These animals did not receive histopathological examinations and, therefore, have not been included in the analyses in this report

ANALYSES

Survival Analyses

Both male and female mice showed statistically significant increasing trends for mortality with increasing doses of Pyrasulfotole, as well as a significant pair-wise comparison of the 4000 ppm dose group with the controls, all at $p < 0.01$ (Table 1 for males; Table 4 for females).

Tumor Analyses

Male mice had statistically significant trends, and significant pair-wise comparisons of the 4000 ppm dose group with the controls, for urinary bladder transitional cell carcinomas, and papillomas and carcinomas combined, all at $p < 0.01$. There was a statistically significant trend for urinary bladder transitional cell papillomas at $p < 0.05$. In addition, although there was only one urethral transitional cell carcinoma at the high dose, there was a statistically significant trend at $p < 0.05$ due to increased mortality at the high dose. In conversations with Dr. John Pletcher, EPA's consulting pathologist, this urethral transitional cell carcinoma should be considered the same tumor type as the transitional cell carcinomas in the urinary bladder. The statistical analyses of the tumors in male mice were based upon Peto's Prevalence Test since there were statistically significant survival disparities among the dose groups (Tables 2 and 3).

Female mice had statistically significant trends for urinary bladder transitional cell papillomas, carcinomas, and papillomas and carcinomas combined, all at $p < 0.01$. There were significant pair-wise comparisons of the 4000 ppm dose group with the controls for urinary bladder transitional cell papillomas and carcinomas, both at $p < 0.05$, and for urinary bladder transitional cell papillomas and carcinomas combined at $p < 0.01$. The statistical analyses of the tumors in female mice were based upon Peto's Prevalence Test since there were statistically significant survival disparities among the dose groups (Table 5).

Table 1. Pyrasulfotole – C57BL/6J Mouse Study (MRID 46801909)

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

	Weeks				
Dose (ppm)	1-26	27-52	53[e]	53-81[f]	Total
0	2/60	1/58	9/57	5/48	8/51 (16)**
100	1/60	3/59	9/56	5/47	9/51 (18)
1000	2/59[a]	3/57	10/54	2/44	7/49 (14)
4000	4/60	10/56	9/46	13/37	27/51 (53)**

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

[e]Interim sacrifice at week 53.
[f]Final sacrifice at weeks 79-81

[a]One accidental death at week 20, dose 1000 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.
If *, then $p < 0.05$. If **, then $p < 0.01$.

Table 2. Pyrasulfotole – C57BL/6J Mouse Study (MRID 46801909)

Male Urinary Bladder Transitional Cell Tumor Rates[+]
and Peto's Prevalence Test Results

	Dose (ppm)			
	0	100	1000	4000
Papillomas (%)	0/46 (0)	0/44 (0)	0/43 (0)	3[a]/33 (9)
p =	0.02155*	-	-	0.06781
Carcinomas (%)	0/47 (0)	0/44 (0)	0/43 (0)	8[b]/34 (24)
p =	0.00000**	-	-	0.00011**
Combined (%)	0/47 (0)	0/44 (0)	0/43 (0)	11/34 (32)
p =	0.00000**	-	-	0.00002**

[+] Number of tumor bearing animals/Number of animals examined, excluding those that died before observation of the first tumor.

[a]First papilloma observed at week 71, dose 4000 ppm.
[b]First carcinoma observed at week 69, dose 4000 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 $p < 0.01$.

Table 3. Pyrasulfotole – C57BL/6J Mouse Study (MRID 46801909)

Male Urethral Transitional Cell Tumor Rates[~]
and Peto's Prevalence Test Results

	Dose (ppm)			
	0	100	1000	4000
Carcinomas# (%)	0/43 (0)	0/42 (0)	0/42 (0)	1[a]/24 (4)
p	0.01400*	-	-	0.09036

[~]Number of tumor bearing animals/Number of animals examined, excluding those that died before observation of the first tumor.

#No adenomas observed.

[a]First carcinoma observed at the final sacrifice at week 79, dose 4000 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 $p < 0.01$.

Table 4. Pyrasulfotole – C57BL/6J Mouse Study (MRID 46801909)

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

	Weeks				
Dose (ppm)	1-26	27-52	53	53-81[f]	Total
0	3/60	3/57	10/54	9/44	15/50 (30)**
100	0/60	4/60	9/56	5/47	9/51 (18)
1000	2/60	4/58	9/54	3/45	9/51 (18)
4000	14/60	7/46	7/39	12/32	33/53 (62)**

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

[f]Interim sacrifice at week 53.
[f]Final sacrifice at weeks 79-81

( )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.
If *, then $p < 0.05$. If **, then $p < 0.01$.

Table 5. Pyrasulfotole – C57BL/6J Mouse Study (MRID 46801909)

Female Urinary Bladder Transitional Cell Tumor Rates[+] and Peto's Prevalence Test Results

	Dose (ppm)			
	0	100	1000	4000
Papillomas (%)	0/35 (0)	0/40 (0)	0/42 (0)	2[a]/19 (11)
p =	0.00042**	-	-	0.02632*
Carcinomas (%)	0/35 (0)	0/40 (0)	0/42 (0)	2[b]/19 (11)
p =	0.00042**	-	-	0.02632*
Combined (%)	0/35 (0)	0/40 (0)	0/42 (0)	4/19 (21)
p =	0.00000**	-	-	0.00260**

[+]Number of tumor bearing animals/Number of animals examined, excluding those that died before observation of the first tumor.

[a]First papilloma observed at the final sacrifice at week 79, dose 4000 ppm.
[b]First carcinoma observed at the final sacrifice at week 79, dose 4000 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 $p < 0.01$.

42

References

Cox, D.R. (1972) Regression Models and Life Tables (with discussion). J. Royal Stat. Soc. Ser. B. 34, 187-220.

Gart, J.J., D. Krewski, P.N. Lee, R.E. Tarone, and J. Wahrendorf (1986) The Design and Analysis of Long-Term Animal Experiments. In: Statistical Methods in Cancer Research, Volume III. IARC Scientific Publications No. 79. Lyon, France: International Agency for Research on Cancer. p. 18.

Peto, R., M. Pike, N. Day, R. Gray, P. Lee, S. Parish, J. Peto, S. Richard, and J. Wahrendorf (1980) Guidelines for Simple, Sensitive, Significant Tests for Carcinogenic Effects in Long-Term Animal Experiments. In: Monographs on the long-term and short-term screening assays for carcinogens: a critical appraisal. IARC Monographs, Supplement 2. Lyon, France: International Agency for Research on Cancer, pp. 311-426.

Thomas, D.G., N. Breslow, and J.J. Gart (1977) Trend and Homogeneity Analyses of Proportions and Life Table Data. Computers and Biomedical Research 10. 373-381

DER may not be find'13

Combined Chronic Toxicity/Carcinogenicity study in the rat

Report:	**KIIA 5.5.2/01, Wason, S.; 2006**
Title:	6-Month toxicity, chronic toxicity and carcinogenicity study of AE 0317309 in the Wistar rat by dietary administration.
Laboratory:	Bayer CropScience, 355 rue Dostoievski, BP 153, 06903 Sophia Antipolis Cedex, France
Study duration:	28 January 2003 – 21 October 2005
Report No.:	SA 02453
Document No.:	*M-267037-01-2*
Guidelines:	**OECD 453 (May, 1981); EEC 88/302/EEC, Method B.33 (Nov., 1987); OPPTS 870.4300 (Aug. 1998); MAFF No. 12 Nousan no 8147 (Nov. 2000)**
GLP/QA	**Yes**

Materials and Methods

AE 0317309 was incorporated into rodent diet at concentrations of 0, 25, 250, 1000, and 2500 ppm and administered for up to 24 months to groups of 75 male and 75 female Wistar rats. At study initiation the rats were 6 weeks old, the males being approximately 286 g - 289 g and the females 193 g - 196 g of body weight. They were sourced from R. Janvier, Le Genest St Isle, France.

The above dietary concentrations corresponded to doses of approximately 0, 1.0, 10, 41, and 104 mg/kg bw/d for males and 0, 1.4, 14, 57, and 140 mg/kg bw/d for females. The stability, homogeneity and concentration of the test substance in the diet were verified as acceptable. The animals were housed individually in suspended stainless-steel wire mesh cages. Food and water were available *ad libitum* except for overnight fasting prior to blood collection. Body weight and food consumption were measured weekly for the first 13 weeks of the study, then every 4 weeks through the remainder of the study until necropsy. Ophthalmoscopic examinations were conducted on all animals during acclimatization and at months 3, 6, 12, 18, and 24 of treatment. Blood for hematological and clinical chemical examination was collected after 6, 12, and 24 months from overnight-fasted rats by venipuncture of the retro-orbital venous plexus under isoflurane anesthesia. Urine was collected overnight at 3, 6, 12, 18, and 24 months from animals fasted overnight of food and water. The following table shows which animals were used for blood and urine collection at each time point

Experimental design

Time point	Sacrifice group		
	6-month sacrifice	**12-month sacrifice**	**Terminal sacrifice**
Blood collection			
6 months	All survivors	All survivors	First 10
12 months		All survivors	First 10
18 months			First 10
24 months			First 10
Urine collection			
3 months	All survivors	All survivors	First 10
6 months	All survivors	All survivors	First 10
12 months		All survivors	First 10

18 months			First 10
24 months			First 10

Hematological parameters studied were Hct, Hb, WBC, RBC, PLT, prothrombin time, white blood cell differential count, MCH, MCHC, MCV and reticulocyte count. Clinical chemistry parameters measured were calcium, chloride, inorganic phosphorus, potassium, sodium, alkaline phosphatase, alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma glutamyltransferase (GGT), albumin, creatinine, urea, total cholesterol, glucose (fasting), total bilirubin, total protein, triglycerides. Urinalysis covered appearance, volume, specific gravity/osmolality/refractive index, pH, sediment (microscopic), protein, glucose, ketones, bilirubin, blood/red blood cells, urobilinogen, creatinine.

Animals were sacrificed by exsanguination after pentobarbital anesthesia and subjected to gross necropsy at 6 months (10/dose group), 12 (10/dose group) and 24 months (55/dose group). Selected organs were weighed, and organs and tissues were preserved for histopathological examination. The following tissues were collected for histopathology: tongue, submaxillary (salivary) gland, esophagus, stomach, duodenum, jejunum, ileum, cecum, colon, rectum, liver, pancreas, trachea, lung, nasal cavities, aorta (thoracic), heart, bone marrow (sternum), lymph node (mesenteric), lymph node (submaxillary), spleen, thymus, kidney, urinary bladder, testis, epididymis, prostate gland, seminal vesicle, ovary, uterus (with cervix), brain, sciatic nerve, spinal cord (cervical, thoracic, lumbar), eyes (retina), optic nerves, pituitary gland, adrenal gland, lachrymal exorbital gland, parathyroid gland, thyroid gland, Harderian gland, bone (sternum), skeletal muscle, skin, all gross lesions and masses (including their lymph nodes if possible), pharynx, larynx, mammary gland, vagina, articular surface (femerotibial joint). The following were also weighed: liver, heart, spleen, kidney, testis, epididymis, ovary, uterus (with cervix), brain, adrenal gland. Paired organs were weighed together.

Findings

Mortalities: Mortality was statistically significantly increased in the high dose group males at 24 months. However, mortality rates were generally elevated, being >50% by 24 months in the control females and in all male groups except at 250 ppm.

Comment [r1]: This is acceptable based on OPPTS 870.4300

Mortalities in absolute numbers and as percentages

Month	Dose (ppm)									
	Males					Females				
	0	25	250	1000	2500	0	25	250	1000	2500
Mortalities, N										
6	1	1	0	1	4	0	0	0	0	0
12	2	6	2	2	8	2	1	0	1	0
24	30	38	25	29	40*	28	19	25	22	26
% mortalities										
6	1.3	1.3	0.0	1.3	5.3	0.0	0.0	0.0	0.0	0.0
12	3.1	9.2	3.1	3.1	12.3	3.1	1.5	0.0	1.5	0.0
24	54.5	69.1	45.5	52.7	72.7	50.9	34.5	45.5	40.0	47.3

*p< 0.05

Clinical signs: Treatment-related clinical signs included white area on the eye and soiled fur in one or more areas. An increased incidence of these findings was observed at all time points at 250, 1000, and 2500 ppm in both sexes and there were indications of a dose-response relationship.

Comment [r2]: Need a table with incidences

Body weight and body weight gain: Generally, body weight in males was decreased during the study at 1000 (≤10%) and 2500 ppm (≤12%), often reaching statistical significance. Body weights in the males at 250 ppm also tended to be somewhat reduced (≤7%), reaching statistical significance at day 540. Body weight gains generally reflected this pattern, except late in the study (days 540-708) when body weights declined in all groups including controls. There were also indications of depressed final body weights (6%; NSS) and body weight gains (9%) in the female rats at and above 250 ppm.

Body weight and body weight gain in rats

Time	Dose of AE 0317309, dietary concentration in ppm									
	Males					Females				
	0	25	250	1000	2500	0	25	250	1000	2500
Body weight, g										
Day 1	287	287	288	289	286	196	193	193	194	193
Day 8	336	336	330	329	327*	214	216	212	210	209
Day 92	548	565	539	526*	529	297	302	300	296	299
Day 176	632	645	624	602**	597**	320	328	330	327	331
Day 372	704	714	688	664*	661**	367	367	369	357	366
Day 540	737	733	688*	664**	647**	437	432	423	410	423
Day 708	651	636	624	619	576	452	443	423	409	415
Body weight gain, g										
Days 1-8	49	50	42**	40**	41**	17	22**	20	17	16
Days 1-92	261	279*	252	237**	243*	102	109	107	102	105
Days 92-176	83	76	86	77	70**	22	26	30**	31**	33**
Days 176-372	70	70	64	62	65	47	39	40	29**	33*
Days 372-540	33	19	1**	-2**	-14**	70	65	54	53	57
Days 540-708	86	-97	-64	-45	-71	15	11	0	-1	-8
Overall	365	349	336	331	290	254	249	231	217	223

* $p < 0.05$; ** $p < 0.01$

Food consumption: There was no treatment-related effect on food consumption.

Ophthalmoscopic examination: Various effects (corneal opacity, neovascularization of the cornea, oedema of the cornea, and "snow flake" corneal opacities) were observed in males at 250 ppm and above after 6, 12, and 24 months of treatment. At 6 months, there was some indication of a dose-response relationship in these effects (except for the "snow flake" corneal opacities). Later in the study there was no dose-response relationship, but the incidence was elevated in all dose groups at 250 ppm and above. At 24 months, there were indications of an increased incidence of corneal opacity and neovascularization of the cornea in the males at 25 ppm. Similar effects were observed in females, but with a general tendency to less sensitivity. It is possible that some of these effects were age-related though they were uncommon in the controls at all stages of the study, and historical control data which would have helped

in assessing the significance of these ocular findings were not provided.

Examination of the individual ophthalmoscopic data indicates that corneal opacity, neovascularisation of the cornea and oedema of the cornea tended to occur together. When the "snowflake" corneal opacity was present, it was typically in the presence of the other abnormalities.

Ophthalmoscopic findings (number of animals affected) at 6, 12, and 24 months

Parameter	Dose (ppm)									
	Males					Females				
	0	25	250	1000	2500	0	25	250	1000	2500
6 months										
N	75	74	75	74	73	75	75	75	75	75
Corneal opacity	0	1	32	39	47	0	0	0	18	37
Neovascularization of cornea	0	1	38	41	47	0	0	4	34	53
Oedema of the cornea	0	0	32	36	45	0	0	0	16	32
"Snow flake" corneal opacity	0	0	6	3	2	0	0	10	17	22
12 months										
N	63	61	64	63	58	64	64	65	65	65
Corneal opacity	1	1	40	47	44	0	0	2	25	39
Neovascularization of cornea	1	1	45	49	45	0	0	5	37	49
Oedema of the cornea	0	0	41	48	44	0	0	1	25	39
"Snow flake" corneal opacity	0	0	6	3	3	0	0	8	12	12
24 months										
N	26	21	29	29	18	30	38	35	35	33
Corneal opacity	1	4	20	24	15	1	1	3	18	23
Neovascularization of cornea	1	4	26	27	17	1	2	9	28	27
Oedema of the cornea	2	3	25	27	17	0	1	3	24	26
"Snow flake" corneal opacity	0	0	9	5	2	0	0	7	11	8

Hematology: There were no treatment-related effects on hematology at any dose or time point in either male or female rats.

Clinical chemistry: Plasma cholesterol was biologically and significantly increased in both sexes at 250 ppm and above at 7 months (≥20%), and in males at 12 months (≥52%), and was biologically and/or statistically significantly increased in males at 1000 and 2500 ppm at 18 and 24 months (≥33%), and at 2500 ppm in females at 24 months (63%). These effects on plasma cholesterol were considered to be the only treatment-related clinical chemistry findings. Although plasma cholesterol was statistically significantly increased in males at 25 ppm at the 7-month time point (22%), this value was within the historical control range (2.02±0.47) and cholesterol concentrations in this dose group returned to near control levels by the 12-month time point; therefore, this increase was considered not to be treatment-related. Triglyceride levels were also increased (NSS) by 124% and 135% in males and females, respectively, at 2500 ppm.

Total cholesterol concentrations at 6, 12, 18, and 24 months in rats

Month	Dose (ppm)									
	Males					Females				
	0	25	250	1000	2500	0	25	250	1000	2500
7	1.90	2.32**	2.40**	2.42**	2.47**	1.86	2.02	2.23**	2.15*	2.13*
12	2.23	2.61	3.40**	3.29**	3.69**	2.01	2.21	2.39	2.17	2.24
18	2.71	2.91	3.47	3.92*	4.24**	2.18	2.61	2.62	2.77	2.57
24	3.61	3.45	4.30	4.81	5.32	2.60	3.34	3.09	3.04	4.23

* $p < 0.05$; ** $p < 0.01$

Urinalysis: Based on a semi-quantitative analysis, increased levels of ketones were noted in both sexes at 1000 ppm and 2500 ppm at all collection periods, and in males at 250 ppm at months 19 and 24 only. Urine pH was decreased at 250 ppm and above in males at all time points, while in females at 250 ppm and above urine pH was decreased only at the 3-month time point. Urinary protein was increased in males in all treatment groups from 6 months onwards.
As noted by the study authors in relation to another study (Report no. KIIA 5.10/04), it is possible that the increased level of ketones apparently observed were related simply to excretion of the test substance, which is a ketone-bearing molecule.

Organ weights. Absolute and relative liver and kidney weights were elevated in males at and above 250 ppm from 6 months onwards (liver: ~20%; kidney: >15%), although the effects on the kidney at 250 ppm did not reach statistical significance until later in the study. There were some indications of a dose-related increase in thyroid weight (NSS) at 6 and 12 months, but not at 24 months. There were no significant effects on organ weights in females.

Terminal body weights and selected absolute and relative organ weights in male rats

Month	Parameter	Dose of AE 0317309, dietary concentration in ppm				
		0	25	250	1000	2500
6	Terminal body weight, g	598.7	617.8	597.4	577.6	581.0
12		658.9	668.8	661.8	654.7	642.7
24		639.6	621.9	594.2	590.8	565.2
6	Brain weight, g	2.20	2.21	2.19	2.18	2.17
12		2.32	2.31	2.21	2.26	2.27
24		2.37	2.37	2.31	2.26	2.25
Liver						
6	Organ weight, g	11.87	13.04	14.49**	14.03*	14.84**
	% body weight	1.980	2.113	2.425**	2.426**	2.551**
	% brain weight	538.5	591.6	661.2**	643.7**	682.7**
12	Organ weight, g	13.45	14.05	16.10*	14.43**	15.96**
	% body weight	2.042	2.102	2.430**	2.511**	2.486**
	% brain weight	580.6	610.9	729.0**	727.3**	704.1*
24	Organ weight, g	12.93	13.28	15.53**	15.83**	15.09
	% body weight	2.035	2.154	2.626**	2.694**	2.676**
	% brain weight	544.3	560.1	673.8**	699.5**	670.7**
Kidney						
6	Organ weight, g	2.92	3.14	3.36	3.56**	4.10**
	% body weight	0.494	0.509	0.564	0.628**	0.713**

	% brain weight	133.4	142.4	153.5	164.6**	189.6**
12	Organ weight, g	3.44	3.67	3.99	4.17**	4.21**
	% body weight	0.524	0.550	0.607**	0.634**	0.656**
	% brain weight	148.5	159.4	180.8**	184.1**	185.7**
24	Organ weight, g	3.84	4.01	4.46*	5.01**	4.98*
	% body weight	0.608	0.654	0.753**	0.854**	0.882**
	% brain weight	161.5	169.0	193.4**	221.3**	220.7**
Thyroid						
6	Organ weight, g	0.0219	0.0231	0.0246	0.0269	0.0284
	% body weight	0.0037	0.0038	0.0041	0.0046	0.0049*
	% brain weight	0.995	1.052	1.116	1.230	1.303
12	Organ weight, g	0.0257	0.0245	0.0289	0.0317	0.0308
	% body weight	0.0039	0.0037	0.0044	0.0048	0.0048
	% brain weight	1.107	1.066	1.312	1.406	1.354
24	Organ weight, g	0.0378	0.0309	0.0343	0.0308	0.0325
	% body weight	0.0060	0.0050	0.0058	0.0052	0.0058
	% brain weight	1.595	1.303	1.488	1.364	1.443

*p< 0.05; ** p< 0.01

Macroscopic findings: Possible treatment-related findings observed in the eyes, liver, and kidney are tabulated below. Eye opacities were more prevalent in both sexes at 250 ppm and above, with some indications of a dose-response relationship and increased incidence over time. There is some indication of an increased incidence of opacity in females in the 25 ppm dose group at "24 months" (10/55 animals, combining scheduled and unscheduled sacrifices, compared with 4/55 in the controls). Enlarged liver was observed in males at 6 months in all treated groups and at 12 months at 250 ppm and above (although no dose-response relationship was observed). Prominent lobulation of the liver was seen in all groups, including controls and is of doubtful significance. At 24 months, there was an increased incidence among males of pale kidneys and irregular surface of the kidney at 1000 and 2500 ppm.

Macroscopic findings in eyes, liver and kidneys of rats (unscheduled and scheduled sacrifices for 6 and 12 months are summed)

Month	Finding	Dose of AE 0317309, dietary concentration in ppm									
		Males					Females				
		0	25	250	1000	2500	0	25	250	1000	2500
Eye(s)											
6	Total N	10	10	10	10	10	10	10	10	10	10
	Opacity	0	1	3	3	3	0	0	0	0	2
12	Total N	10	10	10	10	10	10	10	10	10	10
	Opacity	0	0	1	5	5	0	1	0	5	7
24-US[a]	Total N	32	38	26	29	40	28	20	25	23	28
	Opacity	2	3	9	14	26	1	5	2	14	14
24-S[b]	Total N	23	17	29	26	15	27	35	30	32	27
	Opacity	3	1	22	22	12	3	5	8	24	24
Liver											
6	Total N	10	10	10	10	10	10	10	10	10	10
	Obviously large	0	2	4	1	3	0	0	0	0	0
	Prominent lobulation	1	3	4	4	2	1	0	1	0	1
12	Total N	10	10	10	10	10	10	10	10	10	10
	Obviously large	0	0	3	3	3	0	0	0	0	0
Kidney(s)											
24-US[a]	Total N	32	38	26	29	40	28	20	25	23	28
	Irregular surface	2	2	4	9	16	1	1	2	1	2
	Pale	2	3	2	5	8	0	1	0	0	2
24-S[b]	Total N	23	17	29	26	15	27	35	30	32	27
	Irregular surface	0	1	2	14	7	1	1	1	2	4
	Pale	1	0	1	5	3	2	2	1	2	2

[a]24-US = unscheduled sacrifice at 24 months; [b]24-S = scheduled sacrifice at 24 months.

Non-neoplastic microscopic findings: There were treatment-related microscopic findings in the eyes, liver, pancreas, thyroid gland, and kidneys (see table below).

In the eyes, the incidence of corneal inflammation was significantly increased at all time points in males at dietary concentrations of 250 ppm and above, and in females at all time points at 1000 and 2500 ppm. At 24 months, there was a possible slight increase in corneal inflammation in males at 25 ppm. Regenerative hyperplasia of the cornea was increased in males at all time points at 250 ppm and above, and in females at all time points at 1000 and 2500 ppm. Neovascularization of the cornea was increased in males at 6, 12, and 24 months at 250 ppm and above, in females at 6 and 12 months at 1000 and 2500 ppm, and in females at 24 months at 250 ppm and above. At 24 months, there was an increase in males in the incidence of mucous metaplasia of the cornea at 250 ppm and above. Also at 24 months, corneal atrophy was increased in males at 250 ppm and above and in females at 1000 and 2500 ppm, and peripheral retinal atrophy was statistically increased in both males and females at 250 ppm and above, as well as occurring at a higher incidence in females at 25 ppm. Age-related peripheral retinal atrophy has been observed in Fischer 344 rats; however in the absence of historical control data on the strain of rats used in this study, the effects at 24 months in the present study must be regarded as significant, especially as the incidence in the control groups was relatively low.

Non-neoplastic microscopic findings in the eyes (number of animals affected)

Finding	Dose (ppm)									
	Males					Females				
	0	25	250	1000	2500	0	25	250	1000	2500
6 months										
N examined	9	10	10	10	10	10	10	10	10	10
Inflammation, cornea, unilateral or bilateral	0	0	6	8	8	0	0	0	6	7
Hyperplasia, cornea, regenerative, unilateral or bilateral	0	0	5	8	7	0	0	0	5	6
Neovascularization, cornea, unilateral or bilateral	0	0	3	7	6	0	0	0	3	6
12 months										
N examined	10	10	9	10	8	9	10	10	9	10
Inflammation, cornea, unilateral or bilateral	0	0	6	9	8	0	1	0	5	7
Hyperplasia, cornea, regenerative, unilateral or bilateral	0	0	6	9	8	0	1	0	4	6
Neovascularization, corneal, unilateral or bilateral	0	1	5	8	8	0	1	0	4	7
24 months										
N examined	55	55	55	55	55	55	55	54	55	55
Inflammation, cornea, unilateral or bilateral	1	3	39**	49**	44**	0	1	2	28**	32**
Hyperplasia, cornea, regenerative, unilateral or bilateral	0	0	31**	38**	32**	1	0	2	24**	25**
Neovascularization, cornea, unilateral or bilateral	2	3	46**	49**	53**	0	0	13**	41**	42**
Vacuolation, cornea, focal	0	0	2	0	2	1	0	0	5	5
Metaplasia, mucous, cornea	0	0	2	9**	11**	0	0	0	0	0
Atrophy, cornea	0	0	11**	25**	24**	0	0	0	5*	6*
Atrophy, retina, peripheral, unilateral or bilateral	2	3	17**	15**	17**	1	4	8*	20**	13**

*p<0.05; ** p< 0.01

At 6 months, centrilobular hepatocellular hypertrophy was increased in males at all doses and in females from 250 ppm onwards. However, at 12 and 24 months in males this finding was reported only from 250 ppm onwards. In females, there was no centrilobular hepatocellular hypertrophy at 12 months, and at 24 months it was only reported in one animal at 2500 ppm. Centrilobular hepatocellular vacuolation was increased in incidence in males at 6 and 12 months from 250 ppm, but was not observed in females at these time points or in either sex at 24 months. The most consistent finding was centrilobular hepatocellular hypertrophy, which was observed at all time points in males at 250 ppm and above, although without a dose-response relationship. Nevertheless the persistent high incidence of this effect in males at 250

ppm and above suggests that this was treatment-related

Non-neoplastic microscopic findings in the liver of rats (number of animals affected)

Finding	Dose of AE 0317309, dietary concentration in ppm									
	Males					Females				
	0	25	250	1000	2500	0	25	250	1000	2500
6 months										
N examined	9	10	10	10	10	10	10	10	10	10
Hypertrophy, hepatocellular, centrilobular	0	8	10	10	10	0	3	3	4	5
Vacuolation, hepatocellular, centrilobular	1	1	5	3	4	0	0	0	0	0
12 months										
N examined	10	10	9	10	8	9	10	10	9	10
Hypertrophy, hepatocellular, centrilobular	0	0	6	10	6	0	0	0	0	0
Vacuolation, hepatocellular, centrilobular	0	0	2	5	5	0	0	0	0	0
24 months										
N examined	55	55	55	55	55	55	55	54	55	55
Hypertrophy, hepatocellular, centrilobular	0	0	17**	11**	13**	0	0	0	0	1

*p < 0.05., ** p < 0.01

In the pancreas, diffuse acinar degeneration/atrophy was reported at all time points in treated groups; at 6 months, the incidence of this finding was clearly increased in males and females at 2500 ppm. Only single male animals were recorded with this finding at 12 months at 1000 and 2500 ppm, but the incidence was clearly increased in females at 2500 ppm. At 24 months, there were indications of a dose-response relationship in the incidence of diffuse acinar degeneration/atrophy in both sexes, with statistical significance in males at 1000 and 2500 ppm. Also at 24 months, the incidence of focal acinar degeneration/atrophy was increased in females at 2500 ppm. Other findings included fibrosis, inflammation, and interstitial oedema; however these were generally of a low or sporadic incidence (see table below).

Non-neoplastic microscopic findings in the pancreas (number of animals affected)

Findings	Dose of AE 0317309, dietary concentration in ppm									
	Males					Females				
	0	25	250	1000	2500	0	25	250	1000	2500
6 months										
N examined	9	10	10	10	10	10	10	10	9	10
Degeneration / atrophy, acinar, diffuse	0	0	0	0	7	0	0	0	1	8
12 months										
N examined	10	10	9	10	8	9	10	10	9	10
Degeneration / atrophy, acinar, diffuse	0	0	0	1	1	0	0	0	0	7

Fibrosis, interstitial, diffuse	0	0	0	0	5	0	0	0	0	3
Inflammation, acute	0	0	0	1	0	0	0	0	0	5
24 months										
N examined	55	55	54	54	54	55	55	54	55	55
Degeneration / atrophy, acinar, diffuse	0	0	1	8**	9**	0	1	2	4	4
Degeneration / atrophy, acinar, focal	34	34	34	30	30	18	20	25	26	34**
Oedema, interstitial	1	1	0	6	1	0	1	4	6*	2

*p <0.05; ** p < 0.01.

In the thyroid, the incidence of altered colloid was consistently increased in males in all treatment groups. The incidence of increased follicular diameter was increased at 6 and 12 months in males from 250 ppm, but this finding was not observed in males at 24 months or in females at any time point. Pigment deposition in the follicular cells was increased in males at 6 and 12 months from 250 ppm, and at 24 months was statistically increased in both sexes in all treated groups with some suggestion of a dose-response relationship. Focal follicular cell hyperplasia was observed in males at 12 months at 2500 ppm, and at 24 months the incidence was increased above that in control males from 250 ppm, although there was no dose-response relationship. The incidence of diffuse follicular cell hypertrophy was consistently increased in both sexes at 12 and 24 months from 250 ppm, again in the absence of a clear dose-relationship.

Non-neoplastic microscopic findings in the thyroid (number of animals affected)

Findings	**Dose of AE 0317309, dietary concentration in ppm**									
	Males					**Females**				
	0	**25**	**250**	**1000**	**2500**	**0**	**25**	**250**	**1000**	**2500**
6 months										
N examined	9	10	10	10	10	10	10	10	10	10
Altered colloid, basophilic deposits	1	4	8	6	8	0	0	0	0	0
Increased follicular diameter	0	0	1	1	5	0	0	0	0	0
Pigment, brown, follicular cells	0	0	1	1	4	0	0	0	0	0
12 months										
N examined	10	10	9	10	8	9	10	10	9	10
Hyperplasia, follicular cell, focal	0	0	1	0	4	0	0	0	0	0
Hypertrophy, follicular cell, diffuse	0	0	4	5	7	0	0	1	2	1
Altered colloid, basophilic deposits	2	4	8	10	8	0	0	1	1	1
Increased follicular diameter	0	0	5	5	8	0	0	0	0	0
Pigment, brown, follicular cells	1	1	6	8	8	0	0	1	1	0
24 months										
N examined	55	55	54	55	55	55	55	54	55	55
Altered colloid, basophilic deposits	21	24	36**	30	33*	4	3	9	13*	8
Pigment, brown,	3	14**	39**	33**	38**	0	7**	14**	14**	18**

follicular cells										
Hypertrophy, follicular cell, diffuse	2	2	5	8*	4	0	0	3	7**	2
Hyperplasia, follicular cell, focal	3	2	9	12*	8	0	1	0	1	1

*p<0.05; ** p<0.01

Further information on the incidence of altered colloid and brown pigment in the follicular cells is given below. The rats that died unscheduled would have had less exposure to the test substance, and it is possible to compare them with the animals sacrificed at 24 months, in order to assess the progression of this abnormality. The percent incidence of brown pigment was clearly higher in the animals sacrificed on schedule at 24 months than in the unscheduled death animals, including in the control males but not the control females. There were some indications of a dose-response relationship in the incidence of brown pigment in both sexes and in both the unscheduled and scheduled death groups. The incidence of brown pigment at 25 ppm was clearly increased over control levels in both males and females sacrificed on schedule at 24 months.

Altered colloid in the thyroid in the carcinogenicity phase group: unscheduled (premature) deaths and scheduled sacrificed groups (at 24 months)

Findings	Dose (ppm)									
	Males					Females				
	0	25	250	1000	2500	0	25	250	1000	2500
Unscheduled deaths (number of animals)	32	38	26	28	40	28	20	24	23	28
Number of animals affected	11	13	12	12	24	1	0	2	3	1
Percent incidence	34	34	46	43	60	4	0	8	13	4
Scheduled deaths (number of animals)	23	17	29	27	15	27	35	30	32	27
Number of animals affected	10	11	24	18	9	3	3	7	10	7
Percent incidence	43	65	83	67	60	11	9	23	31	26

Brown pigment in the follicular cells in the thyroid in the carcinogenicity phase group: unscheduled (premature) deaths and scheduled sacrifice groups (at 24 months)

Findings	*Dose (ppm)*									
	Males					Females				
	0	25	250	1000	2500	0	25	250	1000	2500
Unscheduled deaths (number of animals)	32	38	26	28	40	28	20	24	23	28
Number of animals affected	0	1	12	12	23	0	1	2	1	5
Percent incidence	0	3	46	43	58	0	5	8	4	18
Scheduled deaths (number of animals)	23	17	29	27	15	27	35	30	32	27
Number of animals affected	3	13	27	21	15	0	6	12	13	13
Percent incidence	13	76	93	78	100	0	17	40	41	48

The incidence of chronic progressive nephropathy was slightly increased in males at 6 months at 1000 and 2500 ppm and was clearly increased at 12 months from 250 ppm. At 24 months, chronic progressive nephropathy was common in controls, but the incidence was greater in all treated groups of both sexes, achieving statistical significance in the males. The incidence of hyperplasia of the collecting ducts was increased in males at 24 months in all treated groups, reaching statistical significance at 1000 and 2500 ppm (see table below).

Non-neoplastic microscopic findings in the kidneys (number of animals affected)

Findings	Dose of AE 0317309, dietary concentration in ppm									
	Males					Females				
	0	25	250	1000	2500	0	25	250	1000	2500
6 months										
N examined	9	10	10	10	10	10	10	10	10	10
Nephropathy, progressive, chronic	2	2	2	3	5	0	0	0	0	0
12 months										
N examined	10	10	9	10	8	9	10	10	9	10
Nephropathy, progressive, chronic	3	5	9	10	8	0	0	1	1	0
24 months										
N examined	55	55	55	55	55	55	55	54	55	55
Nephropathy, progressive, chronic	44	51*	54**	52*	51*	37	42	45	45	42
Hyperplasia, collecting ducts	5	11	11	19**	19**	7	9	8	7	5

*p< 0.05; ** p< 0.01

More detailed information on chronic progressive nephropathy is given in the table below. Chronic progressive nephropathy is clearly a common observation even in control animals, reaching 100% in male controls (scheduled sacrifice) at 24 months. However, as these tables indicate, there was a tendency for treated males to show an increased incidence of the condition earlier in the study. It is possible that the test substance has accelerated the development of this abnormality.

Chronic progressive nephropathy in the carcinogenicity phase group: unscheduled (premature) deaths and scheduled sacrificed animals (at 24 months)

Findings	Dose (ppm)									
	Males					Females				
	0	25	250	1000	2500	0	25	250	1000	2500
Unscheduled deaths (number of animals)	32	38	26	28	40	28	20	25	23	28
Number of animals affected	21	33	26	25	37	16	12	18	15	18
Percent incidence	66	87	100	89	93	57	60	72	65	64
Scheduled deaths (number of animals)	23	17	29	27	15	27	35	30	32	27
Number of animals affected	23	17	28	27	14	21	30	27	30	24

Percent incidence	100	100	97	100	93	78	86	90	94	89

Other non-neoplastic findings included an increased incidence ($P<0.05$) of focal hyperplasia of the pars distalis of the pituitary that was observed at 2500 ppm in males (32/55) and females (36/55), relative to controls (21/55 and 22/55, respectively). Increased incidences of depletion of ovarian corpora lutea and follicles were observed at ≥250 ppm. Squamous gland metaplasia of the uterus and vaginal granular cell hyperplasia were also observed at 2500 ppm. Focal mineralization in the brain was observed in high-dose males.

Neoplastic findings: The only treatment-related neoplastic finding was that of squamous cell tumors of the cornea (one papilloma, one carcinoma) observed in two males in the 2500-ppm dose group. It was suggested by the study authors that these tumors resulted from the corneal inflammation and regenerative hyperplasia resulting from tyrosinemia, and are not relevant to human risk assessment. Other tumors which were observed showed no relationship to dose or were considered to be consistent with those tumors found in ageing rats, and were not evaluated as related to treatment.

Conclusions and Critique

Only limited historical control data were included with the report.

Treatment-related findings were seen in the eyes, liver, kidney, thyroid, and pancreas.

Findings in the eye were considered by the study authors to be related to the biochemical mechanism of AE 0317309 as an HPPDase (4-hydroxyphenylpyruvate dioxygenase) inhibitor leading to increased tyrosinemia in rats; and it was proposed that they were not relevant to humans Tyrosine levels were not measured in the study. Tyrosinemia is thought to result from inhibition of HPPDase, which is a key enzyme in the tyrosine catabolic pathway. The study authors state that humans are more capable of metabolizing and excreting excess tyrosine than rats, and that the corneal effects of HPPDase inhibitors have been considered to be relevant for rats but not for man. A citation to a report of the EU Scientific Committee on Plants in 2000 is given in support of this conclusion and some data on species differences in the metabolism of tyrosine have been supplied in Report no. KIIA 5.10/01. However this remains a debateable issue given the range of effects of the test substance on the eyes even at 25 ppm in the present study (increased incidence of corneal opacity and neovascularization of the cornea in the males in the ophthalmoscopic observations at 24 months; a possible slight increase in corneal inflammation in males and an increased incidence of retinal atrophy in female rats at 24 months in the microscopic findings; and increased observations of opacity in female rats at 24 months in the macroscopic findings). Of particular concern is the observation of ocular effects not involving the cornea (retinopathy), since the effects of HPPDase inhibitors claimed by the study authors to be observed in rats but not significant for humans are the effects on the cornea. USEPA considers the ocular effects in rats to be relevant to humans.

There were histopathological changes in the liver associated with increased liver weights, but these effects were only prevalent at 250 ppm and above. Chronic progressive nephropathy was a common finding in all animals, including the controls; however there was a tendency for treated males to show a higher incidence of chronic

progressive nephropathy earlier in the study. It is possible that the test substance has accelerated its development. Kidney weights tended to be increased in males at higher doses (250 ppm and above), particularly later in the experiment. Semi-quantitative data indicated increased levels of protein in the urine in all treated groups of males suggesting effects on the kidney. However fully quantitative data on protein levels in the urine were not obtained. The study authors contend that this was a minor observation with no corresponding histopathological findings. However hyperplasia of the collecting ducts in the kidneys was somewhat more common in all treatment groups of males at 24 months relative to controls, although this did not reach statistical significance at the lower doses. Also, hyperplasia of the collecting ducts is not likely to be directly associated with proteinurea.

The findings of colloid alteration and pigment deposition in the thyroid were considered to be specific to the rat and to be normal findings in ageing rats by the study authors (further discussion is in report KIIA 5.5.4/01). However it should be noted that an increased incidence of colloid alteration was observed from 6 months onwards in all treated groups in the males. Also, there is a suggestion of a dose-response effect associated with the increased occurrence in all treated groups of brown pigment in both sexes at 24 months; with the effect tending to be greater ("slight" rather than "minimal") at higher doses (Report no. KIIA 5.5.4/01). Moreover, in a 90-day oral study in rats (Report KIIA 5.3.2/01), males at 1000 ppm and above showed loss of colloid in the thyroid and follicular cell hypertrophy or hyperplasia. Follicular cell hypertrophy, follicular cell hyperplasia and increased follicular diameter were also associated with treatment at the higher doses in males (and follicular cell hypertrophy in females) in the present study. Thyroid effects were also observed at 30 ppm and above in male and female parental animals in the two generation reproductive toxicity study in the rat (Report no. KIIA 5.6.1/01).

There were some indications of a dose-related increase in thyroid weight in the present study at 6 and 12 months, but not at 24 months.

It is clear that the test compound is having an effect on the thyroid. These effects appeared at the lowest dose in the present study. For example, the incidence of brown pigment at 25 ppm was clearly increased over control levels in both males and females sacrificed on schedule at 24 months.

The incidence of diffuse acinar degeneration/atrophy in the pancreas was raised at higher doses throughout the study. The study authors suggest that this may be a tyrosine-linked effect, since these findings were not observed in the mouse or dog, which they state are not as prone to tyrosinemia.

The study authors also suggest that the only treatment-related neoplastic findings, squamous cell tumors of the cornea in the highest dose group, were probably due to a non-genotoxic proliferative mechanism and secondary to rat-specific increased plasma tyrosine concentrations not relevant to man.

The study authors proposed a NOAEL of 25 ppm (1.0 mg/kg bw/day in males and 1.4 mg/kg bw/day in females) and stated that there was no indication of neoplastic findings which are relevant to man. The study authors considered 250 ppm to be the

LOAEL (10 mg/kg bw/day in males and 14 mg/kg bw/day in females), apparently based on raised cholesterol levels at this level.

However it does not appear that a NOAEL/NOEL can be established for this study (NOAEL/NOEL<1.0 mg/kg bw/d), because there is some evidence of effects of the test compound on the eyes of male and female rats at the lowest dose, 25 ppm, and insufficient evidence has been supplied to permit such effects to be dismissed as irrelevant to humans. Other significant findings at 25 ppm included high levels of protein in the urine in males, colloid alteration in the thyroid in males and brown pigment in the thyroid in both sexes, and an increased incidence in the males of collecting duct hyperplasia in the kidneys.

Furthermore, according to the OECD Guideline 453, which is cited in the present study, negative findings in a carcinogenicity test in rats are only acceptable if survival in each dose group is 50% or more at 24 months. In the present study, survival at 24 months was less than 50% in four of the five male study groups, including controls, as well as in the female control group. Therefore, this study is not acceptable from the point of view of assessing carcinogenicity.

Comment [r3]: survival is >25% at 24 months, therefore acceptable according to OPPTS 870.4300

Mechanistic Studies

Mechanism of action and supporting data

Report:	**KIIA 5.5.4/01, Mann, P. C.; 2005**
Title:	Scientific advisory group review of the thyroid from SA 02453: A 6-month toxicity chronic toxicity and carcinogenicity study of AE 0317309 in the Wistar rat by dietary administration - SAG report
Laboratory	Experimental Pathology Laboratories, Inc., PO Box 12766, Research Triangle Park, NC, 27709, USA
Study date	26 October 2005
Report No.:	393-043
Document No.:	M-266373-01-2
Guidelines:	**None**
GLP/QA	**No**

Histopathological sections of the thyroid from male and female rats from the above-described rat chronic/oncogenicity study (Report no. 5.5.2/01) were examined by an independent panel of senior pathologists to determine whether the morphologic changes observed in the thyroid represented an adverse effect of AE 0317309 in the thyroid. Bayer CropScience sponsored Experimental Pathology Laboratories, Inc. to establish a scientific advisory group consisting of independent consultant pathologists to review several issues involving the thyroid arising from toxicology studies with AE 0317309.

The incidence and severity of the findings of colloid alteration and pigment deposition are shown in the following tables.

Incidence and severity of colloid alteration and pigment deposition in the thyroid of rats in the carcinogenicity phase ("24 months"): unscheduled (premature) deaths

Findings	Dose in ppm									
	Males					Females				
	0	25	250	1000	2500	0	25	250	1000	2500
N	32	38	26	28	40	28	20	24	23	28
Colloid alteration: Total (% incidence)	11 (34%)	13 (34%)	12 (46%)	12 (43%)	24 (60%)	1 (4%)	0 (0%)	2 (8%)	3 (13%)	1 (4%)
Minimal	7	13	8	5	16	1	0	1	3	1
Slight	4	0	4	6	7	0	0	1	0	0
Moderate	0	0	0	1	1	0	0	0	0	0
Pigment deposition: Total (% incidence)	0 (0%)	1 (3%)	12 (46%)	12 (43%)	23 (58%)	0 (0%)	1 (5%)	2 (8%)	1 (4%)	5 (18%)
Minimal	0	1	6	7	15	0	1	2	1	5
Slight	0	0	6	5	8	0	0	0	0	0

Incidence and severity of colloid alteration and pigment deposition in the thyroid of rats in the carcinogenicity phase ("24 months"): scheduled deaths (at 24 months)

Findings	Dose in ppm									
	Males					Females				
	0	25	250	1000	2500	0	25	250	1000	2500
N	23	17	29	27	15	27	35	30	32	27
Colloid alteration: Total (% incidence)	10 (43%)	11 (65%)	24 (83%)	18 (67%)	9 (60%)	3 (11%)	3 (9%)	7 (23%)	10 (31%)	7 (26%)
Minimal	6	7	13	6	3	3	3	5	5	4
Slight	4	4	11	9	4	0	0	2	5	3
Moderate	0	0	0	3	2	0	0	0	0	0
Pigment deposition: Total (% incidence)	3 (13%)	13 (76%)	27 (93%)	21 (78%)	15 (100%)	0 (0%)	6 (17%)	12 (40%)	13 (41%)	13 (48%)
Minimal	3	13	8	6	8	0	6	12	8	11
Slight	0	0	19	15	7	0	0	0	5	2

The pathology expert group noted that the colloid alterations were present in all

groups including controls and that the morphology was similar between control and treated groups; the primary difference being an increase in the number of follicles affected in treated groups. Additionally, colloid changes were seen in the absence of follicular cell hypertrophy, and were not considered to indicate a persistent alteration in thyroid function in this study. Similarly, the brown pigment observed in the follicular cells was considered to be similar in morphology between control and treated animals. This pigment was evaluated to be suggestive of lipofuscin, which is a normal pigment often associated with aging and seen in a number of organs under untreated conditions.

It was the opinion of the pathology expert group that the colloid alteration and pigment deposition observed in rats administered AE 0317309 for two years were representative of normal age-related physiologic changes specific to the rat, and that these findings were not adverse.

While noting this opinion that the changes resemble normal ageing effects in the thyroid, it may nonetheless be significant that the test substance appears, in a number of studies, to accelerate and increase the effects relative to controls. For example, it is clear that in the rats sacrificed on schedule at 24 months the percentage incidence of brown pigment deposition is higher in the treated groups (including at 25 ppm) in both sexes. This question is discussed further in the report on the chronic study in rats (Report no KIIA 5.5.2/01).

DER may not be final

Carcinogenicity study in the mouse

Report:	**KIIA 5.5.3/01, Steiblen, G.; 2006**
Title:	AE 0317309 – Study type: Carcinogenicity feeding – mouse – Carcinogenicity study of AE 0317309 in the C57BL/6J mouse by dietary administration
Laboratory	Bayer CropScience, 355 rue Dostoievski, BP 153, 06903 Sophia Antipolis Cedex, France
Study duration	3 September 2003 – 7 October 2005
Report No.:	SA 03172
Document No.:	M-267521-01-2
Guidelines:	**OECD 451 May, 1981); EEC Directive 88/302/EEC, Method B.32 (November, 1987); US OPPTS Series 870.4200 (August, 1998); MAFF no. 12 Nousan no 8147 (November, 2000)**
GLP/QA	**Yes**

Materials and Methods

AE 0317309 (batch Op. 1-4, purity 95.7%) was incorporated into rodent diet and administered at 0, 100, 1000 or 4000 ppm to groups of 60 male C57BL/6J mice. Groups of 60 females received 0, 100, 1000, and 6000 ppm. These doses were selected based on 28 day (SA 02080) and 90 day dietary (SA 03015) studies. In the 28 day study, urothelial hyperplasia was observed in males at 5000 ppm. No compound related effects were observed in males or females upto 3000 ppm. Based on this information the above doses for carcinogenicity study were selected. In the carcinogenicity study, females receiving 6000 ppm in the diet for the first 10 weeks of the study exhibited excessive mortality; therefore, this dose was decreased to 4000 ppm from week 11 onwards. These concentrations resulted in doses of 0, 13.6, 137, and 560 mg/kg bw/d for males and 0, 16.7, 168, and 713 mg/kg bw/d for females. After 52 weeks, 10/sex from each group allocated to the chronic phase of the study were sacrificed and necropsied. The remaining 50 animals/sex/group, allocated to the carcinogenicity phase of the study, continued treatment until the scheduled final sacrifice of the study after at least 78 weeks of treatment.

The stability of the test substance at 2 and 15000 ppm diets was verified for up to 82 and 95 days in a study, SA 02017, respectively, when kept at ambient temperature. The homogeneity and concentration of the test substance in the diet were also verified as acceptable. The mice were housed singly in suspended stainless steel wire cages, and diet and water were provided *ad libitum* except for fasting overnight prior to blood sampling. Body weights were measured on the first day of treatment, weekly for the first 13 weeks of the study, and then at 4-week intervals through the end of the study. Clinical signs and mortality were monitored twice daily on weekdays and once daily on weekends and holidays. Ophthalmoscopic examinations were conducted on all animals during the acclimatisation phase and on all surviving animals at the end of the study. Ophthalmoscopy was performed on 24 animals/sex/dose (all animals from the interim sacrifice phase and the first surviving 14 animals from the final sacrifice phase) after approximately 3, 6, 9, and 12 months, and on all surviving animals at approximately 18 months.

At the end of the one-year treatment period, blood was collected for hematology from the surviving animals in the one-year sacrifice group and from the first 10 surviving

animals of the terminal sacrifice group. At the end of the study, blood was collected from the first 20 surviving animals of the terminal sacrifice group. At both time points, blood was collected after overnight fasting by puncture of the retro-orbital venous plexus under isoflurane anesthesia. The following hematological parameters were measured : hematocrit (Hct), haemoglobin (Hb), leukocyte count (WBC), erthyrocyte count (RBC), platelet count (PLT), leukocyte differential count, mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), mean corpuscular volume (MCV). At the 12-month and terminal sacrifices, animals were anesthetized by pentobarbital injection and sacrificed by exsanguination. A gross necropsy was conducted, selected organs were weighed, and organ and tissue samples were taken for histopathological examination. The following tissues and organs were sampled: tongue, submaxillary (salivary) gland, esophagus, stomach, duodenum, jejunum, ileum, cecum, colon, rectum, liver, gall bladder, pancreas, trachea, lung, nasal cavities, pharynx, larynx, aorta (thoracic), heart, bone marrow (sternum), lymph node (mesenteric), lymph node (submaxillary), spleen, thymus, kidney, urinary bladder, testis, epididymis, prostate gland, seminal vesicle, ovary, uterus (with cervix), mammary gland, vagina, brain, sciatic nerve, spinal cord, eyes (retina), optic nerve, pituitary gland, adrenal gland, lachrymal exorbital gland, parathyroid gland, thyroid gland, Harderian gland, bone (sternum), skeletal muscle, skin, all gross lesions and masses, including their regional lymph node if possible, articular surface (femorotibial joint). The following organs were weighed, paired organs being weighed together: liver, heart, spleen, kidney, testis, epididymis, ovary, uterus (with cervix), brain, and adrenal gland.

Statistical Analysis

Appropriate methods were employed to analyze body weights, food consumption, absolute and relative organ weights, hematology parameters, survival analysis, and tumor analysis. The methods employed as appropriate include Bartlett test, ANOVA, Dunnett test,, Kruskal-Wallis test, Kaplan-Meier estimates procedure, Cox's and Tarone's test, Cochran-Armitage and Fisher's exact test. The methods employed are adequate.

Findings

In all sections, the female high dose group will be referred to as having received AE 0317309 at a dietary concentration of 4000 ppm, although these animals received a dietary concentration of 6000 ppm for the first 10 weeks of the study.

Mortalities: Both at 12 and 18 months, there was increased mortality in males (23% and 50%, respectively) and females (35% and 60%, respectively) at 4000 ppm. At 18 months, the mortality in males at 100 and 1000 ppm was comparable to concurrent controls, whilst that in females at these doses was about 50% below the controls.

Mortality (% animals dying unscheduled) in mice

Study Section	Dose (ppm)							
	Males				Females			
	0	100	1000	4000	0	100	1000	4000
To 12 months	5%	8%	10%	23%	8%	7%	10%	35%
To 18 months	14%	16%	16%	50%	30%	16%	16%	60%

Data obtained from page 35, in the report.

Clinical signs: There were a number of treatment-related clinical signs, some of which were (according to the study author) probably related to the excretion of parent compound in the urine. These included hardness in the area of the urinary bladder, soiled fur, reduced motor activity, labored or rapid respiration, and red urine. These observations were mostly occurred at the high dose. Urinalysis was not performed in this study and no characterization of the red color in the urine was apparently attempted (see table below).

Incidence of clinical signs (number of animals)

Finding	Dose (ppm)							
	Males				Females			
	0	100	1000	4000	0	100	1000	4000
N	60	60	60	60	60	60	60	60
Soiled fur, either general or localized	1	0	2	43	2	0	3	9
Reduced motor activity	1	0	2	8	3	1	3	12
Prostration	1	0	0	5	0	0	1	3
Tremors	0	1	1	4	0	1	0	1
Red urine	0	0	0	4	0	0	0	1
Labored respiration	0	0	0	4	0	0	0	2
Rapid respiration	1	0	1	0	0	0	0	4
Cold to touch	1	0	0	6	3	0	1	5
Abdomen distended	0	1	1	9	0	1	1	3
Hardness in the urinary bladder area	0	0	0	52	0	1	0	33

Data obtained from page 34, in the study report.

Body weight and body weight gain: Body weight and body weight gain were decreased at 4000 ppm. Body weight was unaffected at 1000 ppm in males, however, body weight gain was statistically significantly decreased in this group over the first year of the study; and there was some evidence of a dose-response relationship. Because the males at 1000 ppm were slightly heavier at the beginning of the study than their concurrent controls, decreased body weight gain over the course of the study resulted in similar terminal body weights. This effect was considered to be due to treatment. Mean body weight and body weight gains of females were not affected at 1000 ppm. The mean terminal body weights and body weight gains of females at 4000 ppm decreased 8% and 15.7%, respectively and were statistically significant ($p \leq 0.05$ or 0.01). Decreases in body weight and body weight gains at 4000 ppm were considered to be treatment-related (see table below).

Body weight and body weight gain in mice fed diets containing AE 0317309

Study day/period	Dose (ppm)							
	Males				Females			
	0	100	1000	4000	0	100	1000	4000
Body weight, g								
1	20.9	21.3	21.4	21.3	17.8	17.8	17.8	17.9
8	21.8	21.8	21.9	21.5	17.9	17.7	17.6	17.5
92	27.4	27.9	27.5	26.8	22.2	22.6	22.5	22.3
176	29.7	30.2	29.8	28.7**	24.2	24.7	24.3	24.0
370	32.2	32.5	31.5	29.9**	27.3	27.3	27.7*	26.8
540	32.0	32.5	32.0	29.4**	28.0	27.8	27.4	26.5**

Body weight gain, g								
1-8	0.9	0.6**	0.5**	0.3**	0.1	-0.1	-0.2	-0.4**
1-92	6.5	6.7	6.1*	5.5**	4.4	4.8	4.7	4.4
92-176	2.4	2.3	2.3	1.9**	2.0	2.1	1.7	1.7
176-344	2.4	2.4	1.7**	1.3**	3.0	2.6	3.5	2.8
344-540	-0.3	-0.0	0.6**	-0.4	0.5	0.4	-0.2	-0.2
1-540	11.0	11.3	10.7	8.0**	10.0	9.9	9.6	8.6*

Data obtained from page36, in the study report
*p<0.05. **p<0.01

Food consumption: There was no effect of treatment on food consumption at any dose level.

Ophthalmoscopy: There were no treatment-related ophthalmoscopic findings.

Hematology: RBC, Hb, Hct, and MCHC were decreased in females at 4000 ppm at 18 months (showing indications of dose-response relationships), with decreases in most parameters also at 12 months. MCV was slightly increased in females at 4000 ppm at 18 months. In males at 4000 ppm, similar hematological effects were generally observed at 18 months. The perturbations seen at 4000 ppm were considered to be treatment-related.

Selected hematological findings in mice fed diets containing AE 0317309

Parameter	Month	Dose (ppm)							
		Males				Females			
		0	100	1000	4000	0	100	1000	4000
RBC	12	9.60	9.53	9.46	9.20	9.50	9.44	9.40	9.00
10^{12}/L	18	9.85	9.79	9.61	9.14*	9.17	8.97	8.82	7.70**
Hb	12	14.2	14.3	14.2	14.0	14.5	14.4	14.5	13.6**
g/dL	18	13.9	14.2	13.7	13.0	13.8	13.6	13.2	11.6**
Hct	12	0.471	0.472	0.470	0.463	0.481	0.478	0.478	0.461*
	18	0.448	0.455	0.448	0.428	0.441	0.435	0.429	0.385**
MCV	12	49	50	50	50**	51	51	51	51
fl	18	46	47	47	47**	48	49	49	50**
MCH pg	12	14.7	15.0	15.0*	15.2**	15.3	15.3	15.5	15.1
	18	14.1	14.5*	14.3	14.2	15.1	15.1	15.0	15.1
MCHC g/dL	12	30.1	30.3	30.2	30.2	30.1	30.1	30.4	29.5**
	18	30.9	31.1	30.6	30.2*	31.4	31.2	30.8	29.9**
WBC	12	3.3	3.2	3.2	2.1*	3.2	3.5	3.7	3.7
10^9/L	18	2.8	2.2	2.4	2.1	2.6	3.0	3.0	24.3

Data obtained from Table 7, pages 148 – 156, in the study report.
* $p < 0.05$.** $p < 0.01$.

Organ weights: The majority of statistically significant organ weight changes were restricted to 4000 ppm mice sacrificed at 18 months. Kidney weights in males were increased at 12 and 18 months in the 4000 ppm dose group. Absolute brain weight was reduced at 4000 ppm at 18 months in males, although it was actually significantly increased as a percentage of body weight. Relative liver weights were increased in both males and females at 18 months, reaching statistical significance in all treated males, with no clear dose-response associated effects on absolute liver weights. Absolute and relative spleen weights were increased in males and relative spleen weights in females at 18 months in 4000 ppm group. Organ weight changes occurred

64

at 4000 ppm were considered to be treatment-related (see table below).

Absolute and relative organ weights

Parameter	Dose (ppm)							
	Males				Females			
	0	100	1000	4000	0	100	1000	4000
12-month sacrifice								
Terminal body wt, g	27.6	28.5	26.5	26.1	22.3	23.4	23.3	22.9
Brain weight, g	0.460	0.463	0.456	0.450	0.470	0.477	0.476	0.463
Brain weight, % body weight	1.67	1.63	1.73	1.73	2.11	2.05	2.06	2.03
Liver weight, g	1.17	1.20	1.12	1.23	1.12	1.17	1.16	1.21
Liver weight, % body weight	4.24	4.22	4.19	4.72	5.04	5.00	5.01	5.28
Kidney weight, g	0.419	0.432	0.402	0.762*	0.342	0.347	0.358	0.376
Kidney weight, % body weight	1.52	1.52	1.52	2.91**	1.54	1.49	1.54	1.64
Spleen weight, g	0.055	0.059	0.053	0.059	0.094	0.088	0.077	0.108
Spleen weight, % body weight	0.200	0.206	0.202	0.226	0.421	0.379	0.326	0.470
18-month sacrifice								
Terminal body wt, g	28.1	28.6	28.0	25.9**	24.7	24.4	24.1	23.2**
Brain weight, g	0.462	0.463	0.456	0.446**	0.481	0.480	0.474	0.470*
Brain weight, % body weight	1.65	1.63	1.63	1.73*	1.96	1.98	1.97	2.03
Liver weight, g	1.19	1.25*	1.29**	1.22	1.34	1.36	1.39	1.39
Liver weight, % body weight	4.23	4.38*	4.61**	4.72**	5.43	5.59	5.77**	6.01**
Kidney weight, g	0.474	0.472	0.465	0.901**	0.405	0.405	0.420	0.432
Kidney weight, % body weight	1.687	1.656	1.663	3.464**	1.643	1.662	1.742	1.865
Spleen weight, g	0.052	0.059*	0.060	0.056*	0.118	0.122	0.116	0.210
Spleen weight, % body weight	0.186	0.208	0.216	0.218**	0.478	0.501	0.480	0.900**

Data obtained from pages39, 40, 41, 157- 169, in the report
* $p < 0.05$, ** $p < 0.01$

Macroscopic findings: Prior to the 12-month sacrifice, one male and three females out of the animals dedicated to the 12-month satellite group at 4000 ppm were found dead (male on day 240, females on days 60, 61, and 75, respectively). All of these animals had yellow urinary bladder stones. In the 12-month satellite animals sacrificed as scheduled, one male and four out of 7 females at 4000 ppm were found to have stone(s) in the renal pelvis. Both males and females at 4000 ppm showed enlarged or small kidneys, dilation of the renal pelvis, pale kidneys, or renal cyst(s). Stones or gritty content were also observed in the urinary bladders of nearly all animals sacrificed at 4000 ppm at 12 months, along with bladder distention in the majority of these animals.

Gallstones were observed in 100 and 1000 ppm males, and 1000 and 4000 ppm females, among animals sacrificed as scheduled at 12 months and the incidence in concurrent controls was zero; however, there was no dose-response relationship in their incidence (see table below).

Macroscopic observations at 12 months

Parameter	Dose in ppm							
	Males				Females			
	0	100	1000	4000	0	100	1000	4000
N	9	9	10	9	10	9	9	7
Kidney								
Obviously large	0	1	0	7	0	0	1	2
Obviously small	0	0	0	1	0	0	0	4
Stone(s)	0	0	0	1	0	0	0	4
Pelvic dilation	0	0	0	6	0	0	0	3
Pale	0	0	0	1	0	0	0	3
Cyst(s)	0	0	0	1	0	0	0	2
Urinary bladder								
Gritty content (stone(s))	0	0	0	8	0	0	0	6
Distended	0	0	0	8	0	0	0	4
Gallbladder								
Stone(s)	0	2	1	0	0	0	2	1

Data obtained from pages 42-43, in the report.

Among the animals in the 18-month study group, the majority of those which died unscheduled at 4000 ppm were found to have died due to acute or chronic renal failure, due to urinary tract blockage or chronic kidney and/or urinary bladder inflammation, respectively. Stones were found in the kidney and/or urinary bladder of these animals; other findings at necropsy of unscheduled deaths were enlarged or small kidneys, renal pelvic dilation, pale kidneys, renal cyst(s), distention of the urinary bladder, and gallbladder stones or concretions. Similar findings were observed in animals sacrificed at 18 months. Increased incidence of gallstones was a relatively common observation in all treated groups at scheduled sacrifice, although there was no dose-response relationship. Other observations in both the scheduled and unscheduled death groups tended to be restricted to the medium or high dose. Renal effects observed at the high dose could probably be related to treatment.

Macroscopic findings (number of animals affected) in mice either found dead, sacrificed humanely, or sacrificed at 18 months

Organ	Finding	Dose (ppm)							
		Males				Females			
		0	100	1000	4000	0	100	1000	4000
Unscheduled deaths									
N		7	8	8	25	15	8	8	30
Kidney	Obviously large	0	0	8	6	0	0	1	1
	Obviously small	0	0	0	2	0	0	0	6
	Stone(s)	0	0	0	7	0	0	0	10
	Pelvic dilation	0	0	0	11	0	0	0	13
	Pale	0	1	0	9	1	0	1	7
	Cyst(s)	0	0	1	7	0	0	0	7
Urinary bladder	Stone(s)	0	0	0	22	0	0	0	18
	Distended	1	2	1	10	0	1	0	11
Gall bladder	Stone(s)	0	0	3	0	0	0	0	0
Scheduled sacrifice									
N		43	42	42	25	35	42	42	20
Kidney	Cyst(s)	0	0	0	16	0	0	1	7

	Stone(s)	0	0	0	7	0	0	0	13
	Pale	0	1	0	7	0	0	3	8
	Obviously small	0	0	0	3	0	0	0	7
	Pelvic dilation	0	0	1	9	0	0	1	0
Urinary bladder	Stone(s)	0	0	0	24	0	0	0	11
	Distended	0	0	0	13	0	0	0	0
Gall bladder	Stone(s)	1	10	9	7	0	3	7	2

Data obtained from pages 43-45, in the report.

Non-neoplastic microscopic findings: Microscopic examination was not conducted at 12 months. At 18 months, histopathologic findings included a dose-related increase in the incidence of minor to moderate centrilobular hepatocellular hypertrophy in males and females at 1000 and 4000 ppm; which was statistically significant in the males at 1000 and 4000 ppm and the females at 4000 ppm. This was considered to be treatment-related. Most of the other findings were observed in the urinary system (kidney, urinary bladder, and ureters) at the high dose in males and females and prostate males and were associated with stones and concretions observed in the urinary system at the same dose. An exception is the occurrence of gallstones, which were observed at an increased incidence in all treatment groups in both sexes, although no dose-response relationship was present.

Non-neoplastic microscopic findings in all mice at 18 months

Finding	Dose (ppm)							
	Males				Females			
	0	100	1000	4000	0	100	1000	4000
Liver								
N (includes unscheduled and scheduled deaths)	50	50	50	50	50	50	50	50
Centrilobular hepatocellular hypertrophy	0	1	14**	25**	0	0	3	7**
Hepatocellular vacuolation: diffuse	30	26	20	15	37	39	39	23
Hepatocellular vacuolation: mainly periportal, diffuse	0	0	0	7	0	0	0	6
Interstitial mixed cell infiltrate: focal / multifocal	14	19	18	26**	19	20	25	17
Gall bladder								
Gallstones	4	19**	22**	19**	0	5	14**	5**
Epithelial hyperplasia focal / multifocal	1	2	6	3	0	4	1	3
Kidney								
Pelvic stones: unilateral	0	0	0	10**	0	0	0	21**
Pelvic stones: bilateral	0	0	0	2	0	0	0	3
Collecting ducts hyperplasia: unilateral	0	0	0	7**	2	0	2	13**
Collecting ducts hyperplasia: bilateral	0	0	0	2	0	1	1	0
Pelvic epithelium hyperplasia: unilateral: focal / multifocal	0	0	1	10**	0	0	1	11**
Pelvic epithelium hyperplasia: bilateral: focal / multifocal	0	0	0	1	0	0	1	4**
Papillary fibrosis / atrophy: unilateral	0	0	2	13**	0	0	1	19**

Papillary fibrosis / atrophy: bilateral	0	0	0	12**	0	0	0	4**
Atrophy / fibrosis / scar: cortex / medulla: unilateral	0	0	1	18**	7	10	20**	26**
Atrophy / fibrosis / scar: cortex / medulla: bilateral	0	1	0	21**	1	0	1	9**
Suburothelial mixed cell infiltrate: focal / multifocal	0	0	0	8*	0	0	0	5**
Interstitial hemorrhage(s): focal / multifocal	0	1	0	8**	0	0	0	2
Glomerular chamber dilatation: focal / multifocal	0	0	1	8**	2	3	4	17**
Tubular dilatation: cortex: diffuse	1	3	3	20**	10	4	8	26**
Pelvic dilatation: unilateral	2	1	2	6	2	0	1	12**
Pelvic dilatation: bilateral	0	1	1	33**	1	0	0	15**

Kidney								
Papillary necrosis: unilateral: focal / multifocal	1	0	1	5	0	1	2	4
Papillary necrosis: bilateral: focal / multifocal	0	0	0	2	0	0	0	0
Papillary necrosis: unilateral / bilateral: focal / multifocal	1	0	1	7*	0	1	2	4
Cortical basophilic tubules: unilateral	8	27**	15	14	18	20	13	23*
Collecting duct concretions: unilateral / bilateral	0	0	0	22**	1	3	3	15**
Cyst(s)	5	3	5	7	1	4	3	15**
Medullary tubular mineralization: focal / multifocal	2	1	1	0	0	1	0	6
Arteritis / periarteritis: focal / multifocal	0	0	0	6*	0	0	0	6**
Urinary bladder								
Stones: intraluminal	0	0	0	17**	0	0	0	8**
Stones: intraglandular	0	0	0	2	0	0	0	0
Urothelial hyperplasia: simple: multifocal / diffuse	0	0	1	20**	0	0	0	31**
Urothelial hyperplasia: nodular / glandular: multifocal / diffuse	0	0	0	40**	0	0	0	13**
Urothelial hyperplasia: squamous: multifocal / diffuse	0	0	0	28**	0	0	0	9**
Urothelial hyperplasia: atypical: focal / multifocal	0	0	0	1	0	0	0	2
Distention	11	7	5	37**	4	4	5	19**
Muscular hemorrhage(s) / necrosis: focal / multifocal	0	0	0	5	0	0	0	8
Vascular congestion: focal / multifocal	0	0	0	8*	0	0	0	10*
Interstitial edema: diffuse	0	0	0	42**	0	0	0	27**
Adenomyosis: focal / multifocal	0	0	0	6*	0	0	0	0
Intramuscular inflammatory cell infiltrate: focal / multifocal	3	0	0	27**	1	1	1	20**
Suburothelial mixed cell infiltrate: focal / multifocal	1	0	1	11*	0	0	1	4
Interstitial mixed cell infiltrate:	0	0	0	17**	0	0	0	8**

focal / multifocal								
Serosal mixed cell infiltrate: focal / multifocal	0	1	1	11**	0	0	0	12**
Prostate								
Intra-urethral stones	0	0	0	3				
Urethral urothelial hyperplasia: simple focal / multifocal	0	0	0	8**				
Adenomyosis: focal / multifocal	0	0	0	3				
Ureters								
Stones	0	0	0	2	0	0	0	0
Urothelial hyperplasia: simple: multifocal / diffuse	0	0	0	3	0	0	0	1
Urothelial hyperplasia: nodular / glandular: multifocal / diffuse	0	0	0	2	0	0	0	2
Dilatation	0	0	1	4	0	0	0	1
Adenomyosis: focal / multifocal	0	0	0	2	0	0	0	0

Data obtained from Tables 10a , b, c, pages 47 -57, and 186 - 247, in the study report.
* $p < 0.05$; ** $p < 0.01$.

Further information on the incidence of gallstones in treated animals is given in the table below. In both animals that died during the study (or were sacrificed moribund) and those that were sacrificed at termination, the incidence of gallstones was elevated in all treated groups relative to controls. There was no dose-response relationship in either sex. However, given that the animals having unscheduled deaths were exposed to the test substance for a shorter period, comparison of data from the unscheduled and scheduled sacrificed animals gives some indications of a progression of this abnormality over time. In particular, in the unscheduled deaths group, the incidence of gallstones was above control levels in the mid- and high-dose males and the high dose females only; whereas by the time of the scheduled deaths at 18 months, the incidence was above control levels in all treated groups in both sexes (see table below). It is possible that the incidence of gallstones in the high dose groups would have been higher but for the death of susceptible individuals from the development of stones in the urinary system before gallstones could develop.

Incidence (number of animals affected) with gallstones: unscheduled and scheduled deaths (microscopic pathology)

Finding	**Dose (ppm)**							
	Males				**Females**			
	0	**100**	**1000**	**4000**	**0**	**100**	**1000**	**4000**
Unscheduled deaths (number of animals)	7	8	8	25	15	8	8	30
Gallstones	1	0	5	5	0	0	0	1
Percent incidence	14	0	63	20	0	0	0	3
Scheduled deaths (number of animals)	43	42	42	25	35	42	42	20
Gallstones	3	19	17	11	0	5	14	4
Percent incidence	7	45	40	44	0	12	33	20

Neoplastic microscopic findings: Treatment-related neoplastic findings were limited to the urinary tract of males and females at 4000 ppm, and were comprised of

transitional cell carcinomas (which occurred at a statistically significant level) and papillomas of the urinary bladder and urethra. The study authors hypothesized that these tumors were probably related to the presence of urinary tract stones, and were the result of a non-genotoxic proliferative mechanism associated with the concurrent presence of secondary inflammation and hyperplastic findings in the same tissues. Also, in males, treatment-related urethral transitional cell carcinoma of prostate was seen in 1/48 vs 0/50 in controls, was observe, though the incidence was low, it could be considered the secondary effect of urethral stones. The relevant data are given in the table below.

Treatment-related neoplastic microscopic findings

Finding	Dose (ppm)							
	Males				Females			
	0	100	1000	4000	0	100	1000	4000
N	50	49	50	50	49	47	50	49
Urinary bladder								
M – transitional cell carcinoma	0	0	0	8**	0	0	0	2
B – transitional cell papilloma	0	0	0	3	0	0	0	2
Urethra (prostatic)								
M – urethral transitional cell carcinoma	0	0	0	1 (N=48)				

** $p < 0.01$.

Conclusions

The study authors suggested a NOAEL of 100 ppm (13.6 mg/kg bw/day in males, 16.7 mg/kg bw/day in females), apparently based on increased liver weight and centrilobular hepatocellular hypertrophy in males at 1000, together with gallstones which were associated with epithelial hyperplasia in the gall bladder at this dose.

As well as having some effects at the mid and high dose in the liver (centrilobular hepatocellular hypertrophy in males and females), there were effects in the kidney ("atrophy/ fibrosis/scar: cortex/medulla: unilateral" in females).

Dietary administration of pyrasulfatole resulted in increased incidence of stones in the urinary tract at 4000 ppm. Of more concern is the increased incidence of gallstones in all treated groups. In the summary of studies provided by the applicant, it is stated that the gallstones were composed of cholesterol. No details on the biochemical mechanism of this gallstone formation or any proposed explanation for their occurrence was supplied by the study authors. Consequently, although there was no clear dose-response relationship in their occurrence, the observation of increased incidence of gallstones in treated mice relative to controls must be regarded as toxicologically significant. There was some incidence of gallstones in control males (although not females), which suggests the possibility that this strain of mice may be prone to gallstone formation. However no historical control data were provided.

Based on increased incidence in gallstones in all treated groups, no systemic NOAEL could be established for this study (NOAEL<14 mg/kg bw/d)..

Treatment-related increased incidence of transitional cell carcinomas and papillomas

of urinary bladder (males and females) and urethra (males) were observed in the 4000 ppm dose group. Based on the lack of NOAEL and increased incidence of urinary tract tumors, the dosing was considered adequate and reached MTD for testing carcinogenic potential of the chemical.

Comment [r1]: This conclusion has not been vetted by EPA cancer assessment cmte.

Additionally, according to OECD Guideline 451 for carcinogenicity testing, which is cited in this study, in order for a negative result to be acceptable, survival of all groups should be no less than 50 per cent at 18 months in mice. Survival in the high dose female group in this study was below 50 per cent. Therefore this study was not considered to be suitable for the assessment of carcinogenicity. However, the study is acceptable for regulatory purposes, since survival was above 25%, which meets the US OPPTS Series 870.4200.

DER may not be final

KIIA 5.3.2 Oral 90-day toxicity (rodents) (KIIA 5.3.2/01 rat)

Report:	KIIA 5.3.2/01, Langrand-Lerche, C.; 30, July 2003
Title:	90-day toxicity study in the rat by dietary administration
Test Substance:	AE 0317309: Pyrosulfatole – batch H2235, purity 97.4% w/w
Laboratory:	Bayer CropScience, 355, rue Dostoievski, BP 153, F-06903 Sophia Antipolis Cedex
Report No.: **Document No.:**	SA02017 M-102924-01-2
Study Duration:	13 February to 24 May 2002
Guidelines:	O.E.C.D. 408 (1998); E.E.C. directive 92/69 - Annex V - Method B26 (1992); US E.P.A. OPPTS 870.3100 (August, 1998) M.A.F.F. In Japan 59 NohSan No. 4200 (1985)
GLP/QA	Yes

Materials and Methods

Test substance was ground to a fine powder and incorporated into rodent diet by dry mixing to provide the required concentrations. Diets were prepared every three weeks. When not in use they were stored below minus 15°C. The stability of the test substance in the diet was demonstrated before the start of the study at concentrations of 2 and 15000 ppm for a time which covered the period of storage and usage in the study.

The homogeneity of the test substance in the diet was determined on the first preparation the lowest and highest concentrations. Dietary levels of the test substance were determined for each concentration in each dietary preparation.

One hundred and fifty (75 male and 75 female) Rj:WI(IOPS HAN) Wistar rats obtained from R. Janvier, Le Genest St Isle, France, were acclimatized to laboratory conditions (temperature: 20-24°C; humidity: 40-70%; air changes: 15/hr; photo period: 12 hr on/12 hr off) for 7 days prior to treatment and were 6-7 weeks old at the beginning of treatment. One hundred and twenty rats were selected (60 male rats weighed 191-215 g and 60 females 160-187 g) to be within 20% of the mean body weight for each sex on study day 1. Rats were randomly assigned to six groups of 20 (10/sex) and fed treated diet at 0, 2, 30, 1000, 7000 and 12000 ppm for 90 days. The mean achieved dosage intake per sex per group (mg/kg bw/day) was calculated on a weekly basis.

Rats were housed individually in wire cages throughout the study period. Cage-side observations were conducted twice daily on weekdays and once daily on weekends and public holidays, while detailed clinical examinations were conducted at least weekly during the study.

Neurological (grasping, righting, corneal, papillary, auditory startle and head shaking reflex) examinations were carried out pretest and during the twelfth week of the study. Body weights were measured during acclimatization, on the first day of the feeding period, and weekly for the remainder of the study. Food consumption was measured on a weekly basis. Ophthalmoscopic examinations were carried out prior to the start of the study and in weeks 2, 4, 8, and 12. Blood was collected by puncture of the retro-orbital venous plexus on study days 91, 92, 93, 94 prior to sacrifice for haematology and clot activator for clinical chemistry, and on sodium citrate for coagulation parameters. Rats were fasted overnight and anaesthetized by inhalation of isoflurane prior to bleeding.

RBC, Hb, Hct, MCV, MCH, MCHC, WBC, differential cell and platelets were assayed. A blood smear was prepared and stained with Wright stain. Prothrombin time was assayed. Any significant change in the general appearance of the plasma and the serum was recorded. The following clinical chemistry parameters were determined: total bilirubin, glucose, urea, creatinine, total cholesterol, total protein, albumin, triglycerides, chloride, sodium, potassium, calcium, and inorganic phosphorus concentrations were assayed. AST, ALT, AP and GGT activities were assayed.

On study days 85, 86 or 87, in the morning, overnight urine samples were collected from all rats. An approximately equal number of rats randomly distributed amongst all groups were sampled on each day. Food and water were not accessible during urine collection.

Any significant change in the general appearance of the urine was recorded, and the following parameters were determined: quantitative parameters (urinary volume, and pH), semi-quantitative parameters (glucose, bilirubin, ketone bodies, occult blood, protein and urobilinogen) and microscopic examination of the sediment (presence of RBC, WBC, epithelial cells, bacteria, casts and crystals).

On study Days 91, 92, 93 or 94, all surviving rats from all groups were sacrificed by exsanguination under deep anaesthesia (pentobarbital, intraperitoneal injection of approximately 60 mg/kg body weight). An approximately equal number of rats randomly distributed amongst all groups were sampled on each day. Rats were fasted overnight prior to sacrifice. All rats, either found dead or killed for humane reasons, were necropsied. The necropsy included the examination of all major organs, tissues and body cavities. Macroscopic abnormalities were recorded and sampled. Adrenal gland, brain, epididymis, heart, kidney, liver, ovary, pituitary gland, prostate gland, spleen, testis, thymus, thyroid gland (with parathyroid) and uterus (including cervix) were weighed fresh at scheduled sacrifice only. Paired organs were weighed together.

The following organs or tissues were sampled for pathological examination: adrenal gland, aorta, articular surface (femoro-tibial), bone (sternum), bone marrow (sternum) brain, epididymis, oesophagus, eye and optic nerve, exorbital lachrymal gland, gallbladder, heart, Harderian (lachrymal) gland, intestine (duodenum, jejunum, ileum, caecum, colon, rectum), kidney larynx/pharynx, liver, lung, lymph nodes (submaxillary, mesenteric), mammary gland, nasal cavities, ovary, pancreas, pituitary gland, prostate gland, sciatic nerve, seminal vesicle, skeletal muscle, skin, (cervical, thoracic, lumbar), spleen, stomach, submaxillary (salivary gland), testis, thymus, thyroid gland (with parathyroid gland), tongue, trachea, urinary bladder, uterus (including cervix) and vagina. A bone marrow smear was prepared from a rib, stained with May-Grunwald Giemsa, but not examined. Samples were fixed by immersion in 10% neutral buffered formalin with the exception of the eye, optic nerve, epididymis and testis that were fixed in Davidson's fixative. All the above mentioned samples, with the exception of larynx/pharynx, nasal cavities and exorbital gland were embedded in paraffin wax. Histological sections, stained with hematoxylin and eosin, were prepared for all organs from all rats (except Group 2, 2 ppm). Sections from significant gross findings observed at necropsy were prepared from all rats.

Histopathological examinations were performed on all rats, either found dead or killed for humane reasons, in the control and Groups 3, 4 and 5. Liver, thyroid gland, lung, kidney, urinary bladder and significant macroscopic findings of all the rats in the study were also examined (except Groups 2 and 6).

Mean and standard deviations and statistical analyses were calculated for the following variables for each sex separately for each group: body weight, food consumption, haematology, clinical chemistry, quantitative urinalysis and organ weight parameters.

Findings

All results for homogeneity and concentration were within a range of 90 to 105% of the nominal concentration. All values were therefore within the target range of 85 to 115% of the nominal concentration. The test substance was found to be stable in the rodent diet at concentrations of 2 and 15000 ppm over a 82 days or 95 days period respectively at room temperature.

The mean achieved dosage intakes of test substance were 0.0, 0.13, 1.96, 66, 454, and 830 mg/kg bw/day for males and 0.0, 0.15, 2.32, 77, 537, and 956 mg/kg bw/day in females.

Mortalities: There were no mortalities in the groups receiving 0, 2, 30, or 1000 ppm test substance. At 7000 ppm, one male was found dead on day 8, one male was sacrificed for humane reasons on day 70, and one male was killed for humane reasons on day 83. At 12000 ppm, one female was sacrificed on day 13 for humane reasons. Four males in this treatment group were found dead on days 15, 41, 45, and 72, and two males were killed for humane reasons on days 43 and 64, prior to unscheduled termination of this treatment group in males on day 72.

Clinical signs:

Two males at 7000 ppm and 6 males and one female at 12000 ppm were found dead or killed for humane reasons during the dosing period. Clinical signs observed in these rats consisted of intense yellow coloured urine, piloerection, soiled fur, general pallor, wasted appearance and/or no faeces. Treatment related clinical signs were observed in a large number of rats at 7000 and 12000 ppm in both sexes. The signs extended over a period of time and consisted of intensely yellow coloured urine associated on a few occasions with soiled anogenital area. Other treatment related clinical signs noted on less occasions were: few or no faeces, wasted appearance, cold to touch, piloerection, reduced motor activity, laboured respiration, hunched posture, increased salivation and soiling around the mouth. White areas on eyes were noted two males at 7000 ppm and in one male and four females at 12000 ppm. At 1000 ppm, yellow coloured urine was also noted for all males on a few days and one female presented a white area on eyes. No treatment related clinical signs were noted at 2 or 30 ppm.

No abnormalities were detected during the neurotoxicity assessment. At 12000 ppm, one female had no pupillary reflex; this finding was probably a consequence of corneal opacity.

Body weight and body weight gain: Mean body weight gain was less than that of controls in both males and females at concentrations at and above 7000 ppm. At 12000 ppm, a reduction in mean body weight gain of 70% was recorded in males during the first week of exposure. At this concentration, the depressions in body weight gain ranged from 11.5 to 56% over days 22 to 70. These decreases in mean body weight gain were significantly different from controls ($p < 0.01$) at all time points from day 8 to 70. In females at 7000 ppm and 12000 ppm, the depressions in mean body weight gain were 12.5 and 15.6% respectively, relative to controls at the end of the 90 day period. Body weight and body weight gain data are given in the following table.

Data on body weight and body weight gain[1]

Endpoint	Study week	Dose (ppm) 0	2	30	1000	7000	12000[a]
Males							
Body weight, g	0	203±7	202±7	203±7	202±6	202±7	201±6
	13	517±49	510±26	532±37	524±56	489±36	ND
Body weight gain, g		314	308	329	323	287	ND
% of control		100.0	98.1	104.8	102.9	91.4	ND
Females							
Body weight, g	0	175±6	176±6	174±8	174±7	174±7	174±6
	13	303±9	306±19	301±22	301±20	286±14	281±25
Body weight gain, g		128	130	127	127	112	108*
% of control		100.0	101.6	99.2	99.2	87.5	84.4

1- Data extracted from Table 2, pp. 50-4, and Table 3, pp. 55-59.
ND = No data; [a]Males in this group were sacrificed on humane grounds on day 72. *$p < 0.05$

Food consumption: At 2, 30 and 1000 ppm food consumption was unaffected.

At 7000 ppm, a reduction of food consumption was noted during the first week in both males (28%) and females (15%), the difference with controls reaching statistical significance in males only. Very slight reductions thereafter were also observed in both sexes but were not statistically significant. At 12000 ppm food consumption in males was lower than control values throughout the study. The largest reduction (29%) occurred in Week 1 and statistically significance was reached during several intervals from Days 1 to 70. In females at 12000, the mean food consumption was lower than control value on the first week of treatment only (reduction of 28%) without reaching statistical significance.

Ophthalmoscopy: Neovascularization of the cornea and characteristic "snowflake" corneal opacities were noted at 7000 and 12000 ppm in males, and 1000 ppm, and 12000 ppm in females. Snowflake" corneal opacity was also observed in females at 7000 ppm (see table below).

Data on neovascularization of the cornea and characteristic "snowflake" corneal opacities

Endpoint	Dose (ppm) 0	2	30	1000	7000	12000[a]
Males						
Corneal Opacity "snowflake"	0/10	0/10	0/10	0/10	2/10	1/10
Neovascularization	0/10	0/10	0/10	0/10	2/10	1/10
Females						
Corneal opacity "snowflake"	0/10	0/10	0/10	1/10	1/10	4/10
Neovascularization	0/10	0/10	0/10	1/10	0/10	3/10

[1]- Data extracted from pp. 45-49 of the study report.

Haematology: There were no treatment related effects on hematological parameters at any dose.

Clinical chemistry: In males, bilirubin, AST and ALT, urea, and creatinine were increased (but not statistically significantly so) at the dietary concentration of 7000 ppm, while cholesterol and triglycerides were statistically significantly increased at 1000 and 7000 ppm. The increases in these parameters were not dose related. AP was decreased at 1000 ppm and above when compared to controls. These parameters showed less divergence from control values in females. The relevant clinical chemistry parameters are shown in the following table.

Selected clinical chemistry parameters in male and female rats1

??	Dose (ppm)					
Endpoint	**0**	**2**	**30**	**1000**	**7000**	**12000[a]**
Males						
Bilirubin, µmol/L	1.9±0.4	2.0±0.8	1.5±0.4	1.8±0.2	2.3±1.2	ND
AST, IU/L	54±13	88±93	55±15	56±21	110±146	ND
ALT, IU/L	27±11	62±93	24±4	32±13	54±71	ND
AP, IU/L	79±16	85±16	69±10	63±10*	63±17	ND
Urea, mmol/L	4.88±0.54	5.08±0.46	5.26±0.65	5.01±0.52	7.89±5.81	ND
Creatinine, µmol/L	33±3	34±5	36±5	34±3	48±33	ND
Cholesterol, mmol/L	1.82±0.34	2.14±0.51	1.94±0.50	2.64±0.65**	2.74±0.69**	ND
Triglycerides, mmol/L	0.82±0.22	1.02±0.19	0.81±0.22	1.74±1.06**	1.38±0.42**	ND
Females						
Bilirubin, µmol/L	2.0±0.3	2.3±0.5	2.1±0.4	1.8±0.8	2.0±0.4	1.8±0.3
AST, IU/L	49±7	48±6	54±14	55±10	58±9	50±5
ALT, IU/L	22±5	23±5	22±11	23±3	22±7	24±6
AP, IU/L	48±9	43±8	46±11	47±8	47±10	48±2
Urea, mmol/L	5.33±0.67	5.19±0.98	5.10±0.78	4.64±0.98	6.00±1.35	7.98±5.70
Creatinine, µmol/L	35±2	38±5	37±3	34±4	40±5	51±33
Cholesterol, mmol/L	1.99±0.31	3.04±2.61	1.98±0.27	2.29±0.42	2.39±0.23	2.59±0.49*
Triglycerides, mmol/L	0.60±0.21	0.99±0.94	0.63±0.21	0.91±0.46	0.81±0.22	0.69±0.20

1-Data extracted from Table 7, pp. 74-76.
ND = No Data.
[a]Males in this group were sacrificed on humane grounds on day 72.;
* $p<0.05$; ** $p<0.01$.

Urinalysis: In urinalysis, ketone levels were increased from 1000 ppm in both males and females. This is likely due to detection of the diketone structure of the test substance itself, as the vast majority of the parent molecule is excreted in the urine unchanged. There was an increased incidence of occult blood, erythrocytes, leukocytes, and epithelial cells in the urine in both males and females at 7000 ppm and in females at 12000 ppm (males in the 12000 ppm group did not survive until the end of the study and urine was therefore not collected).

Organ weights: Mean absolute and relative liver weights were increased in both males and females at 1000 ppm concentration and above. At 1000 and 7000 ppm concentration in males, the relative liver to body weight in males was statistically significantly increased by 22 and 26% respectively. The respective increases for males of the relative kidney to body weight were 3.5 and 38.6%, the latter increase being statistically significant.

For females the respective percentage increases at 1000, 7000 and 12000 ppm concentration in relative liver weight were 8.7, 13 and 8.7% (all statistically significant) and in relative kidney weight were 8, 25.4 and 30%.

Liver and kidney weights are summarized in the following table.

Mean absolute and relative liver and kidney weights[1]

Parameter	Dose (ppm)					
	0	**2**	**30**	**1000**	**7000**	**12000[a]**
Males						
Terminal body weight, g	494.4±47.6	489.6±24.4	510.2±	500.7±36.4	465.7±55.4	ND[a]
Liver weight, g	11.6±1.1	11.7±0.9	12.1±1.4	14.2±1.7	13.4±1.6	ND
Liver wt as % body wt	2.3±0.11	2.4±0.17	2.4±0.20	2.8±0.22**	2.9±0.22**	ND
Liver wt as % brain wt	540±57.7	554±40.7	574±64.9	682±87.3**	646±106.4*	ND
Kidney weight, g	2.82±0.29	2.66±0.27	2.83±0.22	2.95±0.33	3.59±0.57**	ND
Kidney wt as % body wt	0.57±0.03	0.54±0.03	0.55±0.03	0.59±0.04	0.79±0.20**	ND
Kidney wt as % brain wt	131.2±12.4	126.0±8.66	134.9±14.7	142.2±18.4	172.2±25.7**	ND
Females						
Terminal body weight, g	290.1±8.1	293.6±19.8	288.1±22.2	287.4±20.0	272.0±12.4**	269.1±24.8
Liver weight, g	6.7±0.5	6.9±0.8	6.9±0.8	7.3±0.7	7.1±0.5	6.9±0.5

Liver wt as % body wt	2.3±0.14	2.4±0.21	2.4±0.16	2.5±0.13**	2.6±0.14**	2.5±0.12*
Liver wt as % brain wt	330±15.4	337±38.8	345±40.7	369±32.0*	370±28.4*	352±19.0
Kidney weight, g	1.81±0.11	1.89±0.18	1.83±0.11	1.96±0.17	2.16±0.76	2.22±0.75
Kidney wt as % body wt	0.63±0.05	0.64±0.06	0.64±0.04	0.68±0.04	0.79±0.25*	0.82±0.31
Kidney wt as % brain wt	89.8±5.68	92.0±7.25	91.2±5.70	99.0±7.98	113.6±40.7*	113.2±38.5

1-Data extracted from Table 11, pp. 94-102.
ND = no data. [a]Males in this group were sacrificed on humane grounds on day 72 ; *p<0.05; **p<0.01.

Macroscopic pathology: In male rats either found dead or sacrificed prior to the end of the study (3 at 7000 ppm and 10 at 12000 ppm), the principal cause of death was considered to be related to calculi in the urinary tract. Clearly treatment-related macroscopic findings seen in these rats included gritty content in and dilation of the renal pelvis, enlarged kidneys, pale or mottled color of the kidneys, and foci on the kidneys, and red or gritty content in the urinary bladder, distension of the urinary bladder, and red foci in the bladder, and enlarged liver. Other findings which are less clearly related to treatment included dilation, dark content, and black foci of the stomach, soiled fur, and dark content in the intestines.

Treatment related macroscopic findings in male rats found dead or sacrificed prior to the end of the study[1]

Organ	Finding	Dose (ppm)					
		0	2	30	1000	7000	12000
	N	0	0	0	0	3	10
Kidney(s)	Pelvic dilatation	0	0	0	0	1	9
	Obviously large	0	0	0	0	0	3
	Pale	0	0	0	0	0	2
	Mottled	0	0	0	0	0	1
	Focus(i), white	0	0	0	0	0	1
	Focus(i), red	0	0	0	0	1	1
	Pelvis: gritty content	0	0	0	0	0	6
Liver	Obviously large	0	0	0	0	0	2
Stomach	Dilatation	0	0	0	0	0	1
	Dark content	0	0	0	0	1	1
	Focus(i), black	0	0	0	0	1	3
Urinary bladder	Red content	0	0	0	0	1	1
	Gritty content	0	0	0	0	1	9
	Distended	0	0	0	0	1	5
	Focus(i), red	0	0	0	0	0	1
External appearance	Soiled fur	0	0	0	0	1	3

Intestines	Dark content	0	0	0	0	1	1

1-Data extracted from Table 12, pp.103-107.

In rats which survived to the end of the study, treatment-related macroscopic findings were seen beginning at 1000 ppm (corneal opacity in one female) and above. In both males and females from 7000 ppm, these findings included corneal opacities, abnormal shape of the kidneys, mottled kidneys, dilation of and gritty content in the renal pelvis, gritty content and distension of the urinary bladder, and gritty content of the ureters. A few females at 7000 and 12000 ppm were noted with small kidneys, and one female at 12000 ppm showed gritty content in the urethra. A few treatment related changes observed in final-sacrifice rats were within the urinary tract and the liver in both sexes, and the eyes and thyroid gland in males. Males had enlarged liver at 1000 ppm, with prominent lobulation at 1000 and 7000 ppm. One male had an enlarged thyroid at 1000 ppm.

Treatment related macroscopic findings in male and female rats at study termination[1]

Organ	Finding	Dose (ppm)					
		0	**2**	**30**	**1000**	**7000**	**12000**[a]
Males							
	N	10	10	10	10	7	0
Eye(s)	Opacity	0	0	0	0	2	0
Kidney(s)	Abnormal shape	0	0	0	0	1	0
	Pelvic dilatation	0	1	0	0	2	0
	Mottled	0	0	0	0	2	0
	Pelvis: gritty content	0	0	0	0	4	0
Liver	Obviously large	0	0	0	3	0	0
	Prominent lobulation	0	1	0	2	1	0
Thyroid gland	Obviously large	0	0	0	1	0	0
Urinary bladder	Gritty content	0	0	0	0	4	0
	Distended	0	0	0	0	1	0
Ureter(s)	Gritty content	0	0	0	0	3	0
Females							
	N	10	10	10	10	10	9
Eye(s)	Opacity	0	0	0	1	0	2
Kidney(s)	Abnormal shape	0	0	0	0	2	2
	Pelvic dilatation	0	0	0	0	4	2
	Obviously small	0	0	0	0	2	2
	Mottled	0	0	0	0	1	1
	Pelvis: gritty content	0	0	0	0	4	4
Urinary bladder	Gritty content	0	0	0	0	1	2
	Distended	0	0	0	0	1	1
Ureter(s)	Gritty content	0	0	0	0	5	4
Urethra	Gritty content	0	0	0	0	0	1

1-Data extracted from Table 12, pp.107-111.
[a]Males in this group were sacrificed for humane reasons on study day 72.

Microscopic pathology: According to the study author, the dose of 12000 ppm was above the Maximum Tolerated Dose in the rat and therefore, histopathological examinations were not

conducted at this dose. Additionally, as 30 ppm was observed to be free of treatment related effects, histopathological examination was not conducted at 2 ppm.

The treatment related changes observed in final-sacrifice rats were within the urinary tract and the liver in both sexes, and the eyes and thyroid gland in males. The following tables summarize treatment related findings in males and females sacrificed at the end of study. The abnormalities noted at 30 ppm were not regarded as treatment related as they were single incidences. They were also noted in control females.

Number of male rats showing microscopic findings following the administration of test substance in diet for 90 days[1]

Organ		Dose (ppm)			
	Finding	**0**	**30**	**1000**	**7000**
	Number male rats in group	10	10	10	7
Eye(s)	Keratitis, mixed cellular, focal, unilateral	0	0	0	2
Liver	Hepatocellular hypertrophy, centrilobular, diffuse	0	0	9	6
Kidney(s)	Urolithiasis, pelvis	0	0	0	1
	Urothelial hyperplasia, simple, diffuse	0	0	1	1
	Urothelial hyperplasia, simple, focal / multifocal	0	0	0	4
	Dilated renal pelvis, unilateral	0	1	2	2
	Dilated renal pelvis, bilateral	0	0	0	2
	Interstitial fibrosis, multifocal to diffuse	0	0	0	3
	Dilated tubules, cortical, focal / multifocal	0	0	1	3
Urinary bladder	Urolithiasis	0	0	0	2
	Urothelial hyperplasia, simple, multifocal to diffuse	0	0	0	4
Thyroid gland	Follicular cell hypertrophy / hyperplasia, diffuse	0	0	5	2
	Loss of colloid, diffuse	0	1	9	5
Ureter(s)	Urolithiasis	0	0	0	3
	Urothelial hyperplasia, simple, diffuse	0	0	0	2
	Ureteritis, mixed cellular, diffuse	0	0	0	1

[1] Data extracted from Table 13, pp. 112-125.

Number of female rats showing microscopic findings following the administration of test substance in diet for 90 days[1]

Organ	Finding	Dose (ppm)			
		0	**30**	**1000**	**7000**
	Number of male rats in groups	10	10	10	10
Liver	Hepatocellular hypertrophy, centrilobular, diffuse	0	0	0	1
	Periportal vacuolation, hepatocellular, diffuse	0	0	3	8
Kidney(s)	Urolithiasis, pelvis	0	0	0	3
	Urothelial hyperplasia, simple, diffuse	0	1	0	5
	Urothelial hyperplasia, simple, focal / multifocal	1	1	1	2
	Dilated renal pelvis, unilateral	0	0	0	5
	Dilated renal pelvis, bilateral	0	1	0	0
	Interstitial fibrosis, multifocal to diffuse	0	0	0	2
Urinary bladder	Urothelial hyperplasia, simple, multifocal to diffuse	1	1	0	4
Ureter(s)	Urolithiasis	0	0	0	4
	Urothelial hyperplasia, simple, diffuse	0	0	0	5
	Ureteritis, mixed cellular, diffuse	0	0	0	2
	Serosal mixed cellular infiltrate, local	0	0	0	2

[1] Data extracted from Table 13, pp. 112-125.

Conclusion

The LOAEL is 1000 ppm (77 mg/kg bw/day) in females and 3000 ppm (454 mg/kg bw/day) in males based on increased incidences of neovascularization of the cornea and "snowflake" corneal opacity. The NOAEL is 30 ppm (2.32 mg/kg bw/day) in females and 1000 ppm (66 mg/kg bw/day) in males.

Comment:

The study authors indicated that the Maximum Tolerated Dose (MTD) had been exceeded in this study but the basis for the selection of the doses used was not discussed. 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.

Oral 90-day toxicity (rodents) (KIIA 5.3.2/02 mouse)

Report:	KIIA 5.3.2/02, Steiblen G.; 2003
Title:	90-day toxicity study in the mouse by dietary administration
Test Substance:	AE 0317309: Pyrasulfotole - batch Op. 1-4, purity 95.7% w/w
Laboratory:	Bayer CropScience, 355, rue Dostoievski, BP 153, F-06903 Sophia Antipolis Cedex
Report No.: **Document No.:**	SA03015 M-103284-01-4
Study Duration:	07 February to 21 November 2003
Guidelines:	OECD 408 (1998); EEC 92/69 Annex V, method B26 (1992); US-EPA OPPTS 870.3100 (1998); JMAFF 12 Nousan 8147 (1985)
GLP/QA	Yes

Materials and Methods

Test substance was ground to a fine powder and incorporated into rodent diet by dry mixing to provide the required concentrations. Diet formulations were prepared twice during the study, for each concentration. When not in use diets were stored at minus 18°C. The stability of the test substance in the diet had previously been demonstrated in the 90-day rat study (SA02017) where samples of the diet at 2 and 15 000 ppm were found to be stable over 95 days, respectively, at ambient temperature. The homogeneity of the test substance was determined on the first formulation at the lowest and highest concentrations. Dietary levels of the test substance were determined for each concentration in each dietary preparation.

One hundred and twenty C57BL/6 J@ Ico mice obtained from Charles River Laboratories, Domaine des Oncins, L'Arbresle Cedex, France, were acclimatized to laboratory conditions for 7 days prior to treatment and were six weeks old at the beginning of treatment. One hundred mice were selected (male mice weighed 17.8 - 21.2 g and females 14.9 - 19.0 g) to be within 20% of the mean body weight for each sex on study day 1. Mice were randomly assigned to five groups of 20 animals per group (10/sex/group) and fed treated diet at 0, 100, 1500 and 3000 ppm for 90 days. The mean achieved dosage intake per sex per group (mg/kg bw/day) was calculated on a weekly basis.

Mice were housed individually in stainless steel wire mesh cages. Diet and tap water were available *ad libitum* except before blood and urine collection. Mice were checked twice daily on weekdays and once daily on weekends and holidays for mortalities and clinical signs. Body weight and food consumption were measured at weekly intervals throughout the study. Mice were fasted overnight prior to blood collection, and blood for clinical chemistry was drawn by puncture of the retro-orbital venous plexus under isoflurane inhalation anesthesia immediately prior to necropsy. Urine samples were collected overnight on the night prior to blood sampling and necropsy. Mice were sacrificed by exsanguination after deep anesthesia and subjected to gross necropsy. The blood and urine samples were drawn on study days 91, 92, 93 or 94, in the morning prior to necropsy.

Any significant change in the general appearance of the plasma and the serum was recorded. Total bilirubin, urea, total protein, albumin and total cholesterol concentrations and AST, ALT and AP activities were assayed. Any significant change in the general appearance of the urine was recorded. The following urine parameters were checked: quantitative parameters (pH), microscopic examination of the sediment was performed, and the presence of RBC, WBC, epithelial cells, bacteria, casts and crystals was graded.

On study Days 91, 92, 93 and 94, all surviving mice from all groups were sacrificed by exsanguination under deep anaesthesia. All mice were necropsied. The necropsy included the examination of all major organs, tissues and body cavities. Macroscopic abnormalities were recorded and sampled. Adrenal gland, brain, epididymis, heart, kidney, liver, ovary, pituitary gland, prostate gland, spleen, testis, thymus, thyroid gland (with parathyroid gland) and uterus were weighed fresh. Paired organs were weighed together.

The following organs or tissues were sampled for pathological examination: adrenal gland, aorta, articular surface (femoro-tibial), bone (sternum), bone marrow (sternum), brain, epididymis, oesophagus, eye and optic nerve, exorbital lachrymal gland, gallbladder, heart, Harderian gland, intestine (duodenum, jejunum, ileum, caecum, colon, rectum), kidney, larynx/pharynx, liver, lung, lymph nodes (submaxillary, mesenteric), mammary gland, nasal cavities, ovary, pancreas, pituitary gland, sciatic nerve, seminal vesicle, skeletal muscle, skin, (cervical, thoracic, lumbar), spleen, stomach, submaxillary (salivary gland), testis, thymus, thyroid gland (with parathyroid gland), tongue, trachea, urinary bladder, uterus (including cervix) and vagina. A bone marrow smear was prepared from a rib, stained with May-Grunwald Giemsa, but not examined.

Samples were fixed by immersion in 10% neutral buffered formalin with the exception of the eye, optic nerve, epididymis and testis that were fixed in Davidson's fixative. All the above mentioned samples, with the exception of larynx/pharynx, nasal cavities and exorbital gland were embedded in paraffin wax. Histological sections, stained with hematoxylin and eosin, were prepared from all the mice in the control and high dose groups. The liver, lung, kidney and thyroid gland were examined in all mice in the study. Significant macroscopic findings were also examined in all intermediate dose groups.

Mean and standard deviations and statistical analyses were calculated for the following variables for each sex separately for each group: body weight, body weight gain/day, average food consumption/day, clinical chemistry, urinary pH (females only), terminal body weight, absolute and relative organ weights. Incidences of histopathology were not analyzed statistically.

Findings

The results for homogeneity and concentration of test substance ranged from 87 to 103% of nominal concentration and thus were within the experimental target range of 85 to 115% of nominal concentration. The overall mean achieved intake for each concentration of test substance was 0, 16.5, 124, 259, and 500 mg/kg bw/day for males and 0, 19.7, 152, 326, and 617 mg/kg bw/day for females. The weekly mean achieved intake rates were provided but are not reproduced here.

Mortalities: One male mouse at 100 ppm was sacrificed for humane reasons on day 29. Prior to sacrifice, the mouse had shown reduced motor activity, prostration, and wasting. Necropsy revealed a marked hydrocephalus which was considered to be a spontaneous developmental defect and not related to treatment. There were no other mortalities.

Clinical signs: There were no treatment related clinical signs in either males or females.

Food consumption, body weight and body weight gain: There was no effect on any of these parameters in either males or females at any dose level.

Clinical chemistry: There were no treatment related changes in any clinical chemistry parameters.

Urinalysis: Urine acidity was statistically significantly ($p<0.05$) decreased at 3000 ppm in females (6.3 cf controls 6.0). Due to the small number of values obtained, urinary pH was not measured in 3000 ppm males. Examination of the individual animal data revealed that urinary pH for the other male dose groups was similar to controls (~6.0). There were no other treatment related findings at urinalysis.

Organ weights: There were no treatment related effects of test substance on organ weights.

Gross necropsy: One male, sacrificed on day 29, was found to have a marked hydrocephalus which was considered to be due to a spontaneous developmental defect, and was therefore evaluated as not related to administration of test substance. There were no other findings at gross necropsy which could be attributed to the test substance.

Microscopic findings: There were no treatment related findings in either males or females.

Conclusions

The only treatment related finding observed in this strain of mice after dietary administration of test substance, was an increase in urinary pH in females at 3000 ppm. The effect of the test substance on the urinary pH in males remained indeterminate.

On the basis of this decrease in urinary acidity in females at 3000 ppm, the NOEL for this study was 1500 ppm (259 mg/kg bw/day for males and 326 mg/kg bw/day for females).

The NOAEL was 3000 ppm (500 mg/kg bw/day for males and 617 mg/kg bw/day for females).

PEK may not be final

KIIA 5.3.1 Oral 28-day toxicity (KIIA 5.3.1/01 mouse)

Report:	KIIA 5.3.1/01, McElligott A.; 2002
Title:	Preliminary 28 day toxicity study in the mouse by dietary administration
Test Substance:	AE 0317309: Pyrasulfotole – batch H2235, purity 97.4% w/w
Laboratory:	Bayer CropScience, 355, rue Dostoievski, BP 153, F-06903 Sophia Antipolis Cedex
Report No.: **Document No.:**	SA02080 M-211308-01-3
Study Duration:	26 February 2002 to 16 September 2002
Guidelines:	US EPA OPPT no. 870.3200
GLP/QA	No. This study was not designed to meet regulatory requirements

Materials and Methods

After being ground to a fine powder, the test substance was incorporated by dry mixing into rodent diet to provide concentrations of 0, 200, 1000, and 5000 ppm and administered *ad libitum* in the diet to groups of 10 male and 10 female C57BL/6J mice/dose for 28 days. The mean achieved doses in mg/kg bw/day are shown below.

The stability of the dietary formulations was determined in a separate study (KIIA 5.3.2/01, SA 02017, Document M-102924-01-2) under conditions similar to those of this study. The homogeneity of the test substance in the diet was determined for the lowest and highest concentrations used. When not in use the diet formulations were stored at approximately minus 18°C

Treatment began after a 7 day acclimatization period at which time the mice were 5-6 weeks old. All mice were examined in the acclimatization phase. On the day before treatment all suitable mice were weighed. The 80 selected mice (40 males, 40 females) were in a weight range from 17.5 to 21.4 g for males and 13.5 to 17.6 g for females i.e. within 20% of the mean body weight on the day of random assignation to treatment groups.

Mice were housed individually in suspended wire-mesh cages. Powdered diet and filtered and softened water were available *ad libitum* throughout the study, except for overnight fasting prior to blood sampling for clinical chemistry on the last day of the study.

Clinical signs (including mortality) were monitored twice daily on weekdays, and once daily on weekends and holidays. Observed clinical signs were recorded at least once daily for all animals. Detailed physical examinations were performed weekly during the treatment period. The nature, onset, severity, reversibility and duration of clinical signs were recorded. Cages and cage-trays were inspected daily for evidence of ill-health such as blood or loose faeces.

Body weight was measured on day 3 of the acclimatization period and on the day of assignment to treatment groups, on the first day of dosing, and then weekly. Food consumption was measured weekly.

Mice were fasted overnight prior to blood sampling. On study days 29, 30 or 31, blood samples were collected from all mice by puncture of the retro-orbital venous plexus. Any significant change in the general appearance of the plasma was recorded. Total bilirubin, urea, protein, albumin, cholesterol concentrations, and AST, ALT, and alkaline phosphatase activities were assayed. Haematological examinations were not conducted in this study.

Also on study days 29, 30 and 31, all study mice were randomly sacrificed by exsanguination under deep pentobarbital anesthesia.

All study mice were necropsied. Macroscopic abnormalities were recorded and sampled. At the scheduled sacrifice, brain, adrenal glands, heart, kidneys, liver, spleen, testis and thymus were weighed. Paired organs were weighed together. The following organs or tissues from each study mouse were sampled and prepared for histopathological examination: adrenal gland, articular surface (femorotibial joint), aorta, bone, sternum, bone marrow sternum, brain, epididymis, eosophagus, exorbital lachrymal gland, eye and optic nerve, gallbladder, Harderian gland, heart, intestine (duodenum, jejunum, ileum, caecum, colon, rectum), kidney, larynx, liver, lung, lymph nodes (submaxillary, mesenteric), mammary gland, nasal cavities, ovary, pancreas, pharynx, pituitary gland, prostate, submaxillary (salivary gland), sciatic nerve, seminal vesicle, skeletal muscle, skin, spinal cord (cervical, thoracic, lumbar), spleen, stomach, testis, thymus, thyroid gland (with parathyroid), tongue, trachea, urinary bladder, uterus (including cervix), and vagina.

Histological sections were prepared for all organs from all study mice. Histopathological examinations were conducted on all tissues from all mice in the control and high dose groups, and liver and kidneys and lung from all mice in the intermediate dose groups. Target organs were examined in the intermediate dose groups as necessary to identify the no-effect-level.

The variables analyzed statistically at two levels of significance (0.05 and 0.01) were body weights, food consumption, clinical chemistry parameters and organ weight parameters. Incidences of pathology were not analyzed statistically. Procedures used were Bartlett's test, followed by ANOVA with Dunnett's test or a modified t-test. Means and standard deviations were calculated for each sex separately for each group at each time period.

Findings

The homogeneity of the diet formulations was found to be within acceptable ranges at the lowest (200 ppm) and highest (5000 ppm) concentrations (96-99% of nominal values). The dietary levels of the test substance verified at each of the dose levels of 200, 1000 and 5000 ppm were also within acceptable target ranges (94-97% of nominal concentrations). In a separate study, diet preparations of the test substance at 2 and 15000 ppm were found to be stable over 82 and 95 days, respectively, at ambient temperature. It is noted, however, that the 2 ppm preparation was frozen for the first 18 days of the measurement. All control samples were below the limit of quantification of 1 ppm for the test substance.

The mean achieved dietary intakes of the test substance were 0, 35.8, 192, and 961 mg/kg bw/day in males and 0, 45.0, 233, and 1082 mg/kg bw/day in females.

Mortalities & clinical signs: There were no mortalities during the study, and no treatment-related clinical signs were observed. Damaged eye was observed in 2/10 females at 5000 ppm; however, it was not considered treatment-related.

Food consumption: There was no treatment-related effect on food consumption.

Body weight and body weight gain: There were no treatment-related changes in mean body weight. On day 22, mean absolute body weight gain in males at 200 ppm was statistically significantly increased ($p < 0.05$). In the absence of a dose response and any effects on body weight, the increase was not considered toxicologically significant.

Clinical chemistry: There were no treatment-related effects on any of the clinical chemistry parameters tested.

Organ weight: There were no treatment-related effects on organ weights in either males or females.

Macroscopic pathology: Gritty content was found in the urinary bladder of 2 males at 5000 ppm. This finding was considered to be treatment-related, as analyses of gritty urinary tract content or urinary tract stones found in other studies (a 28 day dog toxicity study, a 90 day rat toxicity study and a mouse carcinogenicity study) have shown that the urinary tract material contains a high concentration of test substance. Pale livers were noted in 5/10 females at 5000 ppm. Spleens with a black focus were also observed in 4/10 females at 5000 ppm (vs. 1/10, 2/10, and 2/10 females at 0, 200, and 1000 ppm, respectively).

Microscopic pathology: In 3 males at 5000 ppm, examination of the urinary bladder revealed diffuse urothelial hyperplasia, diffuse submucosal granulation tissue, and diffuse suburothelial mixed-cell infiltrate. These findings were suggestive of minor irritation as might occur with intraluminal calculi. Urinary calculi were observed in one of these 3 males at 5000 ppm. Multifocal, centrilobular hepatocytic microvacuolation was observed in the livers of 8/10 and 9/10 males at 1000 and 5000 ppm, respectively (vs. 5/10 and 6/10 males at 0 and 10 ppm, respectively). Multifocal, centrilobular hepatocytic microvacuolation was also observed in 5/10 females at 5000 ppm (vs. 3/10, 4/10, and 4/10 females at 0, 200, and 1000 ppm, respectively). Hepatocytic microvacuolation may have been an adaptive effect of the liver; however, since the study authors did not specify the contents of the vacuoles, the increased incidences at ≥1000 ppm (males) and 5000 ppm (females) were considered toxicologically significant. Focal/multifocal subcapsular hyperplasia of the adrenal glands was observed in 6/10 females at 5000 ppm (vs. 3/10, 0/10, and 0/10 females at 0, 200, and 1000 ppm, respectively). Because linear dose response was lacking for this observation and statistical analysis was not performed, the finding was considered a high-dose effect.

Conclusions

Based on treatment-related changes affecting the liver (microvacuolation) at the dose level of 1000 ppm (equivalent to 192 mg/kg/day), the No Observed Adverse Effect Level (NOAEL) of the test substance in male C57BL mice over a 28-day period was considered to be 200 ppm (equivalent to 35.8 mg/kg/day).

Based on treatment-related changes affecting the liver (hepatocyic microvacuolation, pale liver), and adrenal gland (subcapsular hyperplasia) at the dose level of 5000 ppm (equivalent to 1082 mg/kg/day), the No Observed Adverse Effect Level (NOAEL) of the test substance in female C57BL mice over a 28-day period was considered to be 1000 ppm (equivalent to 233 mg/kg/day)

CANCER BRIEFING PACKAGE

PC CODE: 000692 (Pyrasulfatole)

DATE OF PACKAGE: March 22, 2007

SUBMITTED BY: Jessica Kidwell 6/27/07
SIGNATURE AND DATE