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

OFFICE OF PREVENTION, PESTICIDE AND TOXIC SUBSTANCES

OPP OFFICIAL RECORD
HEALTH EFFECTS DIVISION
SCIENTIFIC DATA REVIEWS
EPA SERIES 361

MEMORANDUM

Date: March 25, 2009

SUBJECT: Pyrasulfotole and Metabolite RPA 203328; Review of Submitted Toxicology Studies for Section 3 Registration of Pyrasulfotole

PC Code: 000692, 623001
Decision No.: NA
Petition No.: NA
Risk Assessment Type: NA
TXR No.: 0054227
MRID No.: See MRID Summary Table

DP Barcode: D335563
Registration No.: NA
Regulatory Action: NA
Case No.: NA
CAS No.: NA
40 CFR: NA

Ver.Apr.08

FROM: Robert Mitkus, PhD, Toxicologist
Registration Action Branch 1
Health Effects Division (7509P)

THROUGH: Dana Vogel, Branch Chief
Registration Action Branch 1
Health Effects Division (7509P)

TO: Joanne Miller (RM23)
Registration Division (7505P)

I. CONCLUSIONS

Registration Action Branch I of HED has successfully reviewed the toxicity data submitted by Bayer CropScience in support of its petition. DERs corresponding to the MRIDs listed below are attached.

II. ACTION REQUESTED

Please provide a toxicology assessment for the new ai pyrasulfotole.

III. MRID Summary Table

Rec'd in Scan 4/6/2009

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EPA Reviewer: William B. Greear, MPH, DABT **Signature:** William B. Greear
Registration Action Branch 1, Health Effects Division (7509P) **Date:** 6/5/2008
EPA WAM: PV Shah, PhD **Signature:** P.V. Shah
Registration Action Branch 1, Health Effects Division (7509P) **Date:** 6/5/08

TXR#: 0054227

DATA EVALUATION RECORD

STUDY TYPE: 28-Day Oral Toxicity - [dog]; OPPTS None; OECD None

PC CODE: 000692 **DP BARCODE:** D335563

TEST MATERIAL (PURITY): AE 0317309 (98.1% w/w a.i.)

SYNONYMS: Pyrasulfatole technical

CITATION: Kennel, P. (2002). AE0317309 – Preliminary 28 day toxicity study in the Beagle dog by dietary administration. Bayer CropScience, 355, rue Dostoievski, BP 153, F-06903 Sophia Antipolis Cedex. Laboratory Study No.: SA020503, December 30, 2002. MRID 46801845. Unpublished.

SPONSOR: Bayer AG, Bayer CropScience, Alfred Nobel Str. 50, Monheim, Germany

EXECUTIVE SUMMARY:

In a 28-day oral toxicity study (MRID 46801845), AE 0317309 (98.1% w/w a.i., batch H2238/2) was administered in the diet to groups of 3 male and 3 female Beagle dogs at levels of 0, 5,000, 13,000 or 26,000 ppm (equivalent to 0/0, 174/171, 469/440 and 860/782 mg/kg bw/day in male/female dogs, respectively) for a period of 28 days. There were no compound-related effects on mortality, body weight or ophthalmology.. Clinical examination revealed yellow urine and/or feces in 1 or two dogs in the 26,000 ppm group. Food consumption was decreased by 5% and 16% in females in the 13,000 and 26,000 ppm groups. In males, triglyceride concentrations were increased -11%, 36%, 72% and 151% over pre-test values in the control, 5,000, 13,000 and 26,000 ppm groups, respectively. In females, triglyceride concentrations were increased 9%, 63%, 95% and 298% over pre-test values in the control, 5,000, 13,000 and 26,000 ppm groups, respectively. In females at 26,000 ppm, alkaline phosphatase activity was elevated in all groups but with no dose-response association. Males and females at 26000 ppm were observed to have an increase in the incidence and grading of crystal formation in the urinary sediment. Absolute liver weights were significantly increased in all treated males and females, although in females this did not show a dose relationship. Absolute liver weights were increased 16, 46 and 53% in males in the 5,000, 13,000 and 26,000 ppm groups, respectively. Male liver-to-body weight ratios were increased 12, 49 and 49% in the 5,000, 13,000 and 26,000 ppm groups, respectively. Male liver-to-brain weight ratios were increased 13, 48 and 51% in the 5,000, 13,000 and 26,000 ppm groups,

respectively. Female liver weight increases ranged from 14-32% greater than controls. Prostate gland weights were elevated in males in the 13,000 and 26,000 ppm groups. Midzonal multifocal vacuolation of the liver was increased in 1/3 males at 5,000 ppm, in 3/3 males at 13,000 ppm and in 3/3 males at 26,000 ppm test substance. This lesion was not observed in control males.

The LOAEL is 171 mg/kg bw/day in females and 174 mg/kg bw/day in males based on increases in serum triglyceride and elevated liver weights in both sexes, and a dose-related increase in midzonal multifocal vacuolation of the liver in males. The NOAEL < 171 mg/kg bw/day in females and < 174 mg/kg bw/day in males.

This 28-day oral toxicity study in the dog is **acceptable (nonguideline)** but does not satisfy any guideline requirement.

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

This Executive Summary was prepared for United States Environmental Protection Agency, Office of Pesticides Programs, Health Effects Division use.

The following text was generated by the Australian Pesticides and Veterinary Medicines Authority. However, this document has undergone critical scientific analysis and been modified as appropriate.

Report:	KIIA 5.3.1/01, Kennel P.; 30, December 2002
Title:	Preliminary 28 day toxicity study in the Beagle dog by dietary administration
Test Substance:	AE 0317309: Pyrosulfatole – batch H2238/2, purity 98.1% w/w
Laboratory:	Bayer CropScience, 355, rue Dostoievski, BP 153, F-06903 Sophia Antipolis Cedex
Report No.: **Document No.:**	SA02053 M-1022922-01-2
Study Duration:	30 May 2002 to 22 October 2002
Guidelines:	None
GLP/QA	Yes

Materials and Methods

After being ground to a fine powder, the test substance was incorporated by dry mixing into ground canine diet to provide dietary concentrations of 0, 5000, 13000, and 26000 ppm and administered in diet to groups of 3 male and 3 female Beagle dogs for 28 days. Three hundred grams of diet was given daily to each dog for about 1.5 h in the morning. Dose levels at the start of exposure to the test substance were based on this daily weight of food, and a mean body weight of 7.8 kg.

The stability of the dietary formulations was determined at the low and high concentrations at 10 days at ambient temperature, 29 days at minus 18°C and 39 days at ambient temperature. 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

The concentration of test substance was determined at the intermediate dietary concentration. The mean value obtained from the homogeneity check of the low and high concentrations was used as the measured concentration.

The dogs were 32-35 weeks old on arrival. Treatment began after a 26 day acclimatization period. Within 48 h of arrival, all dogs were examined by a veterinarian for signs of ill-health and were subjected to a detailed clinical examination to assess their physical and behavioural status. Body weight was recorded and food intake determined. In addition, physical and ophthalmological examination, blood analysis (haematology and clinical chemistry) and urinalysis were performed during the acclimatization phase. At the start of treatment body weights ranged from 7.2 to 9.4 kg for males and 7.8 to 9.0 kg for females. Animals were assigned to dosage groups in order to ensure a similar body weight distribution among groups for each sex.

Dogs were kept in climate controlled housing (temperature: 18-21°C; humidity: 40-70%; air changes: 10-15/hr; photo period: 12 hr on/12 hr off) and housed individually in stainless steel kennels with a floor surface area of 1.2 m^2. Supervised exercise in inside runs was permitted daily for dogs of the same sex and treatment group throughout the acclimatization and treatment periods except on weekends and public holidays. Filtered

and softened tap water was available *ad libitum*.

All dogs were checked for evidence of behavioural changes, ill-health and mortality twice daily (once daily on week-ends and public holidays) throughout the study. Daily observations of the kennels were also recorded for presence of vomitus, blood or diarrhoea.

Each dog was weighed prior to feeding weekly during the acclimatization period, on study Day 1, weekly during the treatment period and prior to necropsy. Food intake was recorded on the seven days preceding the start of treatment and daily throughout the study. Each animal was subjected to a physical examination three times prior to treatment and weekly during the treatment period. Particular attention was paid to the following: fur and skin, eyes, ears, teeth, gum, mucous membrane, rectal temperature, gait, stance, general behaviour, chest including heart and respiratory rate, abdomen including palpation, and, external genitalia and mammary glands.

Additionally, a specific neurological examination was performed which included cranial nerves reflexes (pupillary light and consensual light, palpebral blink and corneal reflex), segmental reflexes [flexor (withdrawal) including crossed extensor], postural reactions (placing reactions, visual and tactile).

During the acclimatization phase and at the end of treatment, both eyes of each dog were examined by means of an indirect ophthalmoscope after the instillation of an atropinic agent.

On study Days -4 (Day 23 of the pre-study phase) and 28, blood samples were taken from all dogs in all groups by puncture of the jugular vein. Blood was collected on EDTA for haematology (2.5 ml), lithium heparin (2.5 ml for plasma) and clot activator (4.0 ml for serum) for clinical chemistry and sodium citrate for coagulation parameters (1.8 ml).

RBC, Hb, Hct, MCV, MCH, MCHC, WBC and WBC-differential count evaluation and platelet count were assayed. A blood smear was prepared and stained with Wright stain. The blood smear was examined when the results of Technicon H1 determinations were abnormal. Reticulocytes were stained with brilliant cresyl blue. A smear was prepared but not examined since no significant RBC changes were observed. Prothrombin time and activated partial thromboplastin time were assayed. Any change in the general appearance of the plasma and the serum was recorded.

Total bilirubin, glucose, urea, creatinine, total cholesterol, triglycerides, chloride, sodium, potassium, calcium and inorganic phosphorus concentrations, and aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase and gamma-glutamyl transferase activities were assayed on plasma samples, total protein, albumin concentrations were assayed on serum samples. Globulin and albumin/globulin ratio values were calculated.

On study Days -6 (Day 21 of the pre-study phase) and 25 in the morning, overnight urine samples were collected from all animals in all groups. Access to water was not restricted during urine collection. Any significant change in the general appearance of

the urine was recorded.

a. Quantitative parameters

Urinary refractive index was measured using a refractometer. pH was assayed.

b. Semi-quantitative parameters

Glucose, bilirubin, ketone bodies, occult blood, protein and urobilinogen were assayed using a Clinitek 200+ and Ames Multistix dipsticks.

c. Microscopic examination of the sediment

Microscopic examination of the urinary sediment was performed after centrifugation. The presence of red blood cells, white blood cells, epithelial cells, bacteria, casts and crystals was recorded.

On study Days 29 to 31, all animals from all groups were tranquilized by intramuscular injection of acepromazine and then sacrificed by exsanguination under deep anaesthesia. All dogs 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: adrenal gland, articular surface (femur head), aorta, brain, bone (sternum), bone marrow (sternum), epididymis, oesophagus, eye and optic nerve, gallbladder, heart, intestine (duodenum, jejunum, ileum, caecum, colon, rectum), kidney, larynx, liver, lung, lymph nodes (retropharyngeal, mesenteric), mammary gland, ovary, oviduct, pancreas, pituitary gland, prostate gland, submandibular (salivary) gland, sciatic nerve, skeletal muscle, skin, spinal cord (cervical, mid-thoracic, lumbar), spleen, stomach, 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 that was not processed, were embedded in paraffin wax. Histological sections, stained with hematoxylin and eosin, were prepared from all the animals in all treatment groups. Histopathological examinations were performed on all animals from all groups.

Mean and standard deviations were calculated for the following variables for each sex separately for each group at each time period: body weight, food consumption, hematology, clinical chemistry, urinalysis (pH and refractive index), organ weights.

Due to the small number of animals per sex present in each group (n=3), no statistical analysis was performed for the quantitative parameters. Group incidence summaries for the semi-quantitative parameters assayed were not prepared and no statistical evaluation was performed. For clinical pathology, the results obtained at the end of the treatment

period were compared individually for each animal with the pre-study values, each animal serving as its own control.

Findings

All results for homogeneity and concentration were within a range of 89 to 96% of the nominal concentration (85 to 115% of the nominal concentration). The test substance was stable in the dog powdered diet at concentrations of 5000 ppm and 26000 ppm after 29 days at -18°C and 10 days at ambient temperature (87 and 91% of the nominal concentration, respectively). Hence the test material was stable in the diet over the period of usage on this study.
The mean achieved dietary intakes of the test substance were 0, 174, 469, and 860 mg/kg bw/day in males and 0, 171, 440, and 782 mg/kg bw/day in females.

Mortalities: There were no mortalities during the course of the study

Clinical signs: At 26000 ppm, faeces described as 'coloured' were observed in 1/3 male dogs. At the same concentration, urine described as 'coloured' was observed in 2/3 females. The male did not have 'coloured' urine and the females did not have 'coloured' faeces. These findings of 'coloured' faeces or urine were not observed at any other concentration. The colour observed was described as intense yellow. Its origin was not specified but the description of the test substance used in this study as 'light yellow crystals' suggests that the appearance of the colour is treatment related.

There were no treatment-related ophthalmological findings.

Food consumption: Over the course of the study, mean daily food consumption was slightly reduced in females at 13000 and 26000 ppm. Food consumption was depressed at these dose levels by about 5% and 16%, respectively, compared to controls. Statistical significance was not indicated. No effect on food consumption was observed in males.

Body weight: Over the course of the study, body weights at 5000, 13000 and 26000 ppm remained comparable to that of the control dogs for each sex.

Haematology: There were no treatment-related effects on RBC, Hb, Hct, MCV, MCH, MCHC, WBC and differential count, platelet and reticulocyte counts.

At study day minus 4, the groups intended to be treated at 0, 5000, 13000 and 26000 ppm concentration test substance, showed a mean activated partial thromboplastin time of 11.5, 11.5, 12.0 and 11.3 sec in males and 11.3, 10.7, 11.3 and 11.9 sec in females respectively. At day 28 of the study, the respective mean activated partial thromboplastin time was 10.8 (-6.0%), 10.6 (-7.8%), 10.6 (-11.6%) and 9.6 (-15%) sec in males and in females 10.9 (-3.5%), 10.4 (-5.6%), 10.2 (-9.7%) and 10.4 (-12.6%) sec respectively. The reduction in partial thromboplastin times was more pronounced in males. The reduction in times was considered to be treatment related but their toxicological relevance is equivocal.

Clinical chemistry: Triglyceride concentrations were increased in both sexes in all treated groups in a dose-related manner. In males, triglyceride concentrations were increased 36%, 72% and 151% over pre-test values at 5,000, 13,000 and 26,000 ppm groups, respectively. In females, triglyceride concentrations were increased 63%, 95%

and 298% over pre-test values at 5,000, 13,000 and 26,000 ppm groups, respectively. In females at 26000 ppm, alkaline phosphatase activity was elevated in all groups but with no dose-response association. Other changes were incidental in origin and unrelated to treatment. The treatment related clinical chemistry findings are shown in the following table.

Selected clinical chemistry findings[1]

Parameter	Sampling day	Dose (ppm)			
		0	5000	13000	26000
Males					
Triglyceride, mmol/L	-4	0.38±0.11	0.53±0.17	0.53±0.11	0.49±0.11
	26	0.34±0.07	0.72±0.24	0.91±0.15	1.23±0.36
Triglyceride, % change compared to pre-test values		-11%	+36%	+72%	+151%
Alkaline phosphatase, IU/L	-4	113±59	93±13	84±23	89±19
	26	88±3	118±2	210±1	185±2
Alkaline phosphatase, % change compared to pre-test values		-22%	+27%	+150%	108%
Females					
Triglyceride, mmol/L	-4	0.68±0.31	0.60±0.19	0.65±0.33	0.49±0.13
	26	0.74±0.36	0.98±0.26	1.27±0.57	1.95±0.57
Triglyceride, % change compared to pre-test values		+9%	+63%	+95%	+298%
Alkaline phosphatase, IU/L	-4	127±37	101±33	110±28	162±14
	26	109±24	168±63	150±49	257±34
Alkaline phosphatase, % change compared to pre-test values		-14%	+66%	+36%	+59%

1-Data extracted from Table 7, pp.66-82.

Urinalysis: It was observed however, that in males and females at 26000 ppm, there was an increase in the incidence and grading of crystal formation in the urinary sediment. The crystal formation was graded 0, 1, 2, 3 or 4.

At pretest in males intended to be treated at 26000 ppm, crystal formation was graded. At day 25 of treatment, the grading of one male had increased from 0 to 3, a second from 1 to 3 and the third remained on a grading of 4. The 3 concurrent control males showed a pretest grading of 0, 0 and 0 and a day 25 a grading of 0, 0 and 4, respectively.

At pretest in females intended to be treated at 26000 ppm, crystal formation was graded. At day 25 of treatment, the grading of one female had increased from 0 to 1, a second from 0 to 3 and the third from 0 to 3. The 3 concurrent control females showed a pretest grading of 1, 0 and 0 and a day 25 grading of 2, 4 and 1, respectively.

The increased incidence and grading of crystal formation occurring at the highest dose, cannot be unequivocally attributed to treatment because of the increased occurrence and grading of crystal formation in concurrent control females. However, these findings are

possibly 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 the test substance.

Organ weight: Absolute liver weights were significantly increased in all treated males and females, although in females this did not show a dose relationship. Absolute and relative liver weights are shown in the following table.

Absolute and relative liver weights[1]

Parameter	Dose (ppm)			
	0	5000	13000	26000
Males				
Terminal body weight, kg	8.21 ± 1.07	7.78 ± 1.07	8.02 ± 1.00	8.37 ± 0.98
Brain weight, g	71.7 ± 4.5	73.5 ± 6.7	70.7 ± 5.1	73.2 ± 14.9
Liver weight, g	286.3 ± 23.0	331.0 ± 51.9 (16)[2]	417.7 ± 28.8 (46)	438.5 ± 76.9 (53)
Liver weight, % body weight	3.5 ± 0.5	4.3 ± 0.7 (12)	5.2 ± 0.3 (49)	5.2 ± 0.3 (49)
Liver weight, % brain weight	399.2 ± 7.5	450.6 ± 59.5 (13)	592.1 ± 41.6 (48)	601.5 ± 16.2 (51)
Females				
Terminal body weight, kg	8.54 ± 0.73	8.51 ± 0.44	8.14 ± 0.42	8.17 ± 0.35
Brain weight, g	72.7 ± 5.1	74.3 ± 6.3	73.5 ± 8.5	69.5 ± 4.2
Liver weight, g	314.5 ± 53.3	363.1 ± 22.1 (15)	398.9 ± 75.6 (27)	367.1 ± 64.4 (17)
Liver weight, % body weight	3.7 ± 0.3	4.3 ± 0.3 (16)	4.9 ± 0.7 (32)	4.5 ± 0.6 (21)
Liver weight, % brain weight	431.9 ± 57.3	492.0 ± 60.5 (14)	553.9 ± 165.3 (28)	533.5 ± 128.8 (24)

1-Data extracted from Table 9, pp. 96-100, Table 10, pp. 101-105 and Table 11, pp. 106-110.
2-Percent increase over the control group.

In respect of absolute and relative organ weights, it was observed that at 0, 5000, 13000 and 26000 ppm test substance, the relative prostate weight was 0.063, 0.045, 0.031 and 0.033, respectively. The latter value represents a 47% decrease in relative prostate weight compared to concurrent controls. An examination of the mean absolute prostate gland weights at the respective concentration levels of test substance showed that they were 5.386, 3.578, 2.619 and 2.698 g. A further examination of the prostate gland weights of the 3 male dogs in the concurrent control group showed that one had an exceptionally high prostate gland weight of 8.483 g which contributed to the comparatively high mean prostate gland weight for the group. The mean absolute prostate weight of the other two dogs in the concurrent control group was 3.837. Nevertheless there appears to have been a treatment related decrease in mean prostate

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weights at 13000 and 26000 ppm

Necropsy findings: All findings other than those reported below were incidental and not treatment related. Midzonal multifocal vacuolation of the liver was increased in 1/3 males at 5000 ppm, in 3/3 males at 13000 ppm and in 3/3 males at 26000 ppm test substance. This lesion was not observed in control males.

In the urinary bladder, urolithiasis was observed in 1/3 males at 13000 ppm and 1/3 males at 26000 ppm test substance. One female (of 3) at 26000 ppm also showed urolithiasis.

Treatment related histopathological findings in the kidney(s) are shown in the following table. The changes observed occurred from 13000 ppm with a higher incidence at 26000 ppm in both sexes.

Treatment-related histopathological findings.[1]

	Dose (ppm)			
Finding	**0**	**5000**	**13000**	**26000**
Males				
N	3	3	3	3
Inclusions, intracytoplasmic, globular, eosinophilic, tubular, multifocal	0	0	1	2
Infiltrate, sub- and intra-urothelial, mixed cell, multifocal	0	0	0	2
Mineralization, pelvic, multifocal	0	0	0	1
Erosion, urothelial, focal	0	0	0	1
Females				
N	3	3	3	3
Inclusions, intracytoplasmic, globular, eosinophilic, tubular, multifocal	0	0	0	0
Infiltrate, sub- and intra-urothelial, mixed cell, multifocal	0	0	1	2
Mineralization, pelvic, multifocal	0	0	0	1
Erosion, urothelial, focal	0	0	0	0

1-Data extracted from Table 13, pp. 114-120.

Conclusions

Dietary administration of test substance to the beagle dog at doses of up to 26000 ppm resulted in some findings of toxicological relevance. These included clinical signs of intense yellow urine in two females at 26000 ppm, dose-related increase in serum

triglyceride levels commencing at 5000 ppm, elevated liver weight at all Gritty content and/or stones were found in the urinary tract at 13000 and 26000 ppm, accompanied in the kidneys by histopathological findings at 13000 and 26000 ppm.

Based on dose-related increase in serum triglyceride levels commencing at 5000 ppm (174 and 171 mg/kg/day in males and females, respectively), elevated liver weight at all doses, and a dose-related increase in midzonal multifocal vacuolation of the liver in males, a NOEL could not be established in this study. The effects at all doses tested were considered to be adverse and a NOAEL was also not determinable.

EPA Reviewer: William B. Greear, MPH, DABT **Signature:** William B. Greear
Registration Action Branch 1, Health Effects Division (7509P) **Date:** 6/5/2008
EPA WAM: PV Shah, PhD **Signature:** P.V. Shah
Registration Action Branch 1, Health Effects Division (7509P) **Date:** 6/5/08

TXR#: 0054227

DATA EVALUATION RECORD

STUDY TYPE: 90 (29)-Day Oral Toxicity - [dog];
OPPTS None [§82-1b] (dog); OECD 409

PC CODE: 000692 **DP BARCODE:** D335563

TEST MATERIAL (PURITY): AE 0317309 (95.7% w/w a.i.)

SYNONYMS: Pyrasulfatole technical

CITATION: Eigenberg, D.. (2004). Technical grade AE0317309 - A 90-day subchronic toxicity feeding study in the Beagle dog. Bayer CropScience LP Toxicology, Stilwell, Kansas and Pathology Associates Division, Charles River Laboratories, Inc., Kansas City, Missouri. Laboratory Study No.: 201019-1, March 2, 2006. MRID 46801901. Unpublished.

SPONSOR: Bayer AG, Bayer CropScience, Alfred Nobel Str. 50, Monheim, Germany

EXECUTIVE SUMMARY:

In a 28-day dermal toxicity study (MRID 46801901), AE 0317309 (96.3% w/w a.i., batch Op:1-4) was administered in the diet to 4 Beagle dogs/sex at dose levels of 0, 1,500, 9,000 or 18,000 ppm for a 29-day period (The mg/kg bw/day equivalency was not calculated.)

There were no effects on ophthalmic effects observed.

One male dog at 9,000 ppm was euthanized for humane reasons on study day 23, while two males at 18,000 ppm were euthanized on study day 28 in moribund condition. The 90 day study was terminated early and the remainder of the dogs was euthanized on study day 29 because of urinalysis findings and the likelihood of urinary tract findings in the dogs unaffected to that point. Red urine was noted in the urine of several male dogs in the 9,000 and 18,000 ppm treatment groups. In the three males sacrificed prior to the termination of the study, decreased activity, reduced food consumption, and lethargy were noted. The 9,000 ppm male sacrificed on day 23 lost 605 g during the study. One of the 18,000 ppm males sacrificed on day 28 lost 694 g prior to sacrifice. The 9,000 ppm male stopped eating on day 14 prior to sacrifice on day 23. One of the 18,000 ppm males ate 1-10 g of food on study days 12-14, and showed markedly decreased food

consumption on study days 22, 23, and 25-27. The other 18,000 pm male showed markedly decreased food consumption on study days 22, 23, and 25-27. In males, on study day 18, urea was statistically significantly increased at 18,000 ppm. In all male treatment groups, blood in the urine was statistically significantly increased on study day 24. Urinary protein was significantly elevated in males at 9,000 and 18,000 ppm as a result of the urinary protein elevation in the 1 and 2 dogs that were euthanized early at these concentrations. One female at 9,000 ppm also showed increased urinary protein. The 9000 ppm dog euthanized on study day 23, was found to be dehydrated and to have multiple yellow calculi in the urethra, bloody urine in the bladder, bilateral enlarged kidneys, bilateral moderate renal pelvis dilatation, and other urinary tract findings. One of the 18000 ppm males sacrificed on study day 28 was found to have yellow calculi in the urethra, bilateral dark red kidney capsule with haemorrhage in the subcapsule, bilateral moderate renal pelvis dilatation, bilateral dark gray/discolored/necrotic renal pelvis, and discolored/dark red urinary bladder with very thick wall. The other 18000 ppm male was found to have multiple calculi in the urinary bladder, bilateral marked dilated renal pelvis, bilateral dilated ureter, bloody urine in the bladder, and urinary bladder with very thick wall.

The study could not be used to determine a NOAEL/LOAEL. No data were provided. Only the sponsor's study report provided a brief summary.

This 90-day oral toxicity study in the dog is **Unacceptable (guideline)** and does not satisfy the guideline requirement for a 90-day oral toxicity study (OPPTS 870.3150; OECD 409) in dogs. The study was too short in duration and was not conducted under GLP requirements. No data were presented for evaluation.

COMPLIANCE: Signed and dated GLP, Quality Assurance, and Data Confidentiality statements were **not** provided.

This Executive Summary was prepared for United States Environmental Protection Agency, Office of Pesticides Programs, Health Effects Division use.

The following text was generated by the Australian Pesticides and Veterinary Medicines Authority. However, this document has undergone critical scientific analysis and been modified as appropriate.

Report:	KIIA 5.3.1/03, Eigenberg, D.; 2, March 2006
Title:	A 90-day subchronic toxicity feeding study in the Beagle dog
Test Substance:	AE 0317309: Pyrosulfatole – batch Op. 1-4, purity 95.7% w/w
Laboratory:	Bayer CropScience LP Toxicology 17745 South Metcalf Stilwell Kansas 66085-9104 & Pathology Associates Division Charles River Laboratories Inc 9584 Marion Ridge Drive Kansas City, MO 64137
Sponsor:	Bayer CropScience LP, 2 T.W. Alexander Drive, Research Triangle Park, NC 27709
Report No.: **Document No.:**	201019-1 M-268108-01-1
Study Duration:	28 July 2003 to 26 August 2003 (Report revised 2 March 2006)
Guidelines:	US EPA Health Effects Test Guidelines, OPPTS 870.3150 (1998), OECD Guidelines for Testing of Chemicals, Section 4, Guideline 409, September 1998, JMAFF, Ref No 12 Nousan No 8147, November 2000
GLP/QA	No/No (study was prematurely terminated after 29 days)

Preliminary Note

This study was not continued past the 29th day because of excess toxicity.

Materials and Methods

Corn oil, at 1% by weight of the diet and acetone, were used as vehicle to suspend the test substance which was then mixed in the diet to achieve concentrations of test substance of 0, 1 500, 9000 and 18000 ppm. It was intended to administer the test substance in diet for 90 days but the study was terminated after 29 days.

The rationale for the selection of this concentration range is discussed at the end of this evaluation report.

The proposed methodology for this study was that undertaken in the 90 day dog study (KIIA 5.3.3/01, Document M-257679-01-1). The following description is to be read in the knowledge that this study ceased at day 29. All feed mixtures were prepared weekly and stored under freezer conditions at minus 23°C until used. A sample of each batch of food mixed was taken and retained in a freezer until the study was complete. The homogeneity of the test substance and its stability in food at room temperature for 7 days and freezer temperature for 28 days were determined analytically. The concentration of the active ingredient in the food was determined for study weeks 1, 2, 3, 7, 11 and 15.

Each treatment group contained 4 male and 4 female beagle dogs, which were approximately 4-6 months old at the start of the treatment period. Their body weight range for each sex was not specified. The dogs were acclimatized to the conditions of the study for two weeks prior to the start. In this period, food was available *ad libitum*. Dogs were housed separately in stainless steel runs. Only healthy dogs, free of signs were placed on study. Dogs were exercised in stainless steel runs for 15 minutes twice weekly. Tap water was available *ad libitum*.

Clinical signs were monitored at least twice daily. Body weights were measured on a

weekly basis, and food consumption was to be determined daily, with food made available to the dogs for 3-6 hours each day. Ophthalmological examinations were conducted prior to the first day of dosing and just prior to the end of the study.

Blood was collected from fasted dogs via jugular venipuncture prior to the beginning of the study and again in weeks 4, 8, and 13 for clinical chemistry and hematology determinations. The period of urine collection was 24 hours. At the end of the study, the dogs were to be euthanized by intravenous injection of a standard euthanasia solution, and subjected to gross necropsy.

Haematological parameters examined were:

Haematocrit, erythrocyte count, leukocyte count, leukocyte differential count, haemoglobin, mean corpuscular haemoglobin, mean corpuscular haemoglobin concentration, mean corpuscular volume, platelet count, reticulocyte count, red blood cell distribution width, hemoglobin distribution width, activated partial thromboplastin time and prothrombin time.

Clinical chemistry parameters examined were:

Electrolytes: calcium, chloride, phosphorous, potassium and sodium

Enzymes: alkaline phosphatase, creatine phosphokinase, lactic acid dehydrogenase, alanine aminotransferase, aspartate aminotransferase gamma glutamyl transferase and glutamate dehydrogenase.

Other: albumin, creatinine, urea nitrogen, total cholesterol, globulins, glucose, total bilirubin, total protein, triglycerides, uric acid and bile acids.

Urinalysis parameters examined were:

Appearance, glucose, volume, ketones, specific gravity/osmolality, bilirubin, pH, blood, sediment (microscopic), nitrate, protein, urobilinogen urine creatinine (24 hour), leucocytes.

A complete gross examination was performed on all dogs. The necropsy consisted of a systematic gross examination of each dog's general physical condition, body orifices, external and internal organs and tissues.

Adrenal gland, aorta (thoracic), bone (rib), bone marrow, brain (multiple sections), epididymides, oesophagus, eyes (retina and optic nerve), lachrymal gland*, gallbladder, heart, intestine (duodenum, jejunum, ileum, caecum, colon, rectum), kidney larynx, liver, lung, lymph nodes, mammary gland, nasal cavities*, nasopharynx, ovaries, pancreas, peripheral nerve, pituitary gland, prostate gland, sciatic nerve, seminal vesicle, skeletal muscle*, skin, spinal cord (three levels), spleen, stomach, salivary glands, testes, thymus, thyroid gland (with parathyroid glands), tongue*, trachea, urinary bladder, ureter, uterus (including cervix), vagina and all gross lesions and masses.

All of these organs and tissues were examined histologically except those marked with an asterisk.

Statistical significance was determined at $p < 0.05$ for all tests with the exception of Bartlett's test, in which a probability value of $p < 0.001$ was used. If the data was homogeneous, an ANOVA was performed followed by Student's t-test on parameters

showing a significant effect by ANOVA. If the data were non-homogeneous, a Kruskal-Wallis ANOVA was performed followed by the Mann-Whitney U-test to identify statistical significance between groups. Frequency data, examined statistically, were initially analysed by a Chi-Square procedure. If there was statistical significance using the Chi-square test, each treatment group was compared to the control group using a Fisher's Exact test.

Findings

Other than that discussed below, no results were provided

The stability and homogeneity of the test substance in the diet was not reported. The mean intake of test substance over the duration of the study in mg/kg bw/day for each concentration of test substance was not calculated.

Mortalities: One male dog at 9000 ppm was euthanized for humane reasons on study day 23, while two males at 18000 ppm were euthanized on study day 28 in moribund condition.

The remainder of the dogs was euthanized on study day 29 because of urinalysis findings and the likelihood of urinary tract findings in the dogs unaffected to that point.

Clinical signs: Red urine was noted in the urine of some male dogs in the 9000 ppm and 18000 ppm treatment groups. In the three males sacrificed prior to the termination of the study, decreased activity, reduced food consumption, and lethargy were noted.

Ophthalmological examination: There were no treatment related ophthalmological findings.

Body weight: The 9000 ppm male sacrificed on day 23 lost 605 g during the study. One of the 18 000 ppm males sacrificed on day 28 lost 694 g prior to sacrifice. Other than these two dogs, there were no effects on body weight.

Food consumption: With the exception of the three dogs sacrificed early, there was no effect on food consumption. The 9000 ppm male stopped eating on day 14 prior to sacrifice on day 23. One of the 18000 ppm males ate 1-10 g of food on study days 12-14, and showed markedly decreased food consumption on study days 22, 23, and 25-27. The other 18000 pm male showed markedly decreased food consumption on study days 22, 23, and 25-27.

Haematology: There was no effect on haematological parameters in either males or females.

Clinical chemistry: There were no effects on clinical chemistry parameters noted in females at any dose level. In males, on study day 18, urea was statistically significantly increased at 18 000 ppm.

Urinalysis: In all male treatment groups, blood in the urine was statistically significantly increased on study day 24. Urinary protein was significantly elevated in males at 9 000 and 18000 ppm as a result of the urinary protein elevation in the 1 and 2 dogs that were euthanized early at these concentrations. One female at 9 000 ppm also showed increased urinary protein.

Macroscopic findings: The 9000 ppm dog euthanized on study day 23, was found to be dehydrated and to have multiple yellow calculi in the urethra, bloody urine in the bladder, bilateral enlarged kidneys, bilateral moderate renal pelvis dilatation, and other urinary tract findings. One of the 18000 ppm males sacrificed on study day 28 was found to have yellow calculi in the urethra, bilateral dark red kidney capsule with haemorrhage in the subcapsule, bilateral moderate renal pelvis dilatation, bilateral dark gray/discolored/necrotic renal pelvis, and discolored/dark red urinary bladder with very thick wall. The other 18000 ppm male was found to have multiple calculi in the urinary bladder, bilateral marked dilated renal pelvis, bilateral dilated ureter, bloody urine in the bladder, and urinary bladder with very thick wall. There were no gross observations in non-moribund dogs.

Conclusions

The dietary administration of test substance to male beagle dogs produced precipitation of test substance in the urinary tract, with the precipitate forming into urinary tract stones. The moribundity, clinical signs, and necropsy findings in the moribund dogs were clearly due to the presence of these urinary tract stones.
On the basis of the incomplete data set for this study, no NOEL could be established.

Comment

The nominal concentrations chosen for this study were 0 (concurrent vehicle control) 1500, 9000, and 18000 ppm (4/sex/dose) of technical grade test substance mixed with dog ration. Selection of these concentration levels was based on a 28 day toxicity study in the Beagle dog (KIIA 5.3.1/10, Document M-1022922-01-2), which used concentrations of 0, 5000, 13000 and 26000 ppm for 28 days (3/sex/dose).

Based on the findings of the 28 day toxicity study in dogs, the low-dose (500 ppm) was expected to establish the NOEL; the next dose (9000 ppm) was expected to demonstrate an intermediate level of toxicity, and the high dose 18000 ppm) was expected to be a maximum-tolerated (MTD) dose, without threatening survival.

The severity of many of the outcomes in the prematurely ended 90 day study was not expected.

EPA Reviewer: William B. Greear, MPH, DABT **Signature:** William B. Greear
Registration Action Branch 1, Health Effects Division (7509P) **Date:** 6/5/2008
EPA WAM: PV Shah, PhD **Signature:** P.V. Shah
Registration Action Branch 1, Health Effects Division (7509P) **Date:** 6/5/08

TXR#: 0054227

DATA EVALUATION RECORD

STUDY TYPE: 90-Day Oral Toxicity - [dog];
OPPTS 870.3150 [§82-2] (nonrodent); OECD 409.

PC CODE: 000692 **DP BARCODE:** D335563

TEST MATERIAL (PURITY): AE 0317309 (95.7% w/w a.i.)

SYNONYMS: Pyrasulfatole technical

CITATION: Eigenberg, D. (2005). Technical grade AE0317309 A 90-day subchronic toxicity feeding study in the dog. Bayer CropScience LP Toxicology, Stilwell, KS, 66085-9104, and Pathology Associates Division of Clarles River Laboratories, Inc., Marion, Mo. 64137. Study No.: 03-S76-UG, August 30, 2005. MRID 46801902. Unpublished.

SPONSOR: Bayer CropScience LP, Research Triangle Park, NC 27709

EXECUTIVE SUMMARY:

In a 90-day oral toxicity study (MRID 46801902), AE 0317309 (95.7% w/w a.i., batch Op:1-4) was administered in the diet to groups of 4 Beagle dogs/sex/dose at dose levels of 0, 100, 500, or 1000 ppm (equivalent to 0/0, 3/3, 17/17 and 40/33 mg/kg bw/day in male/female dogs, respectively) for a period of 90 days.

There were no compound-related effects on mortality, clinical signs, body weight, food and water consumption, hematology, clinical chemistry, urinalysis, gross pathology and microscopic pathology.

The LOAEL was not established. The NOAEL is 40 mg/kg bw/day for males and 33 mg/kg bw/day for females.

This 90-day oral toxicity study in the dog is **acceptable/guideline** and satisfies the guideline requirement for a 90-day oral toxicity study (OPPTS 870.3150 OECD 409) in dogs.

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

This Executive Summary was prepared for United States Environmental Protection

Agency, Office of Pesticides Programs, Health Effects Division use.

The following text was generated by the Australian Pesticides and Veterinary Medicines Authority. However, this document has undergone critical scientific analysis and been modified as appropriate.

Report:	KIIA 5.3.3/01, Eigenberg, D. A.; 30, August 2005
Title:	A 90-day subchronic toxicity feeding study in the Beagle dog
Test Substance:	AE 0317309: Pyrosulfatole – batch Op. 1-4, purity 95.7% w/w
Laboratory:	Bayer CropScience LP Toxicology 17745 South Metcalf Stilwell Kansas 66085-9104 & Pathology Associates Division Charles River Laboratories Inc 9584 Marion Ridge Drive Kansas City, MO 64137
Report No.: **Document No.:**	201210 M-257679-01-1
Study Duration:	11 November 2003 to 18 February 2004
Guidelines:	US EPA Health Effects Test Guidelines, OPPTS 870.3150 (1998), OECD Guidelines for Testing of Chemicals, Section 4, Guideline 409, September 1998, JMAFF, Ref No 12 Nousan No 8147, November 2000
GLP/QA	Yes

Materials and Methods

Corn oil, at 1% by weight of the diet and acetone, were used as vehicle to suspend the test substance which was then mixed in the diet (Purina Certified Canine Diet Etts 5006-3) to achieve concentrations of test substance of 0, 100, 500, and 1000 ppm (equivalent to 0/0, 3/3, 17/17 and 40/33 mg/kg bw/day in male/female dogs, respectively).

The selection of the concentrations of test substance used in this study was based on the findings of a 90 day dog study (KIIA 5.3.1/03, Report No 201019-1, Document M-063774-01-2) which used concentrations in diet of 0, 1500, 9000 and 18000 ppm and was terminated on study day 29 due to excessive toxicity. In this study, one mid-dose group dog was euthanized on study day 23 due to a lack of food intake starting on study day 14 and lethargy, despite supplementation with canned dog food and vitamins. In addition, on study day 28, two high-dose group dogs were euthanized in moribund condition. The gross necropsy of these dogs revealed treatment related lesions of the urinary tract. In the low dose group, there was an increase in blood observed in the urine of males and females, indicating possible urinary tract lesions.

All feed mixtures were prepared weekly and stored at minus 23°C until used. A sample of each batch of food mixed was taken and retained in a freezer until the study was complete. The homogeneity of the test substance and its stability in food at room temperature for 7 days and freezer temperature for 28 days were determined. The concentration of the test material in the food was determined for study weeks 1, 2, 3, 7, 11 and 15.

Each treatment group contained 4 male and 4 female beagle dogs obtained from Marshall Farms USA, Inc., North Rose, New York, which were approximately 6-7 months old at the start of the treatment period. This corresponded to body weights of 5.9-7.7 kg for males and 5.1-6.8 kg for females. The dogs were acclimatized to the conditions of the study (temperature: 64-84°F; humidity: 30-70%; air changes: 10/hr; photo period: 12 hr on/12 hr off) for two weeks prior to the start. In this period, food was available *ad libitum*. Dogs were housed separately in stainless steel runs. Tap water was available *ad*

libitum.

Clinical signs were monitored at least twice daily. Body weights were measured on a weekly basis, and food consumption was determined daily, with food made available to the dogs for 3-6 h each day. Ophthalmoscopic examinations were conducted prior to the first day of dosing and just prior to the end of the study.

Blood and urine were collected from fasted dogs prior to the beginning of the study and again in weeks 4, 8, and 13 for clinical chemistry, hematology determinations and urinalysis. Urine was collected over a 24 h period. The blood was collected by jugular venipuncture.

Haematological parameters examined were, Hct, RBC, Hct, RBC, WBC, leukocyte differential count, Hb, MCH, MCHC, MCV, platelet count, reticulocyte count, red blood cell distribution width, hemoglobin distribution width, activated partial thromboplastin time and prothrombin time. Clinical chemistry parameters examined were, electrolytes: calcium, chloride, phosphorous, potassium and sodium levels, AP, creatine phosphokinase, LDH, ALT, AST, gamma glutamyl transferase and glutamate dehydrogenase activities, and albumin, creatinine, urea nitrogen, total cholesterol, globulins, glucose, total bilirubin, total protein, triglycerides, uric acid and bile acid levels.

Urinalysis parameters examined were: appearance, glucose, volume, ketones, specific gravity/osmolality, bilirubin, pH, blood, sediment (microscopic), nitrate, protein, urobilinogen urine creatinine (24 h) and leucocytes.

At the end of the study, the dogs were euthanized by intravenous injection of a standard euthanasia solution (FatalPlus), and subjected to gross necropsy.

All of the following organs and tissues were examined histologically except those marked with an asterisk: adrenal gland, aorta (thoracic), bone (rib), bone marrow, brain (multiple sections), epididymides, oesophagus, eyes (retina and optic nerve), lachrymal gland*, gallbladder, heart, intestine (duodenum, jejunum, ileum, caecum, colon, rectum), kidney larynx, liver, lung, lymph nodes, mammary gland, nasal cavities*, nasopharynx, ovaries, pancreas, peripheral nerve, pituitary gland, prostate gland, sciatic nerve, seminal vesicle, skeletal muscle*, skin, spinal cord (three levels), spleen, stomach, salivary glands, testes, thymus, thyroid gland (with parathyroid glands), tongue*, trachea, urinary bladder, ureter, uterus (including cervix), vagina and all gross lesions and masses.

Statistical significance was determined at $p < 0.05$ for all tests with the exception of Bartlett's test, in which a probability value of $p < 0.001$ was used. If the data was homogeneous, an ANOVA was performed followed by Student's t-test on parameters showing a significant effect by ANOVA. If the data were non-homogeneous, a Kruskal-Wallis ANOVA was performed followed by the Mann-Whitney U-test to identify statistical significance between groups. Frequency data, examined statistically, were initially analysed by a Chi-Square procedure. If there was statistical significance using the Chi-square test, each treatment group was compared to the control group using a Fisher's Exact test.

Findings

The test substance was found to be homogeneously distributed in the food and was stable at both room and freezer conditions. The analytically determined mean concentrations ranged from 96 to 100% of the nominal concentrations.

The mean intake of test substance in mg/kg bw/day calculated using the analytical concentrations of test substance in the food were 0, 3, 17, and 40 mg/kg bw/day for males and 0, 3, 17, and 33 mg/kg bw/day for females.

Mortalities: No dogs died or were sacrificed early during this study.

Clinical signs: There were no treatment related clinical signs in either males or females. There were no ophthalmoscopic findings attributable to treatment.

Food consumption & body weight: There was no treatment related effect.

Hematology, clinical chemistry, urinalysis, organ weights, pathology: There were no treatment related effects on any of these parameters.

Conclusions

In the absence of any findings, the NOEL for this study was the highest concentration of the test substance tested, 1000 ppm, (40 mg/kg bw/day in males and 33 mg/kg bw/day in females).

The reason for the selection of the highest dose as < 1500 ppm was explained but the actual choice for the highest dose (1000 ppm concentration in diet) did not induce any treatment related effects and was therefore too low (OECD Test Guideline 409). The dose range 1000-1500 ppm concentration was apparently not explored for its capacity to induce toxicity. The dose range used was not therefore suitable for establishing a NOEL or NOAEL.

The NOAEL established by the study authors was 1000 ppm (40 and 33 mg/kg bw day for males and females respectively).

EPA Reviewer: William B. Greear, MPH, DABT **Signature:** William B. Greear
Registration Action Branch 1, Health Effects Division (7509P) **Date:** 6/5/2008
EPA WAM: PV Shah, PhD **Signature:** PVShah
Registration Action Branch 1, Health Effects Division (7509P) **Date:** 6/5/08

TXR#: 0054227

DATA EVALUATION RECORD

STUDY TYPE: 90-Day Oral Toxicity - [rat];
OPPTS 870.3100 [§82-1] (rodent); OECD 408.

PC CODE: 000692 **DP BARCODE:** D335563

TEST MATERIAL (PURITY): AE 0317309 (97.4% w/w a.i.)

SYNONYMS: Pyrasulfatole

CITATION: 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.

SPONSOR: Bayer AG, Bayer CropScience AG, Alfred Nobel Str. 50, Monheim, Germany

EXECUTIVE SUMMARY:

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.

The LOAEL is 1000 ppm (77 mg/kg bw/day) in females based on neovascularization of the cornea and "snowflake" corneal opacity; and 7000 ppm (454 mg/kg bw/day) in males based on mortality, neovascularization of the cornea and "snowflake" corneal opacity, and histopathology in the kidney, urinary bladder, thyroid, and ureters. The NOAEL is 30 ppm (2.32 mg/kg bw/day) in females and 1000 ppm (66 mg/kg bw/day) in males.

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

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

This Executive Summary was prepared for United States Environmental Protection Agency, Office of Pesticides Programs, Health Effects Division use.

The following text was generated by the Australian Pesticides and Veterinary Medicines Authority. However, this document has undergone critical scientific analysis and been modified as appropriate.

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]

		Dose (ppm)					
Endpoint	**Study week**	**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/or characteristic "snowflake" corneal opacities were noted at 7000 and 12000 ppm in males, and 1000 ppm, 7000, and 12000 ppm in females.

Data on neovascularization of the cornea and characteristic "snowflake" corneal opacities[1]

	Dose (ppm)					
Endpoint	**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,	0	0	0	2

	multifocal to diffuse				
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.

EPA Reviewer: William B. Greear, MPH, DABT **Signature:** William B. Greear
Registration Action Branch 1, Health Effects Division (7509P) **Date:** 6/5/2008
EPA WAM: PV Shah, PhD **Signature:** P.V. Shah
Registration Action Branch 1, Health Effects Division (7509P) **Date:** 6/5/08

TXR#: 0054227

DATA EVALUATION RECORD

STUDY TYPE: Metabolism Study - [rat]; OPPTS 870.7485 [§85-1]; OECD 417.

PC CODE: 000692 **DP BARCODE:** D335563

TEST MATERIAL (PURITY): AE 0317309 (97.6-100% w/w a.i.)

SYNONYMS: Pyrasulfatole technical

CITATION: Fischer, D. and Roensch, W. (2005). The Metabolism of [Phenyl-UL-14C] and [Pyrazole-3-14C] AE 0317309 in Rats. Bayer CropScience Environmental Research, Stilwell, KS, 66085-9104, Report No.: MEAIX021, August 30, 2005. MRID 46801918. Unpublished.

SPONSOR: Bayer CropScience LP, Research Triangle Park, NC 27709

EXECUTIVE SUMMARY:

In a metabolism study (MRID 46801918), AE 0317309 (97.6-100% a.i., Vial #s C-938, C-939, C-938B, C-1024A, K-1196, K-1267), male Wistar Hanover rats (five rats for each radiolabel) were given single oral doses of [phenyl-UL-^{14}C] AE 0317309 {methanone, (5-hydroxy-1,3-dimethyl-1H-pyrazol-4-yl)[2-(methylsulfonyl)-4-(trifluoro-methyl) phenyl] [phenyl-UL-^{14}C]} or [pyrazole-3-^{14}C] AE 0317309 {methanone, (5-hydroxy-1,3-dimethyl-1H-pyrazol-4-yl)[2-(methylsulfonyl)-4-(trifluoromethyl)phenyl] [pyrazole-3-^{14}C]} at dose rates of 10.0 and 9.88 mg/kg bw, respectively. In two separate experiments, male Wistar Hanover rats with surgically implanted jugular cannula were dosed intravenously with [phenyl-UL-^{14}C] AE 0317309 (four rats) or [pyrazole-3-^{14}C] AE 0317309 (five rats) at dose rates of 9.81 and 9.60 mg/kg bw, respectively.

The [phenyl-UL-^{14}C] AE 0317309 was readily absorbed following oral dosing, with approximately 62% of the administered radioactivity dose being recovered in the urine within 6 h post dose. A total of approximately 73% of the administered radioactivity dose was recovered in the urine by the time of sacrifice (52 h).

The [pyrazole-3-^{14}C] AE 0317309 was readily absorbed following a single oral dose, with approximately 57% of the administered radioactivity dose being recovered in the urine within 6 h post dose. A total of approximately 75% of the dose was recovered in the urine by the time of sacrifice (48 h).

In the case of intravenous administration, approximately 90% of the radioactivity was excreted in the urine and approximately 10% in the feces by 48 h. Most (over 80%) of the intravenous dose was excreted in the urine within 6 h.

In all experiments, <2% of the administered dose remained in the residual carcass and tissues at sacrifice. In all experiments, the highest residues were found in the liver and kidney. There was no apparent difference between the phenyl-UL-^{14}C label and the pyrazole-3-^{14}C label in the distribution of equivalents in the tissues following intravenous dosing. However, concentrations in the tissues were consistently higher in the case of the pyrazole-3-^{14}C label than in the case of the phenyl-UL-^{14}C label following oral dosing. The distribution of radioactivity in tissues did not differ greatly between oral and intravenous dosing, with the exception that the concentration in the residual carcass is somewhat higher in the latter case. Following both oral and intravenous administration, most of the dose was excreted unchanged as AE 0317309. Hydroxymethyl AE 0317309, desmethyl AE 0317309, and AE B197555 were observed as minor metabolites in the urine and feces. Following oral dosing, approximately 70% of the radioactivity was excreted in the urine and 30% in the feces by 48 or 52 h.

This metabolism study in the rat is classified **Acceptable/guideline** and satisfies the Tier 1 guideline requirement for a metabolism study [OPPTS 870.7485, OECD 417] in rats. A repeated dose study should have been incorporated into the protocol and female rats should have been included in the study. Also, a low- as well as a high- dose (two doses) should have been used instead of the single dose used in this study.

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

This Executive Summary was prepared for United States Environmental Protection Agency, Office of Pesticides Programs, Health Effects Division use.

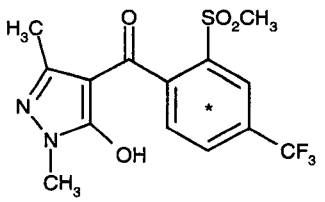
The following text was generated by the Australian Pesticides and Veterinary Medicines Authority. However, this document has undergone critical scientific analysis and been modified as appropriate.

Report:	**KIIA 5.1/01, Fischer, D.R. and Roensch, W.L. ; 2005**
Title:	The Metabolism of [Phenyl-UL-^{14}C] and [Pyrazole-3-^{14}C] AE 0317309 in Rats.
Laboratory; Study duration	Bayer CropScience Environmental Research, Bayer Research Park, 17745 South Metcalf Avenue, Stilwell, KS, USA; the study was initiated on 16 April 2003 and completed on 18 February 2005
Report No.:	MEAIX021
Document No.:	M-258714-01-1
Guidelines:	OPPTS 870.7485
GLP/QA	Yes

Materials and Methods

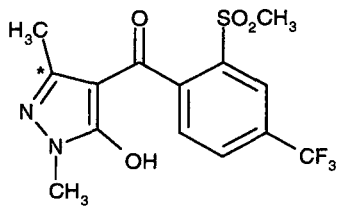
To determine the metabolic fate of the herbicide AE 0317309, 8 week old male Wistar Hanover rats weighing 152-195g (five rats for each radiolabel) were given single oral doses of [phenyl-UL-^{14}C] AE 0317309 {methanone, (5-hydroxy-1,3-dimethyl-1H-pyrazol-4-yl)[2-(methylsulfonyl)-4-(trifluoro-methyl) phenyl] [phenyl-UL-^{14}C]} or [pyrazole-3-^{14}C] AE 0317309 {methanone, (5-hydroxy-1,3-dimethyl-1H-pyrazol-4-yl)[2-(methylsulfonyl)-4-(trifluoromethyl)phenyl] [pyrazole-3-^{14}C]} at dose rates of 10.0 and 9.88 mg/kg bw, respectively. In two separate experiments, 8 week old male Wistar Hanover rats weighing 142-184g with surgically implanted jugular cannulae were dosed intravenously with [phenyl-UL-^{14}C] AE 0317309 (four rats) or [pyrazole-3-^{14}C] AE 0317309 (five rats) at dose rates of 9.81 and 9.60 mg/kg bw, respectively. The test animals were housed in metabolism cages.

[Phenyl-UL-^{14}C] AE 0317309



* indicates the position of the radiolabel

[Pyrazole-3-^{14}C] AE 0317309



* indicates the position of the radiolabel

In all experiments, urine and feces were collected in separate containers cooled with dry ice. Urine was collected at 6, 12, 24 and 48 h post-treatment (and at 52 h in the oral experiment using [phenyl-UL-^{14}C] AE 0317309). Feces were collected at 24 h and 48 h post-treatment (and at 52 h in the oral experiment using [phenyl-UL-^{14}C] AE 0317309). The weight of urine at each interval was determined, and aliquots were radioassayed. Each feces sample was weighed, homogenized and aliquots of the fecal homogenates were oxidized and radioassayed. All urine and feces samples were stored frozen in a freezer (<-18°C) when not in use.

Following completion of each experiment, each metabolism cage was washed with methanol/water (MeOH/H_2O; 1:1), and the washes from each cage were separately radioassayed. The urine and feces collection containers were also washed with MeOH/H_2O (1:1), and the combined washes from the urine and feces containers for each rat were radioassayed.

At the conclusion of each experiment (48 or 52 h after dosing), individual rats were anesthetized with Halothane, and were exsanguinated by cardiac puncture Triplicate blood samples were pipetted into combustion pads for oxidation and radioassay. In all of the experiments, bone, brain, fat, heart, kidney, liver, lung, muscle, skin, spleen, testes, and thyroid were collected and weighed; aliquots of each tissue were oxidized and radioassayed. The gastrointestinal tract (GIT) of each rat was collected and weighed, and the entire sample was homogenized, oxidized and radioassayed. The remaining carcass was dissolved in 3N ethanolic potassium hydroxide (KOH) solution and samples of this were radioassayed.

In an attempt to detect and collect respired $^{14}CO_2$ and all other volatile compounds in the [phenyl-UL-^{14}C] and [pyrazole-3-^{14}C] AE 0317309 oral dose experiments (denoted as O-LDE-M in the following sections and tables), the rats were housed in metabolism cages equipped with a flow-through system which allowed separation and collection of respired gases. Respired gas collection was discontinued 24 h post-treatment. Respired gases were not collected during the [phenyl-UL-^{14}C] and [pyrazole-3-^{14}C] AE 0317309 intravenous experiments (denoted as IV-LDE-M below).

Identification and quantitation of the metabolites in the urine samples and feces extracts were accomplished by using reverse-phase HPLC and LC/MS/MS.

Findings

Absorption:

[Phenyl-UL-^{14}C] AE 0317309:

The [phenyl-UL-^{14}C] AE 0317309 was readily absorbed following oral dosing, with approximately 62% of the administered radioactivity dose being recovered in the urine within 6 h post dose. A total of approximately 73% of the administered radioactivity dose was recovered in the urine by the time of sacrifice (52 h) (see table below: "Recovery of radioactivity after administration of [^{14}C] AE 0317309"). No data were obtained on plasma concentrations.

[Pyrazole-3-^{14}C] AE 0317309:

The [pyrazole-3-^{14}C] AE 0317309 was readily absorbed following a single oral dose, with approximately 57% of the administered radioactivity dose being recovered in the urine within 6 h post dose. A total of approximately 75% of the dose was recovered in the urine by the time of sacrifice (48 h). No data were obtained on plasma concentrations.

Distribution:

In all experiments, <2% of the administered dose remained in the residual carcass and tissues at sacrifice. The radioactive residues calculated as ppm AE 0317309 equivalents in tissues from the oral and intravenous experiments are presented in the table below. In all experiments, the highest residues were found in the liver and kidney. There was no apparent difference between the phenyl-UL-^{14}C label and the pyrazole-3-^{14}C label in the distribution of equivalents in the tissues following intravenous dosing. However, concentrations in the tissues were consistently higher in the case of the pyrazole-3-^{14}C label than in the case of the phenyl-UL-^{14}C label following oral dosing. This is difficult to explain, since the metabolism data given below indicate that the AE 0317309 molecule was largely unmetabolised in the rat and therefore the pyrazole and phenyl moieties should not differ in their distribution. The distribution of radioactivity in tissues did not differ greatly between oral and intravenous dosing, with the exception that the concentration in the residual carcass is somewhat higher in the latter case.

Distribution of radioactivity in rat tissues/organs after a single oral or intravenous dose of [^{14}C] AE 0317309 at 48h or 52 h (for phenyl-UL-^{14}C] Oral).[a1]

	PPM Equivalents[b]

Tissue/organ	[Phenyl-UL-^{14}C] Oral	[Pyrazole-3-^{14}C] Oral	[Phenyl-UL-^{14}C] Intravenous	[Pyrazole-3-^{14}C] Intravenous
Bone	0.004	0.012	0.006	0.006
Brain	0.003	0.006	0.005	0.002
Residual Carcass	0.014	0.026	0.064	0.116
Fat	0.005	0.018	0.004	0.006
GIT	0.052	0.098	0.078	0.047
Heart	0.006	0.011	0.005	0.005
Kidney	0.326	0.410	0.346	0.353
Liver	1.770	1.793	1.538	1.660
Lung	0.014	0.021	0.014	0.013
Muscle	0.006	0.009	0.006	0.006
Skin	0.010	0.023	0.018	0.014
Spleen	0.005	0.015	0.005	0.005
Testes	0.005	0.008	0.004	0.004
Thyroid Gland	0.016	0.028	0.013	0.005

[a] Averages for the dosing group.
[1] Data extracted from Table 2, p. 36 of the study report..
[b] Values are µg of residue (in AE 0317309 equivalents)/g of tissue.

Metabolism:

Identification of residues was accomplished by comparison of mass spectral data with that of authentic reference standards when available. Some residues were tentatively identified by comparison of their HPLC retention times with those of authentic reference standards or components which had been identified in other matrices. (See table below.)

Main metabolites in excreta of rats dosed with [^{14}C] AE 0317309.[a1]

Dose	Percent of Administered Dose			
	[Phenyl-UL-^{14}C] Oral	[Pyrazole-3-^{14}C] Oral	[Phenyl-UL-^{14}C] Intravenous	[Pyrazole-3-^{14}C] Intravenous
Compound				
AE 0317309 (in urine)	66	68	81	86
AE 0317309 (in feces)	23	27	6	4
Hydroxymethyl AE 0317309 (in urine)	1	1	1	1

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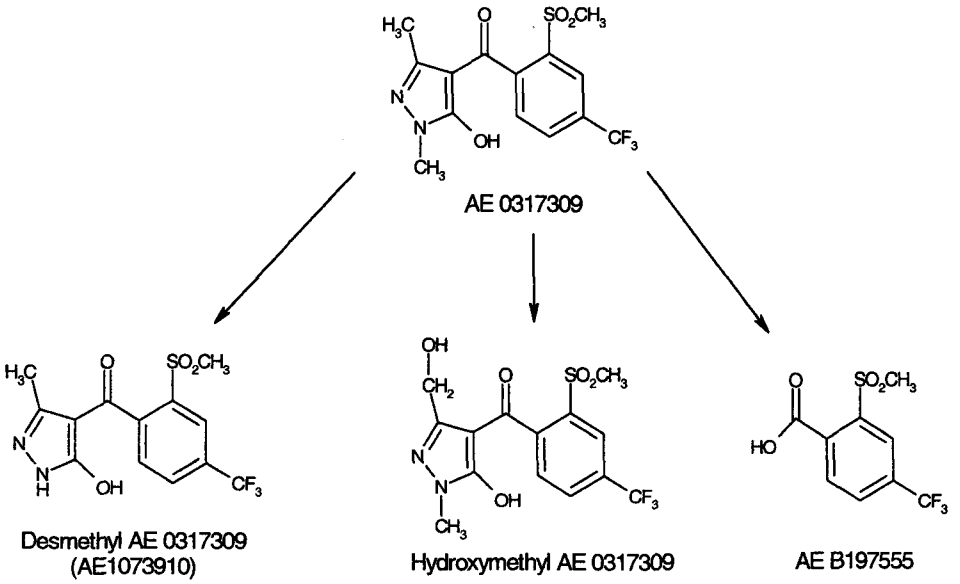
Hydroxymethyl AE 0317309 (in feces)	1	1	0.5	0.3
Desmethyl AE 0317309 (in urine)	4.4	4.5	3.7	4.1
Desmethyl AE 0317309 (in feces)	3.7	3.6	2.7	2.2
AE B197555 (in urine)	0.6	Nil	1.1	Nil
AE B197555 (in feces)	0.9	Nil	0.4	Nil
Nominal total of administered dose recovered in urine and feces	100.6	105.1	96.4	97.6

[a] Reported results are averages from all rats from the dosing group for the experiment.
[1] Data extracted from Table 4, p.38, and pp. 109,115, 121 and 127 of the study report

Following both oral and intravenous administration, most of the dose was excreted unchanged as AE 0317309. Hydroxymethyl AE 0317309, desmethyl AE 0317309, and AE B197555 were observed as minor metabolites in the urine and feces. The metabolic transformations are depicted below.



Excretion:

The recovery of radiolabeled AE 0317309 residues in different experimental groups is shown in the table below. Following oral dosing, approximately 70% of the radioactivity was excreted in the urine and 30% in the feces by 48 or 52 h. The majority (approximately 60%) of the orally administered dose was excreted in the urine within 6 h of dosing. In the case of intravenous administration, approximately 90% of the

radioactivity was excreted in the urine and approximately 10% in the feces by 48 h. Most (over 80%) of the intravenous dose was excreted in the urine within 6 h.

Recovery of radioactivity after administration of [^{14}C] AE 0317309.[a1]

	Radioactive Dose Recovered (% of the administered dose)			
	[Phenyl-UL-^{14}C] Oral	[Pyrazole-3-^{14}C] Oral	[Phenyl-UL-^{14}C] Intravenous	[Pyrazole-3-^{14}C] Intravenous
Expired air	<0.1	<0.1	Not determined	Not determined
Tissues	1.0	1.0	0.8	1.0
Carcass	0.1	0.2	0.4	0.8
Cage wash[b]	2.3	2.5	2.0	3.1
Urine				
0 to 6 h	62.4	56.8	82.7	84.1
6 to 12 h	8.2	15.2	1.8	3.6
12 to 24 h	1.3	2.5	1.5	1.8
24 to 48 h	1.1	0.5	0.7	1.2
48 to 52 h	0.1	Not applicable	Not applicable	Not applicable
Total for urine	73.1	74.9	86.6	90.7
Feces				
0 to 24 h	30.0	31.1	9.6	7.4
24 to 48 h	1.2	0.8	0.8	0.6
48 to 52 h	<0.1	Not applicable	Not applicable	Not applicable
Total for feces	31.2	32.0	10.4	8.0
Total	108	111	100	106

[a] Reported results are averages from all rats from the dosing group for the experiment.
[1] extracted from Table 3, p. 37 of the study report.
[b] Cage wash includes the rinses from the metabolism cages and the urine and feces collection containers.

Conclusions and Critique

The test substance was absorbed readily via the oral route. Following oral administration, approximately 60% of the compound (AE 0317309) was absorbed and excreted in the urine within 6 h. Based on these data, the test material is absorbed rapidly following oral dosing.

Only a small amount of the administered dose (<2%) remained in the residual carcass and tissues at sacrifice (48 or 52 h). The highest residues were found in the liver and kidney.

The compound was found to be poorly metabolized in male rats. A desmethyl derivative was the most abundant, being present at less than 5% in the urine and feces.

Following oral administration, approximately 70% of the radioactive dose was excreted in the urine and approximately 30% in the feces by 48 or 52 h. In the case of intravenous administration, approximately 90 % of the dose was excreted in the urine and approximately 10% was found in the feces over 48 h, the latter indicating biliary excretion.

A possible criticism of this study is that only male rats were used. Also, a repeat dose study would have been desirable. A study provided by the applicants (PA03/010) gives octanol/water partition coefficients at pH 7.0 of 0.043 (P_{ow}) and -1.362 (Log P_{ow}) for pyrasulfotole (AE 0317309). These values indicate that the compound is more likely to be hydrophilic than lipophilic. Despite these observations, it would have been desirable to have a repeat dose study provided to address the issue of possible bioaccumulation of residues in tissues, especially in fat. Finally, according to the OECD Guideline for Testing of Chemicals No. 417, it is desirable to use two dose levels (low and high) in the case of single dose studies.

EPA Reviewer: William B. Greear, MPH, DABT **Signature:** William B. Greear
Registration Action Branch 1, Health Effects Division (7509P) **Date:** 6/5/2008
EPA WAM: PV Shah, PhD **Signature:** P.V. Shah
Registration Action Branch 1, Health Effects Division (7509P) **Date:** 6/5/08

TXR#: 0054227

DATA EVALUATION RECORD

STUDY TYPE: Reproduction and Fertility Effects Study – rat OPPTS 870.3800 [83-4]; OECD 416.

PC CODE: 000692 **DP BARCODE:** D335563

TEST MATERIAL (PURITY): AE 0317309 (95.7% w/w a.i.)

SYNONYMS: Pyrasulfatole technical

CITATION: Eiben, R. (2005) Two generation reproduction study in the Wistar rat by administration in the diet. Bayer HealthCare AG PH-R&D Toxicology 42096, Wuppertal, Germany. Laboratory report number T2063193, December 6, 2005. MRID 46801907. Unpublished.

SPONSOR: Bayer CropScience LP, Alfred Nobel Str. 50, 40789 Monheim, Germany

EXECUTIVE SUMMARY:

In a 2-generation reproduction study (MRID 46801907) AE0317309 (95.7% a.i., batch# Op. 1-4) was administered to 25 Wistar rats/sex/dose in the diet at dose levels of 0, 30, 300 or 3000 ppm (equivalent to 0, 2.5, 26.3, and 272.4 mg/kg bw/day for F_0 males and 0, 3.1, 32.6 and 345.7 mg/kg bw/day for F_0 females, and 0, 3.68, 34.1 and 353.6 mg/kg bw/day in F_1 males and 0, 4.2, 38.9 and 393.4 mg/kg bw/day in F_1 females during the premating period).

There was a dose-related increase in the incidence of either diffuse and/or reticulate corneal opacities, and of corneal neovascularization in both F_0 and F_1 males and females at 300 ppm and 3000 ppm.

There were significant increases in absolute liver (12.9%) and kidney (12.2%) weights in F_1 males in the 3000 ppm group. Absolute thyroid weight was significantly increased in F_1 males in the 300 ppm (28.6%) and 3000 ppm (35.7%) groups. Significant increases in absolute adrenal weights were observed in F1 females at 3000 ppm. There were significant increases in relative liver weight (6.0 and 10.3%, respectively), relative kidney weight (6.2% and 16.4%, respectively), and relative thyroid weight (both 37.9%) in F_1 males at 300 ppm and 3000 ppm.

Treatment related effects were observed in the eyes and thyroid of both males and females of the F_0 and F_1 generations, and in pituitary, kidneys, and liver of F_0 and F_1 males. Findings in the eyes, at 300 ppm and 3000 ppm in both males and females of the F_0 and F_1 generations, included keratitis and vascularization, correlating with in-life observations.

Thyroid findings included colloid alteration at all doses in male F_0 and F_1 rats, and females of the F_0 generation and from 300 ppm in F_1 females, pigment deposition at all doses in F_0 and F_1 males and from 300 ppm in F_0 and F_1 females, and increased follicular cell hypertrophy in F_0 males at 3000 ppm and F_1 males from 3000 ppm and F_0 females at 3000 ppm. It is noted that follicular cell hypertrophy was not observed and that colloid alteration/pigment deposition was minimal at 30 ppm. Eosinophilic inclusions were increased in the anterior pituitary of males at 300 ppm and 3000 ppm in both the F_0 and F_1 generations. Findings in the liver included hepatocellular hypertrophy, cellular alteration, and slight increases in periportal fat accumulation in F_0 and F_1 males at 300 ppm and 3000 ppm. The kidneys of males in the F_0 and F_1 generation had increased incidence and/or severity of basophilic tubules and tubular dilation, in a dose related manner from 300 ppm.

Number of pups born dead appeared to have been elevated at all doses in the F_1 generation and at 30 ppm and 3000 ppm in F_2 generation. However, the data did not show a clear dose-response association or any statistical significance and the live birth index was unaffected in either generation. Treatment related clinical signs in pups included cold to the touch, which was increased in F_1 pups at 3000 ppm only (12 pups), compared to control (5 pups). There was an increased incidence of autolysis and no milk in the stomach in the F_2 pups at 3000 ppm (4-5 pups) compared to controls (1 pup). The incidence of dilated and/or enlarged kidneys was increased at 3000 ppm in F_1 and F_2 pups. The mean viability index was non-significantly reduced in a dose-related manner in F_2 pups at all doses as shown: 92.45±23.326, 83.79±32.046, 78.89±35.016, and 71.60±38.201 at 0, 30, 300 and 3000 ppm, respectively. However, only the index at 3000 ppm of test substance was lower than the historical data presented by the study authors. A benchmark dose analysis for this endpoint by R. Mitkus yielded a BMD_{10}=227.6 ppm and a $BMDL_{10}$=68.13 ppm. This supported the conclusion not to consider the decrease in viability index at the low dose as treatment-related. Examination of pups at the time of weaning on postnatal day 28 showed an increased incidence of corneal opacity and/or corneal neovascularization at 300 ppm and 3000 ppm in both F_1 and F_2 pups, showing a dose-related increase. Both litter (mean body weight for males and females taken together) and pup (mean body weight for males or females, taken separately) body weights were statistically significantly decreased in the F_1 (mean liter weight decr. 7.9%) and F_2 (mean liter weight decr. 11.1%) pups at 3000 ppm at weaning on day 28. In F_2 females at 3000 ppm, body weight was also statistically significantly decreased at lactation day 21. Statistically significant delays of balano-preputial separation at 300 ppm and 3000 ppm, and a statistically significant delay of vaginal opening at 3000 ppm, were observed in F_1 pups. These parameters were not assessed in F_2 pups.

The parental systemic LOAEL is 30 ppm (2.5 mg/kg bw/day in males, 3.1 mg/kg bw/day in females), based on minimal colloid alteration and/or pigment deposition in the thyroid. The parental systemic NOAEL was not observed.

The offspring LOAEL is 300 ppm (26.3 mg/kg bw/day in males, 32.6 mg/kg bw/day in females), based on corneal opacity and/or corneal neovascularization (F1 and F2 generations). The offspring NOAEL is 30ppm (2.5 mg/kg bw/day in males, 3.1 mg/kg bw/day in females).

The reproductive LOAEL is 300 ppm (26.3 mg/kg bw/day in males, 32.6 mg/kg bw/day in females), based on delays in balano-preputial separation in F_1 pups. The reproductive NOAEL is 30ppm (2.5 mg/kg bw/day in males, 3.1 mg/kg bw/day in females).

This study is **acceptable/guideline** and satisfies the guideline requirement for a 2-generation reproductive study (OPPTS 870.3800; OECD 416) in the rat.

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

This Executive Summary was prepared for United States Environmental Protection Agency, Office of Pesticides Programs, Health Effects Division use.

The following text was generated by the Australian Pesticides and Veterinary Medicines Authority. However, this document has undergone critical scientific analysis and been modified as appropriate.

Report:	KIIA 5.6.1/01, Eiben, R.; 2005
Title:	Two-generation reproduction study in the Wistar rat by administration in the diet
Test Substance:	AE 0317309: Pyrosulfatole – batch Op. 1-4, purity 95.7% w/w
Laboratory:	Bayer HealthCare AG, PH-R&D Toxicology, 42096 Wuppertal, Germany
Report No.: **Document No.:**	AT02705 M-263320-01-2
Study Duration:	26 September 2003 to 06 December 2005
Guidelines:	OECD 416 (2001); EPA OPPTS 870.3800 (1998); MAFF 12 Nousan no. 8147 (2000)
GLP/QA	Yes

Materials and Methods

The test substance was incorporated into rodent diet at concentrations of 0, 30, 300, and 3000 ppm and fed to male and female Wistar rats (strain Crl: (WI) WU BR; 25/sex/group) in the F_0 generation throughout pre-mating, mating, gestation, and lactation periods. Following weaning of the F_1 generation, the weanlings were maintained in their same dietary groups through adolescence, mating, gestation, and lactation. Parental generations were sacrificed at 28 days post partum after their litters had been weaned.

Dose selection was based on results of a one-generation pilot study, in which rats received 0, 100, 600 and 3600 ppm in the diet. In the pilot study, the NOAEL was 100 ppm. From 600 ppm onwards F_0 males exhibited reduced body weights. F_0 females showed reduced body weights at 3600 ppm during gestation and lactation. There was an increase in food intake at 3600 ppm in F_0 rats. The pup weights were significantly reduced at 3600 ppm.

Diets were prepared at least weekly. Prior to the start of the study, stability and homogeneity of the test substance was determined. Checks on homogeneity (low and high concentration only) and concentration of the test material in diets were carried out several times during the study.

The F_0 rats were 6-7 weeks old at the start of the study, corresponding to a body weight of 109-149 grams for males and 101-137 grams for females. The F_1 rats were 4 weeks of age at the time of weaning, and body weights were 68-119 grams for males and 65-106 grams for females. All rats were housed singly except during mating periods, in Makrolon cages on low-dust wood shavings for most study phases or on nesting material from day 20 post-coitum to day 7 post partum. Food and water were provided *ad libitum* during all phases of the study.

Mating procedure: Mating was accomplished by natural means by placing a male and a female in a cage over night at a maximum of 12 times during the three-week mating period until sperm cells were observed in vaginal smears taken the next morning. Inseminated females were not further co-housed. Females found sperm-positive after the first mating day but not pregnant (lack of weight gain within 14 days following insemination) were co-housed again over one week with the same male without checking insemination or measuring body weight and food intake during possible further pregnancy. F_0 rats were fed diets containing test substance for approximately 12 weeks prior to mating, with a three-week time period for determination of oestrus. In the F_1 generation, 25 male and 25 female weanlings in each dietary group were fed diets containing test substance for at least a 10-week pre-mating phase, and again a three-week time period was used to determine oestrus. Clinical signs were monitored daily. Body weights were measured on the first day of the study prior to provision of test diets, and then weekly

through to necropsy in males. In females, body weights were measured weekly during pre-mating, and on days 0, 7, 14, and 20 of gestation, on days 0, 7, 14, 21, and 28 of lactation, and at necropsy. Food consumption was measured weekly in males except during mating, and in females weekly during pre-mating, and for days 0-7, 7-14, and 14-20 of gestation, and for days 0-4 and 4-7 of lactation. Spermatological investigations (spermatazoa motility and viability, morphology, determination of spermatazoa in epididymis, and determination of homogenization-resistant spermatid heads in the testis) were conducted at necropsy in the control and 3000 ppm males of the F_0 and F_1 generations. Near or at the day of sacrifice, the eyes of adult males and females were examined.

Litters were culled, where possible, to 4 male and 4 female pups on day 4 of lactation. Number of live pups, pup weight, external findings, and number of dead pups were determined on days 0, 4, 7, 14, 21, and 28 of lactation. For the F_2 litters, ano-genital distance was measured on lactation day 0, and sex of each pup was determined on lactation days 0 and 4.

Ophthalmoscopic examinations were conducted in post-weaning rats in both F_1 and F_2 generations. In the F_1 litters, one male and one female weanling from each litter were randomly selected wherever possible for use as F_1 adults. These selected rats were then monitored for preputial separation or vaginal opening, and body weight was measured on the day of landmark attainment.

Necropsy: The F_0 and F_1 adults were sacrificed by exsanguination under CO_2 narcosis and subjected to a gross necropsy. In females, implantation sites were counted after ammonium sulfide staining. The following organs/tissues of F_0 and F_1 rats were fixed in 10% formalin with the exception of eyes, kidneys and testes which were fixed in Davidson's solution:

Adrenals*#, brain*, epididymides*#, oesophagus#, eyes#, kidneys*#, larynx, liver*#, ovaries and oviducts#, pituitary gland*#, prostate*#, seminal vesicles and coagulating glands*#, skin in mammary region, spleen*, testes*#, thyroid/parathyroids*#, trachea#, ureters#, urethra, urinary bladder#, uterus (inc. cervix)#, vagina#, head with skull cap and gross abnormalities#.

Organs marked with an asterisk above were weighed at necropsy. The organs marked with "#" were microscopically examined at least in the control and high dose group. This also included all F_0/F_1 rats which died intercurrently and those which were killed moribund. Staging of ovarian follicles was done in F_1 females (high and low concentration) only. All reproductive organs of female rats suspected of reduced fertility were investigated.

Those pups sacrificed prior to weaning were sacrificed by CO_2 narcosis, while weanlings were sacrificed by cervical dislocation under CO_2 narcosis.

All pups culled for standardization of litter size, found dead or had to be killed moribund during lactation were necropsied and investigated macroscopically with particular attention on the organs of reproduction except for cases of autolysis or cannibalism. This included also visible skeletal abnormalities as far as possible. A lung flotation in water was performed during the necropsy of pups found dead on the day of the first litter inspection to determine whether pups had breathed at birth or not. All F_1 weanlings not selected for further treatment were killed when they were at least 4 weeks old and examined macroscopically. All F2 weanlings were killed after a 4 week lactation period and examined macroscopically.

Indices: The following reproductive and offspring viability indices (%) were calculated: insemination index, fertility index, gestation index, live birth index, rearing index, viability index and lactation index.

Statistical evaluation: This was performed using:-

a) Analysis of Variance (ANOVA) and in case of significant results Dunnett's test as post hoc test for:-

- Body weights and body weight gains of the male and female rats,
- Food consumption of the male and female rats,
- Number of implantation sites per female• Number of viable pups per female,
- Organ weights at necropsy,
- Number of estruses,
- Time to insemination, and
- Life birth, viability and lactation rate.

b) 2 x N Chi^2 test; in case of significant differences Fisher's exact test with Bonferroni correction for:-
- Number of viable pups per group based on the number of implantations
- Insemination, fertility, gestation and rearing rate

c) Kruskall-Wallis test and in case of significant differences Dunnett's test for the number of prenatal losses per litter.

The sperm and spermatid count data were not evaluated statistically, because there were no meaningful differences occurred between the high dose and control groups. Generally, differences between the control group and groups treated with the test substance groups were considered as statistically significant when $p < 0.05$. Significant differences from the control are indicated with * for $p < 0.05$ and ** for $p < 0.01$

Findings

Homogeneity analysis: In samples taken from the top, middle and bottom of 5 and 5000 ppm mixtures, the test substance was proven to be homogeneously distributed.

Stability analysis: The test substance was stable at animal room temperature for up to 15 days and after 9 weeks of freezer storage.

Concentration analysis: The absence of the test substance in the control diet was confirmed. The test substance concentration (all concentrations including 0 ppm) measured at six time points were in the range of + 20% of the nominal concentrations. The low and high concentrations were proven to be homogenous.

Mean daily dose of test substance in mg/kg bw/day:

Premating phase: The dietary concentrations of 0, 30, 300, and 3000 ppm test substance used, provided doses of 0, 2.5, 26.3, and 272.4 mg/kg bw/day for F_0 males, 0, 3.7, 34.1, and 353.6 mg/kg bw/day for F_1 males, 0, 3.1, 32.6, and 345.7 mg/kg bw/day for F_0 females, and 0, 4.2, 38.9, and 393 mg/kg bw/day for F_1 females.

Gestation and lactation: The dietary concentrations of 0, 30, 300 and 3000 ppm test substance used, provided doses on day 14 to 20 post coitum inclusive of 0, 2.0, 22.3, 228.6 mg/kg bw/day for F_0 females and 0, 2.2, 23.2, 250.4 mg/kg bw/day for F_1 females. The intakes on days 0 to 4 post partum were 0, 3.1, 30.9, 294.2 mg/kg bw/day for F_0 females and 0, 3.2, 29.9, 263.2 mg/kg

bw/day for F_1 females.

Parental Findings

Mortalities: There was no treatment-related increase in mortality in either the F_0 or the F_1 males or females. Two F_1 females at 3000 ppm were sacrificed because they were exhibiting signs of a poor general condition (e.g. piloerection, emaciation, sunken flanks). Histopathological examination did not reveal specific organ lesions.

Clinical signs: The only treatment related clinical sign was increased incidence of eye opacities in F_0 and F_1 rats at 3000 ppm during pre-mating, gestation, and lactation phases.

Ophthalmoscopy: There was a dose-related increase in the incidence of either diffuse and reticulate corneal opacities, and of corneal neovascularization in both F_0 and F_1 males and females at 300 and 3000 ppm.

Incidence of eye opacities in F_0 and F_1 adult rats

Finding	Dose (ppm)							
	Males				Females			
	0	30	300	3000	0	30	300	3000
F_0								
Number examined	25	25	25	25	23	21	21	22
Clinical findings:								
Eye opacity(ies)	0	0	0	15	0	0	0	4
Ophthalmology:								
Diffuse corneal opacity	0	0	3	16	1	0	2	18
Reticulate corneal opacity	0	0	2	11	0	0	13	18
Cornea neovascularization	0	0	2	16	0	0	2	19
F_1								
Number examined	25	25	25	25	25	25	25	23
Clinical findings (during all phases):								
Eye opacity(ies)	0	0	0	15	0	0	0	8
Ophthalmology:								
Diffuse corneal opacity	0	0	5	20	1	0	5	19
Reticulate corneal opacity	0	0	6	20	0	0	10	20
Cornea neovascularization	0	0	5	20	0	0	3	19

Body weight: There was no treatment related effect on body weight in the F_0 males or females.

In F_1 adults, body weight at week 5 and 14 was statistically significantly reduced in males and females at 3000 ppm. Body weight gain over that interval in F_1 males was also statistically significantly reduced at 3000 ppm. A dose-related decrease in body weight gain in F_1 males was observed. Although body weight gain in F_1 females at 3000 ppm was depressed no statistical significance was noted (see table below). There was a reduction in body weight at 3000 ppm at

the end of the pre-mating period, of 12% in males and 7% in females.

Body weight and body weight gain during the pre-mating phase in F_0 and F_1 male and female rats

Phase	Parameter	Dose (ppm)						
		Body weight (g)				% of control		
		0	30	300	3000	30	300	3000
Males								
F_0	Body weight, g, week 1	124.8	123.4	126.1	124.2	98.9	101.0	99.8
	Body weight, g, week 12	412.4	402.5	395.3	402.0	97.6	95.9	97.5
	Body weight gain, g, week 1-12	287.6	279.1	269.2	277.8	97.0	93.6	96.6
F_1	Body weight, g, week 5	94.9	95.5	93.5	80.9**	100.6	98.5	85.2
	Body weight, g, week 14	384.3	383.4	367.4	337.6**	99.8	95.6	87.8
	Body weight gain, g, week 5-14	289.4	288.0	274.0	256.6**	99.5	94.7	88.7
Females								
F_0	Body weight, g, week 1	117.8	118.0	118.6	119.8	100.2	100.7	101.7
	Body weight, g, week 12	235.2	231.4	229.1	231.1	98.4	97.4	98.3
	Body weight gain, g, week 1-12	117.4	112.6	110.5	111.2	95.9	94.1	94.7
F_1	Body weight, g, week 5	86.9	89.5	86.2	77.1**	103.0	99.2	88.7
	Body weight, g, week 14	219.7	225.9	218.7	205.3**	102.8	99.5	93.4
	Body weight gain, g, week 5-14	132.8	136.4	132.5	128.2	102.7	99.8	96.5

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

There was no statistically significant depression in body weight at any dose during gestation in either the F_0 or the F_1 dams. During lactation days 0-4, body weight gain of F_0 dams at 3000 ppm and F_1 dams at 300 and 3000 ppm was statistically significantly reduced compared to controls.

Body weight and body weight gain during gestation and lactation in F_0 and F_1 females

Phase	Parameter	Dose (ppm)						
		Body weight (g)				% of control		
		0	30	300	3000	30	300	3000
Gestation								
F_0	Body weight, g, day 0 p.c.	239.9	230.3	227.2	227.8	96.2	94.9	95.2
	Body weight, g, day 14 p.c.	285.5	273.1	271.3	271.5	95.7	95.0	95.1
	Body weight, g, day 20 p.c.	338.9	323.2	326.3	328.9	95.4	96.3	97.0
	Body weight gain, g, day 14-20	53.4	50.0	55.0	57.3	93.6	103.0	107.3
F_1	Body weight, g, day 0 p.c.	218.8	232.6	223.1	213.1	106.3	102.0	97.4
	Body weight, g, day 14 p.c.	263.6	279.8	267.2	255.4	106.1	101.4	96.9
	Body weight, g, day 20 p.c.	309.8	333.7 **	318.3	308.9	107.7	102.7	99.7
	Body weight gain, g, day 14-20	46.2	53.9*	51.1	53.5*	116.7	110.6	115.8

Lactation								
F_0	Body weight, g, day 0 p.p.	255.9	250.9	251.2	258.4	98.0	98.2	101.0
	Body weight, g, day 4 p.p.	270.6	259.5	259.1	260.0	95.9	95.8	96.1
	Body weight gain, g, day 0-4	14.7	8.7	8.0	1.6**	59.2	54.4	10.9
F_1	Body weight, g, day 0 p.p.	239.6	255.3	250.2	238.9	106.6	104.4	99.7
	Body weight, g, day 4 p.p.	250.6	263.3	249.0	239.5	105.1	99.4	95.6
	Body weight gain, g, day 0-4	11.1	8.0	-1.2**	0.7*	72.1	-10.8	6.3

* $p < 0.05$, ** $p < 0.01$, p.c. = post coitum, p.p = post partum

No statistically significant body weight depression was noted in pregnant F_0 or F_1 females. During lactation, a statistically significant depression in body weight gain was noted in F_0 females at 3000 ppm. Statistically significant body weight depression occurred in F_1 females at 300 and 3000 ppm. These decreases on body weight gain were considered treatment related.

Food consumption: There was no effect on food consumption during the pre-mating period in either male or female rats in the F_0 or the F_1 generation. Food consumption during lactation days 0-4 was slightly reduced (but not statistically significantly) at 3000 ppm in F_0 dams and at 300 and 3000 ppm in F_1 dams. This effect on food consumption was considered related to treatment.

Food consumption during premating by male F_0 and F_1 rats, and during premating, gestation, and lactation phases by female F_0 and F_1 rats

Phase	Time period	Dose (ppm)						
		food intake (g/kg bw/day)				% of control		
		0	30	300	3000	30	300	3000
Males								
F_0	Pre-mating weeks 1-12	87.5	84.8	87.6	90.8	96.9	100.1	103.8
F_1	Pre-mating weeks 5-14	120.3	122.5	113.8	117.9	101.8	94.6	98.0
Females								
F_0	Pre-mating weeks 1-12	107.9	103.0	108.8	115	95.5	100.8	106.6
	Gestation days 14-20	72.5	68.0	74.2	76.2	93.8	102.3	105.1
	Lactation days 0-4	105.1	103.3	103.0	98.1	98.1	98.0	93.3
F_1	Pre-mating weeks 5-14	147.0	139.4	129.6	131.1	94.8	88.2	89.2
	Gestation days 14-20	74.7	73.2	77.5	83.5	98.0	103.7	111.8
	Lactation days 0-4	110.9	104.8	99.5	87.7	94.5	89.7	79.1

Macroscopic findings: There were no treatment related macroscopic findings in F_0 male or female rats at any dose. In F_1 males, one rat at 300 ppm and 14 males at 3000 ppm showed eye opacities. Two F_1 females at 3000 ppm also showed eye opacities. There were no other treatment related findings in F_1 male or female rats.

Organ weights:

In F0 males there were statistically significant perturbations in absolute and/or relative weights.

Of these, only the significant elevations in liver (12.9% abs., 10.3% rel.) and kidney (12.2% abs., 16.4% rel.) weights at 3000 ppm, and thyroid (>28.6% abs.) weights at and above 300 ppm could be attributable to treatment. No significant effects were seen in F0 females. Statistically significant changes in adrenal weights did not show a dose-response association.

Significant increases in adrenal weights were observed in F1 females at 3000 ppm (see tables below). Absolute brain weights were decreased by 3% and 7% in F0 and F1 adult males, respectively, at 3000 ppm. No corresponding changes in absolute weights of other organs were observed and/or they lacked dose-response association.

Weights of selected organs in adult male and female F_0 rats

Parameter	Dose (ppm)							
	Males				Females			
	0	30	300	3000	0	30	300	3000
Absolute weight								
Body wt, g	478.7	473.0	457.1*	461.6	266.1	256.8	265.4	267.7
Brain, mg	1929	1969	1908	1871**	1793	1783	1741*	1756
Kidneys, mg	3063	3032	3107	3436**	2170	2120	2245	2240
Liver, mg	17010	17345	17487	19199**	13669	12676	13836	14290
Thyroid, mg	14	16	18**	19**	11	12	12	12
Adrenals, mg	55	47**	45**	51	66	64	62	66
Relative weight (in mg/100 g body weight)								
Brain	404.5	417.9	418.6	406.7	676.0	697.2	658.1	657.4
Kidneys	640.1	641.6	680.1**	745.1**	816.1	827.1	848.1	838.1
Liver	3551.8	3664.1	3820.9**	4160.0**	5139.8	4919.4	5213.4	5347.9
Thyroid	2.9	3.4	4.0**	4.0**	4.1	4.7	4.6	4.5
Adrenals	11.5	9.9**	9.9**	11.1	25.0	25.2	23.3	24.8

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

Weights of selected organs in adult male and female F_1 rats

Parameter	Dose (ppm)							
	Males				Females			
	0	30	300	3000	0	30	300	3000
Absolute weight								
Body wt, g	457.7	461.5	436.2	407.9**	257.7	268.8	261.2	251.0
Brain, mg	1931	1999**	1904	1796**	1799	1842	1760	1722**
Kidneys, mg	2883	3071*	3090*	2957	1975	2150	2091	2210
Liver, mg	16878	17619	17048	16581	13048	13519	13147	13503
Thyroid, mg	12	16**	16**	15	12	12	11	11
Adrenals, mg	49	47	48	46	59	64	63	68**
Relative weight in mg/100 g body weight								

Body wt, g	457.7	461.5	436.2	407.9**	257.7	268.8	261.2	251.0
Brain	424.3	434.9	437.8	441.2	699.3	688.3	676.0	687.5
Kidneys	630.7	665.3*	709.6**	725.5**	766.8	800.7	800.5	880.6**
Liver	3688.3	3813.4	3908.9*	4069.1**	5060.9	5031.2	5041.0	5387.2
Thyroid	2.7	3.6**	3.7**	3.7**	4.6	4.5	4.2	4.5
Adrenals	10.6	10.2	11.0	11.2	22.7	23.9	24.1	27.2**

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

Histopathological findings: There were no treatment related effects on organs of the reproductive tract.

Treatment related effects were observed in the eyes and thyroid of both males and females of the F_0 and F_1 generations, and in pituitary, kidneys, and liver of F_0 and F_1 males. Findings in the eyes, at 300 and 3000 ppm in both males and females of the F_0 and F_1 generations, included keratitis and vascularization, correlating with in-life observations.

Thyroid findings included colloid alteration at all doses in male F_0 and F_1 rats, and females of the F_0 generation and from 300 ppm in F_1 females, pigment deposition at all doses in F_0 and F_1 males and from 300 ppm in F_0 and F_1 females, and increased follicular cell hypertrophy in F_0 males at 3000 ppm and F_1 males from 3000 ppm and F_0 females at 3000 ppm. The study authors suggested that the increased incidence of colloid alteration and pigment deposition were due to the unique thyroid physiology of the rat, and not indicative of thyroid toxicity. However, no further data were provided.

Eosinophilic inclusions were increased in the anterior pituitary of males at 300 and 3000 ppm in both the F_0 and F_1 generations. Findings in the liver included hepatocellular hypertrophy, cellular alteration, and slight increases in periportal fat accumulation in F_0 and F_1 males at 300 and 3000 ppm. The kidneys of males in the F_0 and F_1 generation had increased incidence and/or severity of basophilic tubules and tubular dilation, in a dose related manner from 300 ppm.

Treatment related histopathological findings in male and female F_0 and F_1 rats

Organ	Finding	Dose (ppm)							
		F_0 rats				F_1 rats			
		0	30	300	3000	0	30	300	3000
Males (n=25)									
Eyes	Keratitis	0	0	2	16	0	0	6	18
	Epithelial hyperplasia	0	0	3	16	0	0	6	16
	Corneal vascularization	0	0	2	14	0	0	6	15
Thyroid	Follicular cell hypertrophy	4	0	4	11	3	4	9	11
	Colloid alteration	11	25	25	25	12	23	25	25
	Pigment deposition	1	11	24	25	0	10	23	24
Pituitary	Incr. eosinophilic inclusions	1	2	21	15	2	4	12	15
Liver	Hepatocellular hypertrophy	0	0	2	17	0	0	9	15

	Cytoplasmic change	0	0	5	17	0	0	15	19
	Fat positive reaction	22	23	24	23	18	18	17	19
	Grade 1	9	13	8	11	15	15	13	10
	Grade 2	12	10	14	8	3	3	4	9
	Grade 3	1	0	2	4	0	0	0	0
Kidneys	Basophilic tubules	21	25	25	24	24	24	25	25
	Grade 1	16	17	8	0	11	10	5	7
	Grade 2	4	7	9	15	8	13	10	8
	Grade 3	1	1	8	9	5	0	10	10
	Grade 4	0	0	0	0	0	1	0	0
	Tubular dilation	17	15	21	24	17	16	20	21
	Grade 1	12	10	10	5	7	8	4	2
	Grade 2	3	5	7	16	10	7	13	11
	Grade 3	2	0	4	3	0	1	3	8
	Hyaline casts	17	17	23	23	15	19	23	23
	Grade 1	12	12	11	3	5	11	7	4
	Grade 2	4	4	9	14	10	7	11	11
	Grade 3	1	1	3	6	0	1	5	8
	Mononuclear cell infiltrates	3	6	9	14	6	9	11	5
Females (n=25)									
Eyes	Keratitis	0	0	6	24	0	0	6	19
	Epithelial hyperplasia	0	0	6	22	0	0	3	19
	Corneal vascularization	0	0	0	9	0	0	2	18
Thyroid	Follicular cell hypertrophy	2	1	0	4	0	1	2	0
	Colloid alteration	9	11	23	24	2	11	21	19
	Pigment deposition	0	0	5	17	0	1	8	8

Reproductive function: There was no treatment-related effect on either oestrus cyclicity in females or any sperm analysis parameters in males in the F_0 or F_1 generations.

Reproductive performance: There were no treatment-related effects on any reproductive performance indices in either the F_0 or F_1 adults. Both gestation and rearing indices were slightly decreased at 3000 ppm in F_1 adults, but these decreases were due to incidental deaths, and therefore were not considered to be related to administration of test substance.

Offspring findings

Mortality & Clinical signs: Number of pups born dead appeared to have elevated at all doses in F1 and at 30 and 3000 ppm in F2 generation. However, the data did not show a clear dose-response association or any statistical significance. The only treatment related clinical sign in

pups was the finding of cold to the touch, which was increased in F_1 pups at 3000 ppm only (12 pups), compared to control (5 pups). There were no treatment related findings in F_2 pups (see table on litter parameters below).

Macroscopic findings: Corresponding with the increased mortality during lactation at 3000 ppm in the F_2 pups compared to controls, treatment related findings in this group at gross necropsy included increased incidence of autolysis and no milk in the stomach. The incidence of dilated and/or enlarged kidneys was increased at 3000 ppm in F_1 and F_2 pups.

Treatment-related findings at necropsy of F_1 and F_2 pups or weanlings

Finding	Dose (ppm)							
	F_1 pups				F_2 pups			
	0	30	300	3000	0	30	300	3000
Autolysis, N	3	0	1	1	1	0	3	5
Affected pups per litter, %	4.63	0.0	0.79	0.51	0.48	0.0	2.38	5.56
No milk in stomach, N	5	4	1	5	1	1	2	4
Affected pups per litter, %	3.92	5.41	0.79	5.56	5.26	2.08	1.19	10.29
Dilated and/or enlarged kidney, N	1	1	1	3	1	1	1	3
Affected pups per litter, %	0.35	0.45	0.79	2.02	0.40	0.83	0.37	1.96

N = number of pups.

Litter parameters: The relevant data are given in the table below. Dietary administration of the test substance did not affect the number of implantations or prenatal loss. There was no statistically significant effect on live birth index or on the sex ratio. Viability and lactation indices were statistically non-significantly reduced in F_2 pups at all doses. The decreases in viability index doses were considered to be dose related; however, only the index at 3000 ppm of test substance ,was lower than the historical data presented by the study authors. The lactation indices were generally within the historical control range, and were due at 30 ppm to total litter loss of two dams before day 4 of lactation.

Though no statistical significance was achieved, pup mortality from day 4 to 21 was increased (2- to 6-fold) in all treated F1 and F2 pups relative to controls.

Litter parameters

Parameter	Dose (ppm)							
	F_1 pups				F_2 pups			
	0	30	300	3000	0	30	300	3000
Mean implantations	12.42	12.09	12.05	12.27	11.58	12.83*	12.35	10.83
Mean prenatal loss	0.96	1.36	1.64	1.32	0.95	1.67	1.04	1.17
Number born	275	236	229	241	202	268	260	174
Number born dead	1	6	5	4	1	4	1	7
% males on day 0	48.86	51.98	52.33	50.39	50.99	52.48	51.52	49.21

Viability index	92.24	89.86	92.94	93.35	92.45± 23.326	83.79± 32.046	78.89± 35.016	71.60±3 8.201
Deaths, day 0 to 4 (%)	7.76	10.14	7.06	8.65	7.55	16.29	21.11	28.40
Lactation index	97.83	85.75	88.47	89.29	98.61± 4.042	95.54± 18.342	92.88± 15.734	89.06± 26.171
Deaths, day 4 to 21 (%)	2.17	4.25	12.57	10.71	1.39	3.46	7.12	10.94
Mean litter size:								
Day 0	11.38	10.45	10.18	10.77	10.58	11.00	11.26	9.28
Day 4, pre-culling	11.04	10.24	9.45	10.05	10.28	10.09	10.25	7.88
Day 4, post-culling	7.74	7.71	7.45	7.91	8.00	7.45	7.40	6.56*
Day 7	7.65	7.67	7.05	7.36	7.94	7.18	7.05	6.53
Day 14	7.61	7.48	7.14	7.09	7.89	7.14	6.95	6.47
Day 21	7.61	7.48	7.10	7.09	7.89	7.14	6.95	6.40
Live birth index	99.31	97.34	97.97	98.47	99.56	98.46	99.60	96.59

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

Ano-genital distance: There was no effect on ano-genital distance in either males or females in the F_1 or the F_2 generations.

Ophthalmoscopic findings: Examination of pups at the time of weaning on postnatal day 28 showed an increased incidence of corneal opacity and/or corneal neovascularization at 300 and 3000 ppm in both F_1 and F_2 pups, with a dose-related increase.

Ophthalmoscopic findings in F_1 and F_2 pups

Finding	Dose (ppm)							
	F_1 pups				F_2 pups			
	0	30	300	3000	0	30	300	3000
Diffuse corneal opacity (%)	0	0	0	10.5	0	0	2.6	12.5
Reticulate corneal opacity (%)	0	0	4.9	30.2	0	0	0	8.9
Cornea neovascularization (%)	0	0	0	2.3	0	0	14.5	50.0

Body weight: Both litter (mean body weight for males and females taken together) and pup (mean body weight for males or females, taken separately) body weights were statistically significantly decreased in the F_1 and F_2 pups at 3000 ppm at weaning on day 28. In F_2 females at 3000 ppm, body weight was also statistically significantly decreased at lactation day 21. These effects on pup and litter body weight were considered to be treatment-related.

Litter and pup body weights (g) of F_1 and F_2 pups

Lactation day	Dose (ppm)							
	F_1 pups				F_2 pups			
	0	30	300	3000	0	30	300	3000
Litter weights, males and females combined								

0	5.40	5.52	5.84*	5.60	5.41	5.69	5.66	5.79
4, pre-culling	8.66	8.88	9.19	8.66	8.87	9.01	8.36	8.26
4, post-culling	8.63	8.93	9.24	8.71	8.84	9.02	8.36	8.26
7	14.06	14.09	14.16	13.28	13.85	14.40	12.95	12.82
14	25.18	26.92	26.36	25.89	28.27	30.01	28.56	27.51
21	41.48	43.80	43.27	39.53	44.00	46.64	44.26	40.37
28	71.79	75.43	73.67	66.13**	76.81	79.96	75.72	68.31**
Males								
0	5.52	5.67	6.00*	5.78	5.59	5.85	5.85	5.96
4, pre-culling	8.84	9.13	9.35	8.83	9.08	9.18	8.68	8.55
4, post-culling	8.82	9.16	9.38	8.84	9.02	9.17	8.68	8.54
7	14.35	14.27	14.38	13.49	14.19	14.61	13.42	13.56
14	25.66	26.99	26.45	26.05	28.73	30.36	29.15	28.73
21	42.33	44.27	43.73	39.89	44.68	47.31	45.13	42.75
28	74.93	78.09	76.07	68.46**	80.16	82.85	78.78	72.86**
Females								
0	5.28	5.40	5.65	5.44	5.21	5.54	5.47	5.65
4, pre-culling	8.48	8.52	8.96	8.52	8.67	8.91	7.96	8.07
4, post-culling	8.44	8.59	9.02	8.59	8.66	8.96	7.93	8.06
7	13.77	13.88	14.25	13.03	13.53	14.60	12.42	12.42
14	24.70	26.61	26.02	25.70	27.83	29.86	27.79	26.68
21	40.63	42.99	42.64	39.09	43.39	45.80	42.90	38.66**
28	68.72	72.15	70.89	64.23**	73.67	77.05	71.47	64.41**

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

Sexual maturation: Statistically significant delays of balano-preputial separation at 300 and 3000 ppm, and a statistically significant delay of vaginal opening at 3000 ppm, were observed in F_1 pups. These parameters were not assessed in F_2 pups.

Mean age (days) at sexual maturation of male and female F_1 pups

Parameter	Dose (ppm)			
	0	30	300	3000
Age at preputial separation	41.0	41.8	44.2**	46.3**
Age at vaginal opening	32.6	32.6	33.6	34.2*

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

Organ weights: Absolute spleen weights were statistically significantly decreased in F_1 male and female weanlings at 3000 ppm, and in F_1 males relative spleen weight was also slightly but statistically significantly decreased. However, this effect was not observed in the F_2 weanlings

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and thus was considered not to be treatment related.

Conclusions

Parental:

The thyroid glands of F_0 and F_1 parental rats exhibited colloidal alteration and pigment deposition in the follicular epithelium at and above 30 ppm, and follicular cell hypertrophy from 300 ppm onwards. In F_0 males the occurrence of colloidal alteration in controls was 11/25. At 30, 300 and 3000 ppm the incidence was 100% of each test group. In F_1 males the same situation prevailed. In F_0 and F_1 females substantial increases were also noted. Substantial increases in pigment deposition in males and females of each generation were noted. In F_1 males, these findings occurred with an increase in relative thyroid weight from 30 ppm onwards. The changes in the thyroids in the F_0 and F_1 generation occurred at and above 30 ppm and were considered treatment related effects.

Because of the effects seen on the thyroid gland in all treated males, it was not possible to set a parental systemic NOAEL.

The study authors considered that in the absence of functional changes to the thyroid or progression to further histopathological changes at 30 ppm, the findings in F_0 and F_1 parents were not adverse and set a parental systemic NOAEL of 30 ppm (2.50 and 3.09 mg/kg bw/day in males and females respectively).

Offspring:

Though not statistically significant, pup mortality was elevated in both generations in all treated groups from Days 4 to 21. Pups in both generations exhibited treatment related diffuse and reticulate corneal opacity at 300 ppm and above. There was a treatment related delay in balano-preputial separation in F_1 weanlings at 300 ppm and above.

The study authors regarded the ophthalmological findings as a rat specific side effect of the test substance class and therefore of no toxicological relevance to humans. The delay in balano-preputial separation was regarded as related to body weight retardation seen at weekly measurements. This reviewer disagrees with both of the study author's conclusions with respect to the ophthalmological findings and the delay in balano-preputial separation. Additional, proof would be required to establish that the delay in balano-preputial separation was not a reproductive effect. On the basis of ocular toxicity at 300 ppm, an offspring NOAEL of 30 ppm (2.5 mg/kg bw/day in males and 3.1 mg/kg bw/day in females) was established.

Reproduction:

Reproductive indices, insemination, fertility, gestation and live birth percentages, were not influenced by treatment with the test substance up to 3000 ppm. A LOAEL for reproductive toxicity was 300 ppm (equivalent to 26.3 mg/kg bw/day in males, 32.6 mg/kg bw/day in females), based on delays of balano-preputial separation in F_1 pups. A NOAEL for reproductive toxicity was 30 ppm (equivalent to 2.5 mg/kg bw/day in males and 3.1 mg/kg bw/day in females).

EPA Reviewer: Guruva B. Reddy, D.V.M., Ph.D. **Signature:** _____ for
Registration Action Branch 1, Health Effects Division (7509P) **Date:** 6/5/08
EPA WAM: PV Shah, PhD **Signature:** PV Shah
Registration Action Branch 1, Health Effects Division (7509P) **Date:** 6/5/08

TXR#: 0054227

DATA EVALUATION RECORD

STUDY TYPE: Prenatal Developmental Toxicity Study - Rat; OPPTS 870.3700a [§83-3a]; OECD 414.

PC CODE: 000692 **DP BARCODE:** D335563

TEST MATERIAL (PURITY): AE 0317309 Technical (95.7% w/w a.i.)

SYNONYMS: 5-Hydroxy-1,3-dimethylpyrazol-4-yl 2-methylsulfonyl)-4-(trifluoromethyl)phenyl ketone

CITATION: Wason, S. (2006) Developmental toxicity study in the rat by gavage. Bayer CropScience, Sophia-Antipolis Cedex, France. Study Report No: SA 03174, February 28, 2006. MRID 46801905. Unpublished.

SPONSOR: Bayer Ag, Bayer CropScience, Alfred Nobel Str. 50, Monheim, Germany

EXECUTIVE SUMMARY: In a developmental toxicity study (MRID 46801905), AE 0317309 (95.7% w/w; Batch# OP. 1-4) in 0.5% methylcellulose 400 was administered via gavage at a dose volume of 10 mL/kg to 25 Sprague Dawley rats/dose group at dose levels of 0, 10, 100, or 300 mg/kg/day from gestation days (GD) 6-20. On GD 21, all dams were euthanized, and the uterus was removed via cesarean section and its contents examined. Fetuses were examined for external, visceral, and skeletal malformations, anomalies, and variations.

All dams survived until scheduled termination, and there were no treatment-related macroscopic findings at termination, except for yellow sediment in the kidney and gritty material in the bladder of one 300 mg/kg/day dose female..

At 300 mg/kg/day, maternal body weight decreased slightly throughout the study, but was not statistically significant. Body weight change was statistically significantly decreased 53 and 84%, at 100 and 300 mg/kg bw/day, respectively during the gestation days 6-8, compared to controls. Thereafter, body weight change was similar across all groups. Maternal corrected body weight change was statistically significantly reduced 8.4% compared to controls at 300 mg/kg /day.

There was a treatment-related, biologically and/or statistically significant decrease in food

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consumption at 300 mg/kg /day between gestation days 6 and 16, compared to controls. The effect was more pronounced between GD 6-8 where there was a 15% reduction in food consumption and 7% reduction during GD 8-10.

The maternal LOAEL is 100 mg/kg/day based on increased incidence of salivation, decreased corrected body weight gain, decreased corrected body weight gain during GD 6-8 and dose-related enlarged placenta. The maternal NOAEL is 10 mg/kg/day.

There were no abortions, premature deliveries, or complete litter resorptions. Furthermore, there were no effects of treatment on numbers of litters, live fetuses, dead fetuses, resorptions (early or late), or on sex ratio or post-implantation loss. There were no treatment-related visceral malformations.

Mean fetal body weights (combined sexes) were significantly ($P \leq 0.01$) decreased by 5.5% at 300 mg/kg/day; male fetal body weights were decreased 2 – 5.5% at > 100 mg/kg/day. In males the body weights were significantly decreased ($P \leq 0.05$ or 0.01) at ≥ 100 mg/kg/day. Fetal body weights at and above 100 mg/kg/day is considered treatment-related. Additionally at 300 mg/kg/day, a total of 4 fetuses from two different litters presented the malformation of hindpaw polydactyly. Three of the fetuses with hindpaw polydactyly, all from the same litter showed an anomaly of kyphosis, forelimb hyperflexion, misshapen digits on the forepaws, and malpositioning of the digits on both the forepaws and the hindpaws. Two of the four fetuses with the external observation of hindpaw polydactyly had malformations including short tibia and femur, supernumerary cartilaginous phalanges, fused cartilage for several metatarsals and phalanges, and unossified 5th metatarsal (presumably the other two fetuses with externally observed hindpaw polydactyly were not included among those subjected to skeletal observation). There was a slight increase at the mid and high dose in the occurrence of 27 pre-sacral vertebrae. Treatment-related variants observed in this study were primarily linked to ossification. Some of these occurred at an increased fetal and/or litter incidence in the low dose group. These were 7th cervical centrum: unossified/normal cartilage; forepaws: 3rd and/or 4th proximal phalanges: unossified/normal cartilage; 1st metatarsal: unossified/normal cartilage; and less than 9 sacrocaudal vertebrae ossified/9 first sacrocaudal vertebrae: normal cartilage. Since the skeletal changes at the low dose were limited to low incidences of variations and since skeletal variations were also observed in the rabbit developmental toxicity study at 75 mg/kg/day (NOAEL = 10 mg/kg/day), they were not considered toxicologically significant.

The developmental LOAEL is 100 mg/kg/day based on increased fetal and/or litter skeletal variations and decreased body weight (males). The developmental NOAEL is 10 mg/kg/day.

This study is classified **acceptable/guideline** and satisfies the guideline requirement for a developmental toxicity study (OPPTS 870.3700; OECD 414) in rats.

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

This Executive Summary was prepared for United States Environmental Protection Agency, Office of Pesticides Programs, Health Effects Division use.

The following text was generated by the Australian Pesticides and Veterinary Medicines Authority. However, this document has undergone critical scientific analysis and been modified as appropriate.

Report:	**KIIA 5.6.10/01, Wason, S.; 2006**
Title:	AE 0317309 - Developmental toxicity study in the rat by gavage
Laboratory	Bayer CropScience, 355 rue Dostoievski, BP 153, 06903 Sophia-Antipolis Cedex, France
Study duration	7 October 2003 – 30 January 2004
Report No.:	SA 03174
Document No.:	M-267036-01-2
Guidelines:	**O.E.C.D. guideline 414 (January, 2001);** **E.E.C. 92/69, annex V - method B31 (1992);** **US E.P.A. OPPTS series 870, no 870.3700 (August 1998);** **M.A.F.F. in Japan notification 12 Nousan no 8147 (November 24, 2000)**
GLP/QA	**Yes**

Materials and Methods

AE 0317309 (batch Op. 1-4, purity 95.7%) was suspended in 0.5% aqueous methylcellulose and administered by gavage to groups of 25 female sperm-positive SD rats at doses of 10, 100, and 300 mg/kg bw/day on gestation days 6 through 20. The homogeneity and concentration of the test substance were confirmed, Its stability in suspension in the vehicle had been determined in a previous study (SA 02020) and it was stable for 29 days at 5°C (± 3°C). Clinical signs were monitored daily, and morbidity and mortality were checked twice daily on weekdays and once daily on weekends. Body weights were measured on gestation days 0, 6, 8, 10, 12, 14, 16, 18, and 21. Food consumption was measured for the periods of gestation days 1-6, 6-8, 8-10, 10-12, 12-14, 14-16, 16-18, and 18-21. On gestation day 21, the rats were sacrificed by CO_2 narcosis and subjected to macroscopic examination. The number of ribs was counted (the data do not appear to have been tabulated in the report) and liver weight was measured. Gravid uterine weight was measured, and the number of corpora lutea, implantations, resorptions (early and late), and live and dead fetuses, and the sex and individual body weights of live fetuses were determined. Live fetuses were sacrificed by subcutaneous injection of sodium pentobarbital and examined for external observations. Approximately half of the fetuses from each litter were preserved for free-hand sectioning, while the other half were skinned, eviscerated, and stained in alizarin red S and alcian blue. Fetal observations are classified as common variants (changes occurring in more than approximately 5% of the control population), minor anomalies (slight, relatively rare structural changes that are not obviously detrimental), and malformations (very rare or obviously lethal changes).

Statistical Analysis

Statistical procedures for analyzing the data were considered appropriate.

Findings

Mortalities: There were no treatment-related mortalities during the study. One control female was sacrificed for humane reasons on gestation day 17 due to trauma.

Clinical signs: Clinical signs observed at 100 and/or 300 mg/kg bw/day included increased salivation, intense yellow urine, and vaginal discharge.

Clinical signs and pregnancy status

Observation	Dose (mg/kg bw/day)			
	0	10	100	300
N	25	25	25	25
Pregnant	25	24	23	25
Increased salivation	0	0	7	25
Intense yellow urine	0	0	0	7
Vaginal discharge	0	0	0	5

[a] Data obtained from page 40, and 42, in the study report

Pregnancy rate: There were no significant differences in pregnancy rate among the control and test groups. Pregnancy rates were 96, 92, and 100% at low, mid- and high-dose groups, compared to controls.

Body weight and body weight gain: At 300 mg/kg bw/day, maternal body weight decreased slightly throughout the study, but not statistically significant. Body weight change was statistically significantly decreased 53 and 84%, at 100 and 300 mg/kg bw/day, respectively during the gestation days 6-8, compared to controls. Thereafter, body weight change was similar across all groups. Maternal corrected body weight change was statistically significantly reduced at 300 mg/kg bw/day.

Maternal body weight, body weight change, and corrected body weight change

Parameter	Time	Dose (mg/kg bw/day)			
		0	10	100	300
Body weight, g	Gestation day 0	256.0	257.8	259.2	259.4
	Gestation day 6	293.3	295.3	296.0	292.0
	Gestation day 8	299.7	301.3	299.0	293.0
	Gestation day 10	310.3	312.0	310.0	303.4
	Gestation day 12	322.4	325.2	321.0	313.2
	Gestation day 14	330.6	332.7	327.6	322.0
	Gestation day 16	348.7	351.0	344.9	340.8
	Gestation day 18	376.0	381.5	375.0	369.4
	Gestation day 21	431.6	439.2	428.4	420.4
Body weight gain, g	Gestation days 0-6	37.3	37.5	36.9	32.6
	Gestation days 6-8	6.4	6.0	3.0*	1.0**
	Gestation days 8-10	10.6	10.8	11.0	10.4
	Gestation days 10-14	20.3	20.7	17.6	18.6
	Gestation days 14-18	45.8	48.8	47.4	47.4
	Gestation days 18-21	55.6	57.7	53.5	51.0
Corrected body weight gain, g		72.8	74.9	70.0	61.3*

Data obtained from pages 44-47 of the study report
* $p < 0.05$;.** $p < 0.01$.

Food consumption: There was a treatment-related, biologically and/or statistically significant decrease in food consumption at 300 mg/kg bw/day between gestation days 6 and 16, compared to controls. The effect was more pronounced between GD 6-8 where there was a 15% reduction in food consumption and 7% reduction during GD 8-10.

Food consumption (g/day)

	Dose (mg/kg bw/day)			
Time period	**0**	**10**	**100**	**300**
Gestation days 1-6	26.2	26.3	26.5	26.0
Gestation days 6-8	26.7	26.2	24.9	22.7**
Gestation days 8-10	26.8	27.2	26.7	24.8*
Gestation days 10-12	27.2	27.9	26.6	26.1
Gestation days 12-14	27.5	27.5	26.5	25.9*
Gestation days 14-16	28.0	28.1	27.5	26.8
Gestation days 16-18	29.9	31.0	30.5	29.8
Gestation days 18-21	30.1	30.6	29.9	29.1

Data obtained from pages 49-50 of the study report
* $p < 0.05$; ** $p < 0.01$.

Macroscopic findings and liver weight: The only possibly treatment-related finding at gross necropsy was observed in one female at 300 mg/kg bw/day, which showed yellow sediment in the kidney and gritty content in the urinary bladder. Liver weight was statistically significantly increased at 300 mg/kg bw/day and somewhat increased at 100 mg/kg bw/day without statistical significance.

Maternal liver weight

Parameter	Dose (mg/kg bw/day)			
	0	**10**	**100**	**300**
N	24	24	23	25
Mean liver weight (g)	14.67	14.66	15.43	15.56*(↑6)

Data obtained from page 52 of the study report
* $p < 0.05$

Litter parameters: There were no differences among the control and test groups in the number of corpora lutea, implantation sites, either early or late resorptions, number of live fetuses, sex ratio, or percent pre- or post-implantation loss per litter. Fetal body weight was biologically and/or statistically significantly decreased at and above 100 mg/kg bw/day.

Mean body weights (g), of live male and female rat fetuses

Group	Dose (mg/kg bw/day)			
	0	**10**	**100**	**300**
Combined sexes	5.44	5.48	5.32	5.14**(↓ 5.5%)
Males	5.58	5.60	5.45*(↓2%)	5.28**(↓5.3%)
Females	5.30	5.34	5.18	5.01**

Data obtained from page 58 of the study report

*p < 0.05; ** p< 0.01.

Fetal external observations: A total of four fetuses at 300 mg/kg bw/day, from 2 different litters, presented the malformation of hindpaw polydactyly. Three of the fetuses with hindpaw polydactyly (300 mg/kg bw/day), all of which were in the same litter, also showed an anomaly of kyphosis, forelimb hyperflexion, misshapen digits on the forepaws, and malpositioning of the digits on both the forepaws and the hindpaws. Historical control data on such observations was not available in the study to aid in assessing their relationship to treatment. There were also some sporadic limb deformities at other dose levels (hindpaw or hindlimb malrotation), as the table below indicates. Reference to relevant historical control data suggests that these were probably only sporadic incidences occurring at the expected rate. There were single cases in the 10 mg/kg bw/day group of forepaw hyperflexion and of umbilical hernia, which were probably also simply sporadic findings, although no historical data were supplied on these conditions. Enlarged placenta was observed at the mid and high dose, with a suggestion of a dose response relationship.

Selected fetal external observations

Observation	Dose (mg/kg bw/day)			
	0	10	100	300
Number of fetuses examined	333 (24)	345 (24)	311 (23)	353 (25)
Hindpaw(s) (unilateral/bilateral): polydactyly (presence of 1 or 2 supernumerary digits).	0	0	0	4 (2)
Kyphosis. Forelimb (bilateral): hyperflexion. Forepaws (bilateral): all digits: misshapen. Forepaws (bilateral): digit 2: malpositioned. Hindpaws (bilateral): all digits: malpositioned.	0	0	0	3 (1)
Hindpaw or hindlimb (unilateral): malrotated: outward or inward.	0	2 (2)	0	1 (!)
Forepaw (unilateral): hyperflexion.	0	1 (1)	0	0
Umbilical hernia.	0	1 (1)	0	0
Placenta: enlarged	0	0	2 (1)	4 (1)

Data obtained from page 61 of the study report
() Number of litters

Fetal visceral observations: Examination of the fetuses for visceral findings revealed no malformations, and no treatment-related variants or anomalies in any group.

Fetal skeletal observations: Two of the four fetuses with the external observation of hindpaw polydactyly had malformations including short tibia and femur, supernumerary cartilaginous phalanges, fused cartilage for several metatarsals and phalanges, and unossified 5th metatarsal

(presumably the other two fetuses with externally observed hindpaw polydactyly were not included among those subjected to skeletal observation). There was a slight increase at the mid and high dose in the occurrence of 27 pre-sacral vertebrae. Treatment-related variants observed in this study were primarily linked to ossification. Some of these occurred at an increased fetal and/or litter incidence in the low dose group. These were 7th cervical centrum: unossified/normal cartilage; forepaws: 3rd and/or 4th proximal phalanges: unossified/normal cartilage; 1st metatarsal: unossified/normal cartilage; less than 9 sacrocaudal vertebrae ossified/9 first sacrocaudal vertebrae: normal cartilage.

None of these data were statistically analysed in the study.

Selected skeletal findings in fetuses (fetal incidence, litter incidence)

	Type[a]	Level[b]	Dose, (mg/kg bw/day)			
			0	10	100	300
Number of fetuses examined:			**172**	**179**	**163**	**182**
Number of litters examined:			**24**	**24**	**23**	**25**
Anterior and/or posterior fontanelle: enlarged	Var	F	0	0	0	2
		L	0	0	0	2
7th cervical centrum: unossified/normal cartilage	Var	F	19	19	40	63
		L	10	14	18	18
7th cervical centrum: unossified / dumbbell or bipartite cartilage	Ano	F	0	0	2	1
		L	0	0	2	1
5th and/or 6th sternebrae: incomplete ossification or bipartite/normal cartilage	Var	F	21	29	44	66
		L	11	11	20	22
5th or 6th sternebra: unossified/normal cartilage	Var	F	1	3	3	5
		L	1	2	2	5
14th thoracic rib(s) (unilateral/bilateral): short	Var	F	2	2	13	19
		L	1	1	7	10
Extra ossification point (unilateral / bilateral) on 14th thoracic vertebra	Var	F	10	5	8	22
		L	9	4	7	10
Presence of 27 pre-sacral vertebrae	Ano	F	0	0	1	2
		L	0	0	1	1
14th thoracic rib(s) (bilateral): short and presence of 27 pre-sacral vertebrae	Ano	F	0	0	1	1
		L	0	0	1	1
5th metacarpal: incomplete ossification / normal cartilage	Var	F	1	0	2	9
		L	1	0	2	5
Forepaws: 3rd and/or 4th proximal phalanges: unossified/normal cartilage	Var	F	0	1	2	4
		L	0	1	2	3
1st metatarsal: unossified/normal cartilage	Var	F	0	11	20	18
		L	0	6	9	9
Tibia (bilateral) and femur (bilateral): short/cartilage normal; supernumerary cartilaginous phalanges (bilateral); and/or 3rd and 4th metatarsal, proximal and distal phalanges (unilateral): cartilage fused; and/or 5th metatarsal: unossified/normal cartilage.	Mal	F	0	0	0	2
		L	0	0	0	1

Less than 9 sacrocaudal vertebrae ossified / 9 first sacrocaudal vertebrae: normal cartilage	Var	F	1	7	7	8
		L	1	4	5	6

Data obtained from pages 65-67 of the study report

[a] Var = Variation; Ano = Anomaly; Mal = Malformation; [b] F = Fetal observations; L = Litter observations

Conclusions

The study authors proposed a NOAEL of 10 mg/kg bw/day for both maternal and fetal effects. This was based on a LOAEL of 100 mg/kg bw/day for dams and fetuses. The relevant effects in the dams included increased salivation and in the fetuses decreased mean body weight.

The increased salivation in the dams at 100 mg/kg bw/day was also observed, with a higher incidence, at the high dose. Salivation as a clinical sign was not observed in any other study in the database. Nevertheless its incidence is clearly increased in a dose-related manner in the mid and high dose dams in this study. Since this is a gavage study, the increased salivation was not related to palatability issues. Additionally, a dose-related increase in enlarged placenta was seen at and above 100 mg/kg bw/d. Based on these findings, the maternal NOAEL (NOEL for Australia) was established at 10 mg/kg bw/day.

There were indications of fetotoxic effects at and above 10 mg/kg bw/d as manifested by increased fetal and/or litter incidence of skeletal variations. However, since the skeletal effects at the lowest dose were variations and not malformations and since skeletal variations were observed in the rabbit developmental toxicity study at 75 mg/kg/day (NOAEL = 10 mg/kg/day), the NOAEL (NOEL for Australia) for fetotoxicity was established for this study at 10 mg/kg bw/d).

EPA Reviewer: Guruva Reddy, D.V.M., Ph.D. **Signature:** [illegible] for
Registration Action Branch 1, Health Effects Division (7509P) **Date:** 6/5/08
EPA WAM: PV Shah, PhD **Signature:** PV Shah
Registration Action Branch 1, Health Effects Division (7509P) **Date:** 6/5/08

TXR#: 0054227

DATA EVALUATION RECORD

STUDY TYPE: Prenatal Developmental Toxicity Study - Rabbit; OPPTS 870.3700b [§83-3b]; OECD 414.

PC CODE: 000692 **DP BARCODE:** D335563

TEST MATERIAL (PURITY): AE 0317309 (95.7% w/w)

SYNONYMS: 5-Hydroxy-1,3-dimethylpyrazol-4-yl 2-methylsulfonyl)-4-(trifluoromethyl)phenyl ketone

CITATION: Wason S. (2006) AE 0317309 Developmental toxicity study in the rabbit by gavage. Bayer CropScience, Sophia Antipolis Cedex, France. Study Report No.: SA 03131, February 22, 2006. MRID 46801906. Unpublished.

SPONSOR: Bayer AG, Bayer CropScience, Research Taingle, NC 27709, USA.

EXECUTIVE SUMMARY: In a developmental toxicity study (MRID 46801906), AE 0317309Technical (95.7% a.i.; Batch # Op. 1-4) in 0.5% aqueous methylcellulose was administered daily via oral gavage to 24 time-mated New Zealand White rabbits/group at a dose volume of 4 mL/kg at dose levels of 0, 10, 75, or 250 mg/kg bw/day from gestation day (GD) 6 through 28 inclusive. All surviving rabbits were killed on GD 29; their fetuses were removed by cesarean section and examined. Liver weight and the number of ribs were noted for each animal. The gravid uterine weight, number of corpora lutea, number of implantations, resorptions (early and late), and live and dead fetuses, and the individual weight of live fetuses, was recorded for each animal. Live fetuses were sacrificed and examined externally. The heads of approximately half the fetuses were fixed for later examination. All fetuses were dissected for visceral findings, fixed, and stained for skeletal examination.

No effects of treatment were observed on mortality, number of corpora lutea, implantation sites, early or late resorptions, number of live or dead fetuses, or percent pre- or post-implantation losses per dam and fetal sex ratio, fetal external, and visceral malformations at any dose level.

At 250 mg/kg bw/day maternal body weight gain decreased (↓230% or 0g gain vs 30g in control) significantly between GD 8-10 and food consumption decreased significantly throughout the study. At necropsy 2/25 does had prominent liver lobulations, however, no histopathology information was available.

The maternal LOAEL is 250 mg/kg bw/day based on decreased body weight gain during GD 8-10 and decreased food consumption during the study. The maternal NOAEL was 75 mg/kg bw/day.

At 250 mg/kg bw/day, fetal body weight was statistically significantly decreased both for combined sexes and for the sexes taken separately. Mean combined fetal body weights decreased 4.7 – 14.9% at ≥ 10 mg/kg bw/day and was dose–related. However, a follow-up historical control analysis revealed that concurrent controls were at the upper end of the historical control range and that body weights at the low and mid doses were within the historical control range. A number of skeletal anomalies and variants were recorded at ≥75 mg/kg bw/day; however; their incidences at 10 mg/kg/day did not exceed rates in historical control data.

The developmental LOAEL was 75 mg/kg bw/day based on an increased incidence of fetal/litter skeletal anomalies/variations. The developmental NOAEL was 10 mg/kg bw/day.

This study is classified **acceptable/guideline (OPPTS 870.3700b)** and satisfy the guideline requirements for a developmental study in the rabbit.

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

This Executive Summary was prepared for United States Environmental Protection Agency, Office of Pesticides Programs, Health Effects Division use.

The following text was generated by the Australian Pesticides and Veterinary Medicines Authority. However, this document has undergone critical scientific analysis and been modified as appropriate.

Report:	**KIIA 5.6.11/01, Wason, S.; 2006**
Title:	AE 0317309 - Developmental toxicity study in the rabbit by gavage
Laboratory	Bayer CropScience, 355 rue Dostoievski, BP 153, 06903 Sophia-Antipolis Cedex, France
Study duration	2 September 2003 – 1 January 2004
Report No.:	SA03131
Document No.:	M-266702-01-2
Guidelines:	**OECD 414 (Jan. 2001); EEC. 92/69 - Annex V - method B31 (1992); OPPTS 870.3700 (Aug. 1998), MAFF 59 No.12 Nousan No8147 (Nov. 24, 2000)**
GLP/QA	**Yes**

Materials and Methods

AE 0317309 (batch Op. 1-4, purity 95.7%) was formulated in 0.5% aqueous methylcellulose and administered by oral gavage in a constant volume of 4 ml/kg to groups of 25 time-mated female New Zealand white rabbits at doses of 0, 10, 75, and 250 mg/kg bw/day from gestations days 6 through 28, inclusive. The homogeneity of the suspensions was checked during the first formulation for the highest and lowest concentrations and all concentrations were checked for all formulations. Stability of the compound in suspension in the vehicle had been determined in a previous study (SA 02020) and it was stable for 29 days at 5°C (± 3°C). The animals were housed individually in polycarbonate cages with a perforated cage floor, and food and water were available *ad libitum*. Clinical signs were monitored and animals were checked twice daily on weekdays and once daily on weekends or holidays for morbidity or mortality. Body weight was measured on gestation days 3, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, and 29. Food consumption was measured on these days as well as for days 3-4, 4-5, and 5-6. On day 29 of gestation, the rabbits were sacrificed and subjected to macroscopic examination. Liver weight and the number of ribs were noted for each animal. The gravid uterine weight, number of corpora lutea, number of implantations, resorptions (early and late), and live and dead fetuses, and the individual weight of live fetuses, was recorded for each animal. Live fetuses were sacrificed and examined externally. The heads of approximately half the fetuses were fixed for later examination. All fetuses were dissected for visceral findings, fixed, and stained for skeletal examination. Fetal observations were classified as common variants (changes occurring in more than approximately 5% of the control population), minor anomalies (slight, relatively rare structural changes that are not obviously detrimental), and malformations (very rare or obviously lethal changes).

Statistical Analysis

Appropriate statistical procedures for analyzing the data were employed. Body weights and body weight gains, food consumption and fetal litter based parameters were analyzed using Bartlett test, ANOVA, Kruskal-Wallis and Dunnett tests. The statistical tests were acceptable.

Findings

Mortalities: There were no mortalities during the study.

Clinical signs: Yellow or beige sediments in the urine of females at 75 and 250 mg/kg bw/day

were noted on several occasions, with the incidence greater at 250 mg/kg bw/day. The study authors suggest that when AE 0317309 is excreted in the urine it forms a yellow sediment. Therefore, although this sediment was clearly treatment-related, it was considered by the study authors to be an indication of compound excretion rather than an adverse effect. However the identity of the sediment does not seem to have been confirmed.

Clinical signs and pregnancy status (%)

Observation	Dose (mg/kg bw/day)			
	0	10	75	250
N	25	25	25	25
Pregnant	24 (96)	23 (92)	23 (92)	22 (88)
Few feces	0	2	3	5
Intense yellow urine	0	0	1	25

[a] Data obtained from page 39 - 40, in the study report
Numbers in the parenthesis is % pregnant

Pregnancy rate: At 0, 10, 75 and 250 mg/kg bw/day, the pregnancy rates were 96, 92, 92, and 88%, respectively.

Body weight and body weight gain: Maternal body weight gain at 250 mg/kg bw/day was statistically significantly reduced between days 8 and 10, with an overall reduction between days 6 and 29 of 31% compared to controls. Corrected maternal body weight at 250 mg/kg bw/day was slightly but statistically non-significantly decreased compared to controls. Body weight changes were not affected at 75 and 10 mg/kg/day.

Body weight and body weight gain (kg)

Parameter	Time	Dose (mg/kg bw/day)			
		0	10	75	250
Body weight, kg	Gestation day 3	3.46	3.47	3.50	3.49
	Gestation day 6	3.49	3.52	3.52	3.51
	Gestation day 8	3.50	3.53	3.53	3.51
	Gestation day 10	3.53	3.56	3.55	3.51
	Gestation day 12	3.56	3.58	3.57	3.52
	Gestation day 14	3.60	3.63	3.63	3.56
	Gestation day 16	3.65	3.68	3.68	3.61
	Gestation day 18	3.67	3.69	3.68	3.62
	Gestation day 20	3.68	3.69	3.70	3.62
	Gestation day 22	3.72	3.73	3.72	3.64
	Gestation day 24	3.76	3.76	3.76	3.67
	Gestation day 26	3.79	3.79	3.80	3.71
	Gestation day 29	3.84	3.85	3.88	3.75
Body weight gain, kg	Gestation days 3-6	0.03	0.04	0.02	0.01
	Gestation days 6-8	0.01	0.02	0.02	0.00
	Gestation days 8-10	0.03	0.02	0.02	0.00**
	Gestation days 10-14	0.07	0.07	0.07	0.05
	Gestation days 14-18	0.08	0.06	0.06	0.06
	Gestation days 18-22	0.05	0.04	0.04	0.02

	Gestation days 22-26	0.06	0.07	0.07	0.07
	Gestation days 26-29	0.05	0.05	0.08	0.04
Corrected body weight gain, kg		-0.11	-0.13	-0.12	-0.21

Data obtained from pages 41-45, in the study report.
* $p < 0.05$; ** $p < 0.01$.

Food consumption: At 250 mg/kg bw/day, food consumption was consistently reduced compared to controls, with the reduction achieving statistical significance on several occasions. Food consumption was reduced 9-24% during GD 6-29. Food consumption was unaffected at 75 and 10 mg/kg/day.

Food consumption, in grams/day, by pregnant rabbits

	Dose (mg/kg bw/day)			
Time period	**0**	**10**	**75**	**250**
Gestation days 3-6	169.3	175.4	173.1	167.8
Gestation days 6-8	169.5	168.1	165.7	144.0**
Gestation days 8-10	171.5	165.7	169.5	143.9**
Gestation days 10-14	161.0	156.4	150.8	131.2**
Gestation days 14-18	158.0	146.0	147.7	120.5**
Gestation days 18-22	162.7	153.4	151.3	130.8**
Gestation days 22-26	139.0	131.9	134.1	117.6
Gestation days 26-29	124.9	117.8	127.7	114.2

Data obtained from pages 46-47, in the study report.
* $p < 0.05$; ** $p < 0.01$.

Liver weight: Mean liver weights are provided in the table below. Liver weight was statistically significantly increased at 250 mg/kg bw/day (125.3 g, $p \leq 0.01$) compared to controls (102.6 g). .

Mean Liver weights (g), and incidence of necropsy findings

Group	Dose (mg/kg bw/day)			
	0	**10**	**75**	**250**
Liver weight	102.6±11.7	99.8±15.4	105.0±14.1	125.3**±15.6 (↑19)
Prominent liver lobulations	0	1	1	2
Cysts (oviduct)	3	10	11	7

Data obtained from Tables 5 and 6, pages 48-49, in the report.

N=25

Data obtained from Table 7, page 52, in the report

Figures in parenthesis (%)

Macroscopic findings: Prominent macroscopic findings are listed above in the table. Prominent lobulation of the liver was noted at 250 mg/kg bw/day in 2 out of 25 females. At 75 and 10 mg/kg/day groups isolated incidences of liver lobulations were observed, however in the absence of liver weight increases, no corresponding histopathology and lack of dose response, liver lobulations in these dose groups are considered equivocal. Oviduct cysts were more common in treated groups, but there was no dose-response effect. Counting ribs in adult female rabbits revealed that the most common number of ribs was 26 (70% of all animals).

Distribution of rib numbers in the dams

Rib count[a]	Dose (mg/kg bw/day)				All animals
	0	10	75	250	
12 / 12	21%	17%	4%	23%	16%
12 / 13	21%	9%	17%	9%	14%
13 / 13	58%	74%	78%	68%	70%
> 12 / 12	79%	83%	96%	77%	84%

Data obtained from Appendix K, pages 203 – 205, in the study report.
a 12 / 12 = 12 ribs per side; 12 / 13 = 12 ribs on one side, 13 ribs on other side; 13 / 13 = 13 ribs per side

Fetal body weight: At 250 mg/kg bw/day, fetal body weight was statistically significantly decreased both for combined sexes and for the sexes taken separately. Moreover, combined-sexes body weight was significantly decreased 4.7 – 14.9% at ≥ 10 mg/kg/day and was dose–related. The study authors stated that these decreases were within historical control ranges; however no relevant data were supplied at the time of study submission. A follow-up historical control analysis revealed that concurrent controls were at the upper end of the historical control range and that body weight at the low and mid doses were within the historical control range.

Mean body weights (g), of live male and female rabbit fetuses

Group	Dose (mg/kg bw/day)			
	0	10	75	250
Combined sexes	38.2±6.5	36.4*±6.4 (↓4.7)	36.2**±5.5 (↓5.2)	32.5**±5.8 (↓14.9)
Males	38.1±7.1	36.4±6.2	36.8±5.2	32.6**±5.8 (↓14.1
Females	38.3	36.4	35.6	32.3**±5.9 (↓15.6)

Data obtained from Table 7, page 52, in the report

Figures in parenthesis (%)

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

Litter parameters: There were no significant differences between groups in the number of corpora lutea, implantation sites, early or late resorptions, number of live or dead fetuses, or percent pre- or post-implantation losses per dam. There was also no effect in any group on the fetal sex ratio.

Fetal external observations: There was no effect of treatment on the incidence of external malformations. Two fetuses were observed to have malformations: one in the control group with gastroschisis median, spina bifida, hindlimb hyperflexion, and acaudate; and one at 75 mg/kg bw/day with umbilical hernia. The occurrence of umbilical hernia was considered not to be treatment-related since one such case in the 208 fetuses in the relevant dose group is within the range of the historical control data supplied. The incidence of runt fetuses was somewhat increased in the treated groups compared with controls. However, the control group showed a low incidence by historical control standards (historical minimum, 15/235 fetuses) and the incidence in the treated groups was below the historical maximum (41/217) at all but the high dose (see table below).

Selected external and skeletal findings in fetuses

Parameter	Type[a]	Level[b]	Dose (mg/kg bw/day) 0	10	75	250
Number of fetuses examined:		**F**	**198**	**197[c]**	**208**	**206**
Number of litters examined:		**L**	**24**	**23**	**23**	**22**
Runt (body weight < 28.0 g)	Var	F	13	23	15	45
		L	6	13	6	13
Atlas centrum: unossified	Var	F	0	3	5	21
		L	0	2	4	11
Extra ossification site between atlas and axis	Var	F	1	5	25	42
		L	1	3	11	13
13th thoracic rib(s) (unilateral or bilateral) or 13th thoracic rib(s) (bilateral, short unilateral)	Var	F	101	138	189	200
		L	23	23	22	22
13th thoracic rib(s) (unilateral/bilateral) and presence of 27 pre-sacral vertebrae	Ano	F	2	2	14	53
		L	2	2	7	14
1st metacarpal: incomplete ossification or unossified	Var	F	43	34	32	78
		L	12	15	11	16
Insertion point(s) (unilateral/bilateral) of pelvic girdle on 2nd sacral vertebra	Var	F	64	90	163	146
		L	19	21	21	22
Pubis (bilateral): incomplete ossification	Var	F	6	5	7	15
		L	4	3	3	5

Data obtained from Tables 8 and 10, pages 54 and 58-60 , in the report.

[a] Var = Variation; Ano = Anomaly;
[b] F = Fetal observation; L = Litter observation
[c] 198 fetuses were recorded in this group but one fetus was not evaluated for skeletal findings.

Fetal visceral findings: The incidence of short innominate artery was somewhat above historical control rates at the low and high dose, but there was no effect at the mid dose.

Fetal skeletal findings: There were no treatment-related malformations. A number of skeletal anomalies and variants were recorded, although their incidences at the low dose did not exceed rates in historical control data. Also, as mentioned above, the incidence of 13th ribs in the rabbits in this study seems to be naturally high (as seen in the dams).

Conclusions

The study authors proposed a maternal NOAEL of 75 mg/kg bw/d and a fetal NOAEL of 10 mg/kg bw/d. The maternal LOAEL was based on decreased food consumption and body weight gain at 250 mg/kg bw/day. The fetal LOAEL was based on altered ossification patterns and other skeletal variants and anomalies at the mid dose (75 mg/kg bw/day).

As well as showing reduced food consumption and increased loss of body weight at 250 mg/kg bw/day, dams at this dose had increased liver weight. The maternal NOEL was therefore set at 75 mg/kg bw/day.

Fetal weight (combined sexes) was decreased in the high-dose group. In addition, there were a number of skeletal effects which showed increased fetal and/or litter incidence at the mid dose

together with a dose-response relationship. Therefore the fetal NOAEL (NOEL for Australia) can be established at 10 mg/kg bw/d.

EPA Reviewer: Guruva B. Reddy, D.V.M., Ph.D. **Signature:** [signature] for
Registration Action Branch 1, Health Effects Division (7509P) **Date:** 6/5/08
EPA WAM: PV Shah, PhD **Signature:** PV Shah
Registration Action Branch 1, Health Effects Division (7509P) **Date:** 6/5/08

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TXR#: 0054227

DATA EVALUATION RECORD

STUDY TYPE: Chronic Toxicity in Dogs (feeding); OPPTS 870.4100b [§83-1b]; OECD 452.

PC CODE: 000692

DP BARCODE: D335563

TEST MATERIAL (PURITY): AE 0317309 (95.7% w/w)

SYNONYMS: 5-Hydroxy-1,3-dimethylpyrazol-4-yl 2-methylsulfonyl)-4-(trifluoromethyl)phenyl ketone

CITATION: Eigenberg, D.A. (2006) A chronic toxicity feeding study in the beagle dog with technical grade AE 0317309. Bayer CropScience LP Toxicology, Stilwell, Kansas, USA. Study Report No.: 201450, February 21, 2006. MRID 46801908. Unpublished.

SPONSOR: Bayer AG, Bayer CropScience, Research Triangle Park, NC 27709, USA.

EXECUTIVE SUMMARY: In a chronic toxicity study (MRID 46801908), AE 0317309 (95.7% w/w, Batch #: OP. 1-4) was administered to 4 beagle dogs/sex/dose in the diet for 52 weeks at doses of 0, 250, 1000, or 3000 ppm (equivalent to 0/0, 7/9, 34/33, and 101/93 mg/kg/day in males/females).

No treatment-related adverse effects were observed on mortality, body weights, body weight gains, food consumption, ophthalmoscopic examination, urinalysis, or clinical chemistry at any dose. LDH levels were decreased in the mid and high dose females at 12months. However, the pretest values were also low in these groups, suggesting that this was not treatment-related. There was no evidence of treatment-related increased blood levels in the urine.

At 3000 ppm, APTT was significantly depressed in males (16%) and females (14-16%) at ≥ 1000 ppm. The prothrombin time was not significantly effected. Since the APTT was decreased rather than increased, the clinical and toxicological significance is unknown.

A dose-related, non-statistically significant, increase in the absolute and relative liver, kidney and thyroid weights increased in males and females. Specifically at the 3000 ppm dose, absolute and relative liver weights in males and females (34-49%), absolute and relative kidney weights in males (26-38%), and absolute and relative thyroid weights in females (18-30%) increased. Microscopically, increased incidence of hepatocytomegaly was observed in 2/4 males and 3/4

females at the high dose, compared to 0/4 in controls. Hepatocytomegaly was observed as 1 (minimal) on a scale of 1 to 5. Kidney tubular dilatation was observed in 1/4, 1/4, 3/4, and 4/4 in the control, 250, 1000 and 3000 ppm males, respectively with a severity grade of 2 in the mid- and high dose groups and grade severity of 1 each in control and low dose groups. Additionally, cataracts were observed one each in 3000 ppm males and females; grade severity of 2. One high-dose male showed inflammation, hemorrhage and debris in bladder. Increased thyroid weights were not associated with any treatment-related micropathology observed.

The LOAEL is 1000 ppm (equivalent to 34 mg/kg/day) in males, based upon the increased incidence and severity of kidney tubular dilatation. The NOAEL was 250 ppm (equivalent to 7 mg/kg/day) in males. The LOAEL is 3000 ppm (equivalent to 93 mg/kg bw/day) in females, based on cataracts. The NOAEL is 1000 ppm (equivalent to 33 mg/kg/day) in females.

This study is classified as **acceptable/guideline** and satisfies the guideline requirement (OPPTS 870.4100b, OECD 452) for a chronic oral toxicity study in dogs.

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

This Executive Summary was prepared for United States Environmental Protection Agency, Office of Pesticides Programs, Health Effects Division use.

The following text was generated by the Australian Pesticides and Veterinary Medicines Authority. However, this document has undergone critical scientific analysis and been modified as appropriate.

Report:	**KIIA 5.3.4/01, Eigenberg, D. A.; 2006**
Title:	A chronic toxicity feeding study in the beagle dog with technical grade AE 0317309
Laboratory	Bayer CropScience LP Toxicology, 17745 South Metcalf, Stilwell, Kansas 66085-9104
Study duration	6 April 2004 – 20 April 2005 (in-life)
Report No.:	201450
Document No.:	M-267321-01-1
Guidelines:	U.S. EPA Health Effects Test Guidelines, OPPTS 870.4100 (1998) OECD Guidelines for Testing of Chemicals, Section 4, Guideline 452, May 1981
GLP/QA	Yes

Materials and Methods

AE 0317309 (batch Op. 1-4, purity 95.7%) was incorporated into diet and administered to groups of 4 male and 4 female beagle dogs at 0, 250, 1000, or 3000 ppm for one year. These concentrations provided doses of 0, 7, 34, and 101 mg/kg bw/d for males and 0, 9, 33, and 93 mg/kg bw/d for females.

Selection of these dose levels was based on a 90-day feeding study in the Beagle dog [Eigenberg, D. A.: "Technical Grade AE 0317309: A 90-Day Subchronic Toxicity Feeding Study in the Beagle Dog", Report Number 201019, March 26, 2004], which used doses of 0, 1500, 9000, and 18000 ppm in the diet and was terminated on study day 29 due to the findings described below and a 90-day feeding study in the Beagle dog, which used 100, 500, and 1000 ppm in the diet [Eigenberg, D. A.: "Technical Grade AE 0317309: A 90-Day Subchronic Toxicity Feeding Study in the Beagle Dog", Report Number 201210, August 30, 2005].

In the first 90-day study, one mid-dose group dog was euthanized on study day 23 because the dog essentially had stopped eating on study day 14. On study day 28, two high-dose group dogs were euthanized in moribund condition. The gross necropsy of these animals revealed compound-related lesions in the urinary tract in all three animals. In the low-dose group, there was an increase in blood in the urine of males and females, indicating urinary tract lesions. In the second 90-day study, no systemic toxicity was observed.

The dogs in the present study were sourced from Marshall Farms USA, Inc, North Rose, New York and were 7-8 months old at the start of the study. They were housed individually in stainless steel runs. The test substance was suspended in acetone and corn oil, and this mixture was added to the diet so that corn oil was 1% of the diet and the required test substance concentration was achieved. The test substance was homogeneously distributed in the diet and was stable in the feed stored at room temperature for 7 days and freezer temperature for 28 days. The concentration of the active ingredient in the feed was verified in study weeks 1, 2, 3, and then every 4 or 5 weeks until the end of the study. The diet was made available to the animals for 3-6 hours each day, and water was available *ad libitum*. Body weights were measured weekly and immediately prior to necropsy, and food consumption was determined daily. Clinical signs were monitored at least twice daily on weekdays and once daily on weekends and holidays. Ophthalmoscopic examinations were conducted prior to the start of the study, and just before the

end of the study. Detailed clinical examinations were conducted at study initiation and weekly thereafter. Blood for hematology and clinical chemistry was drawn once pretest, and in weeks 13, 26, 39, and 52 by jugular vein puncture after overnight fasting. Urine was collected (overnight samples) at these times. At the end of the study, the dogs were sacrificed by intravenous injection of a standard euthanasia solution and subjected to a gross necropsy. Selected organs were weighed, and organs and tissues were collected for microscopic examination.

The following hematology parameters were assessed: Hct, WBC differential count, Hb, MCH, WBC, MCHC, RBC, MCV, platelet count, reticulocyte count, blood cell morphology, activated partial thromboplastin time (APTT), prothrombin time (PT), red blood cell distribution width (RDW), hemoglobin distribution width (HDW). Clinical chemistry included calcium, albumin, chloride, creatinine, urea nitrogen, phosphorous, total cholesterol, potassium, globulins, sodium, glucose, total bilirubin, alkaline phosphatase, total protein, triglycerides, creatine phosphokinase, lactic acid dehydrogenase (LDH), uric acid, ALT, AST, A/G ratio, GGT. Urinalysis covered the following parameters: appearance, glucose, volume, ketones, specific gravity, osmolality, bilirubin, pH, blood, sediment (microscopic), nitrite, protein, urobilinogen, urine creatinine (24 h), leucocytes.

The organs/tissues collected at necropsy were as follows: aorta, brain (multiple sections), salivary glands, heart, esophagus, bone marrow, spinal cord (3 levels), stomach, lymph nodes (mesenteric, retropharyngeal), pituitary, duodenum, spleen, eyes (retina, optic nerve), sciatic nerve, jejunum, thymus, ileum, adrenal gland, cecum, colon, kidneys, parathyroids, rectum, urinary bladder, thyroids, liver, testes, gall bladder, epididymides, bone (sternum and/or femur), pancreas, prostate, skeletal muscle, ovaries, skin, trachea, uterus, physical identifier, lung, mammary gland, all gross lesions and masses, ureter, bone (rib/CCJCT[craniocervical junction]), nasopharynx, fallopian tube (oviduct), larynx, cervix, vagina. In addition, lungs, heart, spleen, kidneys, testes, epididymides, thyroid, brain and adrenal glands were weighed and examined microscopically.

Statistical Analysis

Statistical significance was determined at $P \leq 0.05$ for all tests with the exception of Bartlett's test, in which a probability value of $P \leq 0.01$ was used. All tests were two tailed, except for gross and histology lesions which were one sided. Continuous data were analysed with Bartlett's test for homogeneity followed by ANOVA, if significant Dunnett's test was used. For heterogenous data, a Kruskal-Wallis ANOVA was performed followed by Mann-Whitney U-test used to identify statistical significance between groups.

Frequency data were initially analysed by Chi-Square test, if significant, each treatment group was compared to control group using Fisher's Exact test.

Gross lesions from animals in all dose groups and representative sections of the tissues listed above (with the exception of the physical identifier and vagina) from all control and high-dose group animals were processed, stained with hematoxylin

and eosin (H&E) and examined under the light microscope. If findings were observed in a tissue in the high-dose group the mid-and low-dose groups were evaluated.

Findings

Mortality: No animals were found dead or sacrificed in-extremis during the study.

Clinical signs: Although there was no dose-response association, abnormal color (brownish yellow and reddish brown) urine was more common in treated males. It was no more common in treated females than in control females. There were no other noteworthy clinical observations.

Number of animals showing abnormal color of the urine

Parameter	Dose (ppm)							
	Males				Females			
	0	250	1000	3000	0	250	1000	3000
	N=4	N=4	N=4	N=4	N=4	N=4	N=4	N=4
Abnormal color of the urine	1	4	2	4	3	4	4	3

Body weights: Terminal body weights were not affected. Mean body weights were increased (NS) compared to controls and the increases were not dose-related. The mean body weight gains increased 14-21% in males and 19 – 72% in females.

Mean Body Weights and Body Weight Gains of Beagles[a]

Conc. in Diet (ppm)	Body Weight (g)		Body Weight Gain (g)			52 Week Body Weight Gain	
	0 Weeks	52 Weeks	0-13 Weeks	13-26 Weeks	26-52 Weeks	g	% of Control
Male							
0	8078 ± 757	9946 ± 567	999	263	606	1868	--
250	8670 ±739	11389 ± 2168	1655	423	641	2719	+ 46
1000	8222 ± 480	10344 ± 521	1195	92	835	2122	+ 14
3000	8404 ± 797	10660 ± 1113	1024	15	1218	2257	+ 21
Female							
0	7600 ± 548	8965 ± 648	618	-172	920	1366	--
250	7591 ± 545	9219 ± 1183	998	-77	707	1628	+ 19
1000	7479 ±395	9839 ± 468	1323	87	950	2360	+ 72
3000	7743 ±	9636 ±	1029	10	854	1893	+ 39

	485	348							

[a] Data obtained from Appendix IV, pages 276-300, in the study report

Food consumption: Although it did not generally reach statistical significance, there was a persistent tendency for dogs in the treatment groups to consume more food than the controls (ranging up to 50% greater in the high dose animals).

Ophthalmoscopic findings: There were no treatment-related abnormalities.

Hematology: Most alterations observed were judged not to be related to treatment for various reasons, including a lack of consistency over time, comparable values within the same treatment group in the pre-treatment period as well as during the study, coverage by historical control data, marked alteration in one animal only which led to an alteration in the group mean, or a lack of dose-dependency.

At 12 months, APTT was significantly depressed in males at 3000 ppm (16%) and in females at and above 1000 ppm (14-16%). There were no statistically significant effects on prothrombin time. Since the APTT was decreased rather than increased, the clinical and toxicological significance of the change is doubtful.

Selected hematology parameters in dogs given AE 0317309 in the diet for one year

Parameter	Sampling time	Dose (ppm)							
		Males				Females			
		0	250	1000	3000	0	250	1000	3000
Platelets $10^3/mm^3$	Pretest	341	372	327	406	323	337	348	292
	3	241	315	294	377*	278	303	316	291
	6	248	291	258	338	275	318	336	308
	9	250	277	262	348	286	321	295	297
	12	265	294	282	346	308	316	334	355
WBC $10^3/mm^3$	Pretest	9.1	8.1	9.8	8.4	8.6	9.3	10.4	10.0
	3	8.4	9.2	9.8	9.7	8.8	8.3	10.6	10.7
	6	8.5	8.5	8.3	9.7	7.5	8.2	10.1	11.5*
	9	9.0	9.1	9.5	10.1	8.7	8.4	10.3	10.5*
	12	8.6	9.2	9.7	10.3	7.9	8.1	9.8	9.1
RBC $10^6/mm^3$	Pretest	6.80	6.97	7.41	6.92	7.42	6.93	6.92	6.92
	3	7.04	7.03	7.30	7.02	6.76	6.87	6.77	7.15
	6	7.36	7.06	7.55	7.13	6.97	6.70	6.77	6.92
	9	7.58	7.35	7.58	7.44	6.93	6.88	6.95	7.16
	12	7.48	7.08	7.66	7.31	7.12	7.18	6.65	6.47
Hct %	Pretest	45.0	45.7	49.8*	45.7	49.6	46.2	47.0	47.1
	3	47.0	46.9	47.8	46.9	45.6	46.9	47.1	47.5
	6	49.6	47.7	49.6	47.6	48.2	46.0	46.8	46.1
	9	51.3	50.1	51.2	50.5	47.8	48.0	49.3	48.8
	12	49.2	47.0	50.3	48.6	47.6	48.0	45.4	43.4
APTT (Seconds)	Pretest	19.2	18.7	19.7	18.8	21.8	19.8	19.9	20.6
	3	16.4	16.7	15.1	15.7	18.5	16.9	16.6	17.2
	6	16.0	15.2	15.8	14.8	18.0	15.4	15.9	15.4
	9	16.3	15.1	15.6	14.6	16.9	16.5	15.3	15.0
	12	18.6	16.5	16.3	15.5*	18.8	16.7	15.7*	16.2*

PT	Pretest	8.7	8.7	8.8	9.1	9.0	8.6	8.9	8.9
(Seconds)	3	8.7	8.7	8.8	8.9	8.7	8.5	8.7	8.7
	6	8.9	9.1	9.1	9.4	9.4	8.8	9.2	9.0
	9	9.2	9.4	9.5	9.8	9.7	9.2	9.6	9.5
	12	9.2	9.4	9.3	9.8	9.6	9.2	9.8	9.6

* $p < 0.05$.

Clinical chemistry: There were no treatment-related alterations in clinical chemistry parameters. There were occasional differences from controls which were judged not to be related to treatment for various reasons (lack of consistency over time, comparable values within the same treatment group in the pre-treatment period as well as during the study, values within the historical control data, or marked alteration in one animal only which led to an alteration in the group mean, lack of dose-response relationship). LDH levels were decreased in the mid and high dose females at 12 months. However the pretest values were also low in these groups, suggesting that this was not treatment-related.

Selected clinical chemistry parameters in beagle dogs given AE 0317309 in the diet for one year are presented in the following table:

		Dose (ppm)							
Parameter	**Sampling time (months)**	**Males**				**Females**			
		0	**250**	**1000**	**3000**	**0**	**250**	**1000**	**3000**
Na	Pretest	149	150	151	150	153	151	151	152
mmol/L	3	151	154	154	153	153	156	156	156
	6	149	149	149	148	148	151	150	150
	9	153	153	152	149*	150	150	153*	154*
	12	148	149	148	148	149	148	150	148
K	Pretest	4.5	4.9	4.8	4.8	4.5	4.4	4.4	4.5
mmol/L	3	4.6	4.7	4.7	4.8	4.6	4.6	4.6	4.4
	6	4.7	4.6	4.5	4.7	4.4	4.4	4.5	4.5
	9	5.1	4.8	5.0	4.8	4.5	4.5	4.7	4.7
	12	4.5	4.7	4.5	4.8*	4.5	4.4	4.5	4.4
Cl	Pretest	113	113	114	112*	114	114	115	115
mmol/L	3	115	115	117	113	115	117	116	117
	6	113	115	116	114	116	118	116	118
	9	114	113	116*	113	116	117	116	116
	12	115	113	117*	113	114	118*	114	117
Urea nitrogen	Pretest	17	18	17	17	11	13	15*	12
mg/dL	3	18	18	16	17	16	16	17	14
	6	16	16	14	14	13	15	15	13
	9	18	17	17	15	14	16	16	15
	12	18	17	17	17	15	17	14	15
Glucose	Pretest	104	106	98	102	93	98	101	100
mg/dL	3	101	99	96	99	91	90	95	90
	6	95	101	99	98	100	95	99	92
	9	96	104	92	97	91	92	103*	99
	12	93	96	95	95	91	93	103*	95
Triglycerides	Pretest	32	34	41	56	42	40	47	38
mg/dL	3	39	59	56	79	60	53	60	72
	6	37	52	49	80	51	57	55	54
	9	43	62	55	66	58	76	56	78
	12	29	54	48	73	51	58	47	63

Cholesterol mg/dL	Pretest	124	129	143	163*	129	133	139	131
	3	127	146	145	149	177	174	172	144
	6	139	162	150	155	144	187	196	171
	9	136	156	155	157	167	203	173	159
	12	134	153	147	150	217	195	228	207
Creatine kinase Units/L	Pretest	169	254	275	335	155	182	151	137
	3	180	224	234	212	183	164	195	145
	6	169	181	147	162	142	146	124	110
	9	120	139	137	148	129	157	128	112
	12	129	140	141	151	125	134	124	78
LDH Units/L	Pretest	134	133	114	130	113	72	61*	59*
	3	142	99	134	96	63	69	125	65
	6	138	115	83	72	69	101	81	70
	9	87	102	80	79	80	132	79	60
	12	98	130	96	107	100	111	59	39
Total protein g/dL	Pretest	5.6	5.6	6.0*	5.8	5.6	5.5	5.6	5.5
	3	5.8	6.1	6.2*	6.0*	5.7	5.9	6.0	6.0
	6	5.8	6.3	6.3	6.1	5.5	5.9*	6.1	6.3*
	9	6.0	6.5	6.3	6.4	5.8	6.0	6.0	6.5*
	12	6.0	6.7	6.6	6.3	6.0	6.1	6.2	6.5
Calcium mg/dL	Pretest	10.0	9.9	10.2	10.2	10.1	10.3	10.2	10.2
	3	9.7	10.0	9.6	9.7	9.9	10.1	10.1	10.0
	6	9.6	9.7	9.6	9.8	9.5	9.8*	10.2*	9.8
	9	9.7	9.9	9.8	9.9	9.7	9.8	10.1	10.0
	12	9.7	9.6	9.6	9.5	9.5	9.5	10.1*	9.8*

* $p<0.05$

Urinalysis: There were no treatment-related alterations. There were occasional alterations which were judged to be unrelated to treatment for various reasons, including a lack of consistency over time, comparable values within the same treatment group in the pre-treatment period as well as during the study, coverage by historical control data, marked alteration in one animal only which led to an alteration in the group mean, or the lack of a dose-response relationship.

There were no indications of any treatment-related effects on levels of blood in the urine. However the units and method of measurement of this parameter were not given in the study report.

There are a number of discrepancies between the raw urinalysis data provided in the report and those tabulated in the assessment by the applicant. The latter figures are given in the following table.

Selected urinalysis parameters in treated dogs

Parameter	Sampling time (months)	AE 0317309, dietary concentration in ppm Males 0	250	1000	3000	Females 0	250	1000	3000
Protein Mg/dL	Pretest	53	23	75	40	23	36	25	10
	3	61	44	15	23	11	19	15	54
	6	25	58	4	11	11	0	0	8
	9	15	179*	11	44	54	36	15	23
	12	40	108	19	15	11	4	8	0
Ketones	Pretest	0	0	1	1	0	1	0	0

Mg/dL	3	1	1	0	1	1	5*	5*	10*
	6	0	0	0	1	1	0	1	4
	9	0	3	1	4	1	4	6	13*
	12	0	1	1	1	0	1	3	3
Leukocytes	Pretest	3	1	3	2	1	0	0	0
	3	1	1	0	1	0	0	0	0
	6	2	1	0*	0*	0	0	0	0
	9	2	0	1	1	0	0	0	0
	12	1	0	0	0	0	0	0	0
Urine volume (mL)	Pretest	114.5	185.5	140.5	163.8	41.3	117.0*	70.8	155.3*
	3	122.8	165.3	207.5	260.5	134.8	176.5	153.5	202.5
	6	272.3	172.6	318.8	276.8	86.8	229.0	117.8	181.5
	9	394.3	153.3	111.5	191.8	59.5	221.0	102.3	131.3
	12	127.5	195.8	201.3	223.3	82.0	185.0	97.3	162.8
Blood	Pretest	1.7	1.0	2.0	0.8	1.0	0.3	2.0	0.7
	3	1.5	0.8	2.5	1.3	0.5	0.8	0.0	0.8
	6	0.4	0.8	1.4	0.8	0.0	1.5	0.8	0.0
	9	0.3	0.0	0.8	0.5	0.8	0.0	0.5	0.0
	12	1.3	0.8	0.4	0.8	0.3	0.5	1.3	0.0

* $p < 0.05$.

Organ weights: There were indications of treatment-related changes in the liver, thyroid, and kidney at 3000 ppm, specifically increased absolute and relative liver weight in males and females (34-49%), increased absolute and relative kidney weight in males (26-37%), and increased absolute and relative thyroid weight in females (11- 43%). However, none of these changes achieved statistical significance. Increases in kidney weights in males, and absolute liver weights in females showed a slight dose-response association.

Terminal body weight, and the liver, kidney, and thyroid weights

Parameter	Dose (ppm)							
	Males				Females			
	0	250	1000	3000	0	250	1000	3000
Absolute organ weight, g								
Terminal body weight, g	9769.8	11258.5	10273.8	10734.8	8771.0	9085.0	9843.3	9658.8
Liver weight, g	259.00	340.85	321.17	381.48	242.40	281.60	299.65	362.05
Kidney weight, g	50.720	62.121	62.791	69.975	39.815	43.778	43.173	47.262
Thyroid weight, g	0.782	0.938	0.982	0.901	0.719	0.769	0.771	0.937
Relative organ weight, % body weight								
Terminal body weight, g	9769.8	11258.5	10273.8	10734.8	8771.0	9085.0	9843.3	9658.8
Relative liver weight	2.65	2.99	3.12	3.56	2.771	3.102	3.055	3.766
Relative kidney weight	0.526	0.547	0.610	0.665	0.454	0.483	0.438	0.492
Relative thyroid weight	0.0081	0.0085	0.0096	0.0084	0.0082	0.0084	0.0079	0.0097

* $p<0.05$

Macroscopic findings: There were no observations at gross necropsy which were considered to be related to administration of AE 0317309.

Histopathology: Treatment-related findings were seen in the liver at 3000 ppm, in which the incidence of hepatocytomegaly was increased (2 males, 3 females) compared to controls (0 incidence). These lesions were graded at 1 (where 1 is minimal and 5 is severe). Cataract was

observed in the eyes of one each of the four dogs in the male and female 3000 ppm groups. These lesions were graded at 2. In terms of the urinary system, tubular dilatation in the kidneys was observed in 1/4, 1/4, 3/4 and 4/4 males in the control, 250, 1000 and 3000 ppm, respectively. These abnormalities in the medium and high dose animals were graded 2, whereas those in the control and low dose animals were only graded as 1. One high dose male showed inflammation, hemorrhage and debris in the bladder. There were no lesions in the ureter associated with treatment. There were no noteworthy observations on the thyroid.

Conclusions and Comments:

Because of the observation of blood in the urine in a discontinued 90 day dog study (Eigenberg, D. A.: "Technical Grade AE 0317309: A 90-Day Subchronic Toxicity Feeding Study in the Beagle Dog", Report Number 201019, March 26, 2004), the discoloration in the urine in the present study reported as "brownish yellow" or "reddish brown" is of interest. However, this observation was made in the control animals as well as the treated dogs. Also, no excess blood was detected in treated animals on the occasions when urinalysis was performed. The study authors suggested that the urine discoloration occasionally observed as a clinical sign in the present study was due simply to excretion of the parent compound itself; although no study appears to have been done to confirm this. The dose selection in the present study covers a dose at which blood was observed in the urine in the 90 day dog study referenced above. The high dose in this study (approximately 100 mg/kg bw/d) was rather low. Also, there was apparently no indication of the age of the historical data supplied.

In this one-year study on dogs, administration of the test material was associated with increased liver, kidney and thyroid weights, particularly at the high dose. The increases did not reach statistically significance, although there were some indications of dose-response relationships. These increases were also associated with histopathological effects in the liver and kidney. The incidence and severity of tubular dilatation in the kidney was apparently dose-related in males, being observed at and above 1000 ppm. This effect could be correlated with the apparent dose-response effect on the kidney weight in males.

The study authors proposed a NOAEL for this study of 1000 ppm (34 mg/kg bw/d for males and 33 mg/kg bw/d for females) based on increased organ weights and liver histopathology at the high dose. However, given the increased incidence and severity of tubular dilation in the kidneys of males at 1000 ppm and above, a NOAEL (NOEL for Australia) of 250 ppm is established (7 mg/kg bw/d).

EPA Reviewer: Guruva B. Reddy, D.V.M., Ph.D. **Signature:** [illegible] for
Registration Action Branch 1, Health Effects Division (7509P) **Date:** 6/5/08
EPA WAM: PV Shah, PhD **Signature:** P.V. Shah
Registration Action Branch 1, Health Effects Division (7509P) **Date:** 6/5/08

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DATA EVALUATION RECORD

STUDY TYPE: Carcinogenicity study in mice [dietary]; OPPTS 870.4200b [§83-2b]; OECD 451.

PC CODE: 000692 **DP BARCODE:** D335563

TEST MATERIAL (PURITY): AE 0317309 (95.7% w/w a.i.)

SYNONYMS: 5-Hydroxy-1,3-dimethylpyrazol-4-yl 2-methylsulfonyl)-4-(trifluoromethyl)phenyl ketone

CITATION: 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.

SPONSOR: Bayer AG, Bayer CropScience, Alfred Nobel Str. 50, Monheim, Germany

EXECUTIVE SUMMARY: 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.

There was no effect of treatment on food consumption. Liver and urinary system was identified as target system. Treatment-related effects were observed on mortality, clinical signs, body weight body weight gains, hematology, liver and kidney weights, urolithiasis, and gall stones in males and females. 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 observation in all treated groups at scheduled sacrifice, although there was no dose-response relationship.

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 treatment-related findings were observed in the urinary system (kidney, urinary bladder, and ureters) at the high dose in males and females and were associated with stones and concretions observed in the urinary system at the same dose.

The LOAEL is 100 ppm (equivalent to 13.6/16.7 mg/kg/day [M/F]), based on an increased incidence of gallstones. The NOAEL was not established.

Treatment-related neoplastic findings included urinary bladder transitional cell carcinomas (including urethral transitional cell carcinoma) and papillomas and carcinomas combined in males and urinary bladder transitional cell papillomas, carcinomas, and papillomas and carcinomas combined in females at 4000 ppm. Dosing was considered excessive in both sexes because of significantly increased mortality in males (53% vs 16% in controls) and females (62% vs 30% in controls), much of which occurred in the first year of the study. The increased mortality was due to the presence of urinary bladder stones.

This study is classified as **acceptable/guideline** and satisfies the guideline requirements (OPPTS 870.4200b; OECD 451) for a carcinogenicity study in mice.

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

This Executive Summary was prepared for United States Environmental Protection Agency, Office of Pesticides Programs, Health Effects Division use.

The following text was generated by the Australian Pesticides and Veterinary Medicines Authority. However, this document has undergone critical scientific analysis and been modified as appropriate.

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 0	100	1000	4000	Females 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 0	100	1000	4000	Females 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

		Dose (ppm)							
Parameter	**Month**	**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 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**	4.73**	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
	Cyst(s)	0	0	0	16	0	0	1	7
	Stone(s)	0	0	0	7	0	0	0	13
Kidney	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	Stone(s)	0	0	0	24	0	0	0	11
bladder	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	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:	0	0	1	10**	0	0	1	11**

unilateral: focal / multifocal								
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.
* $p < 0.05$; ** $p < 0.01$.

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 observed, 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.

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. However, the study is acceptable for regulatory purposes, since survival was above 25%, which meets the US OPPTS Series 870.4200.

EPA Reviewer: Robert J. Mitkus, PhD **Signature:** [signature]
Registration Action Branch 1, Health Effects Division (7509P) **Date:** 3/25/09
EPA WAM: PV Shah, PhD **Signature:** [signature]
Registration Action Branch 1, Health Effects Division (7509P) **Date:** 3/25/09

TXR#: 0054227

DATA EVALUATION RECORD

STUDY TYPE: A 14-day comparative toxicity feeding study in male beagle dogs with technical grade AE 0317309; Non-guideline

PC CODE: 000692 **DP BARCODE:** D335563

TEST MATERIAL (PURITY): AE 0317309 (96% w/w a.i.)

SYNONYMS: Pyrasulfotole Technical; 5-Hydroxy-1,3-dimethylpyrazol-4-yl 2-methylsulfonyl)-4-(trifluoromethyl)phenyl ketone

CITATION: Eigenberg DA. (2006). A 14-day comparative toxicity feeding study in male beagle dogs with technical grade AE 0317309. Bayer CropScience LP, Stilwell, Kansas. Laboratory Study No. 04-S16-UV, February 13, 2006. MRID 46801903. Unpublished.

SPONSOR: Bayer CropScience LP, Research Triangle Park, NC

EXECUTIVE SUMMARY:

In a comparative feeding study (MRID 46801903), 5 male Beagle dogs/group were administered 18000 ppm AE 0317309 (batch Op. 1-4) mixed with either dry feed (300g Purina Certified Canine Diet Etts 5006-3) or wet feed (300g Purina Certified Canine Diet 5007 meal + 450 mL tap water) for 1.5 hrs/day for 16 days. Untreated control groups were not included in the study. The study was designed to determine if administration of treated feed with water affects the blood and urinary concentrations of AE 0317309 relative to the blood and urinary concentrations of AE0317309 following administration of treated dry feed. Test substance intake was 318 mg/kg bw/day on days 1-7 and 479 mg/kg bw/day on days 8-16 in the dry feed group and 272 mg/kg bw/day on days 1-7 and 388 mg/kg bw/day on days 8-16 in the wet feed group. Animals were examined for clinical signs of toxicity twice daily. Body weights were measured on study days 0, 7, and 16, and food consumption was measured on a daily basis. Blood urea nitrogen and creatinine, as well as standard urinalysis parameters, were measured pre-test, and on study days 6 and 14. Urine was also collected at 2, 4, 6, 8, 12, and 24 h after the last feeding on day 15 for measurement of urinary AE 0317309 concentration. Blood was collected at 2, 4, and 6 h after the last feeding on day 15 for measurement of plasma AE 0317309 concentration. On study day 16, the animals were sacrificed and subjected to a gross necropsy of the urinary tract with kidney weight measurement.

No mortalities were observed during the study, and there was no difference in food consumption or kidney weight between the wet-feed or the dry-feed group. Treatment-related clinical signs

were limited to red urine in 2/5 dogs of the dry-feed group on or after day 7. A tan substance was noted in the feces of all wet-feed dogs. Mean body weight, relative to day 0, was decreased by 4% (day 7) and 5% (day 16) in the dry-feed group and by 6% (day 7) and 9% (day 16) in the wet-feed group. On days 6 and 14, blood urea nitrogen (BUN) levels were increased 67-87% over pre-test values (15 mg/dl) in the dry-feed group and were more than double pre-test values (11 mg/dl) in the wet-feed group. Blood was observed in the urine in both dietary groups, albeit to a greater extent in the dry-feed group. Urinary calculi were observed in 2/5 dogs in the dry-feed group, and the urinary bladders of these animals showed moderate thickening. These findings were not observed in any of the dogs in the wet-feed group. Plasma concentrations of test substance in the dry-feed group were increased by 52% and 64% over those in the wet-feed group at 2 and 4 hrs. post feeding, but were similar by 6 hrs. post feeding. Urine concentrations of test substance in the dry-feed group were more than double those in the wet-feed group at 12 hrs. post feeding, but were similar by 24 hrs. post dosing. The results of the study are inconclusive, since test substance intake was 20% higher in dogs receiving dry feed than in those receiving wet feed.

The study is classified as **Acceptable/Non-guideline** and is acceptable for assessing the general differences in toxicological effect between dry feed and wet feed treated with AE 0317309 in dogs.

COMPLIANCE: Signed and dated GLP and Data Confidentiality statements were provided. A Quality Assurance statement was not provided.

This Executive Summary was prepared for United States Environmental Protection Agency, Office of Pesticides Programs, Health Effects Division use.

The following text was generated by the Australian Pesticides and Veterinary Medicines Authority. However, this document has undergone critical scientific analysis and been modified as appropriate.

Report:	**KIIA 5.10/04, Eigenberg, D.A.; 2006**
Title:	A 14-day comparative toxicity feeding study in male beagle dogs with technical grade AE 0317309
Laboratory	Bayer CropScience LP, 2 T.W. Alexander Drive, Research Triangle Park, NC 27709, USA
Study duration	27 February 2004 – 18 March 2004
Report No.:	04-S16-UV
Document No.:	M-267237-01-1
Guidelines:	**None**
GLP/QA	**No**

Introduction

This study was conducted to determine whether the type of dietary regimen provided to dogs influenced either the absorption of AE 0317309 or its excretion in the urine. In a previous 28-day study, dogs were fed a mixture of 300 g powdered diet, AE 0317309, and 450 mL water mixed into a slurry. In that study, administration of AE 0317309 in wetted diet at 5000, 13000 or 26000 ppm resulted in the formation of urinary calculi, while no calculi were observed in animals fed the diet containing AE 0317309 at 5000 ppm. In a second dietary study planned for 90 days, administration of 1500, 9000, and 18000 ppm AE 0317309 in dry feed resulted in moribundity and calculus formation at 9000 and 18000 ppm in males, as well as blood in the urine in males of all doses. The study was terminated after 29 days. The finding of blood in the urine at 1500 ppm in males fed AE 0317309 in dry diet, which was below the NOAEL of the 28-day study with wetted diet (5000 ppm), suggested that the amount of water in the diet might be a factor in the difference in toxicity observed between the two studies. Comparison of the effects of the two diets was conducted by examination of relevant clinical chemistry and urinalysis parameters in a further 16-day feeding trial, reported here, along with measurement of AE 0317309 concentration in plasma and urine from the wet-diet and dry-diet groups.

Materials and Methods

AE 0317309 (batch Op. 1-4) was administered at 18000 ppm in the diet to two groups of 5 male beagle dogs. One group received 300 g of a diet including AE 0317309 and 450 mL tap water ("wet feed"), while the other group received 300 g of a diet including the AE 0317309 without added water ("dry feed"). The diet was made available to the animals for 1.5 h each day, and leftover food was removed at the end of that time and weighed. The intake of test substance was somewhat higher in the dry feed group, being 318 mg/kg bw/day on days 1-7 and 479 mg/kg bw/day on days 8-16, compared with the wet feed group with 272 mg/kg bw/day on days 1-7 and 388 mg/kg bw/day on days 8-16. Animals were housed individually in stainless steel runs, and water was provided *ad libitum* to all animals. Clinical signs and moribundity were monitored daily. Body weights were measured on study days 0, 7, and 16, and food consumption was measured on a daily basis. Blood and urine were collected pre-test, and on study days 6 and 14 and measurements of BUN and creatinine, and standard urinalysis parameters, were

conducted at these time points. Urine was also collected on day 15-16 for a 24-h period following the last administration of treated diet, at 2, 4, 6, 8, 12, and 24 h after the last feeding. These samples were used for measurement of urinary AE 0317309 concentration. Blood was collected at 2, 4, and 6 h after the last feeding for measurement of plasma AE 0317309 concentration. On study day 16, the animals were sacrificed by intravenous injection of a standard euthanasia solution and subjected to a gross necropsy of the urinary tract with kidney weight measurement.

Findings

Mortalities: There were no mortalities during the study.

Clinical signs: Treatment-related clinical signs were limited to red urine in two dogs of the dry-feed group, in one dog on study day 7 and in another dog on study days 7, 8, 10, and 12, and a tan substance was noted in the feces of all wet feed dogs.

Body weight: Body weight was significantly reduced, relative to day 0, by 4% (day 7) and 5% (day 16) in the dry-feed group and by 6% (day 7) and 9% (day 16) in the wet-feed group.

Food consumption: There was no treatment-related effect on food consumption in either the wet-feed or the dry-feed group.

Clinical chemistry and urinalysis: There was a treatment-related increase in BUN and blood in the urine in both dietary groups. Urinary ketones were also increased in both groups, but the study authors related this to urinary excretion of AE 0317309 itself (a ketone-bearing molecule) and therefore, this finding was not considered to be related to toxicity of the substance. (The study authors do not indicate the nature of the units of blood in the urine, although it is likely that a semi-quantitative assessment was used.)

Treatment-related clinical chemistry and urinalysis findings in dogs administered AE 0317309 either with (wet feed) or without (dry feed) added water in the diet

Parameter		Treatment regimen	
	Day	Dry feed	Wet feed
Blood urea nitrogen, mg/dL	-1	15	11
	6	25	24
	14	28	26
Blood in the urine	-1	0.4	0.5
	6	2.4	0.8
	14	2.3	1.8

Kidney weights: There was no effect on kidney weight in either group, relative to the other.

Necropsy findings: Two dogs in the dry-feed group showed calculi in several locations in the urinary tract, and the urinary bladders of these animals showed moderate thickening. There were no findings in the other dogs in this group, and no findings in any of the dogs

in the wet-feed group.

Plasma concentrations of AE 0317309: Dogs in the wet-feed group had slightly higher mean plasma concentrations of AE 0317309 at 2 (111 vs. 73 µg/ml) and 4 h (100 vs. 61 µg/ml) after the last feeding than did the dogs in the dry group. However, by 6 h after dosing, mean plasma concentrations were similar (48 vs. 56 µg/ml).

Urine concentrations of AE 0317309: Urine concentrations of the test substance were higher in the dry-feed group at nearly all urine collection time points, as shown in the following table. There was no significant difference in urine volume between the treatment groups.

Concentrations of AE 0317309, in mg/mL, in urine from dogs administered AE 0317309 either with (wet feed) or without (dry feed) added water in the diet.

Time after feeding, hours	Treatment regime	
	Dry feed	Wet feed
2	6.2	6.7
4	6.3	5.1
6	5.8	4.0
8	5.8	4.0
10	5.4	2.9
12	4.4	1.7
24	0.30	0.31
Mean urine volume (mL)	296.4	284.4

Conclusions and comment

The results of this study indicated that the addition of water to the diet slightly increased plasma concentrations of AE 0317309 but apparently reduced the rate of excretion of the test substance in the urine. Although the study authors were inclined to attribute the lower urinary concentrations of the test substance in the wet feed group to the water consumed with the diet, there was no evidence of any increase in urine volume. It is possible that, in the case of the wet feed group, more of the test substance was excreted in the feces. The frequent clinical observation of a "tan substance" in their feces may reflect this, although no relevant data appear to have been collected.

In the dry feed group, urine AE 0317309 concentrations remained elevated for longer, although both groups showed similar urinary concentrations at 24 h after dosing. Additionally, urinary calculi were observed in two animals of the dry-feed group, but not in any of the wet-feed group. As these calculi have been previously demonstrated to be primarily AE 0317309, addition of water to the diet may have reduced formation of calculi in the urinary tract. Increased levels of the test substance in the urine of the dry feed group may have contributed to this difference, as may have increased intake of test compound, which was 20% greater in the dry-feed group relative to the wet-feed group. On the other hand, it should be noted that blood in the urine was observed in the wet feed animals as well as the dry feed group, albeit to a greater degree in the dry-feed group.

The study authors believed that this study demonstrates the reason for the difference between the 28-day study conducted with wet feed (Report no. 5.3.1/02) and the first 90 (29)-day study conducted with dry feed (Report no. 5.3.1/03), which was discontinued on day 28 due to marked toxicity and urinary tract effects at doses lower than those at which such effects had been observed with wet feed. This study does indeed provide some evidence that feed type may be relevant to this question, and it is possible that the higher concentrations of the test substance in the urine of the dry feed group in the present study is associated with the greater evidence of urinary tract stones and blood in the urine. However blood in the urine was also observed in the group given wet feed. Also, the relative toxicokinetics of the test substance in dogs given dry feed and wet feed do not appear to have been fully characterized. For example, the higher concentration of test substance in the dry feed group is not associated with a lower urine volume. Since water was provided *ad libitum*, independent of any water provided with the feed, there is no reason to expect the dogs on dry feed to have been any less well hydrated overall than dogs provided wet feed. A possibility that does not seem to have been addressed is that test substance in wet feed is more readily excreted in the feces.

In the chronic dietary study in dogs (Report no. KIIA 5.3.4/01), the test substance was apparently provided in dry feed, with water available *ad libitum*. This presumably equates to the treatment of the dry feed group in the present study and in the discontinued study (Report no. 5.3.1/03). However, whereas dogs in the discontinued study showed signs such as blood in the urine at 1500 ppm, no such observations were seen at comparable dietary dose levels (1000 and 3000 ppm) in dogs in the chronic study. The difference in these studies cannot be attributed to the mode of presentation of the feed (since it was dry in both cases) and the explanation for the difference remains elusive. Also, in the case of the second 90-day study (Report no. KIIA 5.3.3/01), there were no effects such as blood in the urine observed, despite the test substance being provided in dry feed. However, this may have been due to the low doses given (ie. 1000 ppm being the high dose).

EPA Reviewer: Robert J. Mitkus, PhD **Signature:** [signature]
Registration Action Branch 1, Health Effects Division (7509P) **Date:** 6/5/08
EPA WAM: PV Shah, PhD **Signature:** [signature]
Registration Action Branch 1, Health Effects Division (7509P) **Date:** 6/5/08

TXR#: 0054227

DATA EVALUATION RECORD

STUDY TYPE: Acute Neurotoxicity - Rats OPPTS 870.6200a [§81-8]; OECD 424.

PC CODE: 000692 **DP BARCODE:** D335563

TEST MATERIAL (PURITY): AE 0317309 (95.7% w/w a.i.)

SYNONYMS: Pyrasulfotole technical

CITATION: Gilmore RG, Sheets LP. (2005). An acute oral neurotoxicity screening study with technical grade AE 0317309 in Wistar rats. Bayer CropScience LP, Stilwell, KS. Laboratory Study No.: 04-N12-WL, December 16, 2005. MRID 46801915. Unpublished.

SPONSOR: Bayer CropScience LP, Research Triangle Park, NC

EXECUTIVE SUMMARY:

In an acute neurotoxicity study (MRID 46801915), groups of 12 unfasted, 9-week-old Wistar rats/sex/dose were given a single oral dose by gavage of AE 0317309 (95.7% a.i., batch Op. 1-4) in aqueous 0.5% methylcellulose/0.4% Tween 80 at doses of 0, 200, 500, or 2000 mg/kg bw and observed for 14 days. Cage-side observations were performed at least once daily (once daily on holidays and weekends) for mortality or clinical signs of moribundity. Detailed physical examinations for clinical signs of toxicity were carried out and recorded daily. Animals were weighed weekly as a component of the FOB (functional observational battery) and at sacrifice. Neurobehavioral assessment (FOB and motor activity testing) was performed in all animals (fasted) one week prior to dosing, approximately 30 minutes after dosing on day 0, and on days 7 and 14 after dosing. The 30-min evaluation time was selected based on a preliminary disposition study wherein blood concentrations peaked at approximately 30 minutes post dosing. The FOB included standard parameters including home cage and open field observations, reflex testing, and determination of fore-limb and hind-limb grip strength, and landing foot splay. Motor and locomotor activity were measured over a 60-minute period for each animal. At study termination, all animals were euthanized and 6 rats/sex/dose were perfused *in situ* for neuropathological examination. Of the perfused animals, all were subjected to histopathological evaluation of brain and peripheral nervous system tissues.

There were no treatment-related effects on mortality, body weight, brain weight, gross pathology, or microscopic neuropathology. The FOB revealed no toxicologically significant effects. During clinical observations, an increased incidence of urine staining was observed in all treated male and female groups on the day of treatment, as well as across several days post-

treatment (Appendix 1). Similar staining was reported in high-dose animals only during the FOB. A dose-dependent increase in red nasal stain was observed at ≥500 mg/kg in males and at ≥200 mg/kg in females. The incidence of oral stain or red stained forepaws was also increased in males or females at ≥200 mg/kg. All stainings were considered to be due to excretion of test compound and therefore not toxicologically significant. Mean locomotor activity was significantly decreased (P<0.01) in all treated females on the day of dosing. When compared to both control and pre-test levels, the decrease was dose-dependent. Although not statistically significant, dose-dependent decreasing trends were observed in motor activity (days 0 and 7) and locomotor activity (day 7) in females. The decreases in overall locomotor activity were corroborated by statistically significant decreases in locomotor activity in treated females at several subsessions across days of testing. A 34% (NSS) decrease in mean overall locomotor activity was observed in males at 2000 mg/kg on day 0. The decrease was corroborated by decreased interval locomotor activity in males at 2000 mg/kg on day 0, which reached statistical significance during interval 3.

The LOAEL was 200 mg/kg bw in females and 2000 mg/kg bw in males based on decreased locomotor activity on day 0. The NOAEL was not observed in females and was 500 mg/kg bw in males.

This neurotoxicity study is classified as **acceptable (guideline)** and satisfies the guideline requirement for an acute neurotoxicity study in rats (870.6200; OECD 424).

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

Appendix 1. Summary of incidences of clinical observations[1]

MALES*

Signs	Nominal Dose Level (Mg/Kg)			
	0	200	500	2000
URINE STAIN				
YELLOW	-[b]	8(0-4)	7(0-4)	12(0-10)
BROWNISH, ORANGE	-	-	1(1-6)	9(1-14)
RED NASAL STAIN	-	-	2(0-3)	10(0-4)
ORAL STAIN	-	1(0)	1(0-1)	9(0-4)
RED STAINED FORE PAWS, BOTH	-	-	-	6(0-4)

FEMALES*

URINE STAIN				
YELLOW	-	7(0-14)	12(0-7)	12(0-7)
BROWNISH, ORANGE	-	-	2(2-7)	8(2-14)
RED NASAL STAIN	-	1(1-2)	3(0-2)	8(0-4)
ORAL STAIN	-	-	4(0-1)	9(0-4)
RED STAINED FORE PAWS, BOTH	-	-	1(0-1)	6(0-3)

** Incidence for n=12 (range of days observed - observations performed once daily).*
** Observation not observed.*

This Executive Summary was prepared for United States Environmental Protection Agency, Office of Pesticides Programs, Health Effects Division use.

The following text was generated by the Australian Pesticides and Veterinary Medicines Authority. However, this document has undergone critical scientific analysis and been modified as appropriate.

[1] Reproduced from p. 44 of MRID 46801915

Report:	**KIIA 5.7.1/01, Gilmore, R. G.; Sheets, L. P.; 2005**
Title:	An acute oral neurotoxicity screening study with technical grade AE 0317309 in Wistar rats
Laboratory	**Bayer CropScience LP Toxicology, 17745 South Metcalf Ave, Stilwell, KS 66085-9104, USA**
Study duration	13 September 2004 – 15 October 2004
Report No.:	04-N12-WL
Document No.:	M-263029-01-1
Guidelines:	**US-EPA OPPTS 870.6200; OECD Guideline 424; Health Canada, PMRA DACO No. 4.5.12**
GLP/QA	**Yes**

Materials and Methods

AE 0317309 (batch Op. 1-4, purity 95.7%) was suspended in an aqueous solution of 0.5% methylcellulose/0.4% Tween 80 and administered by oral gavage to Wistar rats (12/sex/group) at doses of 0, 200, 500, and 2000 mg/kg bw. The homogeneity, stability and concentration of the test substance in the solution were confirmed. The rats were at least 9 weeks of age at the time of dosing, and body weights were 225-307 g for males and 159-201 g for females. The animals were housed singly in suspended stainless steel wire-mesh cages and provided rodent lab chow and water *ad libitum*. Food and water were not provided during neurobehavioral testing.

Cage-side observations were performed at least once daily (once daily on holidays and weekends) for mortality or clinical signs of moribundity. Detailed physical examinations for clinical signs of toxicity were carried out and recorded daily. Animals were weighed weekly as a component of the FOB (functional observational battery). Additionally, all animals were weighed on the day of sacrifice for terminal body weight measurement.

It was stated that in a preliminary ADME study (not supplied by the applicants), the time after oral gavage dosing of AE 0317309 (pyrasulfotole) to the peak blood concentration of the test substance was found to be 30 minutes in both males and females. Thus, conduct of the FOB and motor activity studies on day 0 began approximately but no less than 30 minutes after dosing, and was concluded at approximately 2.5 hours after treatment. In total, the FOB and motor activity assessments were conducted one week prior to dosing, approximately 30 minutes after dosing on day 0, and on days 7 and 14 after dosing. The FOB included standard parameters including home cage and open field observations, reflex testing, and determination of fore-limb and hind-limb grip strength, and landing foot splay. Motor and locomotor activity were measured according to standard methods over a 60-minute period for each animal. On day 14 after dosing, all animals were sacrificed. Half of the animals at each dose level were anesthetized and perfused via the left ventricle with a sodium nitrite flush followed by fixative in phosphate buffer. The brain was weighed, and the brain, spinal cord, eyes with optic nerve, sciatic, tibial, and sural nerves, gasserian ganglion, gastrocnemius muscle, both forelimbs, and any gross lesions were removed and preserved for examination. The other half of the animals at each dose level were sacrificed by CO_2 asphyxiation and were not

perfused. All animals were subjected to a gross necropsy. Histopathology was conducted on the nervous tissues from the 6 perfused animals in the control and high dose groups only.

Findings

Mortalities: There were no mortalities at any dose level in this study.

Clinical signs: The treatment-related clinical signs during Days 0-14 were limited to urine stains, nasal and oral stains, and stained forepaws. These stains were seen in all treatment groups, with a general dose-related incidence. The staining had reversed in almost all animals by the time of sacrifice.

Incidence of stained fur in rats administered AE 0317309

Observation	Dose (mg/kg bw)			
	0	200	500	2000
Males				
N	12	12	12	12
Urine stain, yellow	0	8	7	12
Urine stain, brownish / orange	0	0	1	9
Red nasal stain	0	0	2	10
Oral stain	0	1	1	9
Red-stained forepaws, both	0	0	0	6
Females				
N	12	12	12	12
Urine stain, yellow	0	7	12	12
Urine stain, brownish / orange	0	0	2	8
Red nasal stain	0	1	3	8
Oral stain	0	0	4	9
Red-stained forepaws	0	0	1	6

Body weight: There were no effects on body weight in either males or females at any dose level.

FOB: There were no effects on any parameters of the functional observation battery, including grip strength and landing foot splay, in any dose group in either males or females.

Motor and locomotor activity: Motor and locomotor activity were decreased in females at all dose levels on days 0, 7 and 14, reaching statistical significance in all treated groups for locomotor activity on day 0. The decreases in overall locomotor activity were corroborated by statistically significant decreases in locomotor activity in treated females at several subsessions and days of testing. A 34% (non-significant) decrease in mean overall locomotor activity was observed in males at 2000 mg/kg on day 0. The decrease was corroborated by decreased interval locomotor activity in males at 2000 mg/kg on day 0, which reached statistical significance during interval 3.

Motor and locomotor activity (total activity counts for session) and percent difference from control

Day	Dose (mg/kg bw)							
	Males				Females			
	0	200	500	2000	0	200	500	2000
Motor activity, total activity counts per session								
Pre-test	424 ± 195	531 ± 180	383 ± 129	529 ± 246	631 ± 207	538 ± 239	697 ± 165	617 ± 226
0	411 ± 290	466 ± 87	402 ± 139	307 ± 113	488 ± 161	357 ± 109	421 ± 99	306 ± 85
7	434 ± 131	436 ± 89	416 ± 138	448 ± 118	538 ± 176	393 ± 135	405 ± 136	414 ± 119
14	413 ± 158	395 ± 117	350 ± 98	445 ± 111	527 ± 165	493 ± 92	486 ± 143	463 ± 74
Locomotor activity, total activity counts per session								
Pre-test	219 ± 101	291 ± 100	216 ± 83	289 ± 123	265 ± 69	230 ± 107	303 ± 56	290 ± 95
0	232 ± 148	282 ± 49	218 ± 69	153 ± 49	323 ± 129	218* ± 81	229* ± 64	161* ± 53
7	293 ± 112	299 ± 83	274 ± 97	294 ± 92	343 ± 131	239 ± 95	245 ± 106	261 ± 82
14	269 ± 116	264 ± 88	244 ± 84	307 ± 102	335 ± 102	265 ± 70	301 ± 94	299 ± 83
Motor activity, % change relative to control								
Pre-test	---	+ 25	- 10	+ 25	---	- 15	+ 10	- 2
0	---	+ 13	- 2	- 25	---	- 27	- 14	- 37
7	---	+ 0.5	- 4	+ 3	---	- 27	- 25	- 23
14	---	- 4	- 15	+ 8	---	- 17	- 8	- 12
Locomotor activity, % change relative to control								
Pre-test	---	+ 33	- 1	+ 32	---	- 13	+ 14	+ 9
0	---	+ 22	- 6	- 34	---	- 33*	- 29*	- 50*
7	---	+ 2	- 6	+ 0.3	---	- 30	- 29	- 24
14	---	- 2	- 9	+ 14	---	- 21	- 10	- 11

* $p < 0.05$. ** $p < 0.01$.

Organ weights: There was no effect on brain weight (the only organ weight measured) at any dose.

Macroscopic findings: There were no treatment-related lesions observed at any dose in either males or females.

Microscopic findings: There were no treatment-related effects observed at 2000 mg/kg bw when compared to controls, thus histopathological sections were not made at either 200 or 500 mg/kg bw.

Conclusions and Critique

The study authors noted that oral gavage administration of AE 0317309 produced no clinical signs indicative of neurotoxicity. It was considered that motor activity and locomotor activity were decreased in both males and females at 2000 mg/kg bw, but that there was no other effect on any neurotoxicity-related parameter, and therefore no indication of neurotoxicity at any dose level. The NOAEL of the acute neurotoxicity study was therefore proposed by the study authors to be 500 mg/kg bw.

It would have been desirable to be able to examine the study data referred to which reportedly provided evidence that the time after oral gavage dosing of the test compound to the peak blood concentration of the test substance was 30 minutes in both males and females.

Although the oral gavage administration of the test material produced no clinical signs

indicative of neurotoxicity, there was a generally dose-related incidence of stained fur seen in both sexes. This was attributed by the study authors to the excretion of AE 0317309 in the urine leading to intense yellow/orange coloration of the urine and the spread of this discoloration by way of grooming activity (At the same time, the red nasal stain at the higher doses was suggested by the study authors to be within the historical range; however, historical control data were not provided.) While it is possible that the stained fur was due to the excretion of colored test substance in the urine and its being spread by grooming to the nose and forepaws, this was not confirmed by any analysis of the urine.

The observations on motor and locomotor behavior in females were not associated with any other indications of neurological abnormality or any histopathological observations. However there was evidence of an ongoing diminution in such activity in all treated females, which was dose-dependent when compared to both concurrent controls and pre-test levels. Because of the uncharacterised staining of the fur in all dose groups in both sexes and the effects on activity in females in all dose groups, it is arguable that no NOAEL (NOEL for Australia) can be established for systemic toxicity. The LOAEL was 200 mg/kg bw in females and 2000 mg/kg bw in males based on decreased locomotor activity on day 0. The NOAEL was not observed in females and was 500 mg/kg bw in males.

EPA Reviewer: Robert J. Mitkus, PhD **Signature:** [signature]
Registration Action Branch 1, Health Effects Division (7509P) **Date:** 6/5/08
EPA WAM: PV Shah, PhD **Signature:** [signature]
Registration Action Branch 1, Health Effects Division (7509P) **Date:** 6/5/08

TXR#: 0054227

DATA EVALUATION RECORD

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

PC CODE: 000692 **DP BARCODE:** D335563

TEST MATERIAL (PURITY): AE 0317309 (95.7% w/w a.i.)

SYNONYMS: Pyrasulfotole technical

CITATION: 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.

SPONSOR: Bayer AG, Monheim, Germany

EXECUTIVE SUMMARY:

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 and females 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 and females at ≥250 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 included peripheral retinal atrophy, corneal inflammation, corneal hyperplasia, and/or corneal neovascularization at ≥250 ppm in both sexes and an increased incidence of chronic progressive nephropathy in males at ≥250 ppm. Histopathology observed in several other organs at higher doses is summarized in the Australian review (attached). The only treatment-related neoplastic finding was that of rare squamous cell tumors of the cornea (one papilloma, one carcinoma) observed in two separate males at 2500 ppm.

The LOAEL was 250 ppm (10/14 mg/kg [M/F]) based on corneal opacity, neovascularization of the cornea, inflammation of the cornea, regenerative corneal hyperplasia, corneal atrophy, and/or retinal atrophy (both sexes), and hepatocellular hypertrophy along with increased serum cholesterol (males). The NOAEL was 25 ppm (1.0/1.4 mg/kg [M/F]).

A treatment-related increase in the incidence of corneal squamous cell tumors was observed in males of the high dose only. Dosing was considered adequate, but not excessive, based on decreased body weight/body weight gain and non-neoplastic lesions of the eyes, liver, pancreas, thyroid and kidney at the high dose.

This chronic toxicity/carcinogenicity study in the rat is classified as **Acceptable (guideline)** and satisfies the guideline requirement for a chronic toxicity/carcinogenicity study (OPPTS 870.4300; OECD 453) in rat. Although motor activity, grip strength, and reactivity to sensory stimuli were not assessed in the chronic phase of the study as required by the guideline, they were assessed in other, short-term studies.

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

This Executive Summary was prepared for United States Environmental Protection Agency, Office of Pesticides Programs, Health Effects Division use.

The following text was generated by the Australian Pesticides and Veterinary Medicines Authority. However, this document has undergone critical scientific analysis and been modified as appropriate.

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.

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.

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**

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	% 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); however, the observations were not corroborated by histopathology. 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 non-statistically significantly 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	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

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,	0	0	0	1	1	0	0	0	0	7

acinar, diffuse										
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, 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

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, d ietary 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.

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).

EPA Reviewer: Robert J. Mitkus, PhD **Signature:** Robert J. Mitkus
Registration Action Branch 1, Health Effects Division (7509P) **Date:** 6/5/08
EPA WAM: PV Shah, PhD **Signature:** P.V. Shah
Registration Action Branch 1, Health Effects Division (7509P) **Date:** 6/5/08

TXR#: 0054227

DATA EVALUATION RECORD

STUDY TYPE: Developmental Neurotoxicity Study - Rat; OPPTS 870.6300 (§83-6); OECD 426 (draft)

PC CODE: 000692 **DP BARCODE:** D335563

TEST MATERIAL (PURITY): AE 0317309 (95.7% w/w a.i.)

SYNONYMS: Pyrasulfotole technical

CITATION: Gilmore RG, Sheets LP, Hoss HE. (2006). A developmental neurotoxicity screening study with technical grade AE0317309 in Wistar rats. Bayer CropScience LP, Stilwell, KS. Laboratory Study No.: 04-D72-YE, February 2, 2006. MRID 46801917. Unpublished.

SPONSOR: Bayer CropScience LP, Research Triangle Park, NC

EXECUTIVE SUMMARY:

In a developmental neurotoxicity study (MRID 46801917), AE 0317309 (95.7% w/w a.i., batch Op. 1-4) was administered from gestation day 6 through postnatal day 21 to 30 female Wistar rats per dose in the diet at nominal concentrations of 0, 45, 450, or 4500 ppm during gestation and 0/0/0, 24/20/16, 237/196/161, or 2368/1957/1607 ppm during weeks 1/2/3 of lactation. These concentrations provided an average daily intake of 0, 3.8, 37, or 354 mg/kg bw/day over gestation and lactation. Offspring were not dosed directly in this study. Dams were observed for clinical signs at least once daily. FOB (functional observational battery) tests were conducted on GDs 13 and 20 and LDs 11 and 21. Body weight and food consumption were measured on GDs 6, 13, and 20 and on LDs 0, 7, 14, and 21. On LD 4 litters were culled to eight pups, with four male and four female pups wherever possible. Dams were sacrificed on LD 21 following weaning of their litters, and a gross necropsy examination was conducted. Offspring were allocated for FOB and assessment of motor activity, auditory startle reflex habituation, learning and memory (passive avoidance and watermaze testing), and neuropathology (including brain weights). Offspring were monitored daily throughout lactation for clinical signs or morbidity and were weighed individually on LDs 0, 4, 11, 17, and 21 and weekly thereafter. The age of sexual maturation (vaginal opening in females and preputial separation in males) was also recorded.

There were no treatment-related effects on mortality, clinical signs during gestation, or body weight in dams. During lactation, ocular opacities were observed in up to 5/21 dams from LD 9 at 37 mk/kg/day and in up to 14/20 females from LD 10 at 354 mg/kg/day. During the FOB, ocular opacities were observed on LD 11 in 3/10 dams at 37 mk/kg/day and in 7/10 dams at 354

mg/kg/day; and on LD 21 in 2/10 and 7/10 dams at 37 and 354 mg/kg/day, respectively. Food consumption was reduced in dams during weeks 1-2 of lactation at 37 (12-19%) and 354 (9-20%) mg/kg/day, however without a corresponding effect on body weight. The fertility index was decreased (NSS) at 37 mg/kg/day (3%) and 354 mg/kg/day (13.3%); however, this observation was not considered treatment-related, since dosing began on GD 6. All other reproductive parameters were unaffected by treatment.

The maternal LOAEL is 37 mg/kg/day, based on ocular opacities during lactation. The maternal NOAEL is 3.8 mg/kg/day.

There were no treatment-related effects on litter size, viability/mortality, or other litter parameters in offspring. Clinical signs and body weight were unaffected during lactation. During post-weaning, ocular opacities were observed in 6/58 males at 354 mg/kg/day on or after PND 29, as well as in 2/63 females at 37 mg/kg/day and 1/60 females at 354 mg/kg/day on or after PND 30. Body weight was decreased in males and females by 6-9% and 4-8% each at 37 mg/kg/day and by 8-13% and 8-11% each at 354 mg/kg/day. Preputial separation was delayed at 37 mg/kg/day (46.0 days) and 354 mg/kg/day (46.7 days, $P< 0.01$), compared to controls (44.1 days). Vaginal patency was unaffected by treatment. During the FOB, ocular opacity was observed in 1/15 females at both 37 mg/kg/day and 354 mg/kg/day. These changes were first noted on postnatal days 45 and 35, respectively, and persisted in both cases through postnatal day 60.

No treatment-related effects were observed on motor activity or auditory startle response. For PND 22 animals tested for passive avoidance, an increase ($P<0.05$) in the number of trials to criterion, as well as dose-dependent decreases in trials 1 and 2 latencies, were observed at ≥37 mg/kg/day during the learning session. During the retention session, an increase ($P<0.05$) in the number of trials to criterion and reduced latency at trial 1 were observed at 354 mg/kg/day. There were no treatment-related effects on acquisition and retention in either adult males or females during the water maze testing. An increase in the mean time required to complete trial 1 was observed in the retention phase in males at 37 mg/kg/day; however, the result was not considered treatment-related in the absence of dose response and treatment-related effects on the number of trials to reach criterion.

During ophthalmoscopic examinations, retinal degeneration was observed in 4/10 males at 354 mg/kg/day. Retinal degeneration was also observed in 0/13, 1/13, 3/11, and 4/10 females at 0, 3.8, 37, and 354 ($P<0.05$) mg/kg/day, respectively. The increase in one female animal at the low dose was not considered treatment-related based on the low incidence and lack of statistical significance in the current study and since retinal degeneration was not observed at a similar dose in F1 or F2 offspring in the 2-generation reproduction study. Absolute fixed brain weight on PND 21 was statistically significantly decreased for males (8%) at 354 mg/kg/day and for females at 37 mg/kg/day (6%) and 354 mg/kg/day (11%). Absolute fixed brain weight on PND 75 was also decreased for females at 354 mg/kg/day (5%). At necropsy, an increased incidence of opacity was observed in the eyes of 3/10 PND 75 males at 354 mg/kg/day. During macroscopic morphometry at PND 21, cerebellum length was decreased ($P<0.05$) in perfused males (6%) and females (7%) at 354 mg/kg/day. Cerebrum length was also decreased ($P<0.05$) in females at 37 (4%) and 354 (5%) mg/kg/day. At PND 75, cerebellum length was decreased in males (5%, $P<0.05$) and females (4%, NSS) at 354 mg/kg/day. During microscopic morphometry on PND 21, cerebellum height was decreased ($P<0.05$) in males at 37 mg/kg/day

(7%) and 354 mg/kg/day (8%) and in females at 354 mg/kg/day (10%). The hippocampal gyrus was decreased (P<0.05) by 11% and 9% in males and females, respectively, at 354 mg/kg/day on PND 21. On PND 75, cerebellum height was changed at the mid and high doses in males (↑14% and ↓6%, respectively) and females (↑13% and ↓6%); however, in the absence of a consistent change across dose, the findings were not considered toxicologically significant.

The offspring LOAEL is 37 mg/kg/day, based on ocular opacity (post-weaning), decreased body weight, delayed preputial separation (males), increase in the number of trials to criterion and decreases in trial latencies (passive avoidance; PND 22 males), retinal degeneration at ophthalmoscopy (females), decreased brain weight (PND 21 females), decreased cerebrum length (PND 21 females), and decreased cerebellum height (PND 21 males). The offspring NOAEL was 3.8 mg/kg/day.

This study is classified **Acceptable/Non-guideline** and may be used for regulatory purposes. It does not, however, satisfy the guideline requirement for a developmental neurotoxicity study in rats [OPPTS 870.6300, §83-6; OECD 426 (draft)] due to the pending review of the positive control data. The following deficiencies were noted in the report: 1) litter data were reported for only 20-23 dams/group. An explanation as to why litter data were not reported for the remaining 6-8 dams/group (after accounting for non-pregnant animals) that underwent "elective sacrifice" is requested; 2) SE (rather than SD) was used as the measure of variability around sample means.

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

This Executive Summary was prepared for United States Environmental Protection Agency, Office of Pesticides Programs, Health Effects Division use.

The following text was generated by the Australian Pesticides and Veterinary Medicines Authority. However, this document has undergone critical scientific analysis and been modified as appropriate.

Report:	**KIIA 5.7.5/01, Gilmore, R. G.; Sheets, L. P.; Hoss, H. E.; 2006**
Title:	A developmental neurotoxicity screening study with technical grade AE0317309 in Wistar rats
Laboratory	Bayer CropScience LP Toxicology, 17745 South Metcalf Ave, Stilwell, KS 66085-9104, USA
Study duration	7 December 2004 – 18 March 2005
Report No.:	201439
Document No.:	M-266434-01-1
Guidelines:	**U.S. EPA, OPPTS 870.6300** **Health Canada PMRA DACO No. 4.5.14**
GLP/QA	**Yes**

Materials and Methods

AE 0317309 (batch Op. 1-4, purity 95.7%) was incorporated into rodent diet by suspending the test substance in acetone and mixing the suspension into the meal-form chow. Diet was prepared at concentrations of 0, 45, 450, and 4500 ppm (during gestation) and administered to groups of 30 sperm-positive female Wistar rats on gestation day 6 through lactation day 21; dietary concentrations were adjusted during lactation to provide for a constant dosage throughout the treatment period. These concentrations provided an average daily intake of 0, 3.8, 37, and 354 mg/kg bw/day. The homogeneity, stability and concentration of the test substance in the feed were confirmed. The rats were at least 12 weeks of age at the beginning of co-housing with untreated males, and weighed 172.2-198.5 g. During gestation and lactation, the rats were housed individually with corn-cob bedding in plastic cages. Food and water were provided *ad libitum* except during neurobehavioral testing. Dams were observed for clinical signs at least once daily. FOB (functional observational battery) tests were conducted during gestation (GD 13, 20) and lactation (LD 11, 21). Body weight and food consumption were measured on a weekly basis, on gestation days 6, 13, and 20, and lactation days 0, 7, 14, and 21. Maternal body weight was also measured on lactation day 4 and litters were culled on this day to eight pups, with four male and four female pups wherever possible. Dams were sacrificed on lactation day 21 following weaning of their litters, and a gross necropsy examination was conducted.

As soon as possible after pup birth, anogenital distance was measured and pups were tattooed. Surviving pups were counted, sexed, and weighed individually on lactation days 0, 4, 11, 17, and 21. Offspring were monitored daily throughout lactation for clinical signs or morbidity. After weaning on day 21, the pups were monitored twice daily for morbidity and mortality, and were weighed on a weekly basis as well as on the day that vaginal patency or balanopreputial separation were achieved. Food consumption was not measured. Pups were examined daily starting from postnatal day 29 (females) or postnatal day 38 (males) for developmental landmarks, and pupil constriction was tested in all pups on postnatal day 21. The following table summarizes examinations that were conducted on offspring from postnatal day 4 onwards. All tests used at least 10 offspring per sex per dose, and with the exception of learning and memory, no animal was tested more than once in the same test. However, different tests of learning and memory were conducted in weanling and adult animals.

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Timing of neurobehavioral examinations in offspring of females administered AE 0317309 by dietary incorporation during gestation and lactation.

Parameter	Postnatal day											
	4	11	13	17	21	22	29	35†	45†	60†	67†	75‡
Detailed clinical observations / FOB	X	X			X			X	X	X		
Motor activity			X	X	X					X		
Auditory startle habituation						X				X		
Learning and memory						X	X			X	X	
Brain weight					X							X
Neuropathology					X							X

† Date given is ± 2 days.; ‡ Date given is ± 5 days.

Ten to thirteen male and 10-13 female offspring per dose group were selected for ophthalmoscopic examination at approximately 50-60 days of age. On postnatal day 21, 10 male and 10 female offspring were sacrificed and the brains were collected whole for micropathological examination and morphometric analysis and weighed. The animals were anesthetized and perfused via the left ventricle with sodium nitrite in phosphate buffer followed by in situ fixation with universal fixative in phosphate buffer. On postnatal day 75, a further 10 male and 10 female offspring were sacrificed and perfused, and brain, spinal cord, both eyes with optic nerves, bilateral sciatic, tibial, and sural nerves, gasserian ganglion, gastrocnemius muscle, and both forelimbs were collected and fixed. Brains were weighed. On both occasions, prior to sectioning, the anterior-posterior length of the cerebrum and of the cerebellum were measured with Vernier calipers. Other measurements, made after histologic sectioning, were the thickness of the frontal cortex, parietal cortex, and hippocampal gyrus, horizontal width of the caudate putamen, and height of the cerebellum. The offspring not selected for neuropathological examination were sacrificed without examination.

Findings for parental animals

Relevant tables from the study report are attached as an appendix at the end of this review.

Mortalities: There were no mortalities in parental females during either gestation or lactation.

Clinical signs: There were no treatment-related clinical signs observed during gestation. During lactation, ocular opacities were observed in up to 5/21 females from LD 9 at 450 ppm and in up to 14/20 females from LD 10 at 4500 ppm. No staining of the fur was observed (other than red nasal staining in one animal in the high dose group during gestation).

FOB: Ocular opacities, evaluated as related to treatment, were observed during the FOB in 3/10 females at 450 ppm and 7/10 females at 4500 ppm on LD 11 and in 2/10 (450 ppm) and 7/10 (4500 ppm) females on LD 21. This increase at 4500 ppm was statistically significant compared to controls. Red nasal stain was also observed in one high-dose dam on GD 13.

Body weight and body weight gain: Body weight and body weight gain were not

affected during gestation or lactation at any dose.

Food consumption: Food consumption was statistically significantly increased at 4500 ppm during gestation, although this was considered by the study authors to be due to wastage from palatability issues. Installation of grates to reduce wastage in week 3 of gestational treatment markedly reduced food consumption at 4500 ppm, relative to the preceding 2 weeks. During lactation, food consumption was statistically significantly reduced, relative to controls, at both 450 ppm and 4500 ppm during lactation days 0-7 and 7-14.

Mean food consumption (g/animal/day) during gestation and lactation

Days	Dose in ppm[1]			
	0	45	450	4500
Gestation days 6-13	19.7	19.5	21.4	47.6**
Gestation days 13-20	19.6	19.8	19.9	23.0**
Lactation days 0-7	41.7	36.3	33.6*	33.5*
Lactation days 7-14	56.0	54.9	49.5**	50.8*
Lactation days 14-21	66.7	64.3	61.4	55.9

[1] Gestational concentrations in ppm ("doses") are utilized in all tables throughout this review, even though concentrations were increased during lactation to achieve somewhat equivalent doses throughout both gestation and lactation

* $p < 0.05$.** $p < 0.01$.

Reproductive performance: The fertility index was decreased (without reaching statistical significance) at 450 ppm (by 3.3%) and at 4500 ppm (by 13.3%); however, this was not related to dietary administration of AE 0317309, since dosing of dams began on GD 6. Some aspects of the data in the report are unclear (for example, the significance of the parameter "No. of Dams with One Implantation Site"), but it does not appear that there were any other noteworthy observations on reproductive parameters.

Selected reproductive parameters in female rats

Parameter	AE 0317309, dietary concentration in ppm[1]			
	0	45	450	4500
Number of females co-housed	30	30	30	30
Number of females mated	30	30	30	30
Dams not pregnant	0	0	1	4
Mating index	100	100	100	100
Fertility index	100	100	96.7	86.7
Gestation length, days	22.0	22.0	22.1	22.1

[1] Gestational concentrations only

Findings for offspring

Viability: There were no effects of treatment on litter size, viability, or other litter parameters.

Clinical signs: There were no treatment-related signs during lactation. In the post-weaning phase, treatment-related findings were restricted to ocular opacities in 6/58 males on or after PND 29 and 1/60 females at 4500 ppm, and 2/63 females at 450

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ppm.

Mortalities and moribundity: There was no effect of dietary administration of AE 0317309 on the incidence of moribund or found-dead pups.

Body weight: Body weight at birth was similar at all doses. However postnatal pup weights and body weight gains were frequently decreased to a statistically significant extent, particularly in males, at and above 450 ppm.

Body weight (g) of male and female pups

Postnatal Day	Dose in ppm[1]							
	Males				Females			
	0	45	450	4500	0	45	450	4500
0	6.0	6.1	6.1	5.9	5.7	5.8	5.7	5.6
4 (before culling)	10.0	10.0	9.6	9.2	9.6	9.6	9.1	8.9
4 (after culling	10.0	10.1	9.6	9.1* (9)	9.6	9.6	9.1	8.9
11	25.2	25.6	23.5* (7)	22.5* (11)	24.6	24.8	22.7* (8)	22.1** (10)
17	38.2	39.4	35.9* (6)	35.2** (8)	37.4	37.8	34.8* (7)	34.5* (8)
21	48.5	48.8	45.3* (7)	43.3** (11)	47.0	47.2	43.5** (7)	42.1** (10)
28	76.8	75.4	70.1* (9)	67.0* (13)	75.1	74.3	69.3* (8)	66.9* (11)
35	123.8	121.2	113.8* (8)	109.5* (12)	111.7	110.6	104.1* (7)	102.9* (8)
42	170.4	165.7	159.9* (6)	155.6* (9)	134.2	134.7	128.7* (4)	129.5
49	213.1	206.4	199.8* (6)	196.8* (8)	150.5	151.0	145.1	147.5
56	255.8	247.8	240.3* (6)	237.2* (7)	163.9	164.8	159.6	163.5
63	288.0	279.0	271.7* (6)	268.3* (7)	176.9	176.8	171.2	175.8
70	317.6	308.2	299.6* (6)	295.9* (7)	186.6	186.8	181.9	188.4

[1] Maternal gestational concentrations only
* $p < 0.05$.** $p < 0.01$.

Developmental landmarks: Preputial separation was statistically significantly delayed at 4500 ppm (46.7 days, $p \leq 0.01$) and statistically non-significantly delayed at 450 ppm (46.0 days), compared to controls (44.1 days). This increase in time to preputial separation was considered by the study authors to be related to treatment as a secondary effect of reduced body weight. Preputial separation was also delayed in F1 pups at similar doses in the rat 2-generation reproduction toxicity study (Study report KIIA 5.6.1). There was no effect on time to vaginal patency. Pupillary constriction on day 21 in both males and females was not affected by treatment.

FOB: The only treatment-related changes observed at FOB assessment were ocular opacities in 1/15 females at 450 ppm and 1/15 females at 4500 ppm. These changes were first noted on postnatal days 45 and 35, respectively, and persisted in both cases

through postnatal day 60.

Motor and locomotor activity: There were no treatment-related effects observed on motor or locomotor activity in either males or females at any dose level during either lactation or post-weaning phases. Loco/motor activity habituation was observed in control and all treated groups, except in females on PND 60. The expected developmental pattern for motor activity was not observed for control or treated animals.

Auditory startle habituation: There were no treatment-related effects on auditory startle at any time during the study or at any dose level. Peak amplitude at 4500 ppm was decreased by 26% in PND 22 females and by 40% in PND 60 females, however without attaining statistical significance. Peak amplitude at 450 and 4500 ppm was decreased by 36% and 31% in PND 60 males, however without dose response or attaining statistical significance.

Passive avoidance: As the following table (Study Report 5.7.5/01-5) indicates, there is a tendency for reduced performance among males on PND 22 at the mid and high dose in these tests, which sometimes reached statistical significance. This included more "trials to criterion" and reduced latency at trials 1 and 2 during the learning session; and more "trials to criterion" and reduced latency at trial 1 (high dose only) during the retention session.

Passive avoidance performance, as measured on postnatal day 22, in male and female offspring

Session	Parameter	AE 0317309, dietary conc. in ppm[1]			
		0	45	450	4500
Males					
1 (Learning)	Number of animals tested	16	16	16	16
	Number of animals included in analysis	16	16	16	16
	Trials to criterion	2.9±0.3	3.0±0.0	3.5±0.9*	3.4±0.6*
	Latency, trial 1 (sec)	49.0	39.6	31.2	27.5
	Latency, trial 2 (sec)	180±0.0	180±0.0	162.5±42.2	151.0±56.9*
	Failed to meet criterion	0	0	0	0
	Failed to cross during learning phase	2	0	0	0
2 (Retention)	Number of animals tested	14	16	16	16
	Number of animals included in analysis	14	16	16	16
	Trials to criterion	2.2±0.6	2.0±0.0	2.4±0.8	2.8±0.7*
	Latency, trial 1 (sec)	169.4±39.6	180.0±0.0	175.4±18.5	137.7±53.4*
	Latency, trial 2 (sec)	177.0±11.3	180.0±0.0	174.2±18.9	169.7±28.5
Females					
1 (Learning)	Number of animals tested	16	16	16	16
	Number of animals included in analysis	16	16	16	16
	Trials to criterion	3.2	3.5	3.3	3.1
	Latency, trial 1 (sec)	24.8	20.8	45.0	41.8
	Latency, trial 2 (sec)	179.2	171.8	177.8	180.0
	Failed to meet criterion	0	0	0	0
	Failed to cross during learning phase	0	0	1	1

2 (Retention)	Number of animals tested	16	16	15	15
	Number of animals included in analysis	16	16	14	15
	Trials to criterion	2.4	2.7	2.6	2.9
	Latency, trial 1 (sec)	153.2	162.3	139.5	125.1
	Latency, trial 2 (sec)	177.9	166.9	170.2	169.4

[1] Maternal gestational concentrations only
* $p < 0.05$.** $p < 0.01$.

Water maze: There were no treatment-related effects on acquisition and retention in either males or females during the water maze testing. Increases in the mean times required to complete trial 1 were observed in the learning ($P<0.05$) and retention (NSS) phases in males at 450 ppm; however, the results were not considered treatment-related in the absence of dose response and treatment-related effects on the number of trials to reach criterion.

Ophthalmoscopic findings: Retinal degeneration was statistically significantly increased at 4500 ppm, with four males and four females affected at this dose. There was also a dose-response relationship in females, with 0/13, 1/13, 3/11 and 4/10 animals showing retinal degeneration at 0, 45, 450 and 4500 ppm, respectively. The four cases in the 4500 ppm dose group and the three cases in the 450 ppm dose group are considered to be treatment-related, whereas the single instance in the female 45 ppm dose group (NSS) may simply be a sporadic effect of the kind observed in the controls in Study Report no. KIIA 5.7.4/01 (subchronic neurotoxicity study). In addition, retinal degeneration was not observed at a similar dose in F1 or F2 offspring in the 2-generation reproduction study.

Ocular findings in offspring

Observation	Dose (ppm[1])			
	0	**45**	**450**	**4500**
Males				
N	13	13	11	10
Corneal opacity	2	2	3	2
Retinal degeneration	0	1	0	4*
Females				
N	13	13	11	10
Corneal opacity	2	0	0	1
Retinal degeneration	0	1	3	4*

[1] Maternal gestational concentrations only
* $p < 0.05$.

Necropsy: An increased incidence of opacity was observed in the eyes of 3/10 PND 75 males (perfused) at 4500 ppm.

Brain weight: Absolute fixed brain weight was statistically significantly decreased for males (8%) and females (11%) at 4500 ppm and females at 450 ppm (6%) on day 21, and for females at 4500 ppm (5%) on day 75. Relative brain weight in these groups was no different from controls, which the study authors suggested indicated that the decreased absolute brain weight was related to the reduced body weight at these doses. However, brain mass is often considered to be conserved under conditions that lead to reduced body weight.

Gross brain measurements: At day 21, cerebellum length was statistically significantly decreased in perfused males (6%) and females (7%) at 4500 ppm. Cerebrum length was also statistically significantly decreased in females at 450 (4%) and 4500 (5%) ppm. At day 75, cerebellum length was statistically significantly decreased in males at 4500 ppm (5%), and statistically non-significantly decreased (4%) in females at 4500 ppm. There was no effect on cerebrum length in either males or females at day 75. These decreases observed on day 21 and 75 were considered to be related to treatment.

Micropathology brain measurements: On day 21, males at 450 (7%) and 4500 (8%) ppm and females at 4500 ppm (10%) showed a statistically significant decrease in cerebellum height. There were indications of a dose-related decrease for this measurement in both sexes at this time, but this was not the case at postnatal day 75. Cerebellar height on PND 75 was statistically significantly changed at 450 and 4500 ppm in males (↑14% and ↓6%, respectively) and females (↑13% and ↓6%). The hippocampal gyrus was significantly decreased (P<0.05) by 11% and 9% in males and females, respectively, at 4500 ppm on PND 21. In general, concurrent control data were either at the extreme end of the historical range or exceeded the historical range.

Brain measurements, from offspring sacrificed on postnatal day 21 or postnatal day 75.

	Dose in ppm[1]							
	Males				Females			
Parameter	**0**	**45**	**450**	**4500**	**0**	**45**	**450**	**4500**
Postnatal day 21								
Ant/post cerebrum length, mm	13.52	13.67	13.31	13.24	13.63	13.38	13.07*	13.00*
Ant/post cerebellum length, mm	7.20	7.03	7.12	6.80*	7.13	7.06	6.83	6.65*
Frontal cortex, mm	1.728	---[a]	1.769	1.805	1.823	---	1.763	1.749
Parietal cortex, mm	2.008	1.918	1.901*	1.922	1.999	1.939	1.846*	1.850*
Caudate putamen, mm	2.982	---	3.097	3.052	3.029	---	2.974	2.915
Hippocampal gyrus, mm	1.723	---	1.673	1.541*	1.737	---	1.696	1.575*
Cerebellum height, mm	4.367	4.165	4.057*	4.005*	4.292	4.164	4.086	3.869*
Postnatal day 75								
Ant/post cerebrum length, mm	14.56	14.69	14.45	14.64	14.12	14.11	14.24	13.92
Ant/post cerebellum length, mm	7.56	7.69	7.41	7.21*	7.57	7.52	7.47	7.25
Frontal cortex, mm	1.760	---	1.656*	1.713	1.76	---	1.66*	1.79
Parietal cortex, mm	1.863	---	1.808	1.880	1.855	---	1.827	1.833
Caudate putamen, mm	3.345	---	3.302	3.424	3.386	---	3.172*	3.330
Hippocampal gyrus, mm	1.758	---	1.790	1.684	1.631	---	1.731	1.543
Cerebellum height, mm	3.831	---	4.380*	3.618*	3.978	---	4.488*	3.756*

[1] Maternal gestational concentrations only
a measurement not taken
* $p < 0.05$.

Microscopic findings: On histopathological examination of the brains and other nervous tissues, the only treatment-related finding was retinal degeneration in 1/10 males at 4500 ppm.

Conclusions and Critique

The study authors proposed a NOAEL for the dams and offspring of 45 ppm (3.8 mg/kg bw/day). This is basically confirmed in the current assessment, as indicated below.

Based on decreased food consumption during lactation and ocular opacities in dams at 450 and 4500 ppm, the maternal systemic NOAEL (NOEL for Australia) was 45 ppm (3.8 mg/kg bw/day). There were no maternal neurotoxic effects.

Neonatal toxicity included decreased postnatal weights, delayed preputial separation and retinal degeneration in the offspring at 450 and 4500 ppm. The NOAEL (NOEL for Australia) for neonatal systemic toxicity was therefore 45 ppm (3.8 mg/kg bw/day).

There were decreased absolute brain weights, decreased cerebrum length and decreased cerebellum height in male and/or female offspring on postnatal day 21 at 450 and 4500 ppm. There was also diminished performance in males in the passive avoidance test at these doses. Therefore the NOAEL (NOEL for Australia) for neonatal developmental neurotoxic effects was 45 ppm (3.8 mg/kg bw/day). These developmental neurotoxic effects occurred at maternotoxic dose levels.

Appendix

The following tables were extracted from pages 37-60 in the study report (KIIA 5.7.5):

TEXT TABLE 2. Mortality and Maternal Clinical Observations [a]

Observation	Dose (ppm in diet)			
	Control	45 PPM	450 PPM	4500 PPM
Gestation (Days 6-21)				
No. of females examined on Gestation Day 6	30	30	30	30
Nasal stain	0	0	0	1
Hair loss	0	2	0	0
No. of females found dead	0	0	0	0
Lactation (Days 0-21)				
No. of females examined on Lactation Day 0 [b]	30	30	29	26
General Opacity	0	0	5	14
Exophthalmos	0	0	1	0
Hair Loss	0	2	0	2
No. of females found dead	0	0	0	0

[a] Data obtained from pages 72 and 78 in the study report. Observations during gestation (days 6-24). Values are the number of rats with the finding.

[b] The numbers of dams with no pups by Gestation Day 24 were 0, 0, 1, and 4 for the controls, low-, mid-, and high-dose groups, respectively.

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TEXT TABLE 3. Maternal Functional Observations [a]

Observation	Dose (ppm in diet)			
	Control	45 PPM	450 PPM	4500 PPM
Gestation Day 13				
Number of Animals Examined:	30	30	30	30
Handling - Stains Not Observed Red Nasal Stain	 30(100) 0(0)	 30(100) 0(0)	 30(100) 0(0)	 29(97) 1(3)
Handling-Other Not Observed Alopecia	 30(100) 0(0)	 29(97) 1(3)	 30(100) 0(0)	 30(100) 0(0)
Gestation Day 20				
Number of Animals Examined:	30	30	30	30
Handling-Other Not Observed Alopecia	 30(100) 0(0)	 28(93) 2(7)	 30(100) 0(0)	 30(100) 0(0)
Lactation Day 11				
Number of Animals Examined:	10	10	10	10
Handling-Other Not Observed Alopecia	 10(100) 0(0)	 9(90) 1(10)	 10(100) 0(0)	 9(90) 1(10)
Handling-Other Not Observed Opacity	 10(100) 0(0)	 10(100) 0(0)	 7(70) 3(30)	 * 5(50) 5(50)
Lactation Day 21				
Number of Animals Examined:	10	10	10	10
Handling-Other Not Observed Alopecia	 10(100) 0(0)	 9(90) 1(10)	 10(100) 0(0)	 9(90) 1(10)
Handling-Other Not Observed Opacity	 10(100) 0(0)	 10(100) 0(0)	 8(80) 2(20)	 * 3(30) 7(70)

[a] *Data obtained from pages 109-132 in the study report.*
* *Statistically different (p ≤0.05) from the control.*

TEXT TABLE 4. Mean (±SD) Maternal Body Weight and Food Consumption [a]

Observations/study week	Dose (ppm in diet)			
	Control	45 PPM	450 PPM	4500 PPM
Gestation				
Mean body weight (g) Gestation day 0	204.3±2.04 (30)	206.3±1.65 (30)	201.5±1.63 (29)	205.9±2.15 (26)
Mean body weight (g) Gestation day 6	222.6±2.70 (30)	230.2±1.93 (30)	223.1±1.97 (29)	228.7±2.32 (26)
Mean body weight (g) Gestation day 13	245.1±3.56 (30)	252.5±2.07 (30)	242.2±2.28 (29)	246.1±2.83 (26)
Mean body weight (g) Gestation day 20	306.5±4.20 (30)	316.0±3.40 (30)	299.3±3.14 (29)	305.8±3.88 (26)
Mean weight gain (g) Gestation days 0 - 20	102.3±2.79 (30)	109.7±2.42 (30)	97.8±2.65 (29)	99.9±2.36 (26)
Mean food consumption (g/animal/day) Gestation days 6 - 13	19.3±0.59 (29)	19.1±0.49 (29)	21.4±1.29 (29)	47.6**±5.70 (26)
Mean food consumption (g/animal/day) Gestation days 13 - 20	19.6±0.55 (30)	19.8±0.43 (30)	19.9±0.41 (29)	23.0**±0.90 (26)
Lactation				
Mean body weight (g) Lactation day 0	235.6±3.21 (30)	242.0±2.83 (30)	233.8±2.07 (29)	237.1±3.31 (26)
Mean body weight (g) Lactation day 4	252.7±3.65 (27)	260.5±2.92 (26)	249.0±3.09 (21)	256.4±2.94 (20)
Mean body weight (g) Lactation day 7	260.8±3.46 (23)	270.9±2.51 (23)	258.5±2.70 (21)	264.8±3.77 (20)
Mean body weight (g) Lactation day 14	279.9±3.40 (23)	288.2±3.61 (23)	275.7±3.40 (21)	282.8±3.77 (20)
Mean body weight (g) Lactation day 21	269.0±2.84 (23)	273.6±3.11 (23)	267.7±3.88 (21)	272.1±3.48 (20)
Mean food consumption (g/animal/day) Lactation days 0 - 7	41.7±2.97 (23)	36.3±1.51 (23)	33.6*±1.17 (21)	33.5*±1.06 (20)
Mean food consumption (g/animal/day) Lactation days 7 - 14	56.0±1.66 (23)	54.9±1.06 (23)	49.5**±0.96 (21)	50.8*±1.05 (20)
Mean food consumption (g/animal/day) Lactation days 14 - 21	66.7±3.00 (23)	64.3±2.13 (23)	61.4±1.33 (20)	55.9**±1.07 (20)

[a] *Data obtained from pages 74-76, 80-82 in the study report. Values are mean ± standard error (n). Means for gestation period include only dams known to deliver pups (either alive or dead).*
* *Statistically different from control, p ≤ 0.05.*
** *Statistically different from control, p ≤ 0.01.*

TEXT TABLE 5. Mean Maternal Test Substance Intake (mg/kg body weight/day)[1]

Period	Dose (ppm in diet)		
	45 PPM	450 PPM	4500 PPM
Gestation			
Gestation days 6 - 13	3.7±0.09 (29)	42.9±2.38 (29)	890.9±103.7 [2] (26)
Gestation days 13 - 20	3.5±0.06 (30)	36.9±0.64 (29)	402.9±14.32 (26)
Lactation			
Lactation days 0 - 7	3.8±0.17 (23)	34.7±1.22 (21)	343.2±12.69 (20)
Lactation days 7 - 14	4.2±0.08 (23)	36.0±0.54 (21)	360.9±6.16 (20)
Lactation days 14 - 21	3.6±0.13 (23)	34.8±0.66 (20)	309.7±4.19 (20)

[1] *Data obtained from pages 84-85 in the study report. Values are mean ± standard error (n). Dietary concentrations were reduced during weeks 1-3 of lactation (by factors of 1.9, 2.3 and 2.8, respectively), based on estimated increases in feed consumption (g consumed/kg body wt./day) during lactation).*
[2] *Associated with observed food spillage and considered an unreliable measure of a.i. intake. This value was excluded from the mean average daily intake.*

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TEXT TABLE 7. Litter Size and Viability [a]

Observation	Dose (ppm in diet)			
	Control	45 PPM	450 PPM	4500 PPM
No. of Litters	23	23	21	20
Total No. of Pups Born	259	271	240	228
Total No. of Pups Missing	2	0	3	3
Litters with Pups Missing	2	0	2	3
Total No. of Pups Found Dead	1	3	1	3
Litters with Pups Found Dead	1	3	1	3
Total No. of Pups Cannibalized	0	0	0	0
Litter with Pups Cannibalized	0	0	0	0
Litter Size	11.3±0.29 [11.0] (9.0-15.0)	11.8±0.38 [12.0] (8.0-14.0)	11.4±0.49 [11.0] (7.0-16.0)	11.4±0.36 [11.0] (9.0-16.0)
Stillborn pups: Number % Mean±S.E. [Median] (Range)	 0 0.0 0.0±0.00 [0.0] (0.0-0.0)	 1 0.4 0.0±0.04 [0.0] (0.0-1.0)	 0 0.0 0.0±0.00 [0.0] (0.0-0.0)	 0 0.0 0.0±0.00 [0.0] (0.0-0.0)
Mean No. of Viable Pups Birth Day 4 (Pre-cull)[b] Day 4 (Post-cull)[c] Day 21	 11 11 8 8	 12 12 8 8	 11 11 8 8	 11 11 8 8
Live birth index	100.0±0.00 [100.0] (100-100)	99.5±0.48 [100.0] (89-100)	100.0±0.00 [100.0] (100-100)	100.0±0.00 [100.0] (100-100)
Viability index	98.8±0.69 [100.0] (88.9-100)	99.2±0.55 [100.0] (90.0-100)	98.7±0.75 [100.0] (87.5-100)	98.2±0.83 [100.0] (88.9-100)
Lactation index	100.0±0.00 [100.0] (100-100)	99.5±0.54 [100.0] (88-100)	100.0±0.00 [100.0] (100-100)	98.8±0.86 [100.0] (88-100)

[a] *Data obtained from pages 87 in the study report.*
[b] *Before standardization (culling). Values are mean ± standard error*
[c] *After standardization (culling).*

TEXT TABLE 10. Mean (±SD) Age of Sexual Maturation (days) [a]

Parameter	Dose (ppm in diet)			
	Control	45 PPM	450 PPM	4500 PPM
N (M/F)	23	23	21	20
Preputial separation % Pups Reaching Criteria	44.1±0.52 (23) 100	43.6±0.31 (23) 99	46.0±0.58 (21) 100	46.7**±0.63 (20) 100
Vaginal opening % Pups Reaching Criteria	32.8±0.83 (23) 100	33.0±0.65 (23) 99	34.7±0.83 (21) 100	33.7±0.30 (20) 100

[a] *Data obtained from pages 104 in the study report.*
** *Statistically different from control, p≤0.01 Values are mean ± standard error (N)*

TEXT TABLE 11. Functional Observational Battery Results (incidence)

Observation	Dose (ppm in diet)			
	Control	45 PPM	450 PPM	4500 PPM
Males				
Handling - Stain - Lacrimal -PND # 45				
Not Observed	16 (100)	15 (100)	16 (100)	14 (93)
Red	0 (0)	0 (0)	0 (0)	1 (7)
Handling - Stain - Lacrimal -PND # 60				
Not Observed	16 (100)	15 (100)	16 (100)	14 (93)
Red	0 (0)	0 (0)	0 (0)	1 (7)
Females				
Handling - Opacity -PND # 35				
Not Observed	16 (100)	16 (100)	16 (100)	15 (94)
Present	0 (0)	0 (0)	0 (0)	1 (6)
Handling - Opacity -PND # 45				
Not Observed	16 (100)	16 (100)	15 (94)	15 (94)
Present	0 (0)	0 (0)	1 (6)	1 (6)
Handling - Opacity -PND # 60				
Not Observed	16 (100)	16 (100)	15 (94)	15 (94)
Present	0 (0)	0 (0)	1 (6)	1 (6)

Values represent the number of animals and % incidence (in parentheses) with no observation.
** Data obtained from pages 134-189 in the study report.*
*N = 10/sex/dose * Statistically different from control, p≤0.05*

TEXT TABLE 12. Mean (±S.D.) Motor Activity Data (total activity counts for session) [a]

Test Day	Dose (ppm in diet)			
	Control	45 PPM	450 PPM	4500 PPM
Males				
PND 13	78±74 (16)	87±76 (16)	70±47 (16)	73±59 (16)
PND 17	144±73 (16)	200±107 (16)	178±106 (16)	162±103 (16)
PND 21	299±114 (16)	268±103 (16)	243±56 (16)	296±130 (16)
PND 60	554±136 (16)	524±147 (16)	510±176 (16)	552±159 (16)
Females				
PND 13	89±107 (16)	59±40 (16)	75±49 (16)	49±35 (16)
PND 17	130±71 (16)	172±108 (16)	225±110 (16)	179±115 (16)
PND 21	313±116 (16)	291±65 (16)	271±99 (16)	261±114 (16)
PND 60	717±182 (16)	633±160 (16)	617±156 (16)	749±240 (16)

** Data obtained from pages 191-192 in the study report.*

TEXT TABLE 13. Mean (±S.D.) Locomotor Activity Data (total activity counts for session) [a]

Test Day	Dose (ppm in diet)			
	Control	45 PPM	450 PPM	4500 PPM
Males				
PND 13	12±21 (16)	9±10 (16)	6±6 (16)	4±3 (16)
PND 17	38±23 (16)	50±30 (16)	46±30 (16)	36±26 (16)
PND 21	88±33 (16)	86±36 (16)	82±20 (16)	89±37 (16)
PND 60	379±93 (16)	369±131 (16)	349±151 (16)	394±145 (16)
Females				
PND 13	12±29 (16)	5±6 (16)	9±14 (16)	4±3 (16)
PND 17	37±26 (16)	47±32 (16)	58±42 (16)	46±34 (16)
PND 21	91±34 (16)	87±22 (16)	87±34 (16)	86±32 (16)
PND 60	455±147 (16)	413±107 (16)	376±133 (16)	509±193 (16)

** Data obtained from pages 1954195 in the study report.*

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TECHNICAL GRADE AE 0317309
Summary Interval Motor Activity for Male Rats, Postnatal Day 13
Study Number 04-D72-YE

Group	Interval 1	Interval 2	Interval 3	Interval 4	Interval 5	Interval 6
0 PPM	11 ± 12	16 ± 23	12 ± 16	7 ± 11	14 ± 25	19 ± 31
45 PPM	26 ± 26	15 ± 18	15 ± 21	16 ± 29	7 ± 10	7 ± 11
450 PPM	21 ± 22	19 ± 16	13 ± 18	3 ± 5	7 ± 9	6 ± 10
4500 PPM	11 ± 14	13 ± 18	16 ± 22	9 ± 16	12 ± 18	12 ± 14

TECHNICAL GRADE AE 0317309
Summary Interval Motor Activity for Male Rats, Postnatal Day 17
Study Number 04-D72-YE

Group	Interval 1	Interval 2	Interval 3	Interval 4	Interval 5	Interval 6
0 PPM	64 ± 26	33 ± 33	20 ± 19	15 ± 22	7 ± 13	6 ± 10
45 PPM	86 ± 45	32 ± 26	18 ± 17	21 ± 27	23 ± 30	20 ± 27
450 PPM	58 ± 36	39 ± 31	20 ± 18	21 ± 33	24 ± 33	16 ± 21
4500 PPM	41 ± 29	28 ± 29	18 ± 18	18 ± 24	24 ± 28	34* ± 37

TECHNICAL GRADE AE 0317309
Summary Interval Motor Activity for Male Rats, Postnatal Day 21
Study Number 04-D72-YE

Group	Interval 1	Interval 2	Interval 3	Interval 4	Interval 5	Interval 6
0 PPM	107 ± 31	58 ± 28	44 ± 25	37 ± 33	29 ± 23	25 ± 26
45 PPM	111 ± 41	45 ± 30	41 ± 22	26 ± 23	19 ± 20	26 ± 33
450 PPM	100 ± 22	48 ± 24	31 ± 19	23 ± 22	20 ± 17	22 ± 23
4500 PPM	99 ± 41	57 ± 29	36 ± 24	41 ± 29	29 ± 27	35 ± 25

TECHNICAL GRADE AE 0317309
Summary Interval Motor Activity for Male Rats, Postnatal Day 60
Study Number 04-D72-YE

Group	Interval 1	Interval 2	Interval 3	Interval 4	Interval 5	Interval 6
0 PPM	110 ± 19	96 ± 26	102 ± 30	81 ± 33	91 ± 40	73 ± 31
45 PPM	107 ± 18	85 ± 30	95 ± 27	90 ± 40	73 ± 38	74 ± 28
450 PPM	116 ± 21	86 ± 37	79 ± 30	87 ± 47	72 ± 36	72 ± 39
4500 PPM	125 ± 29	94 ± 34	97 ± 35	96 ± 31	77 ± 37	62 ± 26

TECHNICAL GRADE AE 0317309
Summary Interval Motor Activity for Female Rats, Postnatal Day 13
Study Number 04-D72-YE

Group	Interval 1	Interval 2	Interval 3	Interval 4	Interval 5	Interval 6
0 PPM	22 ± 21	21 ± 30	12 ± 21	10 ± 16	8 ± 18	15 ± 28
45 PPM	19 ± 15	11 ± 13	8 ± 10	13 ± 20	8 ± 15	1 ± 2
450 PPM	13 ± 21	14 ± 18	18 ± 17	11 ± 15	7 ± 10	11 ± 21
4500 PPM	12 ± 17	7 ± 12	8 ± 11	8 ± 9	11 ± 16	4 ± 8

TECHNICAL GRADE AE 0317309
Summary Interval Motor Activity for Female Rats, Postnatal Day 17
Study Number 04-D72-YE

Group	Interval 1	Interval 2	Interval 3	Interval 4	Interval 5	Interval 6
0 PPM	55 ± 31	23 ± 21	14 ± 17	15 ± 24	5 ± 10	18 ± 34
45 PPM	75 ± 43	39 ± 31	24 ± 22	13 ± 21	11 ± 16	12 ± 18
450 PPM	62 ± 36	47 ± 24	40 ± 28	36 ± 35	24 ± 27	15 ± 18
4500 PPM	59 ± 45	37 ± 31	24 ± 19	20 ± 21	21 ± 26	19 ± 24

TECHNICAL GRADE AE 0317309
Summary Interval Motor Activity for Female Rats, Postnatal Day 21
Study Number 04-D72-YE

Group	Interval 1	Interval 2	Interval 3	Interval 4	Interval 5	Interval 6
0 PPM	116 ± 29	66 ± 35	42 ± 23	39 ± 32	30 ± 30	21 ± 25
45 PPM	114 ± 24	62 ± 25	34 ± 21	33 ± 20	33 ± 22	26 ± 36
450 PPM	99 ± 24	50 ± 24	36 ± 24	34 ± 27	28 ± 30	24 ± 19
4500 PPM	101 ± 30	49 ± 32	30 ± 23	34 ± 25	23 ± 26	24 ± 23

TECHNICAL GRADE AE 0317309
Summary Interval Motor Activity for Female Rats, Postnatal Day 60
Study Number 04-D72-YE

Group	Interval 1	Interval 2	Interval 3	Interval 4	Interval 5	Interval 6
0 PPM	135 ± 43	108 ± 34	112 ± 38	121 ± 48	122 ± 49	119 ± 31
45 PPM	125 ± 27	97 ± 32	102 ± 33	106 ± 36	108 ± 36	96 ± 33
450 PPM	122 ± 38	103 ± 27	107 ± 42	107 ± 36	91 ± 35	87 ± 40
4500 PPM	141 ± 38	121 ± 51	124 ± 50	131 ± 53	126 ± 61	106 ± 36

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TECHNICAL GRADE AE 0317309
Summary Interval Locomotor Activity for Male Rats, Postnatal Day 13
Study Number 04-D72-YE

Group	Interval 1	Interval 2	Interval 3	Interval 4	Interval 5	Interval 6
0 PPM	2 ± 2	2 ± 6	1 ± 2	1 ± 1	2 ± 6	3 ± 7
45 PPM	3 ± 4	1 ± 2	2 ± 3	1 ± 3	1 ± 3	1 ± 2
450 PPM	3 ± 4	1 ± 2	0 ± 1	0 ± 1	1 ± 2	1 ± 2
4500 PPM	1 ± 2	0 ± 1	0 ± 0	1 ± 1	0 ± 1	1 ± 2

TECHNICAL GRADE AE 0317309
Summary Interval Locomotor Activity for Male Rats, Postnatal Day 17
Study Number 04-D72-YE

Group	Interval 1	Interval 2	Interval 3	Interval 4	Interval 5	Interval 6
0 PPM	17 ± 12	8 ± 7	6 ± 7	3 ± 5	2 ± 5	1 ± 3
45 PPM	20 ± 15	8 ± 7	5 ± 6	5 ± 8	6 ± 8	5 ± 9
450 PPM	18 ± 14	9 ± 8	4 ± 4	5 ± 5	6 ± 9	3 ± 5
4500 PPM	8 ± 8	6 ± 7	5 ± 5	4 ± 5	6 ± 7	8 ± 9

TECHNICAL GRADE AE 0317309
Summary Interval Locomotor Activity for Male Rats, Postnatal Day 21
Study Number 04-D72-YE

Group	Interval 1	Interval 2	Interval 3	Interval 4	Interval 5	Interval 6
0 PPM	35 ± 9	14 ± 8	12 ± 7	10 ± 10	9 ± 8	8 ± 9
45 PPM	32 ± 15	14 ± 9	11 ± 7	8 ± 8	6 ± 7	8 ± 10
450 PPM	36 ± 10	15 ± 9	10 ± 6	7 ± 7	7 ± 6	7 ± 8
4500 PPM	33 ± 16	17 ± 8	10 ± 8	11 ± 8	8 ± 7	9 ± 7

TECHNICAL GRADE AE 0317309
Summary Interval Locomotor Activity for Male Rats, Postnatal Day 60
Study Number 04-D72-YE

Group	Interval 1	Interval 2	Interval 3	Interval 4	Interval 5	Interval 6
0 PPM	77 ± 12	65 ± 18	74 ± 23	57 ± 27	59 ± 27	49 ± 23
45 PPM	74 ± 14	58 ± 26	67 ± 26	69 ± 32	52 ± 29	49 ± 23
450 PPM	81 ± 19	59 ± 31	55 ± 25	60 ± 39	46 ± 31	48 ± 31
4500 PPM	88 ± 24	68 ± 27	71 ± 33	71 ± 28	53 ± 31	44 ± 23

TECHNICAL GRADE AE 0317309
Summary Interval Locomotor Activity for Female Rats, Postnatal Day 13
Study Number 04-D72-YE

Group	Interval 1	Interval 2	Interval 3	Interval 4	Interval 5	Interval 6
0 PPM	3 ± 4	2 ± 5	2 ± 7	2 ± 5	2 ± 7	1 ± 3
45 PPM	2 ± 2	1 ± 2	0 ± 0	1 ± 1	1 ± 3	0 ± 0
450 PPM	2 ± 2	1 ± 2	1 ± 4	1 ± 2	1 ± 3	3 ± 8
4500 PPM	2 ± 2	0 ± 1	1 ± 1	1 ± 1	0 ± 1	0 ± 1

TECHNICAL GRADE AE 0317309
Summary Interval Locomotor Activity for Female Rats, Postnatal Day 17
Study Number 04-D72-YE

Group	Interval 1	Interval 2	Interval 3	Interval 4	Interval 5	Interval 6
0 PPM	15 ± 12	7 ± 6	4 ± 4	5 ± 9	1 ± 3	5 ± 9
45 PPM	22 ± 15	8 ± 7	6 ± 5	4 ± 6	3 ± 5	4 ± 7
450 PPM	14 ± 11	12 ± 9	9 ± 8	11 ± 11	8* ± 10	5 ± 6
4500 PPM	15 ± 13	8 ± 8	6 ± 6	5 ± 7	7 ± 9	5 ± 7

TECHNICAL GRADE AE 0317309
Summary Interval Locomotor Activity for Female Rats, Postnatal Day 21
Study Number 04-D72-YE

Group	Interval 1	Interval 2	Interval 3	Interval 4	Interval 5	Interval 6
0 PPM	39 ± 10	17 ± 9	12 ± 8	11 ± 10	7 ± 7	6 ± 7
45 PPM	39 ± 9	19 ± 9	8 ± 6	9 ± 6	5 ± 6	6 ± 6
450 PPM	36 ± 12	15 ± 9	10 ± 8	11 ± 10	8 ± 8	8 ± 6
4500 PPM	37 ± 9	13 ± 7	10 ± 8	11 ± 8	7 ± 9	8 ± 8

TECHNICAL GRADE AE 0317309
Summary Interval Locomotor Activity for Female Rats, Postnatal Day 60
Study Number 04-D72-YE

Group	Interval 1	Interval 2	Interval 3	Interval 4	Interval 5	Interval 6
0 PPM	88 ± 23	64 ± 28	73 ± 32	82 ± 40	81 ± 44	76 ± 30
45 PPM	82 ± 15	59 ± 27	65 ± 29	72 ± 37	72 ± 25	63 ± 23
450 PPM	77 ± 19	58 ± 22	62 ± 35	65 ± 33	58 ± 29	56 ± 34
4500 PPM	97 ± 35	82 ± 43	83 ± 38	89 ± 41	90 ± 48	68 ± 30

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TEXT TABLE 14. Auditory Startle Reflex Peak Amplitude Data (mean ±S.D.) [a]

Block		Dose (ppm in diet)			
		Control	45 PPM	450 PPM	4500 PPM
Males					
PND 22	Block 1	32 ± 14	32 ± 17	26 ± 11	28 ± 8
	Block 2	31 ± 17	29 ± 17	27 ± 18	22 ± 8
	Block 3	29 ± 13	27 ± 13	26 ± 14	22 ± 7
	Block 4	24 ± 10	26 ± 14	20 ± 11	18 ± 7
	Block 5	25 ± 14	25 ± 13	20 ± 11	19 ± 8
	Avg. For Total Session	**28±11**	**28±14**	**24±12**	**22±7**
	No. Of Animals	16	16	16	16
	Body Weight	51	51	48	44
PND 60	Block 1	229 ± 112	162 ± 98	152 ± 136	187 ± 127
	Block 2	252 ± 147	169 ± 109	140 ± 129	165 ± 138
	Block 3	220 ± 126	156 ± 105	140 ± 115	143 ± 114
	Block 4	168 ± 107	124 ± 83	118 ± 94	111 ± 79
	Block 5	140 ± 64	130 ± 98	100 ± 80	94 ± 56
	Avg. For Total Session	**202±92**	**148±74**	**130±103**	**140±93**
	No. Of Animals	16	16	16	16
	Body Weight	274	274	267	255
Females					
PND 22	Block 1	29 ± 12	27 ± 12	23 ± 9	28 ± 13
	Block 2	30 ± 16	25 ± 14	24 ± 10	22 ± 9
	Block 3	27 ± 14	23 ± 10	24 ± 15	18 ± 8
	Block 4	26 ± 15	24 ± 12	20 ± 10	17 ± 8
	Block 5	23 ± 10	19 ± 10	19 ± 9	17 ± 8
	Avg. For Total Session	**27±11**	**24±11**	**22±9**	**20±7**
	No. Of Animals	16	16	16	16
	Body Weight	49	49	44	44
PND 60	Block 1	110 ± 64	81 ± 51	81 ± 54	74 ± 49
	Block 2	110 ± 74	82 ± 62	89 ± 76	66 ± 36
	Block 3	102 ± 69	73 ± 49	78 ± 72	61 ± 40
	Block 4	88 ± 64	54 ± 28	61 ± 57	50 ± 31
	Block 5	82 ± 72	55 ± 35	49 ± 30	43 ± 24
	Avg. For Total Session	**99±61**	**69±38**	**72±55**	**59±33**
	No. Of Animals	16	16	16	16
	Body Weight	170	171	168	169

[a] *Data obtained from pages 215-216 in the study report.*
Values are mean ± standard deviation

TEXT TABLE 15. Auditory Startle Reflex Latency to Peak Data (miliseconds) [a]

Block		Dose (ppm in diet)			
		Control	45 PPM	450 PPM	4500 PPM
Males					
PND 22	Block 1	38±4	39±6	39±5	39±4
	Block 2	39±6	36±2	38±5	38±6
	Block 3	38±5	36±3	37±3	37±4
	Block 4	39±6	36±2	37±5	39±4
	Block 5	39±6	34±2	35±4	36±7
	Avg. For Total Session	38±4	36±2	37±2	38±4
	No. Of Animals	16	16	16	16
	Body Weight	51	51	48	44
PND 60	Block 1	43±3	42±8	40±6	40±4
	Block 2	40±2	41±5	38±6	39±5
	Block 3	40±3	42±4	38±3	39±5
	Block 4	40±3	41±4	40±3	39±4
	Block 5	39±3	41±5	38±4	37±3
	Avg. For Total Session	41±2	41±4	39±3	39±3
	No. Of Animals	16	16	16	16
	Body Weight	274	274	267	255
Females					
PND 22	Block 1	40±7	38±5	37±3	39±7
	Block 2	39±5	38±6	37±4	38±6
	Block 3	37±6	37±6	37±4	38±4
	Block 4	37±4	36±2	37±4	38±5
	Block 5	36±9	38±6	35±5	36±5
	Avg. For Total Session	38±4	37±3	37±2	38±4
	No. Of Animals	16	16	16	16
	Body Weight	49	49	44	44
PND 60	Block 1	42±3	44±6	42±5	43±6
	Block 2	43±5	41±8	38±7	38±4
	Block 3	43±6	39±6	37±4	36±3
	Block 4	43±6	41±7	39±3	37±5
	Block 5	42±6	42±7	39±4	38±5
	Avg. For Total Session	43±4	41±5	39±4	38±3
	No. Of Animals	16	16	16	16
	Body Weight	170	171	168	169

a Data obtained from pages 218-221 in the study report.
Values are mean ± standard deviation

TEXT TABLE 16. Passive Avoidance Performance at PND 22 Offspring (mean ± S.D.) [a]

Session/Parameter		Dose (ppm in diet)			
		Control	45 PPM	450 PPM	4500 PPM
Males					
Session 1 (Learning Phase)	Number of Animals Tested	16	16	16	16
	Number of Animals Included in Analysis	16	16	16	16
	Trials to criterion	2.9±0.3	3.0±0.0	*3.5±0.9	*3.4±0.6
	Latency trial 1 (sec)	49.0±58.7	39.6±35.8	31.2±22.6	27.5±22.9
	Latency trial 2 (sec)	180.0±0.0	180.0±0.0	162.5±42.2	*151.0±56.9
	Failed to Meet Criterion	0 (0%)	0 (0)%	0 (0)%	0 (0%)
	Failed to Cross During Learning Phase	2 (13%)	0 (0)%	0 (0)%	0 (0%)
Session 2 (Retention Phase)	Number of Animals Tested	14	16	16	16
	Number of Animals Included in Analysis	14	16	16	16
	Trials to criterion	2.2±0.6	2.0±0.0	2.4±0.8	*2.8±0.7
	Latency trial 1 (sec)	169.4±39.6	180.0±0.0	175.4±18.5	*137.7±53.4
	Latency trial 2 (sec)	177.0±11.3	180.0±0.0	174.2±18.9	169.7±28.5
Females					
Session 1 (Learning Phase)	Number of Animals Tested	16	16	16	16
	Number of Animals Included in Analysis	16	16	16	16
	Trials to criterion	3.2±0.5	3.5±0.9	3.3±0.8	3.1±0.6
	Latency trial 1 (sec)	24.8±25.2	20.8±11.9	36.0±42.6	41.8±47.8
	Latency trial 2 (sec)	179.2±3.1	171.8±18.4	179.5±2.1	180.0±0.0
	Failed to Meet Criterion	0 (0%)	0 (0%)	0 (0%)	0 (0%)
	Failed to Cross During Learning Phase	0 (0%)	0 (0%)	1 (6%)	1 (6%)
Session 2 (Retention Phase)	Number of Animals Tested	16	16	15	15
	Number of Animals Included in Analysis	16	16	14	15
	Trials to criterion	2.4±0.6	2.7±1.0	2.6±0.8	2.9±0.9
	Latency trial 1 (sec)	153.2±48.4	162.3±44.5	139.5±66.9	125.1±57.9
	Latency trial 2 (sec)	177.9±8.2	166.9±39.7	170.2±24.8	169.4±31.4

[a] *Data extracted from pages 225-224 of the study report.*
Trials to Criterion = Mean # Trials per Group ± S.D.
Latency to Trial 1 = Mean Session 1 duration (seconds) per Group ± S.D.
Latency to Trial 2 = Mean Session 2 duration (seconds) per Group ± S.D.
Failed to Meet Criterion = Number of Animals that received the shock but did not demonstrate acquisition.
Failed to Cross = Number of Animals that never received the shock.
* *Statistically different from control, $p \leq 0.05$*

TEXT TABLE 17. Water Maze Performance in PND 60 (+2 days) Offspring (mean ± S.D.) [a]

Session/Parameter		Dose (ppm in diet)			
		Control	45 PPM	450 PPM	4500 PPM
Males					
Session 1 (Learning Phase)	Number of Animals	16	16	16	15
	Trials to Criterion (Mean±S.D.)	7.8±3.1	6.8±3.2	7.7±2.8	8.5±3.2
	Trial 1 - Errors (Mean±S.D.)	0.8±1.0	0.3±0.4	0.6±0.7	1.0±1.1
	Trial 1 - Duration (sec) (Mean±S.D.)	18.9±15.0	10.9±4.4	*12.9±7.3	19.7±15.8
	Trial 2 - Errors (Mean±S.D.)	0.3±0.8	0.1±0.3	0.4±0.5	0.3±0.5
	Trial 2 - Duration (sec) (Mean±S.D.)	13.4±9.0	11.8±11.9	13.4±7.4	10.6±6.1
	Failed to Meet Criterion	1 (6%)	0 (0%)	0 (0%)	1 (7%)
Session 2 (Retention Phase)	Number of Animals	15	16	16	14
	Trials to Criterion (Mean±S.D.)	5.6±0.8	5.3±0.8	5.4±0.6	5.5±0.9
	Trial 1 - Errors (Mean±S.D.)	0.4±0.9	0.1±0.3	0.8±1.1	0.3±0.5
	Trial 1 - Duration (sec) (Mean±S.D.)	8.9±5.2	7.3±4.0	17.9±13.9	9.6±5.2
	Trial 2 - Errors (Mean±S.D.)	0.3±0.8	0.0±0.0	0.1±0.3	0.0±0.0
	Trial 2 - Duration (sec) (Mean±S.D.)	6.3±5.6	3.4±1.1	4.8±2.5	3.4±0.7
Females					
Session 1 (Learning Phase)	Number of Animals	16	16	16	16
	Trials to Criterion (Mean±S.D.)	7.4 ± 3.0	7.2 ± 3.0	8.8 ± 3.1	8.7 ± 2.6
	Trial 1 - Errors (Mean±S.D.)	1.3 ± 1.8	0.6 ± 0.7	0.9 ± 1.5	1.5 ± 1.3
	Trial 1 - Duration (sec) (Mean±S.D.)	19.9 ± 17.9	14.5 ± 8.2	17.8 ± 13.7	21.9 ± 12.1
	Trial 2 - Errors (Mean±S.D.)	0.3 ± 0.4	0.8 ± 1.4	0.6 ± 0.9	0.5 ± 0.6
	Trial 2 - Duration (sec) (Mean±S.D.)	13.9 ± 7.1	14.2 ± 14.2	13.1 ± 8.2	10.6 ± 5.0
	Failed to Meet Criterion	0 (0%)	1 (6%)	1 (6%)	0 (0%)
Session 2 (Retention Phase)	Number of Animals	16	15	15	16
	Trials to Criterion (Mean±S.D.)	6.6 ± 2.3	6.1 ± 1.9	6.4 ± 2.1	5.5 ± 0.8
	Trial 1 - Errors (Mean±S.D.)	0.4±0.7	0.3±0.5	0.6±0.8	0.5±0.9
	Trial 1 - Duration (sec) (Mean±S.D.)	9.3 ± 6.9	9.7 ± 4.7	12.0 ± 8.2	8.8 ± 6.2
	Trial 2 - Errors (Mean±S.D.)	0.1±0.5	0.1±0.5	0.3±0.6	0.1±0.3
	Trial 2 - Duration (sec) (Mean±S.D.)	5.0 ± 2.2	5.1 ± 2.8	6.1 ± 3.9	4.2 ± 1.8

[a] *Data obtained from pages 226-227 in the study report.*
Values for rats who failed to learn during session 1 were not included in means for session 2.
Values are mean ± standard deviation * *Statistically different from control, $p \leq 0.05$*

TEXT TABLE 18. Mean (±SD) Brain Weight Data [a]

Parameter	Dose (ppm in diet)			
	Control	45 PPM	450 PPM	4500 PPM
Males				
Day 21(Perfused)				
Terminal Body Weight (g)	47.7±2.4 (10)	50.8±3.3 (10)	45.6±3.8 (10)	43.9*±3.4 (10)
Brain, Fixed (g)	1.432±0.102 (10)	1.441±0.061 (10)	1.368±0.050 (10)	1.313*±0.070 (10)
Brain, Fixed/Body Weight (%)	2.999±0.147 (10)	2.843±0.190 (10)	3.016±0.307 (10)	3.002±0.203 (10)
PND 75 (±5) (Termination - Perfused)				
Terminal Body Weight (g)	327.0±20.3 (10)	310.5±24.5 (10)	299.2*±25.7 (10)	304.7±18.5 (10)
Brain, Fixed (g)	1.829±0.080 (10)	1.868±0.070 (10)	1.789±0.037 (10)	1.827±0.083 (10)
Brain, Fixed/Body Weight (%)	0.561±0.031 (10)	0.604±0.036 (10)	0.602±0.051 (10)	0.601±0.039 (10)
PND 75 (±5) (Termination - Non-Perfused)				
Terminal Body Weight (g)	321.4±15.9 (10)	313.9±33.3 (10)	320.3±15.3 (10)	302.1±27.0 (10)
Brain, Fixed (g)	1.909±0.090 (10)	1.957±0.128 (10)	1.876±0.169 (9)	1.866±0.089 (10)
Brain, Fixed/Body Weight (%)	0.596±0.046 (10)	0.627±0.048 (10)	0.585±0.061 (9)	0.624±0.080 (10)
Females				
Day 21(Perfused)				
Terminal Body Weight (g)	47.2±3.7 (10)	47.3±4.6 (10)	42.3±2.9 (10)	40.0*±7.1 (10)
Brain, Fixed (g)	1.409±0.092 (10)	1.373±0.058 (10)	1.318*±0.049 (10)	1.256*±0.060 (10)
Brain, Fixed/Body Weight (%)	2.992±0.228 (10)	2.920±0.234 (10)	3.129±0.232 (10)	3.241±0.634 (10)
PND 75 (±5) (Termination - Perfused)				
Terminal Body Weight (g)	195.4±11.4 (10)	190.0±11.3 (10)	182.9±8.1 (10)	190.7±18.8 (10)
Brain, Fixed (g)	1.723±0.046 (10)	1.717±0.051 (10)	1.707±0.039 (10)	1.644*±0.084 (10)
Brain, Fixed/Body Weight (%)	0.884±0.056 (10)	0.905±0.043 (10)	0.935±0.043 (10)	0.868±0.072 (10)
PND 75 (±5) (Termination - Non-Perfused)				
Terminal Body Weight (g)	188.4±11.6 (10)	188.0±17.2 (10)	187.2±14.7 (10)	188.9±18.0 (10)
Brain, Fixed (g)	1.793±0.072 (10)	1.811±0.059 (10)	1.751±0.089 (10)	1.702*±0.101 (10)
Brain, Fixed/Body Weight (%)	0.954±0.064 (10)	0.969±0.072 (10)	0.939±0.068 (10)	0.905±0.066 (10)

[a] Data obtained from pages 839-840, 842-843, 845-846 in the study report.
* Statistically different from control, $p \leq 0.05$

TEXT TABLE 19. Histopathology Findings [a]

Parameter	Dose (ppm in diet)			
	Control	45 PPM	450 PPM	4500 PPM
Males				
Gross Measurements				
Day 21				
Ant/Post Cerebrum Length (mm)	13.52±0.37 (10)	13.67±0.25 (10)	13.31±0.22 (10)	13.24±0.23 (10)
Ant/Post Cerebellum (mm)	7.20±0.32 (10)	7.03±0.34 (10)	7.12±0.30 (10)	6.80*±0.19 (10)
PND 75 (±5) (Termination - Perfused)				
Ant/Post Cerebrum Length (mm)	14.56±0.47 (10)	14.69±0.45 (10)	14.45±0.38 (10)	14.04±0.35 (10)
Ant/Post Cerebellum (mm)	7.56±0.38 (10)	7.69±0.17 (10)	7.41±0.31 (10)	7.21*±0.25 (10)
Microscopic Measurements				
PND 21				
Frontal Cortex (mm)	1.728±0.027 (10)	--	1.769±0.006 (10)	1.805±0.012 (10)
Parietal Cortex (mm)	2.008±0.018 (10)	1.918±0.004 (9)	1.901±0.005 (10)	1.922±0.013 (10)
Caudate Putamen (mm)	2.982±0.064 (10)	--	3.097±0.021 (10)	3.052±0.020 (10)
Hippocampal Gyrus (mm)	1.723±0.007 (10)	--	1.673±0.003 (10)	1.541±0.025 (10)
Cerebellum (mm)	4.367±0.077 (10)	4.165±0.046 (10)	4.057±0.019 (10)	4.005±0.033 (10)
PND 75 (±5) (Termination - Perfused)				
Frontal Cortex (mm)	1.760±0.009 (10)	--	1.656±0.010 (10)	1.713±0.004 (10)
Parietal Cortex (mm)	1.863±0.003 (10)	--	1.808±0.005 (10)	1.880±0.004 (10)
Caudate Putamen (mm)	3.345±0.023 (10)	--	3.302±0.012 (10)	3.434±0.012 (10)
Hippocampal Gyrus (mm)	1.758±0.023 (10)	--	1.790±0.064 (10)	1.684±0.021 (10)
Cerebellum (mm)	3.831±0.047 (10)	--	4.380±0.052 (10)	3.618±0.052 (10)

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Parameter	Dose (ppm in diet)			
	Control	45 PPM	450 PPM	4500 PPM
Females				
Gross Measurements				
Day 21				
Ant/Post Cerebrum Length (mm)	13.63±0.34 (10)	13.38±0.52 (10)	13.07*±0.19 (10)	13.00*±0.34 (10)
Ant/Post Cerebellum (mm)	7.13±0.56 (10)	7.06±0.20 (10)	6.83±0.30 (10)	6.65*±0.31 (10)
PND 75 (±5) (Termination - Perfused)				
Ant/Post Cerebrum Length (mm)	14.13±0.39 (10)	14.11±0.31 (10)	14.24±0.29 (10)	13.92±0.29 (10)
Ant/Post Cerebellum (mm)	7.57±0.31 (10)	7.52±0.33 (10)	7.47±0.41 (10)	7.25±0.38 (10)
Microscopic Measurements				
PND 21				
Frontal Cortex (mm)	1.823±0.019 (10)	--	1.763±0.008 (10)	1.749±0.011 (10)
Parietal Cortex (mm)	1.999±0.006 (10)	1.939±0.003 (10)	1.846±0.003 (10)	1.850±0.0131 (10)
Caudate Putamen (mm)	3.029±0.054 (10)	--	2.974±0.0178 (10)	2.915±0.013 (10)
Hippocampal Gyrus (mm)	1.737±0.008 (10)	--	1.696±0.003 (10)	1.575±0.005 (10)
Cerebellum (mm)	4.292±0.048 (10)	4.164±0.072 (10)	4.086±0.063 (10)	3.869±0.022 (10)
PND 75 (±5) (Termination - Perfused)				
Frontal Cortex (mm)	1.761±0.004 (10)	--	1.662±0.003 (10)	1.792±0.005 (10)
Parietal Cortex (mm)	1.855±0.001 (10)	--	1.827±0.003 (10)	1.833±0.002 (10)
Caudate Putamen (mm)	3.386±0.021 (10)	--	3.172±0.020 (10)	3.330±0.008 (10)
Hippocampal Gyrus (mm)	1.631±0.025 (10)	--	1.731±0.019 (10)	1.543±0.024 (10)
Cerebellum (mm)	3.978±0.033 (10)	--	4.488±0.093 (10)	3.756±0.057 (10)

* *Data obtained from pages 848-864 in the study report.*
Values are mean ± standard deviation * *Statistically different from control, $p < 0.05$*
-- = not evaluated

EPA Reviewer: Robert J. Mitkus, PhD **Signature:** [signature]
Registration Action Branch 1, Health Effects Division (7509P) **Date:** 6/5/08
EPA WAM: PV Shah, PhD **Signature:** [signature]
Registration Action Branch 1, Health Effects Division (7509P) **Date:** 6/5/08

TXR#: 0054227

DATA EVALUATION RECORD

STUDY TYPE: NTBC: In vitro inhibition of HPPDase using Liverbeads™ from different species; Non-guideline

PC CODE: 000692 **DP BARCODE:** D335563

TEST MATERIAL (PURITY): NTBC [2-(2-chloro-4- methanesulfonylbenzoyl)-cyclohexane-1,3-dione] (99.7% w/w a.i.)

SYNONYMS: MKH 13222

CITATION: Totis M. (2006). NTBC: In vitro inhibition of HPPDase using Liverbeads™ from different species. Bayer CropScience, Sophia Antipolis Cedex, France. Laboratory Study No.: SA 04276, January 12, 2006. MRID 46801920. Unpublished.

SPONSOR: Bayer CropScience, Monheim, Germany

EXECUTIVE SUMMARY:

In an *in vitro* study (MRID 46801920), mouse, rat, rabbit, dog, and human Liverbeads™ (immobilized hepatocytes) were incubated with buffer, buffer + NTBC (30 µM), buffer + tyrosine (550 µM), or buffer + tyrosine (550 µM) + NTBC (30 µM) for 0, 2, or 4 h at 37°C. The study was designed to test the hypothesis that species differences exist in the production of HPLA, a downstream product of tyrosine catabolism, by hepatocytes incubated with NTBC, a known inhibitor of the liver enzyme 4-hydroxyphenylpyruvate dioxygenase (4-HPPD). One experiment consisted of incubation of Liverbeads™ for one species on one 12-well plate. Experiments were performed in duplicate at each time point. Following incubation, Liverbeads™ preparations were dissolved, hepatocytes were sonicated, and the suspension was transferred to vials for storage at -80°C. Concentrations of L-tyrosine and of *p*-hydroxyphenyl lactic acid (HPLA) were then determined by HPLC analysis.

Baseline HPLA concentrations (µg/mg protein) were below the limit of quantitation (LOQ) at each time point for all species except mouse. HPLA concentrations were increased in mice and human hepatocytes at 2 and 4 h following incubation with NTBC. HPLA concentrations returned to baseline levels when hepatocytes were exposed to basal medium supplemented with tyrosine. When Liverbeads™ were incubated with tyrosine and NTBC, HPLA concentrations were increased at 2 h in rat, mouse, and human and at 4 h in all species except dog. Mean tyrosine concentrations in mouse hepatocytes incubated with L-tyrosine alone were slightly lower than concentrations in hepatocytes measured in the other species tested (Appendix 1).

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Mean tyrosine concentrations in mouse hepatocytes incubated with L-tyrosine + NTBC were increased over those in mouse hepatocytes incubated with L-tyrosine alone (Appendix 1). The results suggest that under basal conditions more HPLA is formed in mouse hepatocytes than in hepatocytes of the other tested species and that inhibition of HPPD by NTBC leads to increased concentrations of HPLA in mouse hepatocytes and detectable concentrations of HPLA in human hepatocytes. The results also suggest that HPLA is formed in hepatocytes of all tested species, except dog, under conditions of increased tyrosine concentration and inhibition of HPPD by NTBC.

The study is classified as **Acceptable/Non-guideline** and is acceptable for assessing the species differences in the production of the tyrosine metabolite HPLA following incubation of hepatocytes with NTBC.

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

Appendix 1. Mean (±SD) tyrosine concentrations (mg/L) in Liverbeads™ from different species[1]

	Time period of incubation	Rat	Dog	Rabbit	Mouse	Human
Basal	0h	23.69 (1.574)	26.22 (2.286)	15.25 (1.673)	29.12 (7.990)	24.33 (4.287)
	2h	25.93 (0.424)	26.47 (2.451)	17.95 (2.192)	20.75 (2.051)	23.03 (2.546)
	4h	25.18 (5.209)	26.22 (6.482)	16.27 (2.923)	21.13 (8.391)	24.40 (2.404)
Basal + NTBC	0h	23.82 (1.768)	26.25 (2.192)	15.35 (1.673)	28.48 (7.849)	24.42 (4.266)
	2h	26.87 (0.189)	27.60 (2.404)	17.30 (0.519)	24.63 (2.263)	24.48 (2.663)
	4h	27.60 (5.893)	27.48 (6.529)	16.85 (2.051)	29.25 (10.347)	27.33 (3.253)
L-Tyrosine	0h	82.17 (13.529)	77.18 (0.919)	74.45 (5.350)	69.45 (2.333)	76.07 (0.283)
	2h	74.42 (2.286)	81.18 (0.863)	74.55 (8.415)	60.63 (4.384)	74.03 (2.404)
	4h	74.78 (1.344)	82.60 (7.684)	78.13 (1.414)	54.02 (9.546)	74.47 (6.411)
L-Tyrosine + NTBC	0h	84.92 (16.806)	78.77 (2.263)	75.60 (2.593)	70.12 (4.643)	74.62 (1.249)
	2h	79.07 (0.283)	82.77 (0.283)	76.05 (11.007)	69.52 (3.371)	76.73 (4.808)
	4h	79.30 (2.451)	81.38 (12.893)	79.77 (0.896)	73.17 (9.051)	77.70 (2.451)

(...) Standard deviation value in brackets

[1] Extracted from p. 18 of MRID 46801920

This Executive Summary was prepared for United States Environmental Protection Agency, Office of Pesticides Programs, Health Effects Division use.

The following text was generated by the Australian Pesticides and Veterinary Medicines Authority. However, this document has undergone critical scientific analysis and been modified as appropriate.

Report:	**KIIA 5.10/01, Totis, M.; 2006**
Title:	NTBC - In vitro inhibition of HPPDase using Liverbeads™ from different species
Laboratory:	Bayer CropScience, 355 rue Dostoievski, BP 153, 06903 Sophia Antipolis Cedex, France
Study duration:	17 November 2004 – 15 April 2005
Report No.:	SA04276
Document No.:	M-264099-01-2
Guidelines:	None
GLP/QA	**Yes**

Introduction

The study authors state that the susceptibility of the rat to development of characteristic "snow flake" corneal lesions after administration of a 4-hydroxyphenylpyruvate dioxygenase (HPPDase) inhibitor is well-known. They also assert that it is known that these lesions are not relevant for human risk assessment, as corneal lesions are only rarely seen in man even with extremely high concentrations of plasma tyrosine, and only approximately 2% of human patients administered 2-(2-nitro-4-fluoromethylbenzoyl)-1,3-cyclohexanedione (NTBC) (an HPPDase inhibitor) at 1-2 mg/kg bw/day over the span of several years ever report corneal lesions. (Reports or data on this issue were not provided.) The study authors hypothesized that this difference between humans and rats was due to their relative ability to produce water-soluble metabolites of tyrosine through an inducible, "emergency exit" pathway that could be activated in the presence of an HPPDase inhibitor. Furthermore, it was hypothesized that the experimental species commonly used in testing agrochemical compounds would fall into two separate categories based on their ability to produce these metabolites. Inability to produce water-soluble metabolites of tyrosine under HPPDase inhibition would lead to increased plasma tyrosine concentrations, while these dramatically-increased concentrations would not be achieved in species which could metabolize tyrosine to forms which could be excreted in the urine. In order to test these hypotheses, in vitro metabolism of tyrosine was examined in preparations of liver cells from rat, rabbit, dog, mouse, and man.

Materials and Methods

NTBC (batch MKH13222-3-2, purity 99.7%) was used as a model inhibitor of the HPPDase enzyme and was dissolved in DMSO for incubation with liver cell preparations from rat, mouse, dog, rabbit, and human. The cell preparations were Liverbeads™, immobilized hepatocytes in an alginate matrix. The preparations were isolated from male Wistar rats, male CD-1 mice, male beagle dogs, male New Zealand white rabbits, and a liver sample from a female human. Liverbeads™ were incubated with buffer, buffer plus NTBC (30 µM), buffer plus tyrosine (550 µM), or buffer plus tyrosine (550 µM) plus NTBC (30 µM). After addition of NTBC or solvent (DMSO only) to the incubation buffer, the plates were incubated for 0, 2, or 4 h at 37°C. At the end of the incubation, the Liverbeads™ were dissolved, hepatocytes were sonicated, and the suspension was transferred to vials for storage at -80°C until analysis. Concentrations of L-tyrosine and of p-hydroxyphenyl lactic acid (HPLA) were determined by HPLC analysis.

Findings

It was stated, although no data were presented, that incubation of Liverbeads™ with 3, 10, or 30 µM NTBC in preliminary experiments led to no visible cytotoxicity. Tyrosine concentrations in the incubation medium did not change over the incubation time, nor was there any effect on tyrosine of adding NTBC to the medium. In basal conditions (no added tyrosine), the concentration of 4-hydroxyphenyl lactic acid (HPLA) was below the limit of quantification in the absence and the presence of NTBC in the rat, dog, and rabbit incubations. Concentrations of HPLA were below the limit of quantification in incubations with human Liverbeads™ in basal conditions; however incubation with NTBC for 2 or 4 h increased HPLA concentration to quantifiable limits in a time-dependent manner. In the mouse incubation, low levels of HPLA were detected in basal conditions but did not increase with time of incubation. Addition of NTBC to the mouse incubation markedly increased HPLA concentrations in a time-dependent manner. When tyrosine was added to basal medium, HPLA concentrations were below the limit of quantification in rat, dog, rabbit, and human. Low levels of HPLA, similar to those observed in basal conditions, were observed in mouse liverbeads incubations. Addition of both tyrosine and NTBC to the incubation medium increased the production of HPLA in nearly all species. However, production of HPLA was low in rat and rabbit, and no HPLA was observed at any time point in dog incubations. By contrast, HPLA concentrations were markedly increased in both mouse and human incubations.

Concentration (µg/mg protein) of HPLA in liver cell incubations from rat, dog, rabbit, mouse, and human

Conditions	Time	Species				
		Rat	Dog	Rabbit	Mouse	Human
Basal	0	< LOQ	< LOQ	< LOQ	0.15	< LOQ
	2	< LOQ	< LOQ	< LOQ	0.25	< LOQ
	4	< LOQ	< LOQ	< LOQ	0.25	< LOQ
Basal + NTBC	0	< LOQ	< LOQ	< LOQ	0.18	< LOQ
	2	< LOQ	< LOQ	< LOQ	0.42	0.33
	4	< LOQ	< LOQ	< LOQ	0.69	0.54
Basal + Tyrosine	0	< LOQ	< LOQ	< LOQ	0.17	< LOQ
	2	< LOQ	< LOQ	< LOQ	0.20	< LOQ
	4	< LOQ	< LOQ	< LOQ	0.26	< LOQ
Basal + Tyrosine + NTBC	0	< LOQ	< LOQ	< LOQ	0.12	< LOQ
	2	0.19	< LOQ	< LOQ	0.73	0.54
	4	0.23	< LOQ	0.36	1.31	1.08

Conclusions and comments

The study authors suggest that these results clearly demonstrate that there are differences among species in their ability to metabolize tyrosine both under basal conditions and in the face of complete inhibition of the tyrosine catabolic pathway. The study authors propose that rat, dog, and rabbit have a very low ability to produce HPLA, a "diversionary" metabolite that enables reduction of plasma tyrosine concentrations. By

contrast, in mice and humans, the production of HPLA was induced by addition of NTBC to the system. The production of HPLA under NTBC inhibition was further increased in these two species, compared to basal conditions, when excess tyrosine was present. However, it is noteworthy that in the rat and rabbit, which otherwise have undetectable levels of HPLA, the addition of tyrosine and challenge by NTBC actually induces a detectable and substantial increase in HPLA levels. Therefore, it could be argued that the "diversionary metabolite" HPLA is indeed capable of being formed even in species like the rat. It is also noteworthy that dogs do not appear to be capable of producing HPLA from tyrosine, but despite this the chronic toxicity feeding study in the dog (Report no. KIIA 5.3.4/01) did not disclose any corneal lesions due to presumed tyrosinemia. This is not consistent with the study author's hypothesis associating corneal lesions with inability to produce HPLA as a "diversionary metabolite".

EPA Reviewer: Robert J. Mitkus, PhD **Signature:** _____
Registration Action Branch 1, Health Effects Division (7509P) **Date:** 6/5/08
EPA WAM: PV Shah, PhD **Signature:** _____
Registration Action Branch 1, Health Effects Division (7509P) **Date:** 6/5/08

TXR#: 0054227

DATA EVALUATION RECORD

STUDY TYPE: Preliminary 28-day toxicity study in mice [dietary]; Non-guideline

PC CODE: 000692 **DP BARCODE:** D335563

TEST MATERIAL (PURITY): AE 0317309 (97.4% w/w a.i.)

SYNONYMS: Pyrasulfotole technical

CITATION: 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.

SPONSOR: Bayer AG, Bayer CropScience, Alfred Nobel Str. 50, Monheim, Germany

EXECUTIVE SUMMARY: 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 (28-day dog toxicity study, 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 was not considered toxicologically significant, in the absence of other evidence of hepatotoxicity and since it was not observed at similar doses in the 90-day toxicity study in the rat. 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.

The LOAEL is 5000 ppm (961/1082 mg/kg/day [M/F]), based on gritty content in the urinary bladder and histopathology (urothelial hyperplasia, diffuse submucosal granulation tissue, diffuse suburothelial mixed-cell infiltrate) in the urinary bladder (males) and subcapsular hyperplasia of the adrenal gland (females). The NOAEL is 1000 ppm (192/233 mg/kg/day [M/F]).

This 28-day oral toxicity study in the mouse is **Acceptable (non-guideline)** as a range-finding study and does not satisfy the guideline requirement for a 90-day oral toxicity study (OPPTS 870.3100; OECD 408) in rodents.

COMPLIANCE: A signed and dated Data Confidentiality statement was provided. Signed and dated GLP and Quality Assurance statements were not provided.

This Executive Summary was prepared for United States Environmental Protection Agency, Office of Pesticides Programs, Health Effects Division use.

The following text was generated by the Australian Pesticides and Veterinary Medicines Authority. However, this document has undergone critical scientific analysis and been modified as appropriate.

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 was not considered toxicologically significant, in the absence of other evidence of hepatotoxicity and since it was not observed in the 90-day toxicity study in the rat. 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

The short-term toxicity of the test substance in the mouse was limited. The treatment related effects observed in this study were limited to gritty content in the urinary bladder of 2/10 males at 5000 ppm, and histopathological findings were urothelial hyperplasia, diffuse submucosal granulation tissue and diffuse suburothelial mixed-cell infiltrata in the urinary bladder of 3/10 males at 5000 ppm.

Based on treatment-related changes affecting the urinary bladder in male animals at the highest dose level of 5000 ppm (equivalent to 961 mg/kg/day) the no observed effect level (NOEL) of the test substance in male c57bl mice over a 28-day period was considered to be 1000 ppm (equivalent to 192 mg/kg/day)".

Based on treatment-related changes affecting the 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).

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Registration Action Branch 1, Health Effects Division (7509P) **Date:** 6/5/08
EPA WAM: PV Shah, PhD **Signature:** [signature]
Registration Action Branch 1, Health Effects Division (7509P) **Date:** 6/5/08

TXR#: 0054227

DATA EVALUATION RECORD

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

PC CODE: 000692 **DP BARCODE:** D335563

TEST MATERIAL (PURITY): AE 0317309 (95.7% w/w a.i.)

SYNONYMS: Pyrasulfotole technical

CITATION: 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.

SPONSOR: Bayer AG, Bayer CropScience, Alfred Nobel Str. 50, Monheim, Germany

EXECUTIVE SUMMARY:

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).

The LOAEL was not observed. The NOAEL is 3000 ppm (500/617 mg/kg/day [M/F]).

This 90-day oral toxicity study in the mouse is **Acceptable (guideline)** and satisfies the guideline requirement for a 90-day oral toxicity study (OPPTS 870.3100; OECD 408) in rodents. Deficiencies in the study include a lack of hematological measurements and a

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lack of urinary pH measurements in high-dose males.

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

This Executive Summary was prepared for United States Environmental Protection Agency, Office of Pesticides Programs, Health Effects Division use.

The following text was generated by the Australian Pesticides and Veterinary Medicines Authority. However, this document has undergone critical scientific analysis and been modified as appropriate.

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).

EPA Reviewer: Robert J. Mitkus, PhD **Signature:** [signature]
Registration Action Branch 1, Health Effects Division (7509P) **Date:** 6/5/08
EPA WAM: PV Shah, PhD **Signature:** PV Shah
Registration Action Branch 1, Health Effects Division (7509P) **Date:** 6/5/08

TXR#: 0054227

DATA EVALUATION RECORD

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

PC CODE: 000692 **DP BARCODE:** D335563

TEST MATERIAL (PURITY): AE 0317309 (95.7% w/w a.i.)

SYNONYMS: Pyrasulfotole technical

CITATION: 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.

SPONSOR: Bayer AG, Bayer CropScience, Alfred Nobel Str. 50, Monheim, Germany

EXECUTIVE SUMMARY:

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).

The LOAEL was not observed. The NOAEL is 3000 ppm (500/617 mg/kg/day [M/F]).

This 90-day oral toxicity study in the mouse is **Acceptable (guideline)** and satisfies the guideline requirement for a 90-day oral toxicity study (OPPTS 870.3100; OECD 408) in rodents. Deficiencies in the study include a lack of hematological measurements and a

PV

lack of urinary pH measurements in high-dose males.

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

This Executive Summary was prepared for United States Environmental Protection Agency, Office of Pesticides Programs, Health Effects Division use.

The following text was generated by the Australian Pesticides and Veterinary Medicines Authority. However, this document has undergone critical scientific analysis and been modified as appropriate.

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).

EPA Reviewer: Robert J. Mitkus, PhD **Signature:** [signature]
Registration Action Branch 1, Health Effects Division (7509P) **Date:** 6/5/08
EPA WAM: PV Shah, PhD **Signature:** P.V. Shah
Registration Action Branch 1, Health Effects Division (7509P) **Date:** 6/5/08

TXR#: 0054227

DATA EVALUATION RECORD

STUDY TYPE: 28-Day Dermal Toxicity - [rat];
OPPTS 870.3200 [§82-2] (rodent); OECD 410.

PC CODE: 000692 **DP BARCODE:** D335563

TEST MATERIAL (PURITY): AE 0317309 (96.3% w/w a.i.)

SYNONYMS: Pyrasulfotole technical

CITATION: Kroetlinger F, Schladt L. (2005). AE0317309 - Subacute toxicity study in the rat (4 weeks dermal administration). Bayer HealthCare AG, Wuppertal, Germany. Laboratory Study No.: T7075087, December 7, 2005. MRID 46801904. Unpublished.

SPONSOR: Bayer CropScience AG, Alfred Nobel Str. 50, Monheim, Germany

EXECUTIVE SUMMARY:

In a 28-day dermal toxicity study (MRID 46801904), AE 0317309 (96.3% w/w a.i., batch Op:1-4) was applied to the shaved skin of 10 Wistar HsdCpb:WU rats/sex/dose at dose levels of 0, 10, 100, or 1000 mg/kg bw/day, 6 hours/day for 5 days/week during a 28-day period.

There were no compound-related effects on mortality, clinical signs (including ophthalmology), body weight, food and water consumption, hematology, or gross pathology. Statistically significant increases in cholesterol (27%), triglycerides (46%), and protein (4%) were observed in males, and total bilirubin (17%) in females at 1000 mg/kg bw/day. Since the observed changes were minor and within the limits of the submitted historical control data, they were not considered toxicologically significant. Absolute liver weights were statistically significantly increased in males (13.9%) and females (11.3%) at 1000 mg/kg bw/day. Hepatocellular hypertrophy and hypertrophy of the pars distalis of the pituitary gland were observed in males at 1000 mg/kg bw/day. Focal degeneration of the pancreas was observed in 2/10 males and 1/10 females at 100 mg/kg bw/day and in 8/10 males and 8/10 females at 1000 mg/kg bw/day. An increased incidence of thyroid follicular cell hypertrophy was seen in high-dose males. Alteration of thyroid colloid was seen with increasing incidence and severity at ≥10 mg/kg bw/day in males. However, since the alteration was predominantly minimal at 10 mg/kg, it was

not considered toxicologically relevant at this dose.

The LOAEL is 100 mg/kg/day, based on focal degeneration of the pancreas (both sexes) and alteration of thyroid colloid (males). The NOAEL was 10 mg/kg/day.

This 28-day dermal toxicity study in the rat is **Acceptable (guideline)** and satisfies the guideline requirement for a 28-day dermal toxicity study (OPPTS 870.3200; OECD 410) in mammals.

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

This Executive Summary was prepared for United States Environmental Protection Agency, Office of Pesticides Programs, Health Effects Division use.

The following text was generated by the Australian Pesticides and Veterinary Medicines Authority. However, this document has undergone critical scientific analysis and been modified as appropriate.

Report:	KIIA 5.3.7/01, Kroetlinger, F.; Schladt, L.; 2005
Title:	AE0317309 - Subacute toxicity study in the rat (4 weeks dermal administration)
Test Substance:	AE 0317309: Pyrasulfotole - batch Op: 1-4, purity 96.3% w/w
Laboratory:	Bayer HealthCare AG, PH-R&D Toxicology, 42096 Wuppertal, Germany
Report No.: Document No.:	AT02678 M-263216-01-2
Study Duration:	03 February to 07 December 2005
Guidelines:	OECD 410 (1981); EEC Directive 67/548 - Annex V - Method B9 (1992); US EPA, OPPTS Series 870, Health Effects Test Guidelines, No. 870.3200 (1998); MAFF JAPAN 12 NohSan No 8147 (2001)
GLP/QA	Yes

Materials and Methods

Test substance was moistened with tap water and applied under a semi-occlusive bandage to the shorn skin of 10 male and 10 female Wistar rats (strain HsdCpb:WU) per group randomly allocated to four treatment groups at 0, 10, 100, and 1000 mg/kg bw/day for 4 weeks. At the start of the study the males were 9-10 weeks of age and weighed 222-262 g and the females were 13-14 weeks of age and weighed 202-230 g. They were fed commercial rat diet and supplied with water in carbonate bottles *ad libitum*.

One day before the start of treatment the back and flanks of the rats were shorn. The required amount of undiluted solid test substance was weighed out, moistened with tap water and transferred to a moist gauze-layer of area 30 cm^2. The test substance was applied five days per week for the first three weeks of the study, and seven days per week for the final week, so that all rats were dosed daily for at least the last 8 days of the study. On each treatment day, the application period was 6 h, following which the remaining test substance was washed off with water. Control rats were treated with tap water.

Clinical signs were monitored twice daily on weekdays and once daily on weekends and holidays. Ophthalmoscopic examinations were conducted prior to the start of treatment and at the end of the treatment period, in all high-dose and control rats. Body weight and food and water consumption were measured on a weekly basis.

The shaved area of the skin was examined on each day of treatment. The skin evaluations were conducted on study days 1, 4, 8, 11, 15, 18, 22, 25 and 29. The time of day was not specified. Erythema of the skin was scored according to Draize scale (1959). Skinfold thickness (swelling) was measured at two different locations within the treatment area using skin-fold calipers.

Blood for glucose measurement was collected from non-fasted and non-anesthetized animals by caudal vein puncture. Blood for hematology and other clinical chemistry determinations was collected from non-fasted animals by retro-orbital venus plexus puncture under light carbon dioxide anesthesia, 4 days and 2 days before necropsy for

males and females, respectively.

WBC count, differential blood count, RBC, Hb, Hct, MCV, MCH, MCHC, thrombocytes, reticulocytes and clotting time (hepatoquick) were determined. In addition, the following clinical chemistry parameters were determined: AP, ALT, AST, albumin, protein (total), cholesterol, creatinine, glucose, urea, bilirubin (total), triglycerides, sodium, inorganic phosphate, potassium, calcium and chloride.

At termination, rats were sacrificed by exsanguination under ether anesthesia and were subjected to gross necropsy. Adrenal glands, kidneys, liver, brain, testes epididymides, heart, ovaries, spleen, thyroid gland, thymus and uterus were weighed in the unfixed state. The organs that were fixed and evaluated histologically were adrenal glands, aorta, brain (cerebrum, cerebellum, brain stem), epididymides, eosophagus*, eyes, eyelids, exorbital lacrimal glands, femur, Harderian glands, head (nasal cavity), intestine (incl Peyer's Patches, duodenum, jejunum, ileum, caecum, colon, rectum, remaining intestine), kidneys, larynx, liver*, lungs, lymph nodes (mandibular, mesenteric), optic nerves, ovaries, oviducts, pancreas*, pharynx, pituitary gland*, prostate, salivary glands (parotid, submandibular, sublingual), sciatic nerve, seminal vesicles (incl. coagulation glands), skeletal muscle (thigh), skin (mammary region), spinal cord (cervical, thoracic, lumbar), spleen, sternum, stomach (forestomach and glandular stomach), testes, thymus, thyroid glands (with parathyroids)*, tongue, trachea*, ureters, urethra, urinary bladder*, uterus (with cervix), vagina, Zymbal's gland, skin - treated, skin - untreated, and all organs and tissues with macroscopic findings*. In all groups, those organs or tissues marked with an asterisk (above) were examined histologically. The remaining organs and tissues were examined only in groups 1 and 4 (0 and 1000 mg/kg bw/day respectively).

Findings

Depending on the body weight of the rats and the surface area of the gauze covered by the test item layer (10 mg/kg: 0.25 cm^2, 100 mg/kg bw: 2.25 - 4.0 cm^2, 1000 mg/kg: 14.0 - 18.0 cm^2 in males; and 10 mg/kg: 0.25 cm^2, 100 mg/kg: 2.25 - 4.0 cm^2, 1000 mg/kg: 14.0 cm^2 in females) the mean amount of test item listed in the following table was applied per cm^2 skin.

Applied weekly doses of test substance

Applied doses (mg/cm^2 skin)				
Dose (mg/kg bw)	**Week 1**	**Week 2**	**Week 3**	**Week 4**
Males				
10	9.6	11.2	12.0	12.8
100	10.8	8.9	7.3	7.7
1000	15.5	19.8	16.9	18.0
Females				
10	8.0	8.0	8.4	8.4
100	9.5	7.3	7.5	5.7
1000	15.0	15.2	15.7	16.1

Mortalities and Clinical Signs: There were no mortalities during the study. There were

no treatment related clinical signs or ophthalmological abnormalities in rats of either sex.

Local skin findings: No erythema or increases in skin-fold thickness were observed.

Food and water consumption: There were no treatment-related effects on food and water consumption.

Body weight: There were statistically significant decreases (males at 10 and 100 mg/kg bw/day) or increases (females at 10 mg/kg bw/day) in body weight on day 15 and 22. However, as these findings showed no relationship to dose and were largely temporary, they were considered not to be treatment-related.

Body weight in rats administered test substance by dermal application

Observation day	Dose (mg/kg bw/day)							
	Males				Females			
	0	10	100	1000	0	10	100	1000
1	248	241	241	247	213	217	213	210
8	283	268	268	278	217	222	219	214
15	310	290*	289*	304	219	228*	225	220
22	332	308*	307*	323	225	235*	227	226
29	341	317	316	330	223	231	224	223

* $p < 0.05$.

Haematology: There were no treatment related effects on haematological parameters.

Clinical chemistry: No treatment related changes in clinical chemistry parameters were observed. Statistically significant increases in cholesterol (27%), triglycerides (46%), and protein (4%) were observed in males, and total bilirubin (17%) and calcium in females at 1000 mg/kg bw/day. Statistically significant decreases in AST (24%), AP (21%), creatinine (7%), and sodium in males and sodium in females were observed at 1000 mg/kg.

Historical control data for the HsdCpb:WU strain of the Wistar rat were provided. On the basis that the observed changes were minor and within historical control data limits, they were not considered toxicologically significant.

Selected clinical chemistry parameters in rats administered test substance by dermal application

Parameter	Dose (mg/kg bw/day)							
	Males				Females			
	0	10	100	1000	0	10	100	1000
AST, IU/L	79.0	69.8	70.3	60.4*	81.0	72.6	66.2	71.2
ALT, IU/L	57.9	57.5	59.1	56.0	55.8	59.2	57.2	57.2
AP, IU/L	217	217	209	172**	134	133	111	114
Glucose, mmol/L	5.51	5.43	5.43	5.36	5.49	5.41	5.41	5.32
Cholesterol, mmol/L	1.61	1.73	1.71	2.04*	1.73	1.74	1.76	1.75
Triglycerides, mmol/L	1.36	1.53	1.36	1.99*	1.26	1.24	1.19	1.26
Creatinine, µmol/L	55	55	54	51*	59	59	57	56
Urea, mmol/L	7.54	7.55	7.79	7.38	6.53	6.84	6.39	6.49

Total bilirubin, µmol/L	1.1	0.9	1.1	1.0	1.2	1.3	1.4	1.4*
Protein, g/L	64.9	65.4	65.8	67.7*	66.2	68.4	66.4	68.5
Albumin, g/L	33.6	34.8	34.5	35.3*	35.9	37.1	36.2	37.5
Na, mmol/L	144	144	143*	142**	144	144	143	142**
K, mmol/L	6.3	6.6	6.5	6.6	6.3	6.4	6.2	6.4
Ca, mmol/L	2.80	2.74	2.78	2.81	2.65	2.70	2.68	2.76**
Cl, mmol/L	95	93	95	93	97	97	97	96
P, mmol/L	3.18	2.90*	2.85*	2.88*	2.28	2.23	2.07	2.12

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

Gross necropsy:

Absolute and relative liver weights were statistically significantly increased in both sexes at 1000 mg/kg bw/day. Since there were no significant changes in body weight over the dose range tested, it is apparent that liver weight increased substantially in both sexes (absolute liver weights: 13.9% in males 11.3% in females) at the highest dose. This effect appears to be treatment related. The statistically significant increase in absolute liver weight in females at 10 mg/kg bw/day was not considered treatment related, since the low-dose group had the highest mean body weight of the female groups, the relative liver weight of this group was not statistically different from controls, and dose response was lacking. No other treatment related changes were observed at gross necropsy.

Absolute and relative liver weight in rats administered test substance by dermal application.

Parameter	Dose (mg/kg bw/day)							
	Males				Females			
	0	10	100	1000	0	10	100	1000
Body weight, g	341	317	316	330	227	234	227	226
Brain weight, mg	1986	1980	1927	1951	1865	1881	1852	1901
Brain weight, mg/100g body wt	583	630	611	592	824	806	818	843
Liver weight, mg	14422	13904	13710	16434*	8490	9199*	8502	9453**
Liver weight, mg/100g body wt	4227	4378	4329	4975**	3748	3942	3750	4189**

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

Histopathology:

There was no evidence of any reaction at the application sites. Treatment related histopathological findings were observed in the pancreas, thyroid, and liver.

The table below shows that focal degeneration of the pancreas was observed in 2/10 males and 1/10 females at 100 mg/kg bw/day and in 8/10 males and 8/10 females at 1000 mg/kg bw/day. An increased incidence of thyroid follicular cell hypertrophy was seen in high-dose males. Alteration of thyroid colloid was seen at an increased incidence and severity at ≥10 mg/kg bw/day in males. However, since the alteration was predominantly

minimal at 10 mg/kg, it was not considered toxicologically relevant at this dose.

Hepatocellular hypertrophy and hypertrophy of the pars distalis of the pituitary gland were observed in males at 1000 mg/kg bw/day. Hepatocellular hypertrophy was not reported for females. All other histopathological findings were not considered to be treatment related.

Incidence of selected histopathological findings in rats administered test substance by dermal application.

Organ	Finding and severity	Dose (mg/kg bw/day)			
		0	10	100	1000
Males					
	Number in the group	10	10	10	10
Pancreas	Degeneration, focal, total	0	0	2	8
	Grade 1	0	0	2	7
	Grade 2	0	0	0	1
Thyroid	Follicular cell hypertrophy, total	5	5	4	9
	Grade 1	0	0	0	0
	Grade 2	5	5	2	9
	Grade 3	0	0	2	0
	Colloid alteration, total	1	4	6	10
	Grade 1 (minimal)	1	3	1	3
	Grade 2 (slight)	0	1	4	6
	Grade 3 (moderate)	0	0	1	1
Liver	Hepatocellular hypertrophy, total	0	0	0	7
	Grade 2	0	0	0	7
Pituitary	Pars distalis hypertrophy, total	0	0	0	5
	Grade 1	0	0	0	3
	Grade 2	0	0	0	2
Females					
	Number in the group	10	10	10	10
Pancreas	Degeneration, focal, total	0	0	1	8
	Grade 1	0	0	1	6
	Grade 2	0	0	0	2
Thyroid	Follicular cell hypertrophy, total	4	1	1	4
	Grade 1	1	1	0	4
	Grade 2	3	0	1	0
	Colloid alteration, total	0	1	0	1
	Grade 1	0	1	0	1

Conclusions

Following repeated dermal application of test substance to rats, there were no signs of skin irritation or increase in skin thickness. On this basis the local NOEL was determined to be 1000 mg/kg bw/day.

Based on increased incidence of histopathological alterations in the pancreas of both

sexes at ≥100 mg/kg bw/d and alteration in thyroid colloid in males at and above 100 mg/kg bw/d, the NOEL for systemic effects was 10 mg/kg bw/d in males and females.

EPA Reviewer: Robert J. Mitkus, PhD **Signature:** [signature]
Registration Action Branch 1, Health Effects Division (7509P) **Date:** 6/5/08
EPA WAM: PV Shah, PhD **Signature:** [signature]
Registration Action Branch 1, Health Effects Division (7509P) **Date:** 6/5/08

TXR#: 0054227

DATA EVALUATION RECORD

STUDY TYPE: Tyrosine: Exploratory 14-day (ocular toxicity) study in the rat and mouse; Non-guideline

PC CODE: 000692 **DP BARCODE:** D335563

TEST MATERIAL (PURITY): L-tyrosine (98% w/w a.i.)

CITATION: Esdaile DJ. (1995). Tyrosine: Exploratory 14-day (ocular toxicity) study in the rat and mouse. Rhone-Poulenc Agrochimie, Sophia Antipolis Cedex, France. Laboratory Study No.: SA 94100, December 20, 1995. MRID 46801922. Unpublished.

SPONSOR: Rhone-Poulenc Agrochimie, Lyon Cedex, France

EXECUTIVE SUMMARY:

In an *in vivo* study (MRID 46801922), 5 Crl:CD(SD)BR rats, 5 Brown Norway rats (BN/Crl BR), and 5 Crl:CD-1(ICR)BR mice/sex (6 weeks old) were administered 0%, 2%, or 5% L-tyrosine (batch# 68160-123) in the diet for 14 days (equivalent to 0/0, 2240/1900, or 5600/4750 mg/kg bw/day for SD rats; 0/0, 2443/2182, or 6107/5455 mg/kg bw/day for Brown Norway rats; and 0/0, 4000/4800, or 10000/12000 mg/kg bw/day for mice, respectively [M/F][1]). The study was designed to determine the relative sensitivities of SD rats, Brown Norway rats, and CD-1 mice to the development of corneal lesions after consuming diet supplemented with tyrosine. Clinical signs and mortality were monitored daily. Body weights and food consumption were measured weekly. Ophthalmoscopic examinations were reported for study days 2, 3, 7, 8, and 14. Blood was collected on study day 15 prior to necropsy for determination of plasma tyrosine concentration. Animals were sacrificed by exsanguination under deep anesthesia and subjected to a gross necropsy. Histological sections of the eyes of selected animals were prepared. Plasma samples from all male rats, control and high-dose female SD rats, and control and high-dose male mice were analyzed for tyrosine concentrations.

There were no treatment-related effects on mortality, body weight, or food consumption or at necropsy. Treatment-related clinical signs in SD rats at 5% tyrosine included dark urine in all males in the second week of the study and in 3 females on day 14 only. Corneal opacity was observed in 1/5 male SD rats at 5% tyrosine. This same animal appeared thin and showed slight

[1] Estimated using approximate day 14 food consumption values of 28/19, 16/12, and 6/6 grams per day (M/F) and approximate day 8 body weight values of 250/200, 131/110, and 30/25 grams (M/F) for SD rats, Brown Norway rats, and mice, respectively. Food consumption was comparable on days 0 and 14.

ptosis and moderate piloerection from Days 10 and 13, respectively. At ophthalmoscopic examination, all male SD rats at 5% tyrosine showed "snow flake" corneal opacities by day 7 with progression of the opacity over time. By day 14, two male SD rats also showed signs of edema and vascularization of the cornea. In 3/5 SD males, congestion of the iris was also evident. One male Brown Norway rat at 5% tyrosine had a slight "snow flake" corneal opacity visible on day 14 only. Male SD and Brown Norway rats showed a dose-related increase in mean plasma tyrosine concentrations. One high-dose male Brown Norway rat, which was observed on day 14 to have a corneal opacity, was found to have plasma tyrosine concentrations markedly higher than those observed in the other four male Brown Norway rats. Male CD-1 mice showed no increase in plasma tyrosine concentrations at 5% dietary tyrosine. No changes in plasma tyrosine concentrations were observed in females; however, plasma tyrosine concentrations were only determined for control and high-dose SD females rats. In 2/2 male SD rats at 5% tyrosine, treatment-related microscopic findings included inflammatory reactions in the cornea, edematous or swollen nuclear changes in the corneal epithelial cells, and corneal vacuolation. In 1/2 Brown Norway rats treated with 5% tyrosine, microscopic findings included inflammation of the anterior chamber and cornea. A focal area of moderate vacuolation of the corneal epithelial cells was observed in 1/2 male Brown Norway rats treated at 2%. This was not observed in 2/2 control Brown Norway males or in this animal at ophthalmoscopic examination.

The study is classified as **Acceptable/Non-guideline** and is acceptable for determining the relative sensitivities of SD rats, Brown Norway rats, and CD-1 mice to the development of corneal lesions after consuming diet supplemented with tyrosine.

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

This Executive Summary was prepared for United States Environmental Protection Agency, Office of Pesticides Programs, Health Effects Division use.

The following text was generated by the Australian Pesticides and Veterinary Medicines Authority. However, this document has undergone critical scientific analysis and been modified as appropriate.

Report:	**KIIA 5.10/03, Esdaile D.J.; 1995**
Title:	Tyrosine: Exploratory 14-day (ocular toxicity) study in the rat and mouse
Laboratory	Rhone-Poulenc Agrochimie, Centre de Recherche, 355, rue Dostoievski, BP 153, F-06903 Sophia Antipolis Cedex, France
Study duration	9 March 1994 – 28 July 1994
Report No.:	SA94100
Document No.:	M-210983-01-1
Guidelines:	None
GLP/QA	**Yes**

Introduction

The aim of this investigation was primarily to compare the development of corneal opacities in rats and mice as a result of administration of diets containing dietary tyrosine. A secondary objective was to determine whether there was a difference in sensitivity between strains of rats.

Materials and Methods

L-tyrosine (batch 68160, purity 98%) was incorporated into rodent diet at 0%, 2%, and 5% and fed to groups of 5 male and 5 female CD rats, Brown Norway rats, and CD-1 mice for a period of 14 days. The animals were housed individually in suspended stainless steel wire cages and provided food and water *ad libitum* throughout the study period. Clinical signs and moribundity were monitored daily. Body weights and food consumption were measured on a weekly basis. Ophthalmoscopic examinations were conducted during acclimatization and on study days 2, 3, 7, 8, and 14. Blood was collected on study day 15 prior to necropsy for determination of plasma tyrosine concentration. Animals were sacrificed by exsanguination under deep anesthesia and subjected to a gross necropsy. Histological sections of the eyes of selected animals were prepared. Plasma samples were analyzed from all male rats, control and high-dose female CD rats, and control and high-dose male mice. It was stated that groups were selected on the basis of the presence of eye lesions and where comparisons between control and treated groups were "considered to be useful."

Findings

Mortalities: There were no mortalities during the study.

Clinical signs: Treatment-related clinical signs were observed only in the CD rats receiving 5% tyrosine, with dark urine seen in all males in the second week of the study and in 3 females on day 14 only. Corneal opacity was observed at clinical examination in one of the male CD rats receiving 5% dietary tyrosine. This same animal appeared thin and showed **slight** ptosis and **moderate** piloerection from Days 10 and 13, respectively.

Body weight: There was no effect on body weight in any group.

Food consumption: There were no treatment-related effects.

Ophthalmoscopic findings: No corneal opacities were observed in female rats or in either male or female mice. Corneal opacities were not seen in male rats of either strain at 2% dietary tyrosine. All male CD rats at 5% tyrosine showed corneal opacities by day 7, with progression of the opacity over time. By day 14, two male CD rats also showed signs of edema and vascularisation of the cornea. In three animals, congestion of the iris was evident. One male Brown Norway rat at 5% tyrosine had a slight corneal opacity only visible on day 14. In all cases, the corneal opacities were the characteristic "snow flake" opacities.

Plasma tyrosine concentrations: In both CD and Brown Norway rats, males showed a dose-related increase in plasma tyrosine concentration. In female rats, plasma tyrosine concentrations were only determined for CD rats at 0% and 5% dietary tyrosine. Male CD-1 mice showed no increase in plasma tyrosine concentrations after 2 weeks administration of 5% dietary tyrosine. Plasma tyrosine concentrations were not determined in male mice at 2% dietary tyrosine, or in female mice at any dose level. The male Brown Norway rat which was observed on day 14 to have a corneal opacity was found to have plasma tyrosine concentrations markedly higher than those observed in the other 4 male Brown Norway rats.

Plasma tyrosine concentrations and incidence of corneal opacities in rats and mice

Species and strain	Dietary tyrosine, %	Males		Females	
		Plasma tyrosine, nmol/mL	Corneal opacities	Plasma tyrosine, nmol/mL	Corneal opacities
CD rat	0	115.9	No	71.7	No
	2	325.6	No	-[a]	-
	5	629.1	Yes	342.2	No
Brown Norway rat	0	66.2	No	-	
	2	176.6	No	-	-
	5	927.2	Yes	-	-
CD-1 mice	0	71.7	No	-	-
	2	-	-	-	-
	5	99.3	No	-	-

[a] Data not collected

Macroscopic findings: There were no treatment-related findings at gross necropsy.

Microscopic findings: In CD rats which had corneal opacities, treatment-related microscopic findings included inflammatory reactions in the cornea, edematous or swollen nuclear changes in the corneal epithelial cells, and corneal vacuolation. In the Brown Norway rat treated with 5%, the microscopic findings included inflammation of the anterior chamber and cornea. One focal area of moderate vacuolation of the corneal epithelial cells was observed in 1/2 male Brown Norway rats treated at 2%. This was not observed in 2/2 control Brown Norway males.

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Conclusions and comment

The study authors suggest that this study demonstrates that male rats are more sensitive than female rats to the development of a plasma tyrosine concentration sufficient to lead to corneal opacities after feeding tyrosine in the diet. This may be consistent with the observation in the chronic study in the rat using pyrasulfotole (Report no. KIIA 5.5.2/01) that the females developed ocular pathology more slowly than the males; although there are a range of possible explanations for this. Also, male rats are more sensitive to the effects of dietary tyrosine on plasma tyrosine concentrations than are male mice. This lack of an effect of dietary tyrosine on plasma levels in mice precludes any conclusions as to the effect of tyrosinemia on the development of ocular pathology in mice. It may also be concluded from the reported results that microscopic, rather than ophthalmoscopic, evaluation of the eye may be a more sensitive method of detecting tyrosine-related ocular effects, since vacuolation of the corneal epithelial cells was observed in 1/2 male Brown Norway rats treated at 2%, whereas corneal opacity was not.

It is noteworthy that no evidence of retinopathy was found in this study, which was a finding in the chronic study in the rat using pyrasulfotole (Report no. KIIA 5.5.2/01). However the relatively short duration of this study and the small number of animals involved may have prevented the development of the full range of ocular effects.

EPA Reviewer: Robert J. Mitkus, PhD **Signature:** Robert J. Mitkus
Registration Action Branch 1, Health Effects Division (7509P) **Date:** 3/25/09
EPA WAM: PV Shah, PhD **Signature:** PV Shah
Registration Action Branch 1, Health Effects Division (7509P) **Date:** 3/25/09

TXR#: 0054227

DATA EVALUATION RECORD

STUDY TYPE: Subchronic Neurotoxicity, OPPTS 870.6200b [§82-7] [dietary] - [rat]; (No OECD guideline).

PC CODE: 000692 **DP BARCODE:** D335563

TEST MATERIAL (PURITY): AE 0317309 (95.7% w/w a.i.)

SYNONYMS: Pyrasulfotole technical

CITATION: Gilmore RG, Hoss HE. (2005). A subchronic neurotoxicity screening study with technical grade AE 0317309 in Wistar rats. Bayer CropScience LP, Stilwell, KS. Laboratory Study No.: 04-N72-VU, December 16, 2005. MRID 46801916. Unpublished.

SPONSOR: Bayer CropScience LP, Research Triangle Park, NC

EXECUTIVE SUMMARY:

In a subchronic neurotoxicity study (MRID 46801916), AE 0317309 (95.7% a.i., batch Op. 1-4) was administered to 12 eight-week-old Wistar rats/sex/group at dose levels of 0, 500, 2500, or 5000 ppm (equivalent to 0/0, 32/42, 166/206, or 345/416 mg/kg bw/day in males/females) for 90 days. Clinical signs were assessed twice daily on weekdays and once daily on weekends and holidays. Body weight and food consumption were measured on a weekly basis. Neurobehavioral assessment (functional observational battery and motor activity testing) was performed in all animals/sex/group once in the week prior to the start of the feeding period, and once each during weeks 2, 4, 8, and 13. The FOB included standard parameters including home cage and open field observations, reflex testing, and determination of fore-limb and hind-limb grip strength, and landing foot splay. Motor and locomotor activity were measured over a 60-minute period for each animal. Ophthalmoscopic examinations were conducted in all animals prior to the start of the study and during week 12. At study termination, all animals were euthanized and 6 rats/sex/dose were perfused *in situ* for neuropathological examination. Of the perfused animals, all were subjected to histopathological evaluation of brain and peripheral nervous system tissues.

There were no treatment-related effects on mortality, body weight, brain weight, gross pathology, or microscopic neuropathology. The FOB and loco/motor activity assessments revealed no toxicologically significant effects. During clinical observations, an increased incidence of urine staining was observed in all treated male and female groups (Appendix 1), however without a relationship to dosing in males. Because the urine was not analyzed, the

cause of the staining is unclear, but is hypothesized to be related to unmetabolized parent compound and therefore not toxicologically significant. Similar staining was reported in high-dose animals only during the FOB. Food consumption was slightly increased (≤12%) at 5000 ppm at several intervals, but was not considered toxicologically significant in the absence of adverse effects on any other parameter, including body weight. Increased incidences of corneal opacity (2, 3, and 1 vs. 0 in controls) and corneal neovascularization (1, 2, and 1 vs. 0 in controls) were observed in all treated females. Incidences of retinal degeneration were dose-dependently increased in females (1, 2, and 3 vs. 1 in controls), but not males. The increased incidence of corneal neovascularisation was considered toxicologically significant at the low dose, since the incidence was 0 in control females at ophthalmoscopy in the 90-day toxicity study in rat and 0 in control females at ophthalmoscopy at 6 and 12 months in the chronic toxicity study in rats.

The LOAEL was 500 ppm (42 mg/kg bw/day) in females based on increased incidences of corneal opacity and corneal neovascularization in females and not observed in males. The NOAEL was not observed in females and was 5000 ppm (345 mg/kg bw/day) in males.

The study is classified as **Acceptable (guideline)** and satisfies the guideline requirement for a subchronic neurotoxicity study in rats (870.6200b).

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

Appendix 1. Summary of incidences of clinical observations[1]

MALES[a]

Signs	Nominal Dietary Level (PPM)			
	0	500	2500	5000
URINE STAIN				
YELLOW	-[b]	1(18-25)	-	6(11-88)
BROWNISH, ORANGE	-	1(32-88)	-	5(32-88)
ALOPECIA				
LIMBS-FORE, BOTH	-	-	1(18-88)	-

FEMALES[a]

URINE STAIN				
YELLOW	-	-	2(25,39-88)	7(4-88)
BROWNISH, ORANGE	-	-	-	5(39-88)
BODY SWOLLEN				
PERIGENITAL AREA	-	-	1(46-88)	-

[a] *Incidence for n=12 (range of days observed - observations performed once weekly).*
[b] *Observation not observed.*

This Executive Summary was prepared for United States Environmental Protection Agency, Office of Pesticides Programs, Health Effects Division use.

The following text was generated by the Australian Pesticides and Veterinary Medicines Authority. However, this document has undergone critical scientific analysis and been modified as appropriate.

[1] Reproduced from p. 42 of MRID 46801916

Report:	**KIIA 5.7.4/01, Gilmore, R. G.; Hoss, H. E.; 2005**
Title:	A subchronic neurotoxicity screening study with technical grade AE 0317309 in Wistar rats
Laboratory	Bayer CropScience LP Toxicology, 17745 South Metcalf Ave, Stilwell, KS 66085-9104, USA
Study duration	2 August 2004 – 17 November 2004
Report No.:	04-N72-VU
Document No.:	M-262680-01-1
Guidelines:	**U.S. EPA OPPTS 870.6200** **Japanese Testing Guideline; Notification No. 12-Nousan-8147** **OECD TG 424** **Health Canada, PMRA DACO No. 4.5.11**
GLP/QA	**Yes**

Materials and Methods

AE 0317309 (batch Op. 1-4, purity 95.7%) was incorporated into rodent meal at concentrations of 0, 500, 2500, and 5000 ppm and provided to 12 male and 12 female Wistar rats per group for 90 days. The dietary concentrations of AE 0317309 (pyrasulfotole) prepared provided doses of approximately 0, 32, 166, and 345 mg/kg bw/day for males and 0, 42, 206, and 416 mg/kg bw/day for females. The homogeneity, stability and concentration (throughout the study) of the test substance in the feed were confirmed. The rats were no more than 8 weeks old at the beginning of treatment, with body weights of 194.0-261.7 g for males and 137.0-183.2 g for females. The animals were housed individually in suspended stainless steel wire-mesh cages and provided diet and water *ad libitum* except during neurobehavioral testing. Clinical signs were assessed twice daily on weekdays and once daily on weekends and holidays. Body weight and food consumption were measured on a weekly basis. The FOB (functional observational battery) and motor activity were assessed in all animals on five occasions, once in the week prior to the start of the feeding period, and once each during weeks 2, 4, 8, and 13. The FOB included standard parameters including home cage and open field observations, reflex testing, and determination of fore-limb and hind-limb grip strength, and landing foot splay. Motor and locomotor activity were measured according to standard methods over a 60-minute period for each animal. Ophthalmoscopic examinations were conducted in all animals prior to the start of the study and during week 12. After at least 90 days dietary administration of AE 0317309, all animals were sacrificed. Half of the animals at each dose level were anesthetized and perfused via the left ventricle with a sodium nitrite flush followed by fixative in phosphate buffer. The brain was weighed, and the brain, spinal cord, eyes with optic nerve, sciatic, tibial, and sural nerves, gasserian ganglion, gastrocnemius muscle, both forelimbs, and any gross lesions were removed and preserved for examination. The other half of the animals at each dose level were sacrificed by CO_2 asphyxiation and were not perfused. All animals were subjected to a gross necropsy. Histopathology was conducted on the nervous tissues from the 6 perfused animals in the control and high dose groups only.

Findings

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Mortalities: There were no mortalities during the study.

Clinical signs: The treatment-related clinical signs observed in this study were limited to urine staining in both males and females, with indications of a dose-response relationship in the case of yellow urine stains in females.

Staining observed in rats administered AE 0317309 by dietary incorporation for 90 days

Observation	Dose (ppm)			
	0	500	2500	5000
Males				
N	12	12	12	12
Urine stain, yellow	0	1	0	6
Urine stain, brownish-orange	0	1	0	5
Females				
N	12	12	12	12
Urine stain, yellow	0	0	2	7
Urine stain, brownish-orange	0	0	0	5

Food consumption: Food consumption was statistically significantly increased in males at 5000 ppm on days 21-28, days 28-35, days 70-77 and days 77-84.

Food consumption in rats administered AE 0317309 by dietary incorporation

Days	Dose (ppm)							
	Males				Females			
	0	500	2500	5000	0	500	2500	5000
0-7	22.45	22.28	23.53	24.20	17.50	17.40	16.88	16.79
7-14	22.20	22.34	23.32	24.63	18.23	18.30	17.86	18.68
14-21	22.70	22.59	22.51	24.46	18.08	19.08	18.21	19.22
21-28	21.80	22.23	21.67	24.39*	17.27	18.21	17.32	17.55
28-35	22.02	22.96	21.55	24.21*	17.83	18.78	18.32	18.55
35-42	21.80	22.29	20.88	23.81	16.94	17.97	17.66	17.82
42-49	21.35	22.04	20.57	22.96	16.24	16.60	16.45	17.49
49-56	20.37	21.05	20.27	21.28	15.88	16.58	16.45	16.81
56-63	20.11	20.99	20.32	22.25	16.42	17.01	17.21	17.03
63-70	20.88	21.21	20.15	22.78	16.95	17.32	17.18	17.69
70-77	20.36	21.24	20.18	22.51*	16.38	16.63	17.09	17.56
77-84	20.09	21.48	20.55	22.41*	16.28	16.97	16.68	17.07
84-91	19.76	20.60	20.15	21.90	16.74	17.02	16.16	17.69

* $p < 0.05$.

Body weight and body weight gain: There was no treatment-related effect on body weight in either males or females at any dose level.

Ophthalmoscopy: There were increased incidences of corneal opacity and corneal neovascularization in the treated groups of females and of retinal degeneration in some treated groups in both sexes (cf. Study no. KIIA 5.7.5/01) relative to controls. Increased incidences of retinal degeneration in females were dose-dependent. The increased

incidence of corneal neovascularisation at the low dose was considered toxicologically significant, since the incidence was 0 in control females at ophthalmoscopy in the 90-day rat study and 0 in control females at ophthalmoscopy at 6 and 12 months in the chronic toxicity study in rats.

Ocular findings in rats administered AE 0317309 by dietary incorporation for 90 days

Observation	Dose (ppm)			
	0	500	2500	5000
Males				
N	12	12	12	12
Corneal opacity	0	0	0	0
Retinal degeneration	1	3	1	2
Corneal neovascularization	0	0	0	0
Females				
N	12	12	12	12
Corneal opacity	0	2	3	1
Retinal degeneration	1	1	2	3
Corneal neovascularization	0	1	2	1

FOB: There was no effect on any FOB parameters including grip strength and landing foot splay, in either males or females at any point during the study.

Motor activity and locomotor activity: There were no treatment-related alterations in either motor or locomotor activity in either males or females at any dose. There were occasional statistically non-significant increases in motor or locomotor activity at various time points, but they followed no dose-related pattern, were generally limited to one sex, and were opposite to the treatment-related decreases in activity observed in the acute neurotoxicity study. Therefore, these increases were considered not to be treatment-related.

Organ weights: There was no effect on brain weight in either males or females at any dose.

Macroscopic findings: No gross lesions or other macroscopic observations were present in either male or female rats which could be attributed to treatment.

Microscopic findings: There were no histopathological findings in male or female rats at 5000 ppm, relative to control, which were indicative of a treatment-related effect. In the absence of any treatment-related effect at 5000 ppm, tissues were not examined at lower doses.

Conclusions

According to the study authors, there were no indications of a neurotoxic effect of AE 0317309 after 90 days dietary administration, nor were there effects on body weight or food consumption. The NOAEL (NOEL) for this study was therefore proposed by the

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study authors to be 5000 ppm, or 345 mg/kg bw/day in males and 416 mg/kg bw/day in females.

However, males showed increased food consumption during some time periods at the high dose. Also, although the yellow to brownish-orange urine stains observed were considered by the study authors to be due simply to urinary excretion of AE 0317309, no analysis appears to have been done to confirm this. The red staining of the nose and forepaws observed in the acute neurotoxicity study (Report no. KIIA 5.7.1/01) at comparable doses was not observed in this study.

The LOAEL was 500 ppm (42 mg/kg bw/day) in females based on increased incidences of corneal opacity and corneal neovascularization in females and not observed in males. The NOAEL was not observed in females and was 5000 ppm (345 mg/kg bw/day) in males.

EPA Reviewer: Robert J. Mitkus, PhD **Signature:** [signature]
Registration Action Branch 1, Health Effects Division (7509P) **Date:** 3/25/09
EPA WAM: PV Shah, PhD **Signature:** PVShah
Registration Action Branch 1, Health Effects Division (7509P) **Date:** 3/25/09

TXR#: 0054227

DATA EVALUATION RECORD

STUDY TYPE: Effect of tyrosinaemia on pregnancy and embryo-fetal development in the rat; Non-guideline

PC CODE: 000692 **DP BARCODE:** D335563

TEST MATERIAL (PURITY): NTBC [2-(2-chloro-4- methanesulfonylbenzoyl)-cyclohexane-1,3-dione] (99.7% w/w a.i.)

SYNONYMS: MKH 13222; LRS 0299

CITATION: Kennel P. (2006). Effect of tyrosinaemia on pregnancy and embryo-fetal development in the rat. Bayer CropScience, Sophia Antipolis Cedex, France. Laboratory Study No.: SA 05192, January 13, 2006. MRID 46801921. Unpublished.

SPONSOR: Bayer CropScience, Monheim, Germany

EXECUTIVE SUMMARY:

In an *in vivo* study (MRID 46801921), 23 pregnant female SD rats/dose group were administered vehicle alone (demineralized water) by gavage from GD 6-20 or 10 µg/kg bw/day NTBC, 1433 mg/kg bw/day L-tyrosine, or NTBC (10 µg/kg bw/day) + L-tyrosine (1424 mg/kg bw/day) by gavage from GD 6-21. The study was designed to test the hypothesis that maternal tyrosinemia has no effect on fetal skeletal development. Morbidity, mortality, and clinical signs were monitored daily throughout gestation. Body weights and food consumption were measured on gestation days 6, 8, 10, 12, 14, 16, 18, and 21. Maternal plasma tyrosine concentrations were measured at sacrifice (GD 21). At necropsy, the reproductive tract and livers were weighed and the number of corpora lutea, implantations, resorptions, and live and dead fetuses, and the sex and individual body weights of the live fetuses were recorded. Following external examination of all fetuses, a limited number of skeletal endpoints were evaluated from approximately half the fetuses in each litter.

There were no treatment-related effects on maternal mortality, clinical signs, body weight, corrected body weight, food consumption, litter parameters, or fetal external findings. Four of the 23 dams in the group receiving tyrosine + NTBC showed minimal corneal opacity. Mean plasma tyrosine concentrations at GD 21 were 5-, 8-, and 63-fold those of controls in the L-tyrosine, NTBC, or L-tyrosine + NTBC groups, respectively. Mean fetal body weight was reduced by 7% ($P<0.05$) in male and female fetuses whose dams were administered tyrosine + NTBC and was slightly but statistically non-significantly reduced by 2-3% in fetuses whose

dams were administered NTBC alone. Increases (relative to concurrent controls) in the incidences of the following skeletal findings were observed in animals receiving tyrosine + NTBC: 7th cervical centrum: unossified / normal cartilage; 6th sternebra: incomplete ossification / normal cartilage; 5th sternebra: unossified / normal cartilage; Extra ossification point(s) (unilateral / bilateral) on 14th thoracic vertebra; Forepaws: 3rd and/or 4th proximal phalanges: unossified / normal cartilage; 5th metacarpal: incomplete ossification / normal cartilage; 1st metatarsal: unossified: normal cartilage; and Less than 9 sacrocaudal vertebrae ossified / 9 first sacrocaudal vertebrae: normal cartilage. Increases in the incidences of the following skeletal findings were also observed in animals receiving tyrosine alone and NTBC alone: 7th cervical centrum: unossified / normal cartilage; and Extra ossification point(s) (unilateral / bilateral) on 14th thoracic vertebra. Because historical control data were not submitted and statistical analysis was not performed, the increased incidences in each of these groups were considered treatment-related.

The study is classified as **Acceptable/Non-guideline** and is acceptable for demonstrating an association between increased maternal blood tyrosine concentrations, fetal skeletal anomalies, and decreased fetal body weight.

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

This Executive Summary was prepared for United States Environmental Protection Agency, Office of Pesticides Programs, Health Effects Division use.

The following text was generated by the Australian Pesticides and Veterinary Medicines Authority. However, this document has undergone critical scientific analysis and been modified as appropriate.

Report:	**KIIA 5.10/02, Kennel, P.; 2006**
Title:	Effect of tyrosinaemia on pregnancy and embryo-fetal development in the rat
Laboratory	Bayer CropScience, 355 rue Dostoievski, BP 153, 06903 Sophia Antipolis Cedex, France
Study duration	19 July 2005 – 29 September 2005
Report No.:	SA05192
Document No.:	M-263626-01-2
Guidelines:	**OECD guideline 414 (January, 2001).** **EEC Directive 2004/73/EC, Method B.31 (April 30, 2004)** **US EPA, OPPTS Series 870, Health Effects Testing Guidelines, N°870.3700 (August 1998).** **MAFF IN JAPAN notification 12 Nousan N°8147, (November 24, 2000)**
GLP/QA	**Yes**

Introduction

The study authors hypothesized that the skeletal findings of decreased ossification, increased thoracic ribs, and increased pre-sacral vertebrae, which were observed in developmental toxicity studies conducted with HPPDase inhibitors were not a direct result of the administration of AE 0317309, but were secondary to increased maternal plasma tyrosine concentrations. To test this hypothesis, tyrosine and NTBC were administered either singly or in conjunction to pregnant rats in a standard developmental toxicity study design. In the absence of HPPDase inhibition, excess dietary tyrosine is excreted from the body relatively rapidly and has only a modest effect on plasma tyrosine. Additionally, the low solubility of tyrosine in standard dosing solutions (ie. its solubility of approximately 0.5 g/L) would require gavage administration of 700 mL to achieve a daily dose of 1400 mg/kg bw/day, the dose administered by diet in the current study in rats.. Therefore, in order to elevate maternal plasma tyrosine to an expected effective concentration, NTBC was administered at a dose which was expected to have little to no effect on plasma tyrosine by itself, but which would markedly increase plasma tyrosine when administered in conjunction with dietary tyrosine. It was expected that administration of either tyrosine alone, or NTBC alone, would have little effect on either maternal plasma tyrosine concentrations or fetal skeletal findings. However, it was expected that co-administration of tyrosine and NTBC would increase both maternal plasma tyrosine concentrations and the incidence of skeletal findings.

Materials and Methods

NTBC (batch MKH13222-3-2, purity 99.7%) and L-tyrosine (batches 111K0888 and 078H06822, both purity > 99%) were administered singly or in combination to pregnant female SD rats. NTBC was suspended in demineralized water and administered by oral gavage at 10 µg/kg bw/day on gestation days 6 through 20 inclusive, while L-tyrosine was incorporated into the diet at a concentration of 20000 ppm, and the tyrosine-containing diet was provided to the relevant groups on gestation days 6 through 21. The control group and the group receiving only L-tyrosine were administered demineralized

water by oral gavage on gestation days 6 through 20 as a control for NTBC administration. The experimental groups are shown in the following table.

Experimental groups used in the examination of the effect of tyrosinemia on embryo-fetal development in the rat

Group	Test substance	Dose level (s)	Number of animals
1	Vehicle	0 µg/kg bw/day	23
2	L-tyrosine	20000 ppm in the diet	23
3	NTBC	10 µg/kg bw/day	23
4	NTBC + L-tyrosine	10 µg/kg bw/day 20000 ppm in the diet	23

The rats were housed singly in suspended stainless steel wire cages, and food and water were available *ad libitum*. Clinical signs and morbidity and mortality were monitored daily throughout gestation. Body weights and food consumption were measured on gestation days 6, 8, 10, 12, 14, 16, 18, and 21. On gestation day 21, prior to sacrifice, the extreme tip of the tail of each rat was removed with a scalpel and blood samples were collected for determination of plasma tyrosine concentration. The rats were then sacrificed by carbon dioxide inhalation and subjected to gross necropsy, livers were weighed, and liver, thyroid, and pancreas were preserved for possible histopathological examination. The reproductive tract was weighed and the number of corpora lutea, implantations, resorptions, and live and dead fetuses, and the sex and individual body weights of the live fetuses were recorded. Live fetuses were then sacrificed by subcutaneous injection of sodium pentobarbital and examined externally. All fetuses were then skinned, eviscerated, and double-stained with alizarin red S and alcian blue. A limited number of skeletal endpoints were then evaluated from approximately half the fetuses in each litter. Fetal skeletal observations were classified by the registrant as common variants (changes occurring in more than approximately 5% of the control population), minor anomalies (slight, relatively rare structural changes that are not obviously detrimental), and malformations (very rare or obviously lethal changes). However, since there is no consensus among developmental toxicologists surrounding the validity of these classifications, they were not utilized in assessing a treatment-related effect.

Findings

Mortalities: One out of 23 females in the NTBC group was sacrificed on gestation day 13, based on body weight loss of 21 grams between gestation days 10 and 12 as well as reduced motor activity and head tilting on gestation days 12 and 13. There were no macroscopic findings at autopsy, and there was no clear relationship between treatment and the clinical signs observed.

Clinical signs: There were no findings at clinical examination which could be related to treatment.

Pregnancy rate: Pregnancy rate among the study groups ranged from 91% to 100%.

Since treatment occurred during gestation, differences in pregnancy rate were not treatment-related.

Body weights: Administration of L-tyrosine alone had no effect on maternal body weight or body weight gain. In the group administered NTBC by oral gavage, there was a statistically non-significant reduction in body weight gain between gestation days 6 and 8. Body weight gain in this group was then comparable to the control group. In the NTBC + tyrosine group, body weight gain between gestation days 6 and 8 was statistically non-significantly reduced, with overall body weight gain between gestation days 6 and 21 slightly but statistically non-significantly reduced. These slight changes in body weight gain were not reflected in changes in body weight, relative to controls, and were therefore not considered toxicologically significant. Corrected (for gravid uterine weight) body weight gain was similar among the groups.

Body weight, body weight gain, and corrected body weight gain for pregnant female rats administered NTBC, tyrosine, or both substances.

Day / Interval	Treatment group			
	Control	Tyrosine	NTBC	NTBC + tyrosine
Body weight, g				
0	256.1	257.8	258.5	256.6
6	285.2	288.5	288.0	287.5
8	291.8	293.2	290.8	290.6
10	301.0	301.1	299.4	300.0
12	310.2	311.3	308.1	309.2
14	319.0	321.0	318.7	318.3
16	333.4	336.5	332.0	335.0
18	360.4	362.9	358.7	360.5
21	409.8	412.6	408.3	403.6
Body weight change, g				
0-6	29.0	30.7	29.5	30.9
6-8	6.6	4.7	2.8	3.0
8-10	9.3	7.9	8.6	9.4
10-14	18.0	19.8	19.3	18.3
14-18	41.3	41.9	40.0	42.2
18-21	48.5	49.8	49.6	43.1
6-21	123.7	124.1	120.4	116.1
Corrected body weight change, g	45.7	50.7	48.7	45.9

Food consumption: There was a slight decrease (8%) in food consumption over gestation days 18 to 21 in the NTBC + tyrosine group. Tyrosine intake was similar in groups receiving diets containing tyrosine at 20,000 ppm.

Food consumption (g/animal/day) by pregnant female rats administered NTBC, tyrosine, neither or both substances, and achieved dose of tyrosine (in mg/kg bw/day) in those groups receiving diets containing tyrosine

Gestation interval, days	Treatment group			
	Control	Tyrosine	NTBC	NTBC + tyrosine
1-6	22.4	22.3	22.2	22.2
6-8	22.1	22.3	21.5	22.5
8-10	22.0	22.0	21.9	22.6
10-12	23.0	23.0	22.3	22.7
12-14	23.4	23.5	23.0	23.3
14-16	24.2	23.9	23.6	24.2
16-18	25.5	26.2	25.7	25.5
18-21	24.6	25.8	24.8	22.6*
Tyrosine, mg/kg bw/day	0	1433	0	1424

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

Maternal necropsy findings: Mottled kidneys were observed at a very low incidence in all three treatment groups (1-3 animals/group) but not in the control animals. Four of the 23 animals in the group receiving both tyrosine and NTBC showed minimal corneal opacity, which was not observed in any other treatment group. These findings were considered to be related to treatment. There was no effect of treatment on liver weight in any group.

Maternal plasma tyrosine concentration: Plasma tyrosine concentration at GD 21 was increased in all treatment groups when compared to controls, however the effect of either dietary tyrosine or NTBC by gavage alone was minimal compared to the effect of both tyrosine and NTBC together.

Plasma tyrosine concentrations on gestation day 21 in pregnant female rats administered NTBC, tyrosine, neither or both substances.

Parameter	Treatment group			
	Control	Tyrosine	NTBC	NTBC + tyrosine
N	23	22	20	23
Tyrosine concentration, nmol/ml	46.04 ± 11.55	216.4 ± 103.9	388.6 ± 213.3	2888 ± 638.1

Litter parameters: There was no effect of treatment on the number of live fetuses, early or late resorptions, or dead fetuses or on sex ratio. Mean fetal body weight (both for combined sexes and for each sex separately) was statistically significantly reduced by 7% in male and female fetuses whose dams were administered both tyrosine and NTBC, and was slightly but statistically non-significantly reduced by 2-3% in fetuses whose dams were administered NTBC alone.

Fetal body weights, in grams, for pregnant female rats administered NTBC, tyrosine, or both substances.

Group	Treatment group			
	Control	Tyrosine	NTBC	NTBC + tyrosine
Both sexes	5.43	5.39	5.30	5.04**

Males	5.56	5.54	5.43	5.17**
Females	5.31	5.25	5.16	4.93**

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

Fetal external findings: There were no findings observed at external examination which were considered to be related to treatment.

Fetal skeletal findings: The study authors claim that an increased incidence of variant findings was only observed in the group co-administered tyrosine and NTBC, mainly decreases in ossification. However some of the variations were somewhat more common than concurrent controls in the tyrosine-only and NTBC-only groups: 7th cervical centrum: unossified/normal cartilage; extra ossification point(s) (unilateral/bilateral) on 14th thoracic vertebra; 1st metatarsal: unossified: normal cartilage. Because historical control data were not submitted and statistical analysis was not performed, increases (relative to concurrent controls) in the incidences of the following skeletal findings were considered treatment-related in the group co-administered tyrosine and NTBC: 7th cervical centrum: unossified / normal cartilage; 6th sternebra: incomplete ossification / normal cartilage; 5th sternebra: unossified / normal cartilage; Extra ossification point(s) (unilateral / bilateral) on 14th thoracic vertebra; Forepaws: 3rd and/or 4th proximal phalanges: unossified / normal cartilage; 5th metacarpal: incomplete ossification / normal cartilage; 1st metatarsal: unossified: normal cartilage; and Less than 9 sacrocaudal vertebrae ossified / 9 first sacrocaudal vertebrae: normal cartilage. In the absence of historical control data and statistical analysis of the submitted results, increases (relative to concurrent controls) in the incidences of the following skeletal findings were considered treatment-related in the tyrosine-only and NTBC-only groups: 7th cervical centrum: unossified / normal cartilage; and Extra ossification point(s) (unilateral / bilateral) on 14th thoracic vertebra.

Skeletal findings in fetuses of pregnant female rats administered NTBC, tyrosine, or both substances.

Fetal finding	Type[a]	Level[b]	Treatment group			
			Control	Tyr	NTBC	Tyr + NTBC
Number of fetuses examined:		**F**	**175**	**162**	**146**	**170**
Number of litters examined:		**L**	**23**	**22**	**20**	**23**
7th cervical centrum: unossified / normal cartilage	Var	F	1	6	9	72
		L	1	5	6	19
5th sternebra: incomplete ossification / normal cartilage	Var	F	49	36	57	71
		L	20	12	17	21
6th sternebra: incomplete ossification / normal cartilage	Var	F	0	1	0	10
		L	0	1	0	2
5th sternebra: unossified / normal cartilage	Var	F	1	2	1	12
		L	1	2	1	6
14th thoracic rib (unilateral): short	Var	F	2	1	0	3
		L	1	1	0	3
14th thoracic ribs (bilateral): short	Var	F	0	1	0	1
		L	0	1	0	1

14th thoracic rib(s) (unilateral / bilateral): short	Var	F	2	2	0	4
		L	1	2	0	4
Extra ossification point(s) (unilateral / bilateral) on 14th thoracic vertebra	Var	F	2	8	5	20
		L	2	5	4	11
Forepaws: 3rd and/or 4th proximal phalanges: unossified / normal cartilage	Var	F	0	1	0	8
		L	0	1	0	3
5th metacarpal: incomplete ossification / normal cartilage	Var	F	7	7	6	20
		L	5	4	5	8
5th metacarpal: unossified / normal cartilage	Var	F	0	0	0	1
		L	0	0	0	1
1st metatarsal: unossified: normal cartilage	Var	F	1	4	2	6
		L	1	3	2	4
Less than 9 sacrocaudal vertebrae ossified / 9 first sacrocaudal vertebrae: normal cartilage	Var	F	1	0	0	7
		L	1	0	0	4

[a] Var = Variation; Ano = Anomaly; Mal = Malformation

[b] F = Fetal observation; L = Litter observation

Conclusions and comments

In the treatment groups (tyrosine alone, or NTBC alone), in which maternal plasma tyrosine was somewhat elevated relative to controls, there was some effect on fetal skeletal parameters. In the group administered both tyrosine and NTBC, in which maternal plasma tyrosine was markedly increased, there was generally a stronger increase in skeletal findings. Fetal plasma tyrosine concentrations were not measured in the study.

EPA Reviewer: Lisa Austin, Ph.D. **Signature:** [signature]
Registration Action Branch 1, Health Effects Division (7509P) **Date** 6/5/08
EPA WAM: PV Shah, PhD **Signature:** [signature]
Registration Action Branch 1, Health Effects Division (7509P) **Date:** 6/5/08

Template version 02/06

TXR#: 0054227

DATA EVALUATION RECORD

STUDY TYPE: ***In vitro* Bacterial Gene Mutation** *(Salmonella typhimurium)*/ mammalian activation gene mutation assay; OPPTS 870.5100 [§84-2]; OECD 471 (formerly OECD 471 & 472).

PC CODE: 000692 **DP BARCODE:** D335563

TEST MATERIAL (PURITY): AE0317309 (pyrasulfatole, 95.7%)

SYNONYMS: 5-hydroxy-1,3-dimethylpyrazol-4-yl)(2-mesyl-4-trifluoromethylphenyl

CITATION: Herbold, B. (2004). AE0317309-Salmonella/Microsome Test-Plate Incorporation And Preincubation Method; Bayer Healthcare AG, Wuppertal, Germany. Report number: AT01032, March 1, 2004. MRID 46801911. Unpublished.

SPONSOR: Bayer Healthcare AG, Wuppertal, Germany

EXECUTIVE SUMMARY:

In two independent reverse gene mutation assays in bacteria (MRID 46801911), strains TA1535, TA100, TA1537, TA98 and TA102 of *Salmonella typhimurium* were exposed to AE0317309 (95.7 %, Op. 1-4) in dimethylsulfoxide (DMSO) at concentrations of 0, 16, 50, 158, 500, 1581 and 5000 µg/plate in the presence and absence of mammalian metabolic activation in standard plate and preincubation tests. Standard strain-specific mutagens served as positive controls.

AE0317309 was tested up to the limit concentration (5000 ug/plate) with and without metabolic activation. Precipitation was not observed in either trial. Cytotoxicity was observed at ≥ 500 ug/plate in all strains as indicated by titer reductions in the first trial only. No decreases in the revertant colony count or the background lawn of growth occurred at any concentration or any strain in either the first or second trials. Marked increases in the number of revertants were not observed at any concentration in any strain in either trial. The positive controls induced marked increases in revertant colonies compared to controls in both trials. **There was no evidence of induced mutant colonies over background.**

The study is classified as **acceptable/guideline** and satisfies the guideline requirements (OPPTS 870.5100[1]; OECD 471) for *in vitro* mutagenicity (bacterial reverse gene mutations) data.

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

This Executive Summary was prepared for United States Environmental Protection Agency, Office of Pesticides Programs, Health Effects Division use.

The following text was generated by the Australian Pesticides and Veterinary Medicines Authority. However, this document has undergone critical scientific analysis and been modified as appropriate.

[1]870.5100 - Reverse mutation *S. typhimurium* TA98, TA100, TA1535, TA1537, TA102

Report:	KIIA 5.4.1/01, Herbold, B.; 2004
Title:	AE 0317309 - Salmonella/microsome test - Plate incorporation and preincubation method
Test Substance:	AE 0317309: Pyrosulfatole – batch Op. 1-4, purity 95.7% w/w
Laboratory:	Bayer HealthCare AG, PH-PD P Health Care Toxicology, 42096 Wuppertal, Germany.
Report No.: **Document No.:**	AT01032 M-000357-01-3
Study Duration:	28 August 2003 to 5 February 2004
Guidelines:	OECD 471; Directive 2000/32/EC, B.13/14.; US-EPA 712-C-98-247, OPPTS 870.5100
GLP/QA	Yes

Materials and Methods

Solvent: Test substance was suspended in the solvent dimethylsulfoxide (DMSO). Analyses were performed to determined the stability of the test substance in vehicle. The amount of solvent for the test substance and the controls was 0.1 mL/plate.

Strains: Histidine-deficient mutants of *Salmonella typhimurium* LT2 served as indicators to demonstrate point mutagenic effects. Since point mutations can be divided into two basic classes, base-pair substitutions and frameshift mutations, several strains were used which cover both types. These included *S typhimurium* TA 1535, TA 1537, TA 98, TA 100 and TA 102. With the exception of TA 102, all strains have reduced capability to repair DNA-damage which increases the likelihood that such damage results in mutations.

These strains have been maintained at this test facility since 1997. Stock cultures were generated as needed through culturing on nutrient agar followed by overnight incubation in nutrient broth at 37°C. Stock cultures were then checked for crystal-violet sensitivity and UV sensitivity, and were frozen at -80°C until use.

For both the plate incorporation and the preincubation assays reported here, the bacterial suspensions were grown for 17 hours in nutrient broth at 37°C with 90-rpm rotation. The bacterial count used for each assay was not standardized in either assay, but titres were measured in parallel to the actual assays to demonstrate that sufficient numbers of bacteria had been used in each assay. To determine the bacterial titre, the bacterial suspension was diluted at 1:1,000,000, then suspended in soft agar containing histidine at 5-fold greater concentrations than those used in the mutagenicity assay. Plates were then incubated for 48 hours and colonies were counted.

Test conditions:

Metabolic activation: Six male Sprague Dawley rats of 200-300 g body weight were administered 500 mg/kg bw Aroclor 1254 by intraperitoneal injection 5 days prior to sacrifice. The liver homogenate fraction mix was prepared containing 10% S9.

Initial plate incorporation test (Ames test): test substance (0.1 ml), bacteria (0.1 ml), S9 mix or buffer (0.5 ml), and soft agar (2.0 ml) were combined, held in a 45°C waterbath for no more than

30 seconds, and then poured onto a petri dish containing solid agar with only minimal histidine content. The petri dishes were allowed to solidify, then were incubated for 48 hours at 37°C. Colonies were then counted with an automatic counter after gross evaluation of the background growth.

Three plates were prepared for each concentration of test substance, for each strain, and for each incubation condition (with/without S9). Concentrations of test substance examined were 16, 50, 158, 500, 1581 and 5000 µg/plate, both with and without S9.

Independent repeat (preincubation) test, the test substance (0.1 ml), bacteria (0.1 ml), and S9 mix or buffer (0.5 ml) were combined and incubated in a 37°C water bath for 20 minutes. At the end of this time, 2 ml molten soft agar was added to each tube and the contents were plated onto individual petri dishes containing solid agar with minimal histidine content. The petri dishes were allowed to solidify, then were incubated for 48 hours at 37°C. Colonies were then counted with an automatic counter after gross evaluation of the background growth.

Three plates were prepared for each concentration of test substance, for each strain, and for each incubation condition (with/without S9). Concentrations of test substance examined were 16, 50, 158, 500, 1581 and 5000 µg/plate, both with and without S9.

Preliminary cytotoxicity study: was conducted by the plate incorporation method at test substance concentrations of 16, 50, 158, 500, 1581 and 5000 µg/plate, in TA 1535, TA 100, TA 1537, TA 98, and TA 102. The preliminary study was carried out both with and without S9, and three replicate plates were prepared for each concentration, strain, and condition

Positive controls:

The positive control agents used in this assay were, sodium azide (TA 1535 only) at 10 µg per plate, nitrofurantoin (TA100 only) at 0.2 µg per plate, 4-nitro-1,2-phenylene diamine (TA1537 and TA98 only) at 10 and 0.5 µg per plate, for each strain respectively, mitomycin C (TA102 only for plate incorporation) at 0.2 µg per plate, cumene hydroperoxide at 50 µg per plate, (TA102 only for pre-incubation) for the incubations without S9, and 2-aminoanthracene at 3 µg per plate, for the incubations with S9 for all strains and all incubation methods.

Findings

Stability of test substance: The test substance was determined as stable in the vehicle at room temperature at concentrations ranging from 0.01 mg/mL to 250 mg/mL for at least 24 h. This period covered the time range from preparation of the formulation to last treatment.

Preincubation trials: The background revertant levels reported are in line with both historical data from the laboratory, and with that reported by other laboratories using these these bacterial strains. The incidence of positive control-induced revertants is also consistent with both historical data and public data.

Cytotoxicity: There was no effect on bacterial growth at concentrations of up to and including 158 µg/plate; however, growth of TA 1535, TA 100, and TA 1537 was significantly decreased at test substance concentrations of 500 µg/plate and above with S9. In TA 98 and TA 102, bacterial growth was only significantly decreased at concentrations of 1581 µg/plate and above with S9. Precipitation of the test substance was not reported at any concentration.

The positive controls sodium azide, nitrofurantoin, 4-nitro-1,2-phenylene diamine, mitomycin C, cumene hydroperoxide and 2-aminoanthracene increased mutant counts to well over those of the negative controls, and thus demonstrated the systems' sensitivity and the activity of the S9 mix.

Mutagenicity: Neither the plate incorporation nor the preincubation trials with the test substance produced any indication of mutagenicity in any strain, either with or without metabolic activation.

The results from both the initial plate incorporation test (Ames test) (Ipct) and the independent repeat (preincubation) test (Irt) are presented in the following table. The positive control substance used for each strain and study is not specified in the table as this has been listed above.

Number of revertant colonies in various strains of *S. typhimurium* after incubation of bacteria with test substance in the Ames microsomally-mediated mutagenicity assay.

Conc.[a]	S9	Trial	TA 1535		TA 100		TA 1537		TA 98		TA 102	
			M[b]	SD	M	SD	M	SD	M	SD	M	SD
DMSO	-	Ipct	17	2	99	15	7	1	23	3	134	28
		Irt	17	1	138	8	10	1	18	3	183	9
	+	Ipct	13	3	110	9	11	2	30	2	158	8
		Irt	15	4	181	19	12	2	30	6	245	10
16	-	Ipct	17	2	109	8	6	2	17	3	132	20
		Irt	15	6	139	10	7	2	13	5	189	3
	+	Ipct	12	6	117	3	8	1	30	1	151	20
		Irt	16	4	173	10	10	2	29	8	212	20
50	-	Ipct	17	3	109	12	6	1	19	6	144	15
		Irt	20	2	135	6	7	2	15	5	201	17
	+	Ipct	8	1	114	10	8	4	24	3	161	12
		Irt	16	3	157	15	10	5	23	6	237	16
158	-	Ipct	12	4	122	34	5	1	22	8	163	3
		Irt	20	5	135	16	9	2	20	5	187	29
	+	Ipct	9	2	133	39	9	2	33	10	175	14
		Irt	15	5	185	18	9	2	27	7	249	37
500	-	Ipct	14	5	103	11	6	2	19	5	112	13
		Irt	14	2	134	12	7	2	15	4	198	13
	+	Ipct	9	1	107	2	9	2	34	3	160	9
		Irt	17	2	157	12	8	1	33	4	237	3
1581	-	Ipct	14	1	115	2	9	2	24	4	122	8
		Irt	20	5	131	1	7	3	14	2	187	14
	+	Ipct	8	2	95	13	6	1	30	3	156	14
		Irt	11	2	156	14	9	2	23	5	229	3
5000	-	Ipct	13	2	113	10	9	2	17	2	116	14
		Irt	16	3	141	25	6	1	17	3	200	18
	+	Ipct	9	2	94	9	5	1	18	6	150	23
		Irt	11	2	155	4	10	3	32	5	227	11
Pos	-	Ipct	356	22	349	46	109	25	161	12	591	11

Cont[c]		Irt	392	17	440	49	100	19	142	12	509	23
	+	Ipct	145	15	1642	27	261	14	1389	30	542	44
		Irt	159	8	1561	16	255	29	1331	59	507	39

[a] Concentration of test substance in µg/plate.
[b] M = Mean; SD = Standard Deviation.
[c] Positive controls for each strain are defined in the Materials and Methods section.
Ipct: initial plate incorporation test
Irt: independent repeat (preincubation) test

Conclusions

The test substance induced weak cytotoxicity as manifested by growth inhibition at relatively high doses (at and above 500 µg/plate), in the plate incorporation study with some strains. In the preincubation study, there was no cytotoxicity.

The positive control agents used induced the appropriate number of revertant colonies in both the plate incorporation and the preincubation assays, both with and without S9, while the negative controls were also within the expected background range of revertant colony incidence.

The incidence of revertant colonies seen in incubations conducted with the test substance both with and without S9 was not different in any strain from the background incidence. Thus, the test substance was evaluated as not mutagenic in the Ames *Salmonella*/microsome-mediated mutagenicity assay.

EPA Reviewer: Lisa Austin, Ph.D. **Signature:** Lisa Austin
RAB1, Health Effects Division (7509P) **Date:** 6/5/08
EPA WAM: PV Shah, PhD **Signature:** PV Shah
Registration Action Branch 1, Health Effects Division (7509P) **Date:** 6/5/08
Template version 02/06

TXR: 0054227

DATA EVALUATION RECORD

STUDY TYPE: *In Vitro* Mammalian Cells in Culture Gene Mutation assay in Chinese Hamster V79 Cells (HPRT Locus Assay); OPPTS 870.5300 [§84-2]; OECD 476.

PC CODE: 000692 **DP BARCODE:** D335563

TEST MATERIAL (PURITY): AE 0317309 (95.7% a.i.)

SYNONYMS: (5-hydroxy-1,3-dimethylpyrazol-4-yl)(2-mesyl-4 trifluoromethylphenyl) methanone

CITATION: Herbold, B. (2004). AE0317309 (Project: AE0317309) – V79/HPRT-In Vitro for the Detection of Induced Forward Mutations. Bayer HealthCare AG, Wuppertal, Germany. Report number: AT01401A, July 6, 2004. MRID 46801912. Unpublished.

SPONSOR: Bayer CropScience, Monheim, Germany

EXECUTIVE SUMMARY: In independently performed mammalian cell gene mutation assays at the HGPRT locus (MRID 46801912), Chinese hamster V79 cells cultured *in vitro* were exposed for 5 hours to AE0317309 (95.7% a.i., Batch No. Op1-4) in dimethyl sulfoxide (DMSO) at concentrations of 0, 30, 60, 120, 240, 480, and 960 µg/mL with and without metabolic activation (S9-mix). Two independent assays without S9 and three independent assays with S9 were conducted with duplicate cultures at each test material concentration and for each solvent, negative and positive control. The S9-fraction was obtained from Aroclor 1254 induced Sprague-Dawley derived rat liver and was used at a concentration of 40% in the S9 cofactor mix.

AE0317309 was tested up to 960 ug/mL (Study report stated that changes in pH were observed at concentrations ≥312.5 ug/mL, data not reported). Precipitation was not observed at any concentration in any trial with/without S9-mix. A decrease in relative population growth was observed at 480 and 960 ug/mL (79 and 89%, respectively), in one trial without S9; and at 960 ug/mL (92%) in one trial with S9. Relative survival and cloning efficiency of treated cells were

generally comparable to both solvent and negative control values with/without S9. There was no dose related increase in mutation frequency at any concentration with/without S9, and all findings were within the historical control frequency. Solvent, negative and positive control values were appropriate and within historical control ranges.

This study is classified as **Acceptable/Guideline** and satisfies the guideline requirement for Test Guideline OPPTS 870.5300, OECD 476 for *in vitro* mutagenicity (mammalian forward gene mutation) data.

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

This Executive Summary was prepared for United States Environmental Protection Agency, Office of Pesticides Programs, Health Effects Division use.

The following text was generated by the Australian Pesticides and Veterinary Medicines Authority. However, this document has undergone critical scientific analysis and been modified as appropriate.

Report:	KIIA 5.4.3/01, Herbold, B.; 2004
Title:	AE 0317309 (Project: AE 0317309) - V79/HPRT-test in vitro for the detection of induced forward mutations
Test substance:	AE 0317309; Pyrosulfatole – batch Op. 1-4, purity 95.7%
Laboratory	Bayer HealthCare AG, PH-PD Toxicology International, 42096 Wuppertal, Germany.
Report No.: **Document No.:**	AT01401A M-084536-02-2
Study Duration:	21 October 2003 to 6 July 2004
Guidelines:	OECD 476; Directive 2000/32/EC, Method B.17; US-EPA 712-C-98-221, OPPTS 870.5300
GLP/QA:	yes

Materials and Methods

Solvent: The test substance was dissolved in dimethylsulfoxide (DMSO). The test substance was stable in the vehicle at room temperature at concentrations ranging from 0.01 mg/mL to 250 mg/mL for at least 24 h, a time interval which covers the time range from preparation of the formulation to last treatment.

Cells: The test substance was tested for its ability to induce reverse mutations at the HPRT locus in V79 Chinese hamster lung cells. The V79 cells used in this study were gifted by the University of Ulm, Germany. These cells were recloned to maintain karyotypic stability. They have a modal chromosome number of 22 and a rapid population doubling time (10 to 14 h).

The V79 cell stocks were stored in liquid nitrogen. Laboratory cultures were maintained in plastic tissue culture vessels at 37°C in a humidified atmosphere containing approximately 5% CO_2. Exponential growth of cell cultures was maintained by subculturing at least twice a week. For cell detachment in order to subculture, a solution consisting of 0.1% trypsin and 0.04% EDTA (ethylenediamine-N,N,N',N'-tetraaceticacid) in phosphate buffered saline (PBS) has been employed.

To keep the number of spontaneous 6-TG resistant mutants at a low level, cell cultures were sub cloned by plating about 1,000 cells per culture vessel at least every two weeks. If necessary, the spontaneous frequency of HPRT-mutants was additionally reduced by supplementing the culture medium with thymidine (9 ug/mL), hypoxanthine (10 ug/mL), glycine (22.5 ug/mL) and methotrexate (0.3 ug/mL). A 6-TG sensitive subclone was then used for the HPRT-test. In all parts of this study incubation was performed at 37°C in a humidified atmosphere with about 5% CO_2.

Test conditions:

Metabolic activation: S9 mix was used for the simulation of the mammalian metabolism. The

S9 fraction was isolated in house from the livers of Aroclor 1254 induced male Sprague Dawley rats. The S9 fraction was kept frozen at -80°C. Prior to its first use in a V79/HPRT-test, a sample of this batch was tested for contamination and cytotoxicity. For use, the S9 fraction was thawed at room temperature and kept on ice until use and only used on the same day.

Preliminary cytotoxicity test: The test substance in DMSO was incubated in either the presence or the absence of S9 with V79 cells at concentrations of 18.8, 37.5, 75, 150, 300, 600, 1200 and 2400 µg/mL. Osmolality in the test medium did not change at any concentration, but the pH of the culture medium changed at concentrations of test substance greater than 312.5 µg/mL, and thus concentrations used in the definitive tests were 30, 60, 120, 240, 480 and 960 µg/mL.

For determination of cytotoxicity, one culture at each concentration of test substance was incubated either with or without S9 activation for 5 h. After incubation, cell monolayers were washed, trypsinized, and replated prior to incubation for 6-8 days to allow colony growth.

Definitive study: Two cultures/concentration of test substance were used. Cells were incubated with test substance in either the absence or the presence of S9 for 5 h, then rinsed, trypsinized, and replated to one culture flask (for mutagenicity) and 3 petri plates (for cytotoxicity). The petri plates were incubated for 6 days and then colonies were counted. The cells transferred to the flasks were cultured for 3 days, subcultured into further flasks, and after a total of 6 days were seeded into petri dishes and incubated with selective medium containing 6-thioguanidine. These plates were incubated for 6-8 days and colonies were then counted.

The positive control agents were ethyl methanesulfonate (EMS) at a final concentration of 900 µg/mL in the absence of metabolic activation, and dimethylbenzanthracene (DMBA) in DMSO at a final concentration of 20 µg/mL in the presence of metabolic activation.

The parameters determined were as follows:

(1) "Survival to treatment" (%) = mean number of colonies (treated cultures) x 100/mean number of colonies (vehicle control cultures),

(2) "Absolute population growth" was calculated using the following formula:-
Absolute population growth (for each culture) = cell no. count 1 x cell no. count 2.

(3) "Relative population growth" = mean no. of colonies per dish x 100/200. (Shows the cumulative growth of the treated cell population, relative to the vehicle control).

(4) "Mutant frequency" = total number of mutant colonies x 100/no. of evaluated dishes x 3 x 10^5 x C.E.. (Calculated for each group by dividing the total no. of mutant colonies by the no. of cells seeded (usually 8-10 plates at $3x10^5$ cells per plate), corrected for the absolute cloning efficiency. The mutant frequency is expressed as 6-TG resistant mutants per 10^6 clonable cells).

Findings

The cytotoxic effects observed in the preliminary test are expressed in the table below as a % of the vehicle control (relative cloning efficiency). Precipitation of test substance in the culture medium was not observed and no cytotoxicity effect was noted at any concentration.

Results of the preliminary cytotoxicity study conducted in V79 cells

Concentration of test substance (µg/mL)	Duplicate dishes, N	Mean colony number	Absolute cloning efficiency, %	% of vehicle control
Without metabolic activation				
Vehicle control	3	103.3	51.5	100.0
18.8	3	75.0	37.5	72.8
37.5	3	106.7	53.3	103.6
75	3	94.0	47.0	91.3
150	3	89.7	44.8	87.1
300	3	66.3	33.2	64.4
600	3	77.7	38.8	75.4
1200	3	90.0	45.0	87.4
2400	2	120.0	60.0	116.5
With metabolic activation				
Vehicle control	3	114.3	57.2	100.0
18.8	3	86.3	43.2	75.5
37.5	3	77.3	38.7	67.6
75	3	114.3	57.2	100.0
150	3	97.3	48.7	85.1
300	3	145.7	72.8	127.4
600	3	132.3	66.2	115.7
1200	3	78.7	39.3	68.8
2400	2	224.5	112.3	196.4

N = number of duplicate dishes

The results of the mutation assay without metabolic activation are shown in the table below. Two trials were performed. The mutant frequencies of the negative controls and of the vehicle controls were all within the normal range as evidenced by the historical control data provided by the study authors. The positive control EMS induced clear mutagenic and statistically significant effects in all trials.

Concentration related decreases in relative population growth (% vehicle control) were observed. There was no dose related increase in mutation frequency, and all findings were within the historical control frequency. The test substance was evaluated as non-mutagenic in the non-activation trial.

Mutagenicity results for V79 cells incubated with test substance without metabolic activation.

Concen. of test substance (µg/mL)	Test	Mean col. #	% veh. Control	Abs. pop. growth, x 10^{12}	% veh. Control	Dishes, N	Mutant colonies	Abs. clonin g eff., %	Mutant freq., x 10^{-6}

Negative control	1	104.5	103.1	135.38	85.1	8	2	50.8	1.6
	1	110.7	94.2	138.94	85.6	8	1	86.5	0.5
	2	135.7	69.3	115.50	166.5	8	3	67.7	1.8
	2	140.5	73.9	122.50	140.0	8	2	55.3	1.5
Vehicle control	1	101.3	100.0	159.0	100.0	8	1	67.0	0.6
	1	117.5	100.0	162.31	100.0	8	1	71.3	0.6
	2	195.7	100.0	69.38	100.0	7	2	76.0	1.3
	2	190.0	100.0	87.50	100.0	8	1	63.8	0.7
EMS, 900 µg/ml	1	40.7	40.1	18.75	11.8	8	331	32.0	431.0
	1	30.3	25.8	30.19	18.6	8	401	31.8	524.9
	2	41.0	21.0	53.63	77.3	8	195	59.5	136.6
	2	58.5	30.8	57.38	65.6	8	156	56.8	114.5
30	1	85.0	83.9	123.38	77.6	8	1	76.2	0.5
	1	94.3	80.3	104.06	64.1	8	1	83.0	0.5
	2	209.0	106.8	60.38	87.0	8	4	63.3	2.6
	2	211.7	111.4	75.00	85.7	8	1	41.2	1.0
60	1	94.5	93.3	59.13	37.2	8	1	55.5	0.8
	1	105.0	89.4	88.00	54.2	8	3	71.8	1.7
	2	135.0	69.0	45.31	65.3	8	1	65.5	0.6
	2	121.0	63.7	87.50	100.0	7	5	61.2	3.9
120	1	88.0	86.8	106.38	66.9	8	1	59.2	0.7
	1	99.3	84.5	137.50	84.7	8	1	72.0	0.6
	2	154.0	78.7	66.00	95.1	7	3	58.7	2.4
	2	176.0	92.6	56.25	64.3	7	3	56.7	2.5
240	1	98.5	97.2	51.75	32.5	8	1	33.5	1.2
	1	106.7	90.8	63.25	39.0	8	4	27.7	6.0
	2	168.3	86.0	52.50	75.7	8	4	55.0	3.0
	2	205.3	108.1	60.00	68.6	8	2	58.0	1.4
480	1	87.7	86.5	28.00	17.6	8	2	26.3	3.2
	1	97.0	82.6	40.63	25.0	8	1	26.7	1.6
	2	207.7	106.1	47.13	67.9	8	3	49.0	2.6
	2	233.0	122.6	44.56	50.9	8	2	51.3	1.6
960	1	98.5	97.2	20.13	12.7	8	2	28.5	2.9
	1	91.5	77.9	16.00	9.9	8	1	45.3	0.9
	2	190.0	97.1	32.50	46.8	7	9	50.8	8.4
	2	208.5	109.7	40.00	45.7	8	5	57.0	3.7

The results of the mutation assay with metabolic activation are shown in the table below. Three trials were performed with S9 mix. The mutant frequencies of the negative controls and of the vehicle controls were all within the normal range. The positive control DMBA induced a clear mutagenic and statistically significant effect in all trials.

Concentration related decreases in relative population growth (% vehicle control) were observed. There were no dose related increases in mutation frequency. The test substance was evaluated as non-mutagenic with metabolic activation.

Mutagenicity results for V79 cells incubated with test substance and with metabolic activation.

Concen. of test substance (µg/mL)	Test	Mean col. #	% veh. Control	Abs. pop growth, x 10^{12}	% veh. Control	Dishes, N	Mutant colonies	Abs. cloning eff., %	Mutant freq., x 10^{-6}
Negative control	1	130.7	114.3	87.75	109.0	8	8	65.5	5.1
	1	115.3	102.7	78.00	96.7	8	8	62.5	5.3
	2	C[a]	ND[b]	175.31	85.0	8	1	76.2	0.5
	2	C	ND	218.63	76.1	8	1	124.5	0.3
	3	175.7	140.9	134.38	94.8	8	15	53.0	11.8
	3	133.7	95.7	185.50	110.8	5	4	56.0	4.8
Vehicle control	1	114.3	100.0	80.50	100.0	8	6	56.8	4.4
	1	112.3	100.0	80.63	100.0	8	7	52.5	5.6
	2	C	ND	206.25	100.0	8	2	94.3	0.9
	2	C	ND	287.44	100.0	8	1	112.2	0.4
	3	124.7	100.0	141.75	100.0	8	16	62.3	10.7
	3	139.7	100.0	167.44	100.0	8	8	47.0	7.1
DMBA, 20 µg/ml	1	50.0	43.7	96.94	120.4	8	247	48.5	212.2
	1	70.0	62.3	71.25	88.4	8	258	32.7	329.1
	2	C	ND	50.63	25.4	8	64	36.7	72.7
	2	C	ND	78.00	27.1	8	118	61.7	79.7
	3	106.0	85.0	46.00	32.5	8	31	54.3	23.8
	3	71.7	51.3	70.13	41.9	8	36	53.8	27.9

Mutagenicity results for V79 cells incubated with test substance and with metabolic activation (table contd.)

Concen. of test substance (µg/mL)	Test	Mean col. #	% veh. Control	Abs., x 10^{12}	% veh. Control	Dishes, N	Mutant colonies	Abs. cloning eff., %	Mutant freq., x 10^{-6}
30	1	174.7	152.8	135.00	167.7	8	11	61.7	7.4
	1	176.0	156.7	93.00	115.3	8	7	56.3	5.2
	2	C	ND	109.31	53.0	8	1	66.5	0.6
	2	C	ND	87.50	30.4	8	3	39.3	3.2
	3	141.5	113.5	113.44	80.0	8	6	47.5	5.3
	3	95.0	68.0	127.50	76.1	8	13	49.0	11.1
60	1	188.7	165.0	76.56	95.1	8	13	68.7	7.9
	1	179.0	159.3	108.38	134.4	8	8	72.0	4.6
	2	C	ND	99.13	48.1	8	1	38.3	1.1

	2	C	ND	87.75	30.5	8	1	31.3	1.3
	3	114.3	91.7	132.75	93.7	8	22	50.7	18.1
	3	126.7	90.7	136.00	81.2	8	13	50.5	10.7
120	1	202.3	177.0	43.31	53.8	8	11	73.5	6.2
	1	197.0	175.4	133.00	165.0	8	11	53.7	8.5
	2	C	ND	82.50	44.8	8	3	39.2	3.2
	2	C	ND	90.00	31.3	8	4	46.8	3.6
	3	172.3	138.2	185.00	130.5	8	11	55.0	8.3
	3	129.7	92.8	137.81	82.3	7	6	48.8	5.9
240	1	95.5	83.5	78.75	97.8	8	10	73.5	5.7
	1	165.0	146.9	71.25	88.4	8	10	48.2	8.7
	2	C	ND	84.56	41.0	8	3	40.0	3.1
	2	C	ND	53.63	18.7	8	2	58.8	1.4
	3	131.7	105.6	61.88	43.7	8	4	49.8	3.3
	3	149.3	106.9	160.88	96.1	8	19	53.5	14.8
480	1	148.0	129.4	83.38	103.6	8	8	63.5	5.2
	1	158.3	140.9	78.63	97.5	8	13	54.7	9.9
	2	C	ND	43.31	21.0	8	1	36.5	1.1
	2	C	ND	72.50	25.2	8	1	69.0	0.6
	3	148.3	119.0	113.75	80.2	8	7	34.8	8.4
	3	140.0	100.2	88.81	53.0	8	6	44.5	5.6
960	1	99.7	87.2	77.94	96.8	8	11	64.7	7.1
	1	94.0	83.7	45.13	56.0	8	10	60.2	6.9
	2	C	ND	21.38	10.4	8	2	35.3	2.4
	2	C	ND	15.00	5.2	6	2	41.5	2.7
	3	112.3	90.1	106.25	75.0	8	6	45.5	9.8
	3	134.7	96.4	57.00	34.0	7	7	47.5	7.0

a C = contaminated. (The nature of the contamination was not specified).
b ND = no data due to contamination. (The nature of the contamination was not specified)

Conclusion

The *in vitro* mutagenicity study in mammalian cells (Chinese hamster V79 cells) demonstrated that the test substance was not mutagenic.

EPA Primary reviewer: Lisa Austin, Ph.D. **Signature:** Lisa Austin
Registration Action Branch 1, Health Effects Division (7509P) **Date** 6/5/08
EPA WAM: PV Shah, PhD **Signature:** PVShah
Registration Action Branch 1, Health Effects Division (7509P) **Date:** 6/5/08

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DATA EVALUATION RECORD

STUDY TYPE: ***In vitro* Mammalian Cytogenetics** chromosomal aberration assay in Chinese hamster V79 cells [OPPTS 870.5375 (§84-2)] OECD 473

PC CODE: 000692 **DP BARCODE:** D335563

TEST MATERIAL (PURITY): Technical AE0317309(95.7% a.i.)

SYNONYMS: 5-hydroxy-1,3-dimethylpyrazol-4-yl)(2-mesyl-4-trifluoromethylphenyl

CITATION: Thum, M. (2004). AE0317309 (Project AE0317309)- *In vitro* Chromosome Aberration Test with Chinese Hamster V79 Cells. Bayer HealthCare, AG, Wuppertal, Germany. Report No.: AT01285. June 14, 2004. MRID 46801913. Unpublished.

SPONSOR: Bayer CropScience, Monheim, Germany

EXECUTIVE SUMMARY: In a mammalian cell cytogenetic assay (chromosomal aberrations) (MRID 46801913), Chinese hamster V79 cells in culture were exposed to Technical AE0317309 (95.7% a.i., batch No. Op1-4) in dimethyl sulfoxide (DMSO) at concentrations of 0, 500, 1000, 1500, 2000 or 2500 ug/mL for 4 hours with and without metabolic activation (S9) and harvested 18 hours post-treatment; 0, 1500, 2000, or 2500 ug/mL for 4 hours with and without metabolic activation (S9) and harvested 30 hours post-treatment; and 0, 200, 400, 600, 800, or 1000 for 18 hours without metabolic activation (S9) and harvested 18 hours post-treatment. Two hundred cells, 100 per duplicate coded slide, were evaluated for metaphases with structural chromosomal aberrations and polyploidy. The S9-fraction was obtained from Aroclor 1254 induced Sprague-Dawley rat liver.

AE0317309 was tested up to 2500 µg/mL, a cytotoxic concentration. Precipitation was not observed in any trial. Without S9, relative to the solvent control (mitotic index 100%), the mitotic indices in the 4 h treated cultures were reduced at 1500, 2000 and 2500 ug/mL. At 2500 ug/mL the mitotic index was 34.7, 31.3 and 36.9% after harvest times of 8, 18 and 30 h respectively ($p < 0.01$ all three indices). The mitotic indices in the 18 h treated cultures were reduced at 600, 800 and 1000 ug/mL. At 1000 ug/mL, the mitotic index was 65.1 and 48.5% after harvest times of 8 and 18 h respectively ($p < 0.01$ both indices). In comparison to the solvent control (survival index 100%), the survival indices in the 4 h treated cultures were

relevantly reduced at 2000 and 2500 ug/mL. At 2500 ug/mL the survival index was 77.2, 77.1 and 48.5% after harvest times of 8, 18 and 30 h respectively. The survival indices in the 18 h treated cultures were not reduced at any concentration after a harvest time of 8 h. After a harvest time of 18 h, survival indices were not markedly reduced at 600, 800 and 1000 ug/mL. The lowest survival index of 83.8% occurred at 800 ug/mL. An increase in aberrant metaphases or polyploidy relative to controls was not observed.

With S9, in comparison to the solvent control (mitotic index 100%), the mitotic indices in the 4 h treated cultures were reduced at 1500, 2000 and 2500 ug/mL. At 2500 ug/mL the mitotic index was 60.4, 50.9 and 26.2% after harvest times of 8, 18 and 30 h respectively ($p < 0.01$ all three indices). In comparison to the solvent control (survival index 100%), the treated cultures showed a relevant reduction at 2000 ug/mL to 63.1% at 18 h harvest time and at 2000 and 2500 ug/mL to 60.9 and 48.5% respectively at 30 h harvest time.

A statistically significant ($p \leq 0.05$) increase in the percentage of aberrant metaphases (7.5% , including and excluding gaps) relative to controls occurred with S9 at 2500 ug/mL following 4-hours exposure and 30-hours harvest. The most common aberrations were chromosome isodeletions; however, these increases occurred at a cytotoxic concentration. The mitotic and survival indices were reduced by 73.8% and 51.5%, respectively, at 2500 ug/mL relative to controls. There was no increase in polyploidy relative to controls

The solvent and positive controls were appropriate and within historical control range.

There was no evidence of clastogenicity induced over background.

This study is classified **Acceptable/Guideline (OPPTS 870.5375)** and satisfies the guideline requirements for an in vitro mammalian cytogenetics assay.

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

This Executive Summary was prepared for United States Environmental Protection Agency, Office of Pesticides Programs, Health Effects Division use.

The following text was generated by the Australian Pesticides and Veterinary Medicines Authority. However, this document has undergone critical scientific analysis and been modified as appropriate.

Report:	KIIA 5.4.2/01, Thum, M.; 2004
Title:	AE 0317309 - In vitro chromosome aberration test with Chinese hamster V79 cells
Test Substance:	AE 03173309: Pyrosulfatole – batch Op. 1-4, purity 95.7% w/w
Laboratory:	Bayer HealthCare AG, PH-PD Toxicology International, 42096, Wuppertal
Report No.: **Document No.:**	AT01285 M-076036-01-2
Study Duration:	13 February 2004 to 6 May 2004
Guidelines:	OECD 473; Directive 2000/32/EC, Method B.10; US-EPA 712-C-98-223, OPPTS 870.5375
GLP/QA	yes

Materials and Methods

Solvent: The test substance is soluble in dimethylsulfoxide (DMSO) up to 250 mg/mL and was chosen as the solvent.

Cells: Chinese hamster V79 cells were used to assess the clastogenic potential of the test substance. A reason for the choice of V79 cells was not specified. Metaphase chromosomes prepared from cells at two harvest times (18 and 30 h) and two treatment periods (4 and 18 h) were evaluated. Aberration frequencies were determined and compared to results of negative controls. The cells were obtained from Merck AG, Darmstadt. Incubation of the cells was always performed at 37°C in a CO_2-incubator (5% CO_2). Unless reported otherwise, cells were grown in medium containing 10% fetal calf serum. As medium, Eagle's minimal essential medium (MEM, Earle) with the following supplements was used: nonessential amino acids, L-glutamine (2 mM), MEM-vitamins, NaHCOs-solution (final concentration: 0.225 %), penicillin (50 units/mL), streptomycin (50 ug/mL), heat-inactivated fetal calf serum. The karyotype of the V79 cells (modal number of chromosomes: 22) was confirmed on February 16, 2004 and March 19, 2004. A routine check for mycoplasma was performed on August 8, 2002 (when the used frozen permanent was prepared). There was no evidence of mycoplasma contamination. Therefore no check for mycoplasma contamination was needed for the actual culture.

Test conditions:

Metabolic activation: S9 mix was used for the simulation of mammalian metabolism. The S9 fraction was isolated from the livers of Aroclor 1254 induced male Sprague Dawley rats. The S9 mix contained 40% S9 fraction and was kept under refrigeration and used the same day.

Pre-test for concentration selection and cytotoxicity: The selection of concentrations used for the main study was based on the results of pre-tests in which cells were exposed with and without S9 mix for 4 h to various concentrations of the test substance. In addition, cells were exposed without S9 mix for 18 h to various concentrations of the test substance.

The concentrations of test substance examined were 0,10, 50, 125, 250, 500, 1250 or 2500

µg/mL in a 4 h incubation/24 h harvest time study (±S9) and 0, 50, 125, 250, 500, 1000, 1500, 2000 µg/mL in a 18 h incubation/18 h harvest time study (–S9). There was no effect on osmolality of the culture medium, but pH was slightly reduced at concentrations of approximately 625 µg/mL and above. This was considered not to have any relevance for the selection of concentrations for the main test.

As indicators of cytotoxic effects, mitotic indices and the numbers of surviving cells (survival index) were examined. The mitotic index was determined by counting a total of 1000 cells per culture. The results of the solvent controls were set 100% and compared to test substance treated cultures.

Main Study: On the basis of the results of the pre-test, the concentrations examined in the main study were 0, 500, 1000, 1500, 2000 or 2500 µg/mL in a 4 h incubation/18 h harvest time study (±S9), 0, 1500, 2000 and 2500 µg/mL in a 4 h incubation/30 h harvest time study (–S9) and 0, 200, 400, 600, 800, 1000 in an 18 h incubation/18 h harvest time study (–S9).

At the end of the 4-h incubation period, the medium with test substance was replaced with fresh medium, and then cells were harvested after a total time of either 18 or 30 h. The 18 h incubations were harvested immediately after the end of the incubation period. In all cases, colcemid was added to each flask two hours prior to the end of the incubation period.

The positive controls used in the 4 h incubation studies were mitomycin C (0.1 µg/mL) in the absence of metabolic activation and cyclophosphamide (2.0 µg/mL) in the presence of metabolic activation. In the 18 h incubation study, mitomycin C (0.03 µg/mL) without S9 activation was used as the positive control.

After harvesting, the cells were washed, fixed, and put onto slides for examination. Duplicate cultures were tested for every concentration of test substance and for the negative and positive controls. From each culture, the mitotic index was determined by counting 1000 cells per culture and noting the number of mitotic and non-mitotic cells. At each concentration of the test substance, 100 metaphases from each of two parallel cultures were examined, and structural chromosome damage was assessed on both the chromatid and the chromosome level.

Findings

Mitotic index without S9 mix: In comparison to the solvent control (mitotic index 100%), the mitotic indices in the 4 h treated cultures were reduced at 1500, 2000 and 2500 ug/mL. At 2500 ug/mL the mitotic index was 34.7, 31.3 and 36.9% after harvest times of 8, 18 and 30 h respectively ($p < 0.01$ all three indices). The mitotic indices in the 18 h treated cultures were reduced at 600, 800 and 1000 ug/mL. At 1000 ug/mL, the mitotic index was 65.1 and 48.5% after harvest times of 8 and 18 h respectively ($p < 0.01$ both indices).

The cultures treated with mitomycin C at 0.1 µg/mL (4 h treatment, 18 h harvest time) showed a reduction in the mitosis rate to 95.1% and at 0.03 µg/mL (18 h treatment, 18 h harvest time) a reduction to 78.5% was observed.

Mitotic index with S9 mix: In comparison to the solvent control (mitotic index 100%), the mitotic indices in the 4 h treated cultures were reduced at 1500, 2000 and 2500 ug/mL. At 2500 ug/mL the mitotic index was 60.4, 50.9 and 26.2% after harvest times of 8, 18 and 30 h respectively ($p < 0.01$ all three indices).

The positive control cyclophosphamide at 2 ug/mL (4 h treatment, 18 h harvest time) reduced the mitosis rate to 70.3%.
Survival index without S9 mix: In comparison to the solvent control (survival index 100%), the survival indices in the 4 h treated cultures were relevantly reduced at 2000 and 2500 ug/mL. At 2500 ug/mL the survival index was 77.2, 77.1 and 48.5% after harvest times of 8, 18 and 30 h respectively. The survival indices in the 18 h treated cultures were not reduced at any concentration after a harvest time of 8 h. After a harvest time of 18 h, survival indices were not markedly reduced at 600, 800 and 1000 ug/mL. The lowest survival index of 83.8% occurred at 800 ug/mL.

The cultures treated with mitomycin C at 0.1 µg/mL (4 h treatment, 18 h harvest time) showed a reduction in the survival index to 78.9% and at 0.03 µg/mL (18 h treatment, 18 h harvest time) a reduction to 78.5% was observed.

Survival index with S9 mix: In comparison to the solvent control (survival index 100%), the treated cultures showed a relevant reduction at 2000 ug/mL to 63.1% at 18 h harvest time and at 2000 and 2500 ug/mL to 60.9 and 48.5% respectively at 30 h harvest time.

The positive control cyclophosphamide also reduced the survival index to 60.3%.

The positive controls mitomycin C and cyclophosphamide induced clear clastogenic effects and demonstrated the sensitivity of the test system.

Chromosome aberrations

Without S9 mix: No biologically relevant and statistically significant increases of metaphases with aberrations were detected after 4 h treatment and harvest times of 18 or 30 h or after 18 h treatment and harvest time of 18 h.

The treatment with positive control mitomycin C resulted in a clear and statistically significant increase of metaphases with aberrations and demonstrated the sensitivity of the test system.

With S9 mix: No biologically relevant and statistically significant increases of metaphases with aberrations were detected after 4 h treatment and harvest times of 18 or 30 hours. Statistically significant findings were noted at 2500 ug/mL after 4 h treatment and harvest time of 30 h. The percent of metaphases with aberrations including and excluding gaps was 7.5% compared to 2.0% in controls. The most common aberrations were chromosome isodeletions; however, these

increases occurred at a cytotoxic concentration.

The positive control cyclophosphamide induced statistically significant and biologically relevant increases of metaphases with aberrations and demonstrated the sensitivity of the test system and the activity of the used S9 mix.

Conclusion

Based on the results of this study, the test substance was considered not to be clastogenic in Chinese hamster V79 cells *in vitro*.

EPA Reviewer: Lisa Austin, Ph.D. **Signature:** [signature]
Registration Action Branch 1, Health Effects Division (7509P) **Date** 6/5/08
EPA WAM: PV Shah, PhD **Signature:** [signature]
Registration Action Branch 1, Health Effects Division (7509P) **Date:** 6/5/08
Template version 11/01

TRX#: 0054227

DATA EVALUATION RECORD

STUDY TYPE: *In Vivo* Mammalian Cytogenetics - Erythrocyte Micronucleus assay in mice; [OPPTS 870.5395 (§84-2)] OECD 474.

PC CODE: 000692 **DP BARCODE:** D335563

TEST MATERIAL (PURITY): Technical AE0317309 (95.7% a.i.)

SYNONYMS: (5-hydroxy-1,3-dimethylpyrazol-4-yl)(2-mesyl-4 trifluoromethylphenyl) methanone

CITATION: Herbold, B. (2003). AE0317309- Micronucleus Test Using Mice. Bayer HealthCare, PH-PD P Health Care Toxicology, Wuppertal, Germany. Report No.: AT001719, October 22, 2003. MRID 46801914. Unpublished.

SPONSOR: Bayer HealthCare, Wuppertal, Germany.

EXECUTIVE SUMMARY: In a bone marrow micronucleus assay (MRID 46801914), five Hsd/WinNMRI mice/sex/dose (5 additional mice/sex were treated with either 500 or 1000 mg/kg/body weight to replace mice that died during treatment at the highest dose for males and females, respectively) were treated twice via intraperitoneal injections at 24 hours apart with AE0317309 (95.7% a.i., Batch# Op.1-4) in 0.5% aqueous Cremophor at 0, 125, 250 and 500 mg/kg/body weight (males) and 0, 250, 500 and 1000 mg/kg body weight (females). Bone marrow cells were harvested at 24 hours (all groups) post-treatment and scored for the number of micronucleated polychromatic erythrocytes (MPCEs) and normochromatic erythrocytes (NCEs).

AE0317309 was tested up to 500 and 1000 mg/kg/body weight in males and females, respectively. No mortality occurred in males; however, 8/10 females died at 1000 mg/kg/body weight. Clinical signs observed at all doses during treatment included apathy, roughened fur, weight loss, staggering gait, spasm, twitching, stretching of the body, decreased body temperature, and difficulty in breathing in males; and apathy, roughened fur, weight loss, spasm, stretching of the body, difficulty breathing, and slitted eyes in females. Since there were no differences between males and females in the manifestation of clinical signs of toxicity, only slides from the males were analyzed. In treated mice, the proportion of immature erythrocytes to total erythrocytes (PCE:NCE) was decreased in a dose-related manner. There was no statistically significant increase in the incidence of MPCEs over the solvent control value. The solvent and positive control values at the 24 hour harvest time induced the expected result. **There was no**

statistically significant increase in the frequency of micronucleated immature erythrocytes in mouse bone marrow at any dose or harvest time. Therefore, AE0317309 was neither clastogenic nor aneugenic in this *in vivo* mouse micronucleus assay.

This study is classified as **Acceptable/Guideline** and satisfies the guideline requirement for Test Guideline OPPTS 870.5395; OECD 474 for *in vivo* cytogenetic mutagenicity data.

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

This Executive Summary was prepared for United States Environmental Protection Agency, Office of Pesticides Programs, Health Effects Division use.

The following text was generated by the Australian Pesticides and Veterinary Medicines Authority. However, this document has undergone critical scientific analysis and been modified as appropriate.

Report:	KIIA 5.4.4/01, Herbold, B.; 2003
Title:	AE 0317309 - Micronucleus-test using mice
Test substance:	AE 0317309: Pyrosulfatole – batch Op. 1-4, purity 95.7%
Laboratory:	Bayer HealthCare, PH-PD P Health Care Toxicology, Molecular and Genetic Toxicology, D 42096, Wuppertal, Germany
Report No.: **Document No.:**	AT00719 M-110250-01-2
Study Duration:	14 July 2003 to 12 September 2003
Guidelines:	US EPA OPPTS Guideline No. 870.5395; OECD guideline no. 474
GLP	yes

Materials and Methods

Solvent: The test substance was suspended in 0.5% aqueous Cremophor emulsion. The stability of the test substance in this vehicle was determined.

Test animals: The animals used were young adult male and virgin female mice, strain Hsd/Win:NMRI, bred and supplied by Harlan Winkelmann GmbH, Borchen. The females initially weighed 27-32 g and the males 37-43 g and were approximately 6 to 12 weeks of age. Healthy mice were acclimatized to housing conditions for 5 days. Tap water and food were available at all times. These mice were used in a pilot study and in the main study.

Dose selection: The selection of doses for the main study was based on the results of the pilot test. There were substantial differences between the sexes in mortality rates and 500 mg/kg bw test substance was chosen as the MTD for males and 1000 mg/kg bw was chosen as the MTD for females. The doses used were 0, 125, 250, and 500 mg/kg bw for males and 0, 250, 500, and 1000 mg/kg bw for females.

Five males and 5 females were tested at each dose, with an additional 5 replacement mice of each sex, tested at the top doses of 500 (males) or 1000 (females) mg/kg bw. The replacement mice were intended to substitute for mice that died at the highest dose for each sex. The same group size was administered the positive control substance cyclophosphamide. The negative controls received 0.5% aqueous Cremophor emulsion. In all groups the dose volume was 10 mL/kg bw.

Test conditions:

Each dose was administered ip twice at 24 hours apart to mice of test and replacement groups.

The positive control substance cyclophosphamide was dissolved in deionized water and administered to a further group of 5 male and 5 female mice by a single ip injection at 20 mg/kg bw. The negative controls received their emulsion similarly.

The mice were sacrificed 24 h after their second injection and bone marrow was removed from one femur of each animal. Slides were prepared, dried, and stained. From each animal, 2000 polychromatic erythrocytes were counted, and the number of micronucleated polychromatic erythrocytes was determined. Additionally, the number of normochromatic erythrocytes per

2000 polychromatic erythrocytes was counted.

Findings

Stability of test substance in the vehicle:

The test substance was stable in the vehicle at room temperature at concentrations ranging from 10 to 150 mg/mL for at least 4 h, a time interval, which covers the time range from preparation of the formulation to last treatment

Clinical signs: All test group males survived. In males, two ip injections of the test substance at 125, 250, and 500 mg/kg bw produced a number of clinical signs evident at 125 mg/kg bw and above, of systemic exposure, including apathy, roughened fur, weight loss, staggering gait, spasm, twitching, stretching of the body, decreased body temperature, and difficulty in breathing. Eight out of the 10 females at 1000 mg/kg bw died during the test period. No females at other doses died. At 250, 500, and 1000 mg/kg bw, clinical signs of systemic toxicity in females included apathy, roughened fur, weight loss, spasm, stretching of the body, difficulty breathing, and slitted eyes. No signs were reported for mice in the negative control group.

As there were no differences between males and females in the toxicity of test substance as manifest in the clinical signs observed, only slides from the males were read, while slides from the females were archived unread. The results were as follows:-

1. The polychromatic: normochromatic erythrocyte ratio was decreased in a generally dose-related manner in males, demonstrating relevant systemic exposure to the test agent.

2. Micronucleated erythrocytes: There was no increase in micronucleated polychromatic or normochromatic erythrocytes at any dose of test substance. The positive control, cyclophosphamide, induced a marked increase in micronucleated polychromatic erythrocytes.

The data obtained are presented in the table below.

Polychromatic: normochromatic erythrocyte ratio and incidence of micronucleated erythrocytes in male mice treated with test substance

Group	Number PCEs evaluated	Number NCE per 2000 PCE	Micronucleated NCE per 2000 NCE	Micronucleated PCE per 2000 PCE
Negative control	10,000	1457	4.7	4.6
Cyclophosphamide, 20 mg/kg bw	10,000	1241	1.2	16.6
Test substance				
125 mg/kg bw	10,000	1426	5.7	4.6
250 mg/kg bw	10,000	1472	2.5	4.6
500 mg/kg bw	10,000	2147	4.7	4.8

PCE = polychromatic erythrocytes; NCE = normochromic erythrocytes

Conclusion

The results of this study demonstrated that the test substance was not clastogenic in the *in vivo* mouse micronucleus study.

EPA Reviewer: Robert J. Mitkus, PhD **Signature:** Robert J. Mitkus
Registration Action Branch 1, Health Effects Division (7509P) **Date:** 6/5/08
EPA WAM: PV Shah, PhD **Signature:** P.V. Shah
Registration Action Branch 1, Health Effects Division (7509P) **Date:** 6/5/08

TXR#: 0054504

DATA EVALUATION RECORD

STUDY TYPE: Acute Oral Toxicity Study in Rats (OPPTS 870.1100; OECD 401)

PC CODE: 623001 **DP BARCODE:** D335563

TEST MATERIAL (PURITY): RPA 203328 (99.7% a.i.)

SYNONYMS: Metabolite of pyrasulfotole

CITATION: Bigot D. (1995). RPA 203328: Oral limit test in the rat. Rhone-Poulenc Agrochimie, Sophia Antipolis Cedex, France. Laboratory Study No.: SA 95373, November 10, 1995. MRID 43904812. Unpublished.

SPONSOR: Rhone-Poulenc Agrochimie, Lyon, France

EXECUTIVE SUMMARY: In an acute oral toxicity study (MRID 43904812), 5 Sprague Dawley [ICO:OFA SD (IOPS Caw)] rats/sex were administered 5000 mg/kg bw RPA 203328 (99.7%; batch GH 705) by gavage. The test substance was formulated in 0.5% aqueous methylcellulose. Evaluation parameters included clinical signs of toxicity and mortality on the day of dosing and for a subsequent period of 14 days. Body weights and necropsy findings were recorded for all animals.

The oral LD_{50} for male and female rats was >5000 mg/kg bw (Category IV).

No mortalities were observed in the study. Clinical signs included dyspnea, piloerection, soiled fur, mucoid feces, and increased salivation. These signs were observed in two males on day 2 of the study only. One female showed the same clinical signs one hour after treatment and on study days 2 and 3. This animal also showed decreased motor activity, hunched posture, dyspnea, and noisy breathing from days 11 to 13. There was no effect on body weight or body weight gain in either sex. No abnormalities were detected at necropsy.

This acute oral toxicity study in the rat is **Acceptable (guideline)** and satisfies the guideline requirement for an acute oral toxicity study (OPPTS 870.1100; OECD 401) in the rat.

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

This Executive Summary was prepared for United States Environmental Protection Agency, Office of Pesticides Programs, Health Effects Division use.

The following text was generated by the Australian Pesticides and Veterinary Medicines Authority. However, this document has undergone critical scientific analysis and been modified as appropriate.

Report:	**KIIA 5.8/01, Bigot D.; 1995**
Title:	RPA 203328 (99.7% pure) - Oral limit test in the rat
Lab; Duration of study	**Rhone-Poulenc Agrochimie, Sophia Antipolis Cedex, France; 28 August - 29 September 1995**
Report No.:	SA95373
Document No.:	M-170815-01-1
Guidelines:	**EU: 92/69/EEC, Method B1 ; OECD: 401, (1987); USEPA: FIFRA 81-1, (1984)**
GLP/QA	**yes**

Materials and Methods

RPA 203328 (batch GH 705), suspended in 0.5% aqueous methylcellulose, was administered by oral gavage in a dose volume of 20 ml/kg to groups of SD rats (5/sex) at a dose of 5000 mg/kg bw. The rats were 7 weeks old at the time of treatment and weighed from 213-222 g for males and 146-160 g for females. The animals were housed individually in suspended stainless steel wire cages, with food and water available *ad libitum* throughout the study except on the night prior to dosing. After dosing, animals were checked for clinical signs at approximately one hour after dosing and at least once more on the day of dosing. For the remainder of the 14-day observation period, clinical signs were monitored at least once a day, with a second check on weekdays for morbidity and mortality. Body weights were measured on the day before and the day of treatment, and on days 8 and 15 post dose. On day 15 of the study, animals were anesthetized by intraperitoneal injection of pentobarbital, sacrificed by exsanguination, and subjected to gross necropsy.

Findings

There were no mortalities observed. The clinical signs reported included dyspnea, piloerection, soiled fur, mucoid feces, and increased salivation. These signs were reported in two males on day 2 and showed recovery by day 3 of the study. One female showed the same clinical signs beginning one hour after treatment, and on study days 2 and 3. This animal also showed decreased motor activity, hunched posture, dyspnea, and noisy breathing from days 11 to 13. There was no effect on body weight or body weight gain in either sex. No abnormalities were detected at necropsy.

Conclusions

The acute oral toxicity of RPA 203328 was low (acute oral LD_{50} > 5000 mg/kg bw) in rats.

EPA Reviewer: Robert J. Mitkus, PhD **Signature:** Robert J. Mitkus
Registration Action Branch 1, Health Effects Division (7509P) **Date:** 6/5/08
EPA WAM: PV Shah, PhD **Signature:** P.V. Shah
Registration Action Branch 1, Health Effects Division (7509P) **Date:** 6/5/08

TXR#: 0054227

DATA EVALUATION RECORD

STUDY TYPE: Preliminary 28-day toxicity study in rat [dietary]; Non-guideline

PC CODE: 623001 **DP BARCODE:** D335563

TEST MATERIAL (PURITY): RPA 203328 (99.7% w/w a.i.)

SYNONYMS: Metabolite of pyrasulfotole

CITATION: Dange, M. (1995). RPA 203328 (A metabolite of RPA 201772): 28-day toxicity study in the rat by dietary administration. Rhone-Poulenc Agrochimie, Sophia Antipolis Cedex, France. Laboratory Study No.: SA 94097, April 27, 1995. MRID 43904813. Unpublished.

SPONSOR: Rhone-Poulenc Agrochimie, Lyon Cedex, France

EXECUTIVE SUMMARY: In a 28-day oral toxicity study (MRID 43904813), RPA 203328 (99.7% w/w a.i., batch DA 938) was administered to 10 Sprague Dawley rats/sex/dose in the diet at dose levels of 0, 150, 500, 5000, or 15000 ppm (equal to 0/0, 11.1/12.7, 37.6/42.7, 377/421, and 1118/1269 mg/kg bw/day in males/females).

There were no compound-related effects on mortality, clinical signs, body weight, food consumption, hematology, clinical chemistry, ophthalmology, organ weights, or gross and histological pathology. Mean urinary pH was reduced in males at 15000 ppm (6.5, $P<0.01$ vs. 7.28 in controls). In the absence of corroborating evidence of toxicity in any other parameter, the finding was not considered toxicologically significant.

The LOAEL was not observed. The NOAEL is 15000 ppm (1118/1269 mg/kg/day [M/F]).

This 28-day oral toxicity study in the rat is **Acceptable (non-guideline)** as a range-finding study and does not satisfy the guideline requirement for a 90-day oral toxicity study (OPPTS 870.3100; OECD 408) in rodents.

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

This Executive Summary was prepared for United States Environmental Protection Agency, Office of Pesticides Programs, Health Effects Division use.

The following text was generated by the Australian Pesticides and Veterinary Medicines Authority. However, this document has undergone critical scientific analysis and been modified as appropriate.

Report:	**KIIA 5.8/02, Dange M.; 1995**
Title:	RPA 203328 (99.7% pure) - 28-day toxicity study in the rat by dietary administration RPA203328 (a metabolite of RPA201772)
Lab; Duration of study	Rhone-Poulenc Agrochimie, Sophia Antipolis Cedex, France; 31 May to 26 September 1994
Report No.:	SA94097
Document No.:	M-170705-01-2
Guidelines:	OECD: 407, (1981)
GLP/QA	yes

Materials and Methods

RPA 203328 (batch DA 938) was administered via diet to groups SD rats (10/sex/dose) rats at 0, 150, 500, 5000, and 15000 ppm. These concentrations provided doses of 0, 11.1, 37.6, 377, and 1118 mg/kg bw/day in males and 0, 12.7, 42.7, 421, and 1269 mg/kg bw/day in females. The rats were housed individually in suspended stainless steel wire cages, and food and water were available *ad libitum*. The rats were approximately 7 weeks old at the start of the study, with body weights of 240 to 313 g for males and 175-218 g for females. Clinical signs, moribundity, and mortality were checked twice daily on weekdays and once daily on weekends and holidays. Body weight and food consumption were measured weekly throughout the treatment period. Ophthalmoscopic examinations were conducted during the acclimatization period on all animals, and on rats in the control and 15000 ppm groups during weeks 2 and 4. In the last week of the study, animals were fasted overnight on one night prior to blood collection the following morning for haematology (red blood cell count, hemoglobin, hematocrit, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, white blood cell count, platelet count, differential leukocyte count evaluation, reticulocytes and prothrombin time) and clinical chemistry (total bilirubin, glucose, urea, total protein, albumin, total cholesterol, triglycerides and inorganic phosphate, chloride, sodium, potassium, calcium, creatinine, AST, ALT and AP) examinations by puncture of the retro-orbital venous plexus. Urine was collected overnight a few days later from fasted animals for analysis. The rats were sacrificed by intraperitoneal injection of pentobarbital followed by exsanguination under anesthesia, and were subjected to gross necropsy. Selected organs were weighed (adrenals, brain, liver, kidneys, epididymis, heart, pituitary, prostate, spleen, testes, thyroid, and ovaries) and samples of organs and tissues were preserved for histopathological examination (liver, spleen, kidney, urinary bladder, prostate, testis, epididymis, seminal vesicle, uterus, vagina, ovary, brain, spinal cord, heart, aorta, trachea, esophagus, lung, thymus, mesenteric lymph node, submaxillary gland, submaxillary lymph node, pancreas, tongue, skin, mammary gland, stomach, duodenum, jejunum, ileum, cecum, colon, rectum, anus, pituitary gland, adrenal, thyroid, parathyroid, skeletal muscle, sciatic nerve, eye and optic nerve, harderian gland, bone-sternum and bone marrow-sternum).

Findings

There were no mortalities or any clinical signs during the study. There was no effect of RPA 203328 on food consumption and bodyweight. Rats in the 15000-ppm treatment group showed no ophthalmoscopic abnormalities compared to control rats at any time during the study. There were no treatment-related effects on any hematological and clinical chemistry parameters tested.

Mean urinary pH was reduced in males at 15000 ppm (6.5, P<0.01 vs. 7.28 in controls). In the absence of corroborating evidence of toxicity in any other parameter, the finding was not considered toxicologically significant.

There were no effects on organ weights in any treatment group or any abnormalities detected at necropsy. No histopathological findings that could be attributed to treatment were observed.

Conclusions

The NOAEL (NOEL) in this study was 15000 ppm (1118 mg/kg bw/day for males, 1269 mg/kg bw/day for females), the highest dose tested.

EPA Reviewer: Robert J. Mitkus, PhD **Signature:** [signature]
Registration Action Branch 1, Health Effects Division (7509P) **Date:** 6/5/08
EPA WAM: PV Shah, PhD **Signature:** [signature]
Registration Action Branch 1, Health Effects Division (7509P) **Date:** 6/5/08

TXR#: 0054227

DATA EVALUATION RECORD

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

PC CODE: 623001 **DP BARCODE:** D335563

TEST MATERIAL (PURITY): RPA 203328 (99% w/w a.i.)

SYNONYMS: Metabolite of pyrasulfotole; metabolite of isoxaflutole

CITATION: Bigot, D. (1998). RPA 203328: 90-day toxicity study in the rat by dietary administration. Rhone-Poulenc Agrochimie, Sophia Antipolis Cedex, France. Laboratory Study No.: SA 98129, September 23, 1998. MRID 45655903. Unpublished.

SPONSOR: Rhone-Poulenc Agrochemical Company, North Carolina, USA

EXECUTIVE SUMMARY:

In a 90-day oral toxicity study (MRID 45655903), RPA 203328 (99% a.i, batch # NMI874) was administered to 10 Sprague Dawley rats/sex/dose in the diet at dose levels of 0, 1200, 4800, or 12000 ppm (equivalent to 0, 73.2/93.1, 306/371, or 769/952 mg/kg bw/day in males/females, respectively).

There were no compound-related effects on mortality, clinical signs (including reflexes), ophthalmology, body weight, food consumption, hematology, clinical chemistry, urinalysis, organ weights, or gross and histologic pathology. Urinary pH was slightly reduced ($P<0.01$) at the mid and high doses (5.45 vs. 6.11 in controls); however, the findings were not considered toxicologically significant in the absence of corroborating evidence of toxicity in any other measure. **The LOAEL was not observed. The NOAEL is 12000 ppm (769/952 mg/kg/day in males/females).**

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

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

This Executive Summary was prepared for United States Environmental Protection Agency, Office of Pesticides Programs, Health Effects Division use.

The following text was generated by the Australian Pesticides and Veterinary Medicines Authority. However, this document has undergone critical scientific analysis and been modified as appropriate.

Report:	**KIIA 5.8/03, Bigot D.; 1998**
Title:	RPA 203328 (99% pure): 90-Day Toxicity Study in the Rat by Dietary Administration
Lab; Duration of study	Rhone-Poulenc Agrochimie, Sophia Antipolis Cedex, France; 20 March - 24 August 1998
Report No.:	SA98129
Document No.:	M-240662-01-1
Guidelines:	EU: Annex V Method B26; MAFF: NohSan N 4200; OECD: 408; USEPA: FIFRA 82-1
GLP/QA	yes

Materials and Methods

RPA 203328 (batch NMI874) in the diet was given to SD rats (10/sex/dose) at 0, 1200, 4800, and 12000 ppm for 90 days. These concentrations provided doses of 0, 73.2, 306, and 769 mg/kg bw/day for males and 0, 93.1, 371, and 952 mg/kg bw/day for females. Stability, concentration and homogeneity of RPA 203328 in the diet were analysed and proved to be satisfactory (100-108% of nominal concentration). The rats were 7 weeks old at the start of treatment, and body weights ranged from 219 to 249 g in males and from 159 to 189 g in females. Animals were housed individually in suspended stainless steel wire cages, with food and water available *ad libitum* except on the night prior to blood and urine collections. Clinical signs, moribundity, and mortality were checked twice daily on weekdays and once daily on weekends and holidays. Prior to the start of the study and in week 12, the grasping, righting, corneal, pupillary, auditory startle, and head shaking reflexes were tested for each animal. Body weight and food consumption were measured on a weekly basis. Ophthalmoscopic examinations were conducted on all animals prior to the start of the study and on the control and high-dose animals in week 12. Also in week 12, blood was collected for hematology (red blood cell count, hemoglobin, hematocrit, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, white blood cell count, platelet count, differential leukocyte count evaluation, reticulocytes and prothrombin time) and clinical chemistry (total bilirubin, glucose, urea, total protein, albumin, total cholesterol, triglycerides and inorganic phosphate, chloride, sodium, potassium, calcium, creatinine, AST, ALT and AP) by puncture of the retro-orbital venous plexus. Urine was collected overnight on the night prior to sacrifice. Animals were then anesthetized by intraperitoneal injection of 50 or 60 mg/kg bw pentobarbital and sacrificed by exsanguination. Gross examination was conducted, selected organs were weighed (adrenals, brain, liver, kidneys, epididymis, heart, pituitary, prostate, spleen, testes, thyroid, and ovaries), and samples of organs and tissues were preserved for microscopic examination (eye and optic nercve, liver, spleen, kidney, urinary bladder, prostate, testis, epididymis, seminal vesicle, uterus, vagina, ovary, brain, spinal cord, heart, aorta, trachea, esophagus, larynth, lung, thymus, mesenteric lymph node, submaxillary gland, submaxillary lymph node, pancreas, tongue, skin, mammary gland, stomach, duodenum, jejunum, ileum, cecum, colon, rectum, anus, urinary bladder, pituitary gland, adrenal, thyroid, parathyroid, skeletal muscle, sciatic nerve, eye and optic nerve, harderian gland, bone-sternum and bone marrow-sternum).

Findings

There were no mortalities or any clinical signs during the study. There was no effect of RPA 203328 on food consumption and bodyweight. Rats in the 12000-ppm treatment group showed no ophthalmoscopic abnormalities compared to control rats at any time during the study. There were no treatment-related effects on any hematological and clinical chemistry parameters tested. Urinary pH was slightly reduced ($P<0.01$) at the mid and high doses (5.45 vs. 6.11 in controls).

Pathology

There were no treatment-related effects on organ weights at any dose in either males or females. A few macroscopic findings were noted at necropsy in some treated groups without a dose-related pattern, namely dark or yellowish color of the liver, dark kidneys, and marked lobular liver pattern (see Table below). However, there was no histopathological correlate to any of these findings and therefore they were not considered to be treatment-related. There were no histopathological abnormalities that could be attributed to treatment.

Pathology findings at necropsy

Parameter	Male (ppm)				Female (ppm)			
	0	1200	4800	12000	0	1200	4800	12000
Organ weight								
Bodyweight (g)	445	448	427	425	250	257	248	256
Liver								
Absolute (g)	10.6	10.7	9.8	10.1	6.1	6.1	6.0	6.0
Relative (%)	2.4	2.4	2.3	2.4	2.4	2.4	2.3	2.4
Kidney								
Absolute (g)	2.7	2.7	2.6	2.7	1.6	1.5	1.6	1.6
Relative (%)	0.6	0.6	0.6	0.6	0.67	0.67	0.63	0.63
Macroscopic findings								
Liver								
dark	0	2	2	2	0	0	0	0
yellowish	0	0	0	0	0	0	1	1
marked lobular pattern	0	2	1	2	0	0	0	1
Kidneys								
dark	0	0	0	0	0	0	1	1

Conclusions

Because there were no toxicologically significant effects on any of the parameters tested, the NOAEL (NOEL) for this study was 12000 ppm (769 mg/kg bw/day for males and 952 mg/kg bw/day for females), the highest dose tested.

EPA Reviewer: Robert J. Mitkus, PhD **Signature:** [signature]
Registration Action Branch 1, Health Effects Division (7509P) **Date:** 6/5/08
EPA WAM: PV Shah, PhD **Signature:** [signature]
Registration Action Branch 1, Health Effects Division (7509P) **Date:** 6/5/08

TXR#: 0054227

DATA EVALUATION RECORD

STUDY TYPE: ***In vitro* Bacterial Gene Mutation** (Salmonella typhimurium)/mammalian activation gene mutation assay; OPPTS 870.5100 [§84-2]; OECD 471 (formerly OECD 471 & 472).

PC CODE: 623001 **DP BARCODE:** D335563

TEST MATERIAL (PURITY): RPA 203328 (99.7% w/w a.i.)

SYNONYMS: Metabolite of pyrasulfotole; metabolite of isoxaflutole

CITATION: Percy, A. (1994). RPA 203328: Salmonella typhimurium reverse mutation assay (Ames test). Rhone-Poulenc Secteur Agro, Sophia Antipolis Cedex, France. Laboratory Study No.: SA 94057, October 13, 1994. MRID 43904814. Unpublished.

SPONSOR: Rhone-Poulenc Secteur Agro, Lyon Cedex, France

EXECUTIVE SUMMARY:

In a reverse gene mutation assay in bacteria (MRID 43904814), the TA 98, TA100, TA 1535, and TA 1537 strains of *S. typhimurium* were exposed to RPA 203328 (99.7% a.i, batch DA938) in DMSO at concentrations of 100, 250, 500, 1000, 2500, or 5000 µg/plate in the presence and absence of mammalian metabolic activation (S9 derived from Aroclor 1254-induced male rat livers) in the plate incorporation procedure.

RPA 203328 was tested up to cytotoxic concentrations (≥2500 µg/plate). The positive controls induced the appropriate responses in the corresponding strains. **There was no evidence of induced mutant colonies over background either with or without metabolic activation.**

This study is classified as **Acceptable (non-guideline)** and does not satisfy the guideline requirement for Test Guideline OPPTS 870.5100; OECD 471 for *in vitro* mutagenicity (bacterial reverse gene mutation) data, since only four strains of bacteria were tested. The study may be reclassified upon receipt of reverse gene mutation data for RPA 203328 using *S. typhimurium* TA102 or *E. coli* WP2.

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

This Executive Summary was prepared for United States Environmental Protection Agency, Office of Pesticides Programs, Health Effects Division use.

The following text was generated by the Australian Pesticides and Veterinary Medicines Authority. However, this document has undergone critical scientific analysis and been modified as appropriate.

Report:	**KIIA 5.8/04, Percy A.; 1994**
Title:	RPA 203328 (99.7% pure) - Reverse mutation assay (Ames test) Salmonella typhimurium
Lab; Duration of study	Rhone-Poulenc Agrochimie, Sophia Antipolis Cedex, France; 06 May - 06 June 1994
Report No.:	SA94057
Document No.:	M-170668-01-2
Guidelines:	**OECD: 471, (1983); USEPA: FIFRA 84-2, (1984)**
GLP/QA	**yes**

Materials and Methods

RPA 203328 (batch DA938, purity 99.7%) was tested in a standard Salmonella typhimurium microsomally-mediated mutagenicity study. The test substance was dissolved in dimethyl sulfoxide (DMSO). In a preliminary cytotoxicity study, RPA 203328 was tested at concentrations of 1 through 5000 µg/plate with TA100 in the presence and the absence of S9 metabolic activation. Based on the results of that study, concentrations of RPA 203328 in the two independent definitive mutagenicity studies were between 100 and 5000 µg/plate in the presence or absence of S9 metabolic activation. The positive controls used were sodium azide (SA, strains TA100 and TA1535 without S9), 2-nitrofluorene (2-FN, strain TA98 without S9), 9-aminoacridine (9-AA, strain TA1537 without S9), and 2-aminoanthracene (2-AA, all strains with S9). The strains were tested during each assay for their genetic characteristics. The S9 fraction used was obtained from livers of Fischer 344 male rats induced with Aroclor 1254.

Findings

RPA 203328 was found to be cytotoxic at 5000 µg/plate, based on thinning of the background lawn at that concentration, with some cytotoxicity also seen at 2500 µg/plate. There were no relevant increases in the number of revertant colonies with any strain either in the presence or the absence of S9 metabolic activation. The positive controls gave expected results.

Conclusions

RPA 203328 was negative for mutagenicity in this test.

Comments: The OECD Guidelines states that at least 5 strains of bacteria including *E Coli* WP2 should be in the test. This study used only 4 strains of bacteria and therefore, the validity of the findings is reduced.

DATA EVALUATION RECORD

RPA 203328
OPPTS 870.3700 [§83-3]
STUDY TYPE: DEVELOPMENTAL TOXICOLOGY STUDY
MRID 45655906

Prepared for

Health Effects Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
One Potomac Yard
2777 S. Crystal Drive
Arlington, VA 22202

Prepared by

Toxicology and Hazard Assessment Group
Life Sciences Division
Oak Ridge National Laboratory
Oak Ridge, TN 37831
Task Order No. 2008-200

Primary Reviewer: Jennifer L. Rayner, Ph.D.	Signature: Date: MAY 21 2008
Secondary Reviewers: Carol S. Wood, Ph.D., D.A.B.T.	Signature: Date: MAY 21 2008
Robert H. Ross, M.S., Group Leader	Signature: Date: MAY 21 2008
Quality Assurance: Lee Ann Wilson, M.A.	Signature: Date: MAY 21 2008

Disclaimer

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

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

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STUDY TYPE: DEVELOPMENTAL TOXICOLOGY STUDY
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EPA Reviewer: Robert Mitkus, Ph.D. **Signature:** [signature]
Registration Action Branch 1, Health Effects Division (7509P) **Date:** 6/5/08
EPA Work Assignment Manager: Myron Ottley, Ph.D. **Signature:** [signature]
Registration Action Branch 3, Health Effects Division (7509P) **Date:** 6/5/08

Template version 02/06

TXR#: 0054227

DATA EVALUATION RECORD

STUDY TYPE: Prenatal Developmental Toxicity Study - Rat; OPPTS 870.3700 [§83-3]; OECD 414.

PC CODE: 623001 **DP BARCODE:** D335563

TEST MATERIAL (PURITY): RPA 203328 (99% purity a.i.; Batch No. NMI874; white powder with small aggregates)

SYNONYMS: 2-methanesulphonyl-4-trifluoromethylbenzoic acid; metabolite of pyrasulfotole and isoxaflutole

CITATION: Repetto-Larsay, M. (1999) RPA 203328 Developmental Toxicology Study in the Rat by Gavage. Unpublished report by: Rhône-Poulenc Agro, Centre de Recherche, 355, rue Dostoïevski, BP 153, F-06903 Sophia Antipolis Cedex. Laboratory Report No. SA 98427, June 24, 1999. MRID 45655906.

SPONSOR: Aventis Crop Science (formally Rhône-Poulenc Ag Company), Research Triangle Park, NC 27709

EXECUTIVE SUMMARY:

In a developmental toxicity study (MRID 45655906) RPA 203328 (99% purity a.i.; Batch No. NMI874) was administered to 25 Sprague-Dawley rats/dose by gavage at dose levels of 0, 75, 250, or 750 mg/kg bw/day from days 6 through 20 of gestation. Maternal body weight and food consumption were recorded for all females throughout the gestational period. Clinical observations were recorded daily. At sacrifice on gestation day 21, the gravid uterine weight was recorded and the dams evaluated for number of corpora lutea and number and status of implantations. Live fetuses were removed from the uteri, counted, weighed, sexed, and examined externally. Half of each litter was fixed in Bouin's fixative and dissected for internal examination. The remaining half was eviscerated, fixed in alcohol, and stained with alizarin red S for skeletal examination.

Maternal toxicity was evident in the study. An increased incidence of salivation was observed at 250 (6/25) and 750 mg/kg/day (18/25). This finding was associated with red nasal discharge shortly after treatment in 3 animals at 750 mg/kg. Both salivation and nasal discharge were resolved within approximately one hour after treatment. Piloerection was also observed around the time of treatment in 2/25 and 3/25 dams at 250 and 750 mg/kg/day, respectively. Piloerection

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was associated with reduced motor activity in 1 animal at 750 mg/kg. No abnormal clinical signs were observed during the study period in the low dose (75 mg/kg/day) group. Corrected body weight change was significantly reduced by 31% and 37% in dams of the 250 and 750 mg/kg/day groups, respectively. These decreases were supported by significant decreases (>25%) in maternal body weight gain during treatment at the same doses. Administration of the test substance also significantly reduced food consumption during the treatment period in the same groups noted above. **The maternal LOAEL is 250 mg/kg bw/day, based on clinical signs (salivation and piloerection around the time of treatment), decreased body weight change, decreased corrected body weight change, and decreased food consumption. The maternal NOAEL is 75 mg/kg bw/day.**

External, internal, and skeletal examination of the fetuses did not reveal any findings which were treatment-related. **The developmental LOAEL was not observed. The developmental NOAEL is 750 mg/kg bw/day.**

The developmental toxicity study in the rat is classified **acceptable (guideline)** and satisfies the guideline requirement for a developmental toxicity study (OPPTS 870.3700; OECD 414) in rats.

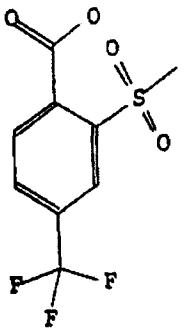
COMPLIANCE: Signed and dated GLP, Quality Assurance, and Statement of No Data Confidentiality Claims statements were provided.

I. MATERIALS AND METHODS:

A. MATERIALS:

1. Test material: RPA 203328

Description: White powder with small aggregates
Lot/batch #: NMI874
Purity: 99% a.i.
Compound stability: Determined in study SA 98333
CAS #of TGAI: 142994-06-7
Structure:



2. Vehicle and/or positive control: 0.5% Methylcellulose 400

3. Test animals:

Species:	Rat
Strain:	Sprague-Dawley Crl: CD (SD) BR
Age/weight at study initiation:	Age not reported; 237-306 g at mating
Source:	Charles River Laboratories, St Aubin les Elbeuf, France
Housing:	Animals were individually housed in suspended, stainless steel wire mesh cages.
Diet:	Certified Rodent pellet diet AO4C (Usine, d'Alimentation Rationnelle, Villermoisson-sur-Orge, France) was available *ad libitum*.
Water:	Water from the municipal supply was provided *ad libitum* with an automatic watering system. Filters servicing the watering system were changed regularly and sterilization of the system was periodically performed. Routine analysis of feed and water indicated that there was no contamination which could have been expected to have compromised the study.
Environmental conditions:	**Temperature:** 20-24°C **Humidity:** 40-70% **Air changes:** 10 /hr **Photoperiod:** 12 hrs dark/ 12 hrs light
Acclimation period:	14 days

B. PROCEDURES AND STUDY DESIGN

1. In life dates: Start: October 20, 1998; End: November 12, 1999

2. Mating: Females were mated on a one-to-one basis with stock males of the same strain and same supplier. Each morning following pairing, rats showing spermatozoa in a vaginal smear or sperm plug *in situ* were considered pregnant. The day on which evidence of mating was found was designated as gestation day 0 (GD0).

3. **Animal assignment:** Animals were assigned to control and treated groups at the end of each week of mating using a body weight dependent procedure (no further details given). Animals were assigned to dose groups as indicated in Table 1.

TABLE 1: Animal Assignment				
Dose (mg/kg bw/day)	0	75	250	750
Number of Females	25	25	25	25

4. **Dose selection rationale:** The range of doses was selected in agreement with the sponsor representative and was based on the results obtained in a previous range finding study in the rat (SA 98333). In the range-finding study, doses were 0, 50, 100, 300, 600, and 1000 mg/kg. Maternal body weight and body weight gain were decreased at 1000 mg/kg/day compared to controls. In addition, corrected body weight changes were significantly decreased at 600 and 1000 mg/kg/day. In the present study, the high dose of 750 mg/kg/day was based on maternal toxicity at 1000 mg/kg/day in the range-finding study. Other doses were chosen to give a graded response.

5. **Dosage preparation and analysis:** Test material-vehicle mixture was prepared periodically by mixing appropriate amounts of test substance with 0.5% methylcellulose 400 with storage at 5°C (± 3°C) temperature. Homogeneity of the suspensions was checked during the first formulation for the lowest and highest concentrations. Stability of the test substance in suspension in the vehicle was determined in the range-finding study (SA 98333). All concentrations were checked for each new formulation.

Results:

Homogeneity analysis: Homogeneity was checked on the formulations at 7.5, 25, and 75 g/L. The sampling was done at three levels, the surface, middle, and bottom. All three samples measured 7.4 g/L for the 7.5 g/L sample (99% of nominal concentration). For the 25 g/L samples, the measurements were 25.7 g/L for surface, 26.0 for the middle, and 25.8 g/L at the bottom. These were 103, 104, and 103% of nominal concentration respectively. The measurements for the surface, middle, and bottom of the 75 g/L samples were 76.7 g/L (102% of nominal concentration), 77.1 g/L (103% of nominal concentration), and 77.6 g/L (103% of nominal concentration) respectively. The results were within the target range (90 to 110% of nominal concentration for concentration ranging from 5 to 250 g/L).

Stability analysis: The stability of preparations was checked in the range-finding study (SA 98333). Preparations were stored at 5°C ± 3°C with daily four-hour periods at ambient temperature (~20°C) under magnetic stirring to reproduce the conditions of utilization. Two samples from these preparations (1.0 and 100 g/L) were taken after 0, 7, 14, and 21 days. The measured concentration was 1.0 g/L at all time points (100% of nominal concentration) for the 1.0 g/L sample. The measured concentration for the 100 g/L sample was 105 g/L, (Day 0, 105% of nominal concentration), 102 g/L (Day 7, 102% of nominal concentration), 105 g/L (Day 14, 105% of nominal concentration), and 102 g/L (Day 21, 102% of nominal concentration). The results were within target ranges.

Concentration analysis: Mean values obtained from the homogeneity checks were used as measured concentrations. The values ranged from 7.4-7.4 g/L for the 7.5 g/L sample, 24.3-25.83 g/L for the 25 g/L sample, and 72.8-77.13 g/L for the 75 g/L sample. The results were within the target range.

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

6. **Dosage administration:** All doses were administered once daily by gavage, on gestation days 6 through 20, in a volume of 10 mL/kg of body weight/day. Dosing was based on the body weight of the most recent body weight determination.

C. OBSERVATIONS:

1. **Maternal observations and evaluations:** The animals were checked for mortality or clinical signs daily. Body weight was recorded on gestation days 0, 3, 6, 8, 10, 12, 14, 16, 18, and 21 and food consumption was recorded during the periods GD 1-3, 3-6, 6-8, 8-10, 10-12, 12-14, 14-16, 16-18, and 18-21. Dams were sacrificed on day 21 of gestation. Examinations at sacrifice consisted of macroscopic examination of the visceral organs, weighing and examination of the reproductive tract including number of corpora lutea, implantation sites, resorption sites, live and dead fetuses, and the sex and weight of live fetuses.

2. **Fetal evaluations:** The fetuses were examined as follows. Half of each litter was immersed in Bouin's fluid for subsequent internal examination following free-hand sectioning. The remaining half was eviscerated and placed in absolute ethanol before staining. A modification of Staples and Schnell staining technique (alizarin red) was used and subsequent skeletal examination was performed. Structural deviations were defined as malformations, anomalies, or variations. Malformations were described as major abnormalities that are rare and/or probably lethal. Anomalies were described as minor differences from normal that are detected relatively frequently and not obviously detrimental. Variations were described as structural changes occurring frequently in the control population.

D. DATA ANALYSIS:

1. **Statistical analyses:** Statistical analyses were performed using SAS programs. Results of maternal body weight changes and corrected body weight change, food consumption, and mean litter weights were compared between the treated groups and the control group by use of Bartlett's test for homogeneity, analysis of variance (ANOVA) when Bartlett's test indicated homogeneous variances, and Dunnett's test if ANOVA was significant. When Bartlett's test indicated heterogeneous variances, the Kruskal-Wallis non parametric one-way ANOVA was performed, followed by the Mann-Whitney test when the Kruskal-Wallis test was significant. Data from non-pregnant, dead or killed "*in extremis*" animals were not included in group mean calculations of all maternal parameters.

   Litter data were analyzed statistically using the Kruskal-Wallis test, followed by the Mann-Whitney test when the Kruskal-Wallis test was significant.

The significance levels for each statistical comparison were 0.05 and 0.01.

2. **Indices:** The following indices were calculated from records of animals in the study:

Body weight (BW) change and food consumption (FC) for interval periods were calculated as follows:
BW (Day 6 to Day 8) = BW D8 - BW D6
FC (Day 6 to Day 8) = (FC D6 + FC D7)/2

Corrected body weight change (CBWC) was calculated as follows:
CBWC = (BW D21 - BW D0) - gravid uterine weight

Pre-implantation loss (%) = ((number of corpora lutea - number of implantations)/ number of corpora lutea) x 100

Post-implantation loss (%) = ((number of implantations - number of viable fetuses)/ number of implantations) x 100

Male sex ratio = number of male fetuses/total number of fetuses

Percentage of live fetuses = (number of live fetuses/ (number of live fetuses+ number of dead fetuses)) x 100

3. **Historical control data:** Historical control data were not provided to allow comparison with concurrent controls.

II. RESULTS:

A. MATERNAL TOXICITY:

1. **Mortality and clinical observations:** There was no treatment-related mortality in the study. At 250 and 750 mg/kg/day, 6 (24%) and 18 (72%) females respectively, had at least one occurrence of transient salivation during the entire treatment period. At 750 mg/kg/day, the salivation was associated with red nasal discharge a few minutes following administration in some animals. The observations disappeared approximately one hour after treatment. Piloerection was also observed on the day of treatment in 2/25 and 3/25 dams at 250 and 750 mg/kg bw/day, respectively. Piloerection was associated with reduced motor activity in 1 animal at 750 mg/kg.

2. **Body weight:** Body weight data are summarized in Table 2. Absolute maternal body weight during the pre-dosing period was similar among all the groups. Body weight parameters at 75 mg/kg/day were comparable to control body weight parameters. During GD10-14 and GD8-10 in the 250 and 750 mg/kg/day groups respectively, mean maternal body weight change was statistically significantly reduced (>25%) compared to control body weight during these same time periods. There was a trend of decreasing body weight due to decreased body weight gain and food consumption in the mid and high dose dams. From treatment day 14 until day 21, dams in the 250 and 750 mg/kg/day groups had a mean body weight that was consistently 5% and 7% respectively, less than control body weight. At 250 and 750 mg/kg/day, corrected body weight changes were statistically significantly reduced by 31% and 37% of control weight respectively, indicating maternal systemic toxicity.

TABLE 2 Mean (±SD) maternal body weight (g), body weight change (g), and food consumption (g/d) [a]

Interval	Dose in mg/kg bw/day (# of Dams)			
	Control (24)	75 (25)	250 (25)	750 (25)
Pretreatment: Day 0	270.5 ± 14.66	270.1 ± 14.38	267.2 ± 13.85	266.6 ± 13.90
Pretreatment: Day 3	291.1 ± 14.79	291.0 ± 17.45	286.5 ± 13.88	287.0 ± 15.10
Treatment: Day 6	306.8 ± 16.43	305.7 ± 19.31	300.4 ± 14.21	301.0 ± 14.62
Treatment: Day 10	325.3 ± 17.78	321.5 ± 21.70	313.4 ± 16.81	309.2 ± 17.87
Treatment: Day 14	346.8 ± 20.91	342.0 ± 24.59	329.4 ± 18.86	325.2 ± 24.36
Treatment: Day 18	390.7 ± 24.88	385.1 ± 31.46	372.0 ± 19.28	367.3 ± 28.20
Posttreatment: Day 21	445.8 ± 30.28	439.9 ± 38.89	423.7 ± 23.95	415.7 ± 29.85
Weight Change: Days 0-3	20.6 ± 5.34	20.9 ± 6.71	19.4 ± 5.22	20.4 ± 5.92
Weight Change: Days 6-8	8.0 ± 4.34	5.7 ± 3.67	5.7 ± 4.28	4.6 ± 6.99
Weight Change: Days 8-10	10.4 ± 2.93	10.1 ± 4.54	7.3 ± 5.98	3.6 ± 9.63**
Weight Change: Days 10-14	21.5 ± 5.13	20.5 ± 5.83	16.0 ± 7.33**	16.0 ± 14.5
Weight Change: Days 14-18	43.9 ± 8.31	43.1 ± 10.24	42.6 ± 8.81	42.1 ± 8.15
Weight Change: Days 18-21	55.1 ± 9.18	54.8 ± 12.26	51.7 ± 12.12	48.4 ± 9.44
Corrected Body Weight Change	68.2 ± 17.02	63.9 ± 19.47	46.8 ± 18.66**	43.1 ± 18.44**
Food Consumption: Days 1-3	27.7 ± 3.18	27.0 ± 3.09	26.8 ± 2.45	26.9 ± 2.60
Food Consumption: Days 6-8	29.5 ± 4.11	28.3 ± 4.20	27.6 ± 3.12	25.9 ± 3.52**
Food Consumption: Days 8-10	29.5 ± 3.40	29.0 ± 4.46	26.4 ± 3.40**	24.7 ± 4.98**
Food Consumption: Days 16-18	29.4 ± 3.61	28.4 ± 3.98	26.3 ± 4.48*	26.2 ± 3.96*
Food Consumption: Days 18-21	28.9 ± 4.05	28.1 ± 4.20	25.2 ± 2.75*	25.4 ± 3.43*

[a] Data obtained from pages 33-40 in the study report.
* Statistically different ($p < 0.05$) from the control.
** Statistically different ($p < 0.01$) from the control.

3. **Food consumption:** Food consumption data are summarized in Table 2. Food consumption at 75 mg/kg/day was comparable to control food consumption. Mean food consumption during GD8-21 was significantly reduced at 250 mg/kg/day and during the entire treatment period at 750 mg/kg/day.

4. **Gross pathology:** There were no treatment-related observations at necropsy.

5. **Cesarean section data:** Data are summarized in Table 3. Twenty-four of the twenty-five females mated in the control group were pregnant. All females in the other study groups were pregnant. Group means and litter average for corpora lutea, implantation losses, and sex ratio were comparable in all experimental groups. There were no dead fetuses. Litter means of fetal body weight per sex and with sex combined were comparable between all study groups.

TABLE 3 Cesarean section observations [a]

Observation	Dose (mg/kg bw/day)			
	0	75	250	750
No. Animals assigned (mated)	25	25	25	25
No. Animals pregnant	24	25	25	25
Pregnancy rate (%)	96	100	100	100
No. Nonpregnant	1	0	0	0
Maternal wastage				
No. died	0	0	0	0
No. Died pregnant	0	0	0	0
No. Died nonpregnant	0	0	0	0
No. Aborted	0	0	0	0
No. Premature delivery	0	0	0	0
Total No. corpora lutea Corpora lutea/Dam	431 18.0 ± 3.57	432 17.3 ± 2.57	445 17.8 ± 2.40	417 16.7 ± 1.25
Total No. implantations Implantations/Dam	398 15.9 ± 2.19	381 15.2 ± 3.31	409 16.4 ± 1.19	396 15.8 ± 1.25
Total No. litters	24	25	25	25
Total No. live fetuses Live fetuses/Dam	354 14.8 ± 2.35	368 14.7 ± 3.32	391 15.6 ± 1.38	377 15.1 ± 1.44
Total No. dead fetuses Dead fetuses/Dam	0 0.0 ± 0.00	0 0.0 ± 0.00	0 0.0 ± 0.00	0 0.0 ± 0.00
Total No. resorptions	28	13	18	19
Early	26	13	15	17
Late	2	0	3	2
Resorptions/Dam				
Early	1.1 ± 1.41	0.5 ± 0.71	0.6 ± 0.71	0.7 ± 0.56
Late	0.1 ± 0.28	0.0 ± 0.00	0.1 ± 0.33	0.1 ± 0.28
Litters with total resorptions	0	0	0	0
Mean fetal weight (g)	5.39 ± 0.350	5.34 ± 0.232	5.26 ± 0.261	5.27 ± 0.302
Males	5.53 ± 0.339	5.52 ± 0.275	5.40 ± 0.296	5.39 ± 0.318
Females	5.26 ± 0.364	5.21 ± 0.221	5.11 ± 0.238	5.13 ± 0.309
Sex ratio (% male)	0.49 ± 0.147	0.46 ± 0.154	0.52 ± 0.133	0.52 ± 0.135
Preimplantation loss (%)	8.9 ± 17.06	11.1 ±18.07	7.0 ± 9.79	4.9 ± 4.90
Postimplantation loss (%)	7.1 ± 9.40	3.4 ± 4.62	4.4 ± 5.31	4.9 ± 3.43

[a] Data obtained from pages 42-45, 67-70 in the study report.
* Statistically different ($p < 0.05$) from the control.
** Statistically different ($p < 0.01$) from the control.

B. DEVELOPMENTAL TOXICITY:

In the control, 75, 250, and 750 mg/kg/day groups, the number of fetuses (litters) examined externally were 354 (24), 368 (25), 391 (25), and 377 (25), respectively; the number examined internally were 173 (24), 179 (25), 189 (25), and 183 (25), respectively; and the number examined for skeletal malformations were 181 (24), 189 (25), 202 (25), and 193 (25), respectively.

1. **External examination:** There were eight fetuses with body weight less than 4.0 g; two in control, one at 75 mg/kg/day, two at 250 mg/kg/day, and three at 750 mg/kg/day. One fetus at 75 mg/kg/day had agnathia and two fetuses at 250 mg/kg/day had anasarca (Table 4a). No effects related to treatment were noted.

2. **Visceral examination:** No treatment-related findings were noted in any group upon internal examination of the fetuses. Malformations, anomalies, and variations, including dilated renal pelvis, dilated ureter, and convoluted ureter, were common findings in fetuses from treated and control groups, were observed with a comparable incidence in all study groups and were not considered treatment-related (Table 4b).

3. **Skeletal examination:** An increased incidence of unossified 7th cervical centrum was observed sporadically without dose response in treated groups. Variations in vertebrae, sternebrae, and ribs were noted in all groups with no dose response relationship (Table 4c). There was an increase in the incidences of short 13th ribs, but the incidence was low, not statistically significant (Fisher's exact test used), and lacked dose-response. No treatment-related effects were observed.

TABLE 4a. External examinations [a]

Observations	Dose (mg/kg bw/day)			
	0	75	250	750
No. Fetuses(litters) examined	354 (24)	368 (25)	391 (25)	377 (25)
No. Fetuses(litters) affected	2 (2)	2 (2)	4 (3)	3 (3)
Body weight < 4.0 g	2 (2)[b]	1 (1)	2 (2)	3 (3)
Agnathia	0 (0)	1 (1)	0 (0)	0 (0)
Anasarca	0 (0)	0 (0)	2 (1)	0 (0)

[a] Data obtained from page 47 in the study report.
[b] Fetal (litter) incidence

TABLE 4b. Visceral examinations [a]

Observations	Dose (mg/kg bw/day)			
	0	75	250	750
No. Fetuses(litters) examined	173 (24)	179 (25)	189 (25)	183 (25)
No. Fetuses(litters) affected	60 (19)	58 (19)	63 (18)	73 (16)
Dilated renal pelvis	14 (9)[b]	17 (8)	17 (9)	23 (12)
Dilated ureter	46 (19)	41 (19)	46 (18)	50 (16)

[a] Data obtained from page 48 in the study report.
[b] Fetal (litter) incidence

TABLE 4c. Skeletal examinations [a]				
Observations [b]	Dose (mg/kg bw/day)			
	0	75	250	750
No. Fetuses(litters) examined	181 (24)	189 (25)	202 (25)	193 (25)
No. Fetuses (litters) with sternebrae variations	32 (17) [c]	31 (17)	36 (20)	40 (22)
No. Fetuses (litters) with vertebrae variations	11 (8)	8 (6)	11 (10)	12 (11)

[a] Data obtained from pages 50-51 in the study report.
[b] Some observations may be grouped together.
[c] Fetal (litter) incidence

III. DISCUSSION AND CONCLUSIONS:

A. INVESTIGATORS' CONCLUSIONS:

The study author concluded that administration of RPA 203328 by gavage to pregnant Sprague-Dawley rats from gestation days 6-20 resulted in signs of maternal toxicity at 250 and 750 mg/kg/day including decreased body weight change, decreased corrected body weight change, and decreased food consumption during the treatment period. None of the litter parameters recorded during cesarean section was affected by treatment. External, internal, and skeletal examination of the fetuses did not reveal any treatment-related findings. The maternal NOEL was 75 mg/kg/day and the developmental NOEL was 750 mg/kg/day.

B. REVIEWER COMMENTS:

1. **Maternal toxicity:** Maternal toxicity was evident in the study. Clinical signs of toxicity were observed in dams around the time of treatment at ≥250 mg/kg/day. Body weight changes and corrected body weight changes were significantly reduced in dams of the 250 and 750 mg/kg/day groups (mid and high-dose). Administration of the test substance significantly reduced food consumption during the treatment period in the same groups noted above. It is likely that test article administration led to decreased food consumption which resulted in decreased weight gain and decreased absolute body weight. The significantly reduced corrected body weight change supports maternal toxicity in the mid and high dose groups since no treatment-related effects were observed on fetal body weight.

   The maternal LOAEL in Sprague-Dawley is 250 mg/kg bw/day, based on clinical signs (salivation and piloerection around the time of treatment), decreased body weight change, decreased corrected body weight change, and decreased food consumption. The maternal NOAEL is 75 mg/kg bw/day.

2. **Developmental toxicity:**

   a. **Deaths/resorptions:** Maternal treatment with test article did not result in an increase in fetal or embryo death.

b. **Altered growth:** No adverse effects of maternal treatment were observed on fetal body weight or ossification rate.

c. **Developmental variations:** The number of litters containing fetuses with developmental variations was not increased as a result of maternal treatment.

d. **Malformations:** No treatment related external, visceral, or skeletal malformations were observed in fetuses from dams treated with RPA 203328.

The developmental LOAEL was not observed. The developmental NOAEL is 750 mg/kg bw/day.

C. STUDY DEFICIENCIES:

A minor deficiency is that the age of the mated females was not listed in the study report and differences in absolute body weight were not analyzed statistically.

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R168684

Chemical Name: Pyrasulfotole Technical
RPA 203328 (a metabolite of isoxaflutole)

PC Code: 000692

623001

HED File Code: 13000 Tox Reviews
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