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

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

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

TXR No. 0050321

MEMORANDUM

DATE: 12/14/2004

SUBJECT: 044309. Clothianidin Toxicology Data Evaluation Records

PC Code: 044309 DP Barcode: D282578

TO: Dan Kenny
RM 01
Registration Division (7505C)

FROM: Pamela M. Hurley, Toxicologist
Reregistration Branch 3
Health Effects Division (7509C)

THRU: Richard Loranger, Senior Scientist
Registration Action Branch 2
Health Effects Division (7509C)

Background and Summary:

The Health Effects Division (HED) was asked to review all the submitted toxicology studies conducted with the new chemical, Clothianidin (TI-435). All the Data Evaluation Records (DERs) reviewed by HED are attached. The acute toxicity studies on the technical material have been previously reviewed by the Registration Division (RD); however, some of the acute toxicity studies conducted with metabolites/degradates were reviewed by HED. They are included in this package.

With the exception of a developmental immunotoxicity study, the toxicological database for Clothianidin is adequate for assessment of risk to infants and children. There is a concern for immunotoxicity following exposure of clothianidin during the period of organogenesis. This concern was based on the decreases in absolute and adjusted thymus and spleen weights observed in several species in various studies. In addition, the available data indicate that the juvenile rats appeared to be more sensitive/susceptible to these effects than adults in the two-generation

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reproduction study. Testing needs to be conducted to assess immune system function in adults and young animals following exposure during the period of organogenesis.

The requirements (CFR 158.340) for food use for Clothianidin are listed in Table 1. Use of the new guideline numbers does not imply that the new (1998) guideline protocols were used. Table 1 lists studies conducted with the technical material and Table 2 lists additional studies conducted with metabolites and degradates.

Table 1. Clothianidin Guideline Requirements: Studies Conducted with Technical Material

Test		Technical		
		MRID	Required	Satisfied
870.1100	Acute Oral Toxicity	45422621	yes	yes
870.1200	Acute Dermal Toxicity	45422634	yes	yes
870.1300	Acute Inhalation Toxicity	45422636	yes	yes
870.2400	Primary Eye Irritation	45422701	yes	yes
870.2500	Primary Dermal Irritation	45422703	yes	yes
870.2600	Dermal Sensitization	45422705	yes	yes
870.3100	Oral Subchronic (rodent)	45422708, 45422809	yes	yes
870.3150	Oral Subchronic (nonrodent)	45422808, -10, -11	yes	yes
870.3200	21-Day Dermal	45422707	yes	yes
870.3250	90-Day Dermal	Not submitted	no	-
870.3465	90-Day Inhalation	Not submitted	no	-
870.3700a	Developmental Toxicity (rodent)	45422710 and -11	yes	yes
870.3700b	Developmental Toxicity (nonrodent)	45422712 and -13	yes	yes
870.3800	Reproduction	45422714 thru -16, 45422825 thru -26	yes	yes
870.4100a	Chronic Toxicity (rodent)	45422719 and -20	yes	yes[a]
870.4100b	Chronic Toxicity (nonrodent)	45422717 and -18	yes	yes
870.4200a	Oncogenicity (rat)	45422719 and - 20	yes	yes
870.4200b	Oncogenicity (mouse)	45422709, 45422721, 45422722	yes	yes
870.4300	Chronic/Oncogenicity	45422719, 45422720	yes	yes
870.5100	Mutagenicity—Gene Mutation - bacterial	45422731 45422732 45422733 45422734	yes	yes
870.5300	Mutagenicity—Gene Mutation - mammalian	45422736 45422737 45422738	yes	yes
870.5375	Mutagenicity—Structural Chromosomal Aberrations	45422740	yes	yes
870.5550	Mutagenicity—Other Genotoxic Effects	45422735 45422739	yes	yes

5.2. Tabulated Summary of Data

Summary of Mean Values Without S9 Mix

Table and Group	Strain				
	TA 1535	TA 100	TA 1537	TA 98	TA 102
1-5 µg/Plate					
0	10	84	9	18	250
16	10	81	6	19	259
50	7	80	6	16	263
158	7	81	5	13	294
500	7	82	6	20	257
1581	10	73	6	12	256
5000	10	63	4	18	196
Na-azide	729				
NF		248			
4-NPDA			97	171	
Cumene					400
6-10 µg/Tube					
0	7	66	9	21	241
16	8	64	9	23	278
50	8	66	10	17	257
158	7	76	10	19	255
500	6	78	10	24	251
1581	8	55	9	17	235
5000	-	41	4	13	132
Na-azide	572				
NF		220			
4-NPDA			169	172	
Cumene					511

237

Test	Technical		
	MRID	Required	Satisfied
870.6100a Acute Delayed Neurotox. (hen)	Not submitted	no	-
870.6100b 90-Day Neurotoxicity (hen)	Not submitted	no	-
870.6200a Acute Neurotox. Screening Battery (rat)	45422801 and -02	yes	yes
870.6200b 90 Day Neuro. Screening Battery (rat)	45422803, 45422825	yes	yes
870.6300 Developmental Neurotoxicity	45422804	yes	yes
Developmental Immunotoxicity	Not yet submitted	yes	no
870.7485 General Metabolism	45422805 and -06 45422807 45422823	yes	yes
870.7600 Dermal Penetration	45868001	no	yes
Special Studies for Ocular Effects Acute Oral (rat) Subchronic Oral (rat) Six-month Oral (dog)	Not submitted	no	-

a Satisfied with combined chronic toxicity/carcinogenicity study

Table 2. Studies Conducted with Clothiandin Metabolites/Degradates

Test	MRID	Metabolite/Degradate Tested
870.1100 Acute Oral Toxicity	45422623	BN0335E2
870.1100 Acute Oral Toxicity	45422624	TZMU
870.1100 Acute Oral Toxicity	45422626	TZNG
870.1100 Acute Oral Toxicity	45422627	TMG
870.1100 Acute Oral Toxicity	45422628	BN0230M
870.1100 Acute Oral Toxicity	45422630	CCMT-Adduct
870.1100 Acute Oral Toxicity	45422631	Hexahydropyrimidine
870.1100 Acute Oral Toxicity	45422632	TI-435-Triazan
870.2500 Primary Dermal Irritation	45422813	CCMT-Adduct
870.2600 Dermal Sensitization	45422815	CCMT-Adduct
870.2600 Dermal Sensitization	45422821	TI-435-Triazan
870.5100 Mutagenicity—Gene Mutation - bacterial	45422817	Benzyltriazinone
870.5100 Mutagenicity—Gene Mutation - bacterial	45422724	BN0335E2
870.5100 Mutagenicity—Gene Mutation - bacterial	45422725	TZMU
870.5100 Mutagenicity—Gene Mutation - bacterial	45422726	Methyl guanidine
870.5100 Mutagenicity—Gene Mutation - bacterial	45422727	TZNG
870.5100 Mutagenicity—Gene Mutation - bacterial	45422728	TMG
870.5100 Mutagenicity—Gene Mutation - bacterial	45422729	BN0230M
870.5100 Mutagenicity—Gene Mutation - bacterial	45422730	MA I
870.5100 Mutagenicity—Gene Mutation - bacterial	45422812	N-Methylnitroguanidin
870.5100 Mutagenicity—Gene Mutation - bacterial	45422816	CCMT-Adduct
870.5100 Mutagenicity—Gene Mutation - bacterial	45422818	Hexahydropyrimidine
870.5100 Mutagenicity—Gene Mutation - bacterial	45422822	TI-435-Triazan

DATA EVALUATION RECORD

TI-435 (CLOTHIANIDIN)

Study Type: §83-1b, Chronic Dietary Toxicity Study in Dogs

Work Assignment No. 4-02-167A Amendment (Formerly 4-01-167A)
(MRIDs 45422717 and 45422718)

Prepared for

Health Effects Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
1921 Jefferson Davis Highway
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Disclaimer

This Data Evaluation Record may have been altered by the Health Effects Division subsequent to signing by Dynamac Corporation personnel.

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Signature: Pamela Hurley
Signature: Ghazi Dannan
Date 12/10/04
Template version 11/01

TXR#: 0050321

DATA EVALUATION RECORD

STUDY TYPE: Chronic toxicity - dog [feeding]; OPPTS 870.4100b [§83-1b]; OECD 452.

PC CODE: 044309 **DP BARCODE:** D282578
SUBMISSION NO.: S614462

TEST MATERIAL (PURITY): TI- 435 (Clothianidin; 95.2-95.5 % a.i.)

SYNONYMS: (E)-1-2-(2-chloro-5-thiazolylmethyl)-3-methyl-2-nitroguanidine

CITATION: Bernier, L. (2000). 52-Week Dietary Chronic Toxicity Study With TI 435 in Dogs. Covance Laboratories, Inc., Vienna, VA. Laboratory Project Id: Covance 6155-113, March 22, 2000. MRID 45422717.

Bernier, L. (2001). 52-Week Dietary Chronic Toxicity Study With TI 435 in Dogs. Covance Laboratories, Inc., Vienna, VA. Laboratory Project Id: Covance 6155-126, April 2, 2001. MRID 45422718.

SPONSOR: Takeda Chemical Industries Ltd., 13-10 Nihonbashi 2-chome, Chuo-ku, Tokyo, Japan

EXECUTIVE SUMMARY: In a chronic toxicity study (MRIDs 45422717 and 45422718), TI-435 (clothianidin; 95.2-95.5% a.i., Lot No. 30037120) was administered in the diet to 4 beagle dogs/sex/dose at dose levels of 0, 325, 650, 1500, or 2000 ppm (equivalent to 0/0, 4.8/8.5, 16.6/15.0, 36.3/40.1 and 46.4/52.9 mg/kg bw/day, respectively in males/females) for 52 weeks.

There were no mortalities. There were no treatment-related differences in clinical observations, body weights, body weight gains, food consumption, ophthalmoscopy, neurobehavior, urinalysis, organ weights, gross pathology, or microscopic pathology.

Dose-related decreases ($p<=0.05$) in alanine aminotransferase (ALT) activity were observed throughout treatment in the 1500 ppm males (decr. 46-128%) and females (decr. 32-73%), and in the 2000 ppm males (decr. 55-71%) and females (decr. 36-73%). The ALT levels noted at the 2000 ppm groups at Week 26 (females only) and at Weeks 39 and 52 (males and females) were below historical controls data range. Decreases in ALT levels may indicate decreased liver mass; however, because liver weights in the treated groups were comparable to controls, the decreases in ALT are considered to be treatment related but not adverse.

Erythrocytes, hemoglobin, and hematocrit were decreased (decr. 15-18%; $p<=0.05$) in the 2000

ppm females at Week 52. The hemoglobin and hematocrit levels were lower than the range of historical controls. The decrease in circulating red blood cells was sufficient to be considered adverse at the high dose in females.

The LOAEL for this study was 2000 ppm (equivalent to 52.9 mg/kg bw/day in females) based on clinical evidence of anaemia. There were no adverse effects seen in male dogs and as such the NOAEL for males was 2000 ppm, and 1500 ppm for females.

The submitted study is classified as **acceptable/guideline** and satisfies the requirements for a chronic oral toxicity study in the dog (OPPTS 870.4100b; OECD 452). Although, no LOAEL was observed; the dose-selection rationale was justifiable based on results from the 4-Week (MRID 45422808) and 13-Week (MRID 45422810) studies.

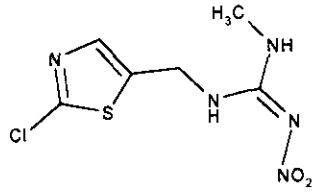
COMPLIANCE: Signed and dated GLP, Quality Assurance, Flagging, and Data Confidentiality statements were provided.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Material: TI-435 (*Clothianidin*)

Description:	Pale yellow powder
Lot #:	30037120
Purity:	95.2 -95.5 % a.i.
Compound Stability:	Stable in the diet for up to 14 days at room temperature
CAS # of TGAI:	210880-92-5



2. Vehicle: Diet

3. Test animals:

Species:	Dog
Strain:	Beagle
Age/weight at study initiation:	Approximately 6-7 months of age/ 9.3-12.8 kg for males and 8.5-11.0 kg for females
Source:	Covance Research Products, Inc., Cumberland, VA
Housing:	Individually, in elevated, stainless-steel cages
Diet:	Certified Canine Diet® Meal # 5007, PMI® Nutrition International, *ad libitum* (except during fasting prior to blood and urine collections)
Water:	Tap water, *ad libitum*
Environmental conditions:	
Temperature:	20.8 - 33.3 °C
Humidity:	30.0 - 72.1%
Air changes:	≥10/hour
Photoperiod:	12 hrs light/ 12 hrs dark
Acclimation period:	At least 2 weeks

B. STUDY DESIGN

1. In life dates - Start: September 4, 1998 End: September 9, 1999

2. Animal assignment - Animals were randomly assigned (stratified by weight) to the test groups shown in Table 1. At study initiation all animals were within ±20 % of the mean body weight for each sex.

Table 1. Study design[a]

Test Group	Conc. in Diet (ppm)	Mean dose (mg/kg bw/day) [M/F]	# of Animals	
			Male	Female
Control	0	0/0	4	4
Low	325	7.8/8.5	4	4
Mid	650	16.6/15.0	4	4
Mid High	1500	36.3/40.1	4	4
High	2000	46.4/52.9	4	4

a Data obtained from the study report (MRID 45422717), pages 17 and 29, and Table 6, page 79.

3. Dose selection rationale - In a 4-Week dietary dog study (MRID 45422808), submitted concurrently with this study, TI-435 was administered in the diet to 3 beagle dogs/sex/group at nominal doses of 0, 1250, 2500, or 5000 ppm for up to 4 weeks. The study author indicated the LOAEL was 2500 ppm based on compromised general health resulting in mortality; decreased body weight and food consumption; and decreased number of erythrocytes and leukocytes, corroborated with bone marrow hypocellularity and depletion of lymphoid tissues.

Thereafter, in a 13-Week dietary dog study (MRID 4522810), also submitted concurrently with this study, TI-435 was administered in the diet to 4 beagle dogs/sex/group at nominal doses of 0, 325, 650, 1500, or 2250 ppm for 13 weeks. The LOAEL was 2250 ppm based on decreases in food consumption, body weight gains, leukocytes, and changes in clinical chemistry. The only findings at 1500 ppm were thinness and decreases in albumin, total protein, and alanine aminotransferase.

Based upon the results of the 4-Week and 13-Week studies, the doses summarized in Table 1 were selected for the 52-Week study.

4. Diet preparation, dosing, and analysis - Test diets were prepared each week by first mixing the appropriate amount of test material with diet to make a pre-mix. This pre-mix was then blended with additional diet to achieve appropriate dietary concentration levels. Prepared diets were stored at room temperature until used. Homogeneity (top, middle, bottom) of the 50 and 5000 ppm formulations was determined prior to the study. Stability for up to 14 days at room temperature was analyzed for the 50, 325, and 2500 ppm diet formulations prior to the study. Concentration analyses were determined for each dose level from diets prepared for study Weeks 1, 13, 26, 39, and 52. All samples were analyzed in duplicate. Additionally during the study, samples of treated food using the same mixing procedures as in the study were analyzed by the Sponsor six months after study initiation, and at the end of the in-life phase at concentrations of 50 and 5000 ppm for stability and concentration. For dosage calculations, the test material was assumed to be 100% pure.

Results

Homogeneity Analysis - (mean±SD % of nominal; %RSD):

50 ppm: 96.5±0.62; 0.64
5000 ppm: 96.3±0.72; 0.75

Stability Analysis - (range as % of nominal after 14 days at room temperature):

50 ppm: 92.3-92.4
325 ppm: 93.1
2500 ppm: 90.1-90.9

Concentration Analysis - (range as mean % of nominal):

325 ppm: 95.5-101
650 ppm: 94.9-101
1500 ppm: 93.6-100
2000 ppm: 92.7-101

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

5. Statistics - Data were analyzed for homogeneity of variances by Levene's test. If variances were heterogenous ($p \leq 0.05$), transformations were attempted to achieve homogeneity. Body weights, body weight gains, food consumption, continuous pathology values, and organ weight data were analyzed using one-way analysis of variance (ANOVA). If ANOVA was significant, then Dunnett's t-test was used to compare treated groups with controls. Alpha levels of 0.05 and 0.01 denoted significance. In general the reviewer considered the statistical methods appropriate; however, in cases of heterogeneous variances, non-parametric analyses (Kruskal-Wallis & Mann Whitney) may have been appropriate.

C. METHODS

1. Observations

a. Cageside Observations - Animals were inspected twice daily for mortality and moribundity and once daily for clinical signs of toxicity.

b. Clinical Examinations - Detailed physical examinations were performed weekly at the same time as the weekly weighing.

c. Neurological Evaluations - Neurological evaluations were performed on each animal during Weeks 26, 38, and 53. Observations included, but were not limited to, changes in skin, fur, eyes, and mucous membranes; secretion and excretion occurrences; autonomic activity (e.g. lacrimation, piloerection, pupil size, respiration); changes in posture, reactivity to handling, clonic or tonic movements, and stereotypies or bizarre behavior. Gait changes were determined by assessing the animal's ability to walk freely in a large area.

2. Body weight and body weight gains - Animals were weighed prior to treatment, weekly during Weeks 1-14, once every 4 weeks thereafter, and finally at Week 53. Group mean body

weight and body weight gains (kg) were reported weekly. Overall (Weeks 1-53) group mean body weight gains were also reported.

3. Food consumption and test material consumption - Food consumption (kg) was recorded weekly for Weeks 1-13, once every 4 weeks thereafter, and finally at Week 52. Food consumption was reported for each of these intervals and for the overall (Weeks 1-53) study. Food efficiency was not reported. Overall (Weeks 1-53) study test material consumption (mg/kg bw/day) was calculated from the consumption, body weight data, and nominal dose.

4. Ophthalmoscopic examination - The eyes of every animal were examined by a veterinarian prior to dosing and during Week 52 using an indirect ophthalmoscope and a slit lamp after dilation of the eyes with a mydriatic agent.

5. Hematology and Clinical Chemistry - Blood was collected from the jugular vein of each animal twice prior to treatment (Weeks -2 and -1), and during Weeks 5 (hematology only), 13, 26, 39, and 52. The animals were fasted (food only) overnight prior to collection. The CHECKED (X) parameters were examined. Additionally, a blood sample was taken from a 2000 ppm male (# G35518) at Week 15 because of high leukocyte count at Week 13.

a. Hematology

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

* Recommended for chronic studies based on Guideline 870.4100.

b. Clinical Chemistry

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

* Recommended for chronic studies based on Guideline 870.4100.

6. Urinalysis - Urine was collected overnight from each animal twice prior to treatment (Weeks -2 and -1), and during Weeks 13, 15, 26, 39, and 52. The animals were fasted (food only) overnight during collection. The CHECKED (X) parameters were examined.

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

* Recommended for chronic studies based on Guideline 870.4100.

7. Sacrifice and Pathology - At study termination, all animals were weighed, anesthetized with sodium thiopental, exsanguinated, and subjected to macroscopic examination. The CHECKED (X) tissues were collected from all animals, preserved in 10% neutral buffered formalin (with the exception of the nose and pharynx which were fixed in Bouin's fixative), embedded in paraffin, sectioned, and stained with hematoxylin and eosin. These tissues (with the exception of the lacrimal gland, nose, pharynx, and larynx) were examined microscopically. The (XX) organs, in addition, were weighed.

	DIGESTIVE SYSTEM		CARDIOVASC./HEMAT.		NEUROLOGIC
	Tongue	X	Aorta*	XX	Brain (multiple sections)*+
X	Salivary glands*	XX	Heart*+	X	Periph. nerve*
X	Esophagus*	X	Bone marrow*	X	Spinal cord (3 levels)*
X	Stomach*	X	Lymph nodes*	XX	Pituitary*
X	Duodenum*	XX	Spleen*+	X	Eyes (with optic nerve)*
X	Jejunum*	X	Thymus		
X	Ileum*				GLANDULAR
X	Cecum*		UROGENITAL	XX	Adrenal gland*+
X	Colon*	XX	Kidneys*+		Lacrimal gland
X	Rectum*	X	Urinary bladder*	XX	Thyroids with parathyroids *
XX[a]	Liver*+	XX	Testes with epididymides*+		
XX[a]	Gall bladder*	XX	Prostate*		OTHER
X	Pancreas*	XX	Ovaries*+	X	Bone
		XX	Uterus*+	X	Skeletal muscle
	RESPIRATORY	X	Mammary gland* (females)	X	Skin*
X	Trachea*	X	Vagina	X	All gross lesions and masses*
XX	Lung*	X	Cervix		
	Nose*+				
	Pharynx*+				
	Larynx*+				

a Liver and drained gall bladder were weighted together.
* Required for chronic studies based on Guideline 870.4100.
+ Organ weight required in chronic studies.

II. RESULTS

A. OBSERVATIONS

1. Clinical signs of toxicity - No treatment-related clinical signs were observed. Thinness was observed in 3/4 females in the 2000 ppm dose group. Several other clinical findings were noted such as yellow feces in all male groups including the control, yellow vomit in the 1500 and 2000 ppm females, and non-formed feces in all male and female groups including controls; however, these parameters were all minimal in incidence and/or frequency. Additionally, there was an increased frequency of redness inside the ears of the 1500 ppm females and 2000 ppm animals (Table 2); however, these observations were minor not considered to be adverse.

Table 2. Redness inside ears in dogs treated with TI-435 in the diet for 52 weeks [a]

Observation	Dose (ppm)				
	0	325	650	1500	2000
Males					
Number of dogs affected [b]	1	1	4	3	4
Females					
Number of dogs affected [b]	2	1	2	4	4

a Data obtained from individual data presented in Appendix 2a on pages 194-233 of the study report (MRID 45422717).

b Data obtained from summary data presented in Table 2a on pages 54-59 of the study report.

2. Mortality - There were no unscheduled deaths during the study.

3. Neurological Evaluations - There were no treatment-related neurological findings in males or females at Days 181, 266, or 365.

B. BODY WEIGHT AND BODY WEIGHT GAIN: Body weights in all treated males and females were similar to controls throughout the study (Table 3). Body weight gains were decreased significantly ($p \leq 0.05$) in the 2000 ppm males at Weeks 1-2; however, the effect was transient. Additionally, body weight gains were decreased in the 650 and 1500 ppm males at Weeks 9-10 and increased in the 650, 1500, and 2000 ppm males at Weeks 10-11; however these sporadic differences either were unrelated to dose or not considered adverse. Weekly body weight gains of females were comparable to controls, and overall (Weeks 1-53) body weight gains were comparable to controls in all male and female dose groups.

Table 3. Mean (±SD) body weights (kg) at selected intervals and overall (Weeks 0-53) body weight gains (kg) in dogs treated with TI-435 for 53 weeks.[a]

Study Week	Dose (ppm)				
	0	325	650	1500	2000
Males					
1	11.4 ± 1.42	10.9 ± 0.88	10.3 ± 0.62	10.7 ± 1.20	11.2 ± 1.31
13	12.4 ±1.77	12.4 ± 1.25	11.6 ± 059	11.4 ± 1.27	11.8 ± 1.53
26	12.3 ± 2.02	12.7 ± 0.91	12.0 ± 1.08	12.1 ± 1.70	12.0 ± 1.62
53	12.7 ± 1.80	12.6 ± 0.74	13.5 ± 1.93	12.5 ± 1.78	12.5 ± 1.71
Overall gain (Weeks 0-53)	1.3 ± 0.58	1.7 ± 0.71	3.3 ± 1.68	1.8 ± 0.81	1.2 ± 0.66
Females					
1	9.4 ± 0.69	9.5 ± 1.01	9.4 ± 0.50	9.3 ± 0.79	9.5 ± 1.02
13	9.9 ± 0.46	10.6 ± 0.65	10.5 ± 0.91	10.0 ± 1.03	9.8 ± 0.68
26	10.0 ± 0.57	10.7 ± 0.62	10.3 ± 1.46	10.0 ± 1.01	10.0 ± 1.04
53	10.9 ± 0.62	11.3 ± 0.70	11.3 ± 1.92	11.0 ± 1.54	10.3 ± 0.87
Overall gain (Weeks 0-53)	1.5 ± 0.68	1.8 ± 1.05	1.9 ± 1.85	1.7 ± 0.82	0.8 ± 0.44

a Data obtained from Tables 3 and 4, pages 65-67, and 71 in the study report (MRID 45422717); n=4.

C. FOOD CONSUMPTION AND TEST MATERIAL CONSUMPTION

1. Food consumption - A transient decrease (↓22%; $p \leq 0.05$) in food consumption was observed in the 2000 ppm females at Week 1-2; however, this decrease did not correlate with lower body weight or body weight gains. Food consumption was similar among treated and control males throughout dosing. Furthermore, overall (Weeks 1-53) food consumption was comparable among treated and control animals.

2. Test material consumption - Group mean test material consumption values (mg/kg bw/day) for the overall (Weeks 1-53) study are reported in Table 1.

D. OPHTHALMOSCOPIC EXAMINATION: No lesions were reported in the eyes of any of the animals after 363 days of treatment.

E. BLOOD ANALYSES

1. Hematology - Erythrocytes, hemoglobin, and hematocrit were significantly decreased (↓15-18%; $p \leq 0.05$) in the 2000 ppm females at Week 52 (Table 4). When compared with historical control data (MRID 45422718) the hemoglobin and hematocrit values were below the range of historical controls. The decrease in circulating red blood cells was sufficient to be considered adverse at the high dose in females. Similar decreases in circulating red blood cell parameters (6-7% decrease) were noted in females treated with 1500 ppm, however these decreases did not reach levels of significance.

Several other hematological parameter decreases ($p \leq 0.05$) were noted in white blood cells in the 2000 ppm females and were deemed related to treatment but not adverse because they did not persist throughout dosing: (i) leukocytes at Weeks 5 (↓46%) and 39 (↓27%), (ii) monocytes at Weeks 5 (↓83%) and 13 (↓67%), (iii) eosinophils at Week 5 (↓75%), and (iv) neutrophils at Week 39 (↓40%). Additionally in females, reticulocytes were decreased at 1500 ppm (↓75-80%) and at 2000 ppm (↓60-75%) at Week 39.

In males, absolute lymphocytes were decreased at 650,1500, and 2000 ppm (↓24-43%) at Week 5; however, these decreases were deemed related to treatment but not adverse because they were transient. No other hematological differences from controls were observed in males.

Table 4. Selected mean hematological findings in female dogs treated with TI-435 in the diet for up to 52 weeks.[a]

Hematological Parameter	Study Week	Dose (ppm)					
		0	325	650	1500	2000	Historical controls[b]
Erythrocyte Count (millions/µL)	Pretest (-2)	6.52±0.530	6.87±0.369	6.56±0.412	6.44±0.390	6.81±0.209	NP
	13	6.82±0.308	7.04±0.580	6.45±0.609	6.47±0.446	6.43±0.183	NP
	52	7.05±0.841	7.27±0.302	6.75±0.474	6.56±0.496	6.00*±0.316(↓15)	5.83-7.28
Hemoglobin (g/dL)	Pretest (-2)	14.2±0.78	14.6±0.34	14.1±0.74	14.1±0.82	14.6±0.47	NP
	13	14.9±0.42	15.2±1.18	14.0±1.28	14.2±1.16	13.8±0.33	NP
	52	15.9±1.45	16.2±0.50	15.0±1.14	14.9±0.94	13.2*±0.21(↓17)	13.8-17.1
Hematocrit (%)	Pretest (-2)	44.1±2.92	45.4±1.53	43.8±2.07	44.3±2.62	45.6±1.25	NP
	13	44.6±1.40	44.5±2.64	41.5±4.41	41.9±3.45	40.5 ±0.84	NP
	52	48.1±4.51	48.7±1.36	45.4±2.99	44.8±3.15	39.4*±1.00(↓18)	40.6-52.8

a Data were obtained from the Table 7 on pages 80-115 of the study report (MRID 45422717); n=4; Percent difference from controls, calculated by the reviewers, is included in parentheses.
b Historical control data were obtained from page 12 of the study report (MRID 45422718); n=23
* Significantly different from the control group at $p \leq 0.05$.
NP Not provided

2. Clinical Chemistry - Dose-related decreases ($p \leq 0.05$) in alanine aminotransferase activity were observed throughout treatment in the 1500 ppm males (↓36-44%) and females (↓45-54%), and in the 2000 ppm males (↓55-71%) and females (↓36-73%; Table 5). There were no historical control data provided for Week 13 for comparison; however, historical control data were included to compare with Weeks 26, 39, and 52. The values observed in females at Week 26 and males and females at Weeks 39 and 52 at the 2000 ppm dose were below historical control ranges.

Creatinine was increased ($p \leq 0.05$) in the 1500 and 2000 ppm males at Week 26 (↑20% each) and in the 2000 ppm males at Week 39 (↑33%); however, the parameter was comparable to controls at Week 52. Cholesterol was increased (↑37%; $p \leq 0.05$) in the 2000 ppm males at Week 13; however, this parameter was comparably increased (↑28%; $p \leq 0.05$) in the 1500 ppm males prior to treatment. There were no other dose-related findings in males or females.

Table 5. Mean alanine aminotransferase (U/L) in dogs treated with TI-435 in the diet for up to 52 weeks.[a]

Study Week	Dose (ppm)					
	0	325	650	1500	2000	Historical controls [b]
Males						
Pretest (-2)	42±10.5	39±8.1	42±12.1	38±7.1	48±10.4	NP
13	42±7.0	34±5.8	34±6.3	27±7.0*(↓36)	17±3.5*(↓60)	NP
26	48±2.5	39±8.3	34±4.1*(↓29)	27±4.6*(↓44)	18±5.1*(↓71)	19-56
39	43±7.6	34±5.4	34±6.4	25±4.8*(↓42)	16±3.2* (↓63)	20-71
52	40±6.3	38±6.4	34±4.5	25±4.1*(↓38)	18±5.9*(↓55)	30-448
Females						
Pretest (-2)	36±10.6	33±4.5	40±11.3	37±3.8	32±2.1	NP
13	38±4.8	30±9.3	26±5.4*(↓32)	21±3.5*(↓45)	14±2.2*(↓63)	NP
26	41±9.9	35±8.9	37±10.4	19±8.8(↓54)	13±3.5*(↓36)	18-58
39	41±7.9	30±9.1	29±10.3	20±4.2*(↓51)	12±3.8*(↓54)	20-58
51	37±7.8	32±10.3	28±6.8	19±6.6*(↓49)	10±3.0*(↓73)	27-61

a Data obtained from Table 8 on pages 124 and 125 in the study report; n=4. Percent differences from controls are included in parentheses, and were calculated by reviewers.

b Historical control data were obtained from pages 14-16 of the study report (MRID 45422718).

* Significantly different from the control group at $p \leq 0.05$.

NP Not provided

F. URINALYSIS: There were no treatment-related findings in urinalyses.

G. SACRIFICE AND PATHOLOGY

1. Organ weight - Relative (to body) adrenal weights were increased significantly ($p \leq 0.05$) in the 2000 ppm females (↑45%); however, there were no macroscopic or microscopic data to suggest an effect of treatment. There were no other significant findings in males or females in any other treated groups.

2. Macroscopic pathology - There were no macroscopic findings in males or females of any group that could be attributed to treatment.

3. Microscopic pathology - There were no treatment-related microscopic findings in males or females. Mononuclear cell infiltrate in the stomach, inflamation of the prostrate, atrophy of the testis, and tubular vacuolization of the testis were observed in a 2000 ppm male (# G35517); however, the effects were only slight to moderate in severity. All other microscopic findings were unrelated to dose.

III. DISCUSSION and CONCLUSIONS

A. INVESTIGATORS' CONCLUSIONS: It was concluded that the NOEL was 325 ppm based on decreased alanine aminotransferase levels in males and females at 650, 1500, and 2000 ppm. Additionally at 2000 ppm, transiently decreased body weight (males), slightly decreased overall body weight gain and food intake (females), localized red erythema inside the ears (both sexes), and transiently decreased total leukocytes and neutrophils were observed. The leukopenia observed at 2000 ppm suggested that the immune system was the likely target organ of toxicity.

B. REVIEWER COMMENTS: Dose-related decreases ($p \leq 0.05$) in alanine aminotransferase (ALT) activity were observed throughout treatment in the 1500 ppm males and females (↓32-128%), and in the 2000 ppm males and females (↓36-73%). The ALT levels noted in the 2000 ppm groups at Week 26 (females only) and at Weeks 39 and 52 (males and females) were below historical control data ranges. Additionally, in the 4-week study, ALT was decreased during weeks 3 and 4 in the 2500 ppm males and females (↓54-73%), and in the 5000 ppm males and females (↓13-57%). Finally, in the 13-week study, ALT was decreased ($p \leq 0.05$) in the 1500 (↓53-55%) and 2250 (↓65-72%) ppm females at weeks 5 and 13. Decreases in ALT levels may indicate decreased liver mass; however, because liver weights in the treated groups were comparable to controls, the decreases in ALT are considered of equivocal toxicological significance.

White blood cells were decreased ($p \leq 0.05$) in the 2000 ppm females, including: (i) leukocytes at Weeks 5 (↓46%) and 39 (↓27%), (ii) monocytes at Weeks 5 (↓83%) and 13 (↓67%), (iii) eosinophils at Week 5 (↓75%), and (iv) neutrophils at Week 39 (↓40%). Additionally, in the 650, 1500, and 2000 ppm males absolute lymphocytes were decreased (↓24-43%) at Week 5. In the 4-Week study, leukocytes, segmented neutrophils, and lymphocytes were generally decreased in the 2500 (↓20-58%) and 5000 (↓31-93%) ppm males and females during weeks 3 and 4. This leukopenia was corroborated by histological evidence of general bone marrow hypocellularity and depletion of lymphoid tissue in the spleen, thymus, and mesenteric lymph node. Finally, in the 13-Week study, decreases ($p \leq 0.05$) were observed in leukocytes (↓52%), more specifically

segmented neutrophils (↓61%) and monocytes (↓75%) in the 2250 ppm females at week 13; however, there were no corroborating microscopic findings in the bone marrow or lymphoid tissues. Thus, the effects of treatment on white blood cells appears to be less severe as the doses are decreased with each longer-term study (from 5000 and 2500 ppm in the 4-Week study, to 2250 ppm in the 13-Week study, and finally, to 2000 ppm in the 52-week study). The decreases in white blood cells in the 52-Week study, although possibly due to treatment, were considered not to be adverse because they did not persist throughout treatment and, were uncorroborated by microscopic effects on the bone marrow and/or lymphoid tissues.

Significant ($p \leq 0.05$) decreases were observed in the 2000 ppm females in erythrocytes (↓15%), hemoglobin (↓17%), and hematocrit (↓18%) at Week 52. The hemoglobin and hematocrit levels were lower than the range of historical controls. In the 4-Week study, erythrocytes, hemoglobin, and hematocrit were decreased (↓5-9%) in the 5000 ppm males at weeks 3 and 4. Again, the bone marrow hypocellularity found in the 2500 and 5000 ppm males and females at weeks 3 and 4 in the 4-week study may corroborate these decreases in red blood cells. Finally, in the 13-Week study, hematocrit was decreased (↓6%; $p \leq 0.05$) in the 2250 ppm males at week 13. The decreases in red blood cells in the 52-Week study were considered to be adverse because they reached the criteria for anaemia (as determined by the Pest Management Regulatory Agency).

The LOAEL for this study was 2000 ppm (equivalent to 52.9 mg/kg bw/day in females) based on clinical evidence of anaemia. There were no adverse effects seen in male dogs and a s such the NOAEL for males was 2000 ppm, and 1500 ppm for females.

The submitted study is classified as **acceptable/guideline** and satisfies the guideline requirements for a chronic oral toxicity study in the dog (OPPTS 870.4100b; OECD 452).

C. **STUDY DEFICIENCIES:** The following deficiencies were noted but do not alter the conclusions of this DER:

Major deficiency -

- An irrefutable adverse effect was not established in this study (i.e., no LOAEL was observed) and the limit dose was not tested; however, the dose selection rationale was justifiable based on the findings in the 4- and 13-Week studies.

Minor deficiencies -

- Magnesium and sorbitol dehydrogenase (in blood serum) were not measured.
- The nose, pharynx, larynx, and lacrimal gland were not examined microscopically at sacrifice.

DATA FOR ENTRY INTO ISIS

Chronic Study - dogs (870.4100b)

PC code	MRID	Study	Species	Duration	Route	Admin	Dose range mg/kg/day	Doses mg/kg/day	NOAEL mg/kg/day	LOAEL mg/kg/day	Target organ	Comments
044309	45422717, 45422718	chronic	dogs	52 weeks	oral	dietary	7.8-52.9	0/0, 4.8/8.5, 16.6/15.0, 36.3/40.1 and 46.4/52.9 [M/F]	46.4	not observed		

HED Records Center Series 361 Science Reviews - File R107689 - Page 21 of 721

CLOTHIANIDIN *SALMONELLA*/MAMMALIAN ACTIVATION; GENE MUTATION (84-2)

EPA Reviewer: Irving Mauer, Ph.D. Date: 06/14/02
Registration Action Branch 3, HED (7509C)
EPA Secondary Reviewer: Nancy McCarroll Date: 11/14/02
Toxicology Branch, HED (7509C)

TXR NO.: 0050321

DATA EVALUATION RECORD

STUDY TYPE: Bacterial system, *e.g.*, *Salmonella*/mammalian activation gene mutation assay; OPPTS 870.5100 [84-2]

DP BARCODE: D279778
P.C. CODE: 044309

SUBMISSION CODE: S607124
TOX. CHEM. NO.: None.

TEST MATERIAL (PURITY): TZMU (99.3% a.i.; Batch No. 89066326)

SYNONYMS: None.

CITATION: Dawkes, N. (1999). TZMU. Reverse Mutation in Five Histidine-Requiring Strains of *Salmonella typhimurium*, performed by Covance Laboratories Ltd., Harrogate, North Yorkshire (ENGLAND)., Covance Laboratories Project ID 586/150-D5140, dated February 10, 1999. MRID 45422725. Unpublished.

SPONSOR: Takeda Chemical Industries Ltd., Tokyo (JAPAN).

EXECUTIVE SUMMARY:

In repeat reverse gene mutation assays in bacteria (MRID 45422725), five histidine-deficient (*his*$^-$) strains of *Salmonella typhimurium* (TA98, TA100, TA1535, TA1537 and TA102) were exposed to TZMU (99.3% a.i.; Batch No. 89066326) in dimethylsulfoxide (DMSO) under standard plate incorporation at concentrations of 8, 40, 200, 1000 and 5000 μg/plate, and at 51.2, 128, 320, 800, 2000 and 5000 μg/plate, both in the presence and absence of mammalian metabolic activation. A preincubation step was included in the S9-activated phase of the second experiment. The frequency of revertant colonies (*his*$^-$ to *his*$^+$) was determined after incubation at 37°C for 3 days in the dark. Additional cultures were treated with the vehicle (DMSO), as well as with strain-specific mutagens, the latter to serve as positive controls.

No evidence of cytotoxicity (as indicated by reduction in background bacterial lawn and/or decrease in the number of revertants) was found in either assay up to 5000 μg/plate. The mean number of revertant colonies or negative control plates all fell within acceptable range, and were significantly elevated by positive control treatments.

However, no TZMU treatment at any dosage in any strain induced an increase in revertants sufficient to be considered indicative of mutagenic activity.

Therefore, TZMU is not mutagenic in five strains of *Salmonella typhimurium* treated up to 5000 μg/plate, both in the presence and absence of metabolic activation.

This study is classified as acceptable/guideline and satisfies the requirement for FIFRA Test Guideline 84-2 for *in vitro* mutagenicity (bacterial reverse gene mutation) data.

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

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Material: TZMU
Description: White powder
Lot/Batch No.: 89066326
Purity: 99.3% a.i.
Stability of compound: Responsibility of the sponsor.
CAS No.: Not reported.
Solvent used: Anhydrous analytical grade dimethylsulfoxide (DMSO).
Other comments: Store at 1-10°C in the dark.

2. Control Materials:
Negative; None
Solvent/Final concentration: DMSO/0.1 mL/plate: plate incorporation assay
DMSO/0.05 mL/plate: preincubation step

Positive Non-activated	
Sodium azide	2.0 µg plate for TA100 and TA1535
2-Nitrofluorene	5.0 µg/plate for TA98
9-Aminoacridine	50.0 µg/plate for TA1537
Other (list):	
Glutaraldehyde	25.0 µg/plate for TA102
Activated:	
2-Aminoanthracene (2-anthramine)	5.0 µg/plate for TA98, TA100/TA1535
Other (list):	None

3. Metabolic Activation: S9 was prepared by Molecular Toxicology, Inc., and derived from male Sprague-Dawley rats:

x	Aroclor 1254	x	induced	x	rat	x	liver
	phenobarbital		non-induced		mouse		lung
	none				hamster		other
	other						other

Describe S9 mix composition (if purchased, give details):
Preparation of 10% S9 mix and Buffer Solution:

Sodium phosphate (pH 7.4)	500 mM
Glucose-6-phosphate, disodium	180 mg/mL
NADP, disodium	25 mg/mL
$MgCl_2$	250 mM
KCl	150 mM
L-histidine	1 mg/mL
d-Biotin	1 mg/mL
S9	10.0

4. Test Organisms: *Salmonella typhimurium* strains (10% S9)

	TA97	x	TA98	x	TA100	x	TA102
	TA104	x	TA1535	x	TA1537		TA1538

List any others: None

Properly maintained? Yes.
Checked for appropriate genetic markers (rfa mutation, R factor)? Yes.

5. Test Compound Concentrations Used:

Cytotoxicity test (only in TA100): 8, 40, 200, 1000, 5000 μg/plate:

Main Assays:

Conditions	Experiment 1	Experiment 2
Non-activated and Activated	8, 40, 200, 1000, 5000 μg/plate	51.2, 128, 320, 800, 2000, 5000 μg/plate

B. TEST PERFORMANCE

According to referenced papers of standard procedures (Ames *et al.* 1975; Maron & Ames, 1983; etc.).

Colonies were counted electronically (Seescan Colony Counter), or manually where there were confounding factors, such as split agar, or very small revertant colonies, (*e.g.*, see Experiment 2 treatments with strain TA102 in the presence of S9, ATTACHMENT) prevented accurate automatic counts. In each case, the background lawn was inspected for signs of toxicity.

1. Type of *Salmonella* Assay:

x	standard plate test
x	preincubation (20 minutes) with S9 only
	"Prival" modification
	spot test
	other (describe)

2. Protocol:

Triplicate plates were used at all test concentrations and with positive controls, and quintriplicates with negative controls.

3. Statistical Treatment:

The m-statistic was calculated to check that the data was Poisson-distributed, and Dunnett's Test was used to compare the counts of each dose with control. The presence or otherwise of a dose-response was checked by linear regression.

Criteria for acceptance and evaluation of results were provided.

II REPORTED RESULTS

A. PRELIMINARY CYTOTOXICITY ASSAY

No evidence of cytotoxicity in treated TA100 cultures (normally indicated by thinning of the background bacterial lawn and/or a marked reduction in revertant number) was found either in the presence or absence of metabolic activation at doses up to 5000 μg/plate.

B. MUTAGENICITY ASSAYS

Again, no evidence of cytotoxicity was seen. A singular small (1.3-fold over the solvent control) but statistical increase in revertants of TA102 (Dunnett's Test, $p < 0.01$) in non-activated (-S9) cultures treated at a maximum concentration (5000 µg/plate) in Experiment 2 was observed, but not reproduced in Experiment 1. None of the test treatments provided clear evidence of TZMU mutagenic activity (increased revertants) over concurrent or historic negative controls (MRID 45422725, pp. 24, 28, 37, 38, ATTACHMENTS). All positive controls responded with extensive increases in revertants.

Therefore, the investigator concluded that TZMU did not induce mutation in *Salmonella typhimurium* when tested under conditions of this study, including treatments up to 5000 µg/plate, both in the presence and absence of rat liver metabolic activaton (S9).

III. REVIEWER'S DISCUSSION/CONCLUSIONS

A. The reviewer agrees with the investigator that TZMU did not induce mutagenicity in this study (as reflected in increased reversion to histidine prototrophy).

B. STUDY DEFICIENCIES

None that would affect the conclusions of this study.

ATTACHMENT

THE FOLLOWING ATTACHMENT IS NOT AVAILABLE ELECTRONICALLY
SEE THE FILE COPY

TZMU: summary of mean revertant colonies (-S-9) - Experiment 1

Substance	Dose Level g/plate	TA98	TA100	TA1535	TA1537	TA102
		Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
DMSO	100 . l	27 ± 4	87 ± 11	20 ± 4	6 ± 4	299 ± 22
TZMU	8	29 ± 9	101 ± 11	14 ± 4	9 ± 5	267 ± 8
	40	31 ± 6	85 ± 23	11 ± 4	7 ± 2	278 ± 37
	200	23 ± 6	82 ± 12	20 ± 3	7 ± 1	289 ± 4
	1000	25 ± 7	108 ± 4	19 ± 6	7 ± 4	289 ± 32
	5000	21 ± 9	120 ± 40	11 ± 4	12 ± 1	205 ± 24
Positive controls	Compound	2NF	NaN3	NaN3	AAC	GLU
	Dose Level	5 . g	2 . g	2 . g	50 . g	25 . g
	Mean ± SD	619 ± 30	357 ± 34	356 ± 15	432 ± 115	451 ± 21

SD Standard deviation

2NF 2-Nitrofluorene
NaN3 Sodium azide
AAC 9-Aminoacridine
GLU Glutaraldehyde

TZMU: summary of mean revertant colonies (+S-9) - Experiment 1

Substance	Dose Level g/plate	TA98	TA100	TA1535	TA1537	TA102
		Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
DMSO	100 l	34 ± 5	102 ± 14	21 ± 8	10 ± 2	328 ± 33
TZMU	8	34 ± 7	120 ± 5	20 ± 6	9 ± 5	317 ± 7
	40	31 ± 5	100 ± 2	20 ± 2	12 ± 5	295 ± 20
	200	36 ± 4	95 ± 20	14 ± 6	8 ± 4	296 ± 23
	1000	29 ± 4	121 ± 19	14 ± 5	9 ± 3	277 ± 21
	5000	24 ± 5	119 ± 34	21 ± 3	6 ± 3	230 ± 12
Positive controls	Compound	AAN	AAN	AAN		
	Dose Level	5 g	5 g	5 g		
	Mean ± SD	938 ± 108	1287 ± 69	251 ± 13		

SD Standard deviation

AAN *2-Aminoanthracene*

TZMU: summary of mean revertant colonies (-S-9) - Experiment 2

Substance	Dose Level g/plate	TA98	TA100	TA1535	TA1537	TA102
		Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
DMSO	100 µl	30 ± 4	94 ± 4	18 ± 4	7 ± 4	336 ± 22
TZMU	51.2	36 ± 1	109 ± 7	14 ± 3	9 ± 4	398 ± 27
	128	26 ± 8	104 ± 8	13 ± 3	9 ± 2	386 ± 25
	320	37 ± 21	108 ± 9	14 ± 5	4 ± 1	393 ± 17
	800	33 ± 2	106 ± 0	24 ± 6	10 ± 2	390 ± 26
	2000	37 ± 11	98 ± 16	19 ± 5	7 ± 4	374 ± 29
	5000	25 ± 13	110 ± 12	16 ± 6	6 ± 1	426 ± 42
Positive controls	Compound	2NF	NaN3	NaN3	AAC	GLU
	Dose Level	5 µg	2 µg	2 µg	50 µg	25 µg
	Mean ± SD	561 ± 50	658 ± 25	429 ± 41	521 ± 101	784 ± 47

SD Standard deviation

2NF 2-Nitrofluorene
NaN3 Sodium azide
AAC 9-Aminoacridine
GLU Glutaraldehyde

TZMU: summary of mean revertant colonies (+S-9) - Experiment 2

Substance	Dose Level g/plate	TA98	TA100	TA1535	TA1537	TA102
		Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
DMSO	50 l	48 ± 4	152 ± 18	22 ± 3	9 ± 4	499 ± 50 (M)
TZMU	51.2	47 ± 8	148 ± 16	16 ± 5	8 ± 2	445 ± 55 (M)
	128	43 ± 24	149 ± 12	18 ± 4	10 ± 1	411 ± 77 (M)
	320	58 ± 12	168 ± 13	21 ± 3	10 ± 3	470 ± 39 (M)
	800	54 ± 9	172 ± 16	18 ± 3	13 ± 3	449 ± 39 (M)
	2000	50 ± 5	147 ± 33	15 ± 2	12 ± 3	473 ± 60 (M)
	5000	48 ± 3	148 ± 9	18 ± 3	14 ± 3	485 ± 17 (M)
Positive controls	Compound	AAN	AAN			
	Dose Level	5 g	5 g			
	Mean ± SD	1422 ± 78	1405 ± 85			

SD Standard deviation

AAN 2-Aminoanthracene

M : Plate counted manually

CLOTHIANIDIN *SALMONELLA*/MAMMALIAN ACTIVATION; GENE MUTATION (84-2)

EPA Reviewer: Irving Mauer, Ph.D. Date: 10/30/02
Registration Action Branch 3, HED (7509C)
EPA Secondary Reviewer: Nancy McCarroll Date: 11/07/02
Toxicology Branch, HED (7509C)

TXR. NO.: 0050321

DATA EVALUATION RECORD

STUDY TYPE: Bacterial system, *e.g.*, *Salmonella*/mammalian activation gene mutation assay; OPPTS 870.5100 [84-2]

DP BARCODE: D279778 SUBMISSION CODE: S607124

P.C. CODE: 044309 TOX. CHEM NO.: None

TEST MATERIAL (PURITY): TI-435 (Lot. No. 30037120, 95.2% a.i.)

SYNONYMS: Clothianidin (iso). Chemically: N-[(2-chloro-5-thiazolyl)methyl]-N^{1}-methyl-N^{11} ~ nitroguanidine.

CITATION: Herbold, B. (1999). TI-435: *Salmonella*/Microsome Test; Plate Incorporation and Preincubation Method, performed at Bayer AG's Department of Toxicology, Wuppertal (GERMANY). Bayer AG Report No. 28849 (Bayer Corporation Agricultural Divison Report No. 109990), Bayer AG Study No.: 8053884, dated June 14, 1999. MRID 45422733. Unpublished.

SPONSOR: Takeda Chemical Industries Agro Company, Tokyo (JAPAN).

EXECUTIVE SUMMARY: In repeat, gene mutation assays in bacteria (MRID 45422733), five histidine-requiring strains (*his*$^{-}$) of *Salmonella typhimurium* (TA1535, TA100, TA1537, TA98 and TA102) were exposed, in the presence and absence of a mammalian metabolic activation system (± S9), to TI-435 (Lot No. 30037120, 95.2% a.i. dissolved in dimethylsulfoxide, DMSO), initially in a plate incorporation procedure at six doses ranging from 16 to 5000 μg/plate (EXPERIMENT 1), followed by a repeat assay (EXPERIMENT 2) using a preincubation step with the same range of doses in four of the test strains (TA1535, TA100, TA1537, and TA98), but at a narrower range of seven doses, from 16 to 112 μg/plate with TA102. In addition to the strains treated with the solvent (DMSO), other cultures were exposed to strain-specific mutagens, to serve as positive controls. S9 was derived from the livers of adult male Sprague-Dawley rats induced by an intraperitoneal injection of Aroclor 1254. All cultures were incubated at 37°C for 48 hours, following which reversion to histidine independence (*his*$^{-}$ to *his*$^{+}$) was compared to solvent controls.

In EXPERIMENT 1 (plate-incorporation), no bacteriotoxic (growth inhibition) was evident up to the highest (limit) dose, 5000 μg/plate.

In EXPERIMENT 2 (preincubation), no clear evidence of cytotoxicity was also seen, other than minor strain-specific growth inhibition above 158 μg/plate. **At no concentration in either assay was any increase in revertants observed.** In contrast, positive control chemicals all induced large increases in frequencies of revertants.

Therefore, under these conditions, TI-435 is considered non-mutagenic in the standard battery of *Salmonella* strains, exposed up to toxic, limiting concentrations (5000 μg/plate) in the presence and absence of S9.

This study is classified as acceptable/guideline and satisfies the requirement for FIFRA Test Guideline 84-2 for *in vitro* mutagenicity (bacterial reverse gene mutation) data.

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

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Material: TI-435
Description: Yellowish powder.
Lot/Batch No.: 30037120.
Purity: 95.2% a.i.
Stability of compound: Not stated..
CAS No.: 205510-53-8.
Solvent used: Dimethylsulfoxide (DMSO).
Other comments: Store at room temperature.

2. Control Materials:
Negative: None
Solvent/Final Concentration: DMSO/0.1 mL
Positive: Non-activated:

Sodium azide	10.0 µg/plate for TA1535
Nitrofurantoin	0.2 µg/plate for TA100
4-NPD[1]	10/0.5 µg/plate for TA1537/TA98
Other (list):	
Cumene hydroperoxide	50.0 µg/plate for TA102

Activated:
2-Aminoanthracene (2-anthramine) 3.0 µg/plate for all strains.

3. Metabolic Activation: S9 was derived from adult male Sprague-Dawley rats.

x	Aroclor 1254	x	induced	x	rat	x	liver
	phenobarbital		non-induced		mouse		lung
	none				hamster		other
	other						other

[1]4-NPD = 4-Nitro-1,2-phenylene diamine.

Describe S9 mix composition:

Ingredient	In 70 mL Cofactor Solution
$MgCl_2$ x 6 H_2O	162.6 mg
KCl	246.0 mg
Glucose-6-phosphate (disodium salt)	179.1 mg
NADP (disodium salt)	315.0 mg
Phosphate buffer	100.0 mM

4. Test Organisms: *Salmonella typhimurium* strains: (obtained from Dr. B. Ames, University of California at Berkeley.)

	TA97	x	TA98	x	TA100	x	TA102
	TA104	x	TA1535	x	TA1537		TA1538

List any others: None.
Properly maintained? Yes.
Checked for appropriate genetic markers (rfa mutation, R factor)? Yes.

5. Test Compound Concentration Used:

Cytotoxicity Test: Not performed.

Main Assays	EXPERIMENT 1 All Strains	EXPERIMENT 2 4 TA Strains	EXPERIMENT 2 TA102
Non-activated and activated conditions	16, 50, 158, 500, 1581, 5000 μg/plate	16, 50, 158, 500, 1581, 5000 μg/plate	16, 32, 48, 64, 80, 96, 112 μg/plate

B. TEST PERFORMANCE:

The initial plate-incorporation test (EXPERIMENT 1); followed Ames *et al.* (1973a, 1975) and Marin and Ames (1983[2,3,4]).

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[2]Ames, B.N., W.E. Durston, E. Yamasaki and F.D. Lee:
Carcinogens are mutagens: A simple test system combining liver homogenates for

This initial experiment showed no mutagenic effects; therefore, the repeat with preincubation was performed with triplicate plates for test cultures, solvent and positive control. Toxicity was assessed and revertant counts were again determined after incubation at 37°C for 48 hours.

Criteria:
Criteria for assay acceptance and evaluation of results were both presented. A reproducible and dose-related increase in mutant counts in at least one strain would be considered a positive (mutagenic) result; for TA1535, TA100, and TA98, this increase should be twice negative (solvent) controls, whereas for TA1537, at least a threefold increase would be considered positive. For TA102, an increase of 150 mutants should be reached. Otherwise, the result is evaluated as negative.

Type of *Salmonella* Assay:

x	Standard plate test (EXPERIMENT 1)
x	Preincubation (20 minutes) (EXPERIMENT 2)
	"Prival" modification
	Spot test
	Other

II REPORTED RESULTS:

A. PRELIMINARY CYTOTOXICITY ASSAY:
(None was performed.)

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activation and bacteria for detection.
Proc. Nat. Acad. Science (USA) 70, 2281-2285, 1973a.

[3]Ames, B.N., J. McCann and E. Yamasaki:
Methods for detecting carcinogens and mutagens with the Salmonella/mammalian-microsome mutagenicity test.
Mutation Res. 31 , 347-364, 1975.

[4]Maron, D.M. and B.N. Ames:
Revised methods for the *Salmonella* mutagenicity test.
Mutation Res. 113, 173-215, 1983.

B. MUTAGENICITY ASSAYS:

None of the tested cultures in EXPERIMENT 1 (plate-incorporation) demonstrated increased reversions to histidine independence at levels up to the limit producing no toxicity (MRID 45422733, pp. 28, 29 - ATTACHMENT). EXPERIMENT 2 cultures showed no clear evidence of cytotoxicity and no increase in revertants. By contrast, positive control chemicals all induced large increases in revertants.

Therefore, the innvestigator concluded that TI-435 was not mutagenic in *Salmonella typhimurium* strains.

III. REVIEWER'S DISCUSSION/CONCLUSIONS:

A. The reviewer agrees with the investigator's conclusions that comprehensive assays of this batch of TI-435 showed no mutgenic activity, which is consistent with a previous Japanese study (MRID 45422732), but at variance with a later (UK) study using another lot (MRID 45422731), which concluded that mutagenic activity was displayed in treated TA 1535 cultures.

B. STUDY DEFICIENCIES:

None.

ATTACHMENT

THE FOLLOWING ATTACHMENT IS NOT AVAILABLE ELECTRONICALLY
SEE THE FILE COPY

TI 435
Salmonella/Microsome Test
Study No. T 8053884
BAYER AG

5.2 Tabulated Summary of Data

Summary of Mean Values Without S9 Mix

Table and group	Strain TA 1535	TA 100	TA 1537	TA 98	TA 102
1-5 µg/plate					
0	9	60	7	27	215
16	8	65	8	24	195
50	8	70	6	22	217
158	6	67	7	23	204
500	6	70	6	28	169
1581	9	57	4	29	200
5000	12	81	8	26	193
Na-azide	820				
NF		203			
4-NPDA			85	155	
Cumene					371
6-10 µg/tube					
0	6	102	6	16	126
16	5	89	4	16	135
50	5	88	4	17	113
158	5	91	3	28	115
500	9	91	2	19	140
1581	9	80	2	20	137
5000	8	---	3	23	120
Na-azide	912				
NF		237			
4-NPDA			63	151	
Cumene					494

TI 435
Salmonella/Microsome Test
Study No. T 8053884
BAYER AG

Summary of Mean Values With S9 Mix

Table and group	Strain TA 1535	TA 100	TA 1537	TA 98	TA 102
1-5 µg/plate					
0	11	101	7	25	254
16	9	92	7	19	271
50	9	94	7	29	266
158	6	95	6	23	224
500	10	90	7	22	179
1581	8	104	6	25	172
5000	11	110	7	21	163
2-AA	80	1267	235	1146	411
6-10 µg/tube					
0	10	100	6	22	148
16	11	107	4	19	186
50	8	101	3	25	251
158	7	101	3	27	228
500	7	127	4	24	146
1581	11	102	2	22	155
5000	16	128	4	28	168
2-AA	137	1211	28	1755	474
11 µg/tube					
0					195
16					203
32					226
48					233
64					187
80					178
96					197
112					201
2-AA					467

CLOTHIANIDIN

SALMONELLA/MAMMALIAN ACTIVATION; GENE MUTATION (84-2)

EPA Reviewer: Irving Mauer, Ph.D. Date: 06/04/02
Registration Action Branch 3, HED (7509C)
EPA Secondary Reviewer: Nancy McCarroll Date: 11/14/02
Toxicology Branch, HED (7509C)

TXR No.: 0050321

DATA EVALUATION RECORD

STUDY TYPE: Bacterial system, *e.g.*, *Salmonella*/mammalian activation gene mutation assay; OPPTS 870.5100 [84-2]

DP BARCODE: D279778
P. C. CODE: 044309

SUBMISSION CODE: S607124
TOX. CHEM. NO.: None

TEST MATERIAL (PURITY): Methyl guanidine (98.8% a.i.; Batch No. FHE02)

SYNONYMS: None

CITATION: Dawkes, N. (1999). Methyl Guanidine: Reverse Mutation in Five Histidine-Requiring Strains of *Salmonella typhimurium*, performed at Covance Laboratories, Ltd., Harrogate, North Yorkshire (ENGLAND), Laboratory Project No.: Covance 586/151-D5140 (Bayer Corporation Agricultural Division Report No. 110201); dated February 10, 1999. MRID 45422726. Unpublished.

SPONSOR: Takeda Chemical Industries Ltd., Agro Company, Tokyo (Japan)

EXECUTIVE SUMMARY:

In repeat reverse gene mutation assays (MRID 45422726), five histidine-deficient (*his*$^-$) strains of *Salmonella typhimurium* (TA98, TA100, TA1535, TA1537 and TA102) were exposed to methyl guanidine (98.8% a.i.; Batch No. FHE02 dissolved in sterile purified water, SPW) in the absence and presence of metabolic activation provided by Aroclor 1254-induced rat liver post mitochondrial fraction (S9), under a standard plate-incorporation procedure at five concentrations ranging from 8 to 5000 μg/plate (EXPERIMENT 1), as well as with a preincubation step (with S9 only) at six narrower series of concentrations ranging from 51.2 to 5000 μg/plate (EXPERIMENT 2). After 3 days of incubation at 37°C, reversion to prototrophy (*his*$^-$ to *his*$^+$) was determined and compared to the frequency of revertants in vehicle controls (SPW) run concurrently. In addition, other cultures were treated with known strain-specific mutagens, to serve as positive controls.

The compound was completely soluble in the aqueous vehicle at all concentrations in both experiments; there was no evidence of a cytotoxic response at any concentration with or without S9 activation.. No test article-treated culture produced an increase in revertant numbers sufficient to be considered as indicative of mutagenic activity. The positive controls all responded with large increases in mutagenics activity.

Therefore, methyl guanidine is non-mutagenic when tested to the limit dose (5000 μg/plate) in the standard battery of *Salmonella typhimurium* strains.

This study is classified as acceptable/guideline and satisfies the requirement for FIFRA Test Guideline 84-2 for *in vitro* mutagenicity (bacterial reverse gene mutation) data.

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

I. MATERIALS AND METHODS

A. MATERIALS:

1. Test Material: Methyl guanidine
Description: White powder
Lot/Batch No.: FHE02
Purity: 98.8% a.i.
Stability of compound: Reported to be the responsibility of the sponsor.
Solvent used: Sterile purified water (SPW)
Other comments: Stored at 1-10°C in the dark.

2. Control Materials:

Negative: None.
Solvent/Final concentration: SPW/100 µL (all treatments).
Positive: **Non-activated**:

Sodium azide	2.0 µg/plate for TA100 and TA1535.
2-Nitrofluorene	5.0 µg/plate for TA98.
9-Aminoacridine	50.0 µg/plate for TA1537
Other (list):	
Glutaraldehyde	25.0 µg/plate for TA102

Activated:

2-Aminoanthracene (2-anthramine) 5.0 µg/plate for at least one strain.

3. Metabolic Activation: S9 (purchased from Molecular Toxicology, Inc., USA) was derived from male Sprague-Dawley rats.

x	Aroclor 1254	x	induced	x	rat	x	liver
	phenobarbital		non-induced		mouse		lung
	none				hamster		other
	other						other

Describe S9 mix composition:

Ingredient	Per 100 mL	mL in 10% S9-mix
Sodium phosphate (buffer, pH 7.4)	500 mM	20.0
Glucose-6-phosphate (disodium)	180 mg/mL	0.845
NADP (disodium)	25 mg/mL	12.6
$MgCl_2$	250 mM	3.2
KCl	150 mM	22.0
L-histidine HCl (in 250 mM $MgCl_2$	1 mg/mL	4.0
d-biotin	1 mg/mL	4.88
S9 (Commercially prepared by Molecular Toxicology, Inc.)	As detailed in APPENDIX 5 of the REPORT	10.0
Water	To volume	To volume

4. Test Organisms: *Salmonella typhimurium* strains

	TA97	x	TA98	x	TA100	x	TA102
	TA104	x	TA1535	x	TA1537		TA1538

Properly maintained? Yes.
Checked for appropriate genetic markers (rfa mutation, R factor)? Yes.

5. Test Compound Concentrations Used:

Cytotoxicity test (in TA100 only): 8, 40, 200, 1000, 5000 μg/plate.

Main Assays	EXPERIMENT 1	EXPERIMENT 2
Non-activated and activated conditions	8, 40, 200, 1000, 5000 μg/plate	51.2, 128, 320, 800, 2000, 5000 μg/plate

B. TEST PERFORMANCE

Methyl guanidine was fully soluble in SPW; solutions were protected from light and used within 3.5 hours of initial formulation. Triplicate plates were prepared at each concentration with/without S9-mix; solvent and positive controls were included in quintuplicate and triplicate, respectively, with/without S9-mix. In Experiment 1, to 2.5 mL molten agar at 46°C were added: 0.1 bacterial culture; 0.1 test article solution or control; and 0.5 mL 10% S9 mix or buffer solution. When set, the plates were inverted, and incubated at 37°C for 3 days in the dark. In Experiment 2, a preincubation step was introduced where the quantities of test article or control solution, bacteria and S9 mix detailed above were mixed together and incubated for 20 minutes at 37°C, with shaking, before addition to the molten agar plates at 46°C.

Colony Counting:

Colonies were counted electronically using a Seescan Colony Counter, or manually where confounding factors such as slight bacterial contamination, split agar or very small revertant colonies (*e.g.*,: EXPERIMENT 2 treatments with strain TA102 in the presence of S9) prevented accurate automated counts. In each case the background lawn of representative plates was inspected for signs of cytotoxicity.

1. Type of *Salmonella* Assay:

x	Standard plate test	**EXPERIMENT 1**
x	Preincubation (20 minutes) with S9 only	**EXPERIMENT 2**
____	"Prival" modification	
____	Spot test	
____	Other (describe)	

NOTE: The nonactivated portion of EXPERIMENT 2 was prepared as described for the plate incorporation test.

2. Analysis of Results:

Individual plate counts were recorded separately and compared to concurrent vehicle controls, the mean and standard deviation of counts for each treatment determined. These were compared to the accepted background normal ranges of spontaneous revertants collected for this laboratory (and presented as APPENDIX 3 of the Report).

3. Statistical Analysis:

The m-statistic was calculated to determine that the data were Poisson-distributed, and Dunnett's Test was used to compare the counts of each dose with the control. The presence or otherwise of a dose-response was checked by linear regression.

4. Both Assay Acceptance Criteria and Evaluation of Results were presented.

Only EXPERIMENT 2 treatment of strain TA1535 in the absence of S9 at 320 μg/plate resulted in a small (1.7-fold) but significant increase in revertants (when analyzed at the 1% level using Dunnett's Test). However, there was no evidence of a dose-related response and this small response only occurred at an intermediate-dose level (APPENDIX 2, Table 15); furthermore, it was not reproducible in EXPERIMENT 1 where no statistically significant increase occurred (APPENDIX 1, Table 5). None of the other treatments provided any clear evidence of methyl guanidine mutagenic activity.

All positive control chemicals induced large increases in revertants.

Therefore, the investigator concluded that methyl guanidine was non-mutagenic when tested under conditions of this study.

II REPORTED RESULTS

A. PRELIMINARY CYTOTOXICITY ASSAY:

The test article was completely soluble in vehicle. There was no evidence of cytotoxicity (normally indicated by thinning of the background lawn and/or reduction in revertants) in TA100 cultures treated up to 5000 μg/plate, either in the absence or presence of S9 mix.

B. MUTAGENICITY ASSAY:

In neither experiment was any evidence of mutagenicity observed up to the limit dose, 5000 μg/plate (MRID 45422726, pp. 24, 25, 37, 38 - ATTACHMENT).

III. REVIEWER'S DISCUSSION/CONCLUSIONS:

A. The reviewer agrees with the investigator that methyl guanidine did not induce increases in revertants in this study with the standard battery of *Salmonella* strains.

B. STUDY DEFICIENCIES:

None.

ATTACHMENT

THE FOLLOWING ATTACHMENT IS NOT AVAILABLE ELECTRONICALLY
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Methyl guanidine: summary of mean revertant colonies (-S-9) - Experiment 1

Substance	Dose Level g/plate	TA98	TA100	TA1535	TA1537	TA102
		Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
WATER	100 . l	18 ± 3	89 ± 6	. 10 ± 4	4 ± 2	268 ± 28
Methyl guanidine	8	18 ± 2	97 ± 27	9 ± 3	6 ± 4	266 ± 9
	40	14 ± 5	75 ± 12	9 ± 4	5 ± 2	308 ± 57
	200	21 ± 5	78 ± 5	11 ± 4	5 ± 2	271 ± 27
	1000	19 ± 1	96 ± 3	14 ± 2	6 ± 2	282 ± 11
	5000	21 ± 3	88 ± 5	9 ± 4	8 ± 2	270 ± 17
Positive controls	Compound	2NF	NaN3	NaN3	AAC	GLU
	Dose Level	5 g	2 g	2 g	50 . g	25 . g
	Mean ± SD	358 ± 37	424 ± 37	386 ± 34	349 ± 23	477 ± 18

SD Standard deviation

2NF 2-Nitrofluorene
NaN3 Sodium azide
AAC 9-Aminoacridine
GLU Glutaraldehyde

Methyl guanidine: summary of mean revertant colonies (+S-9) - Experiment 1

Substance	Dose Level g/plate	TA98	TA100	TA1535	TA1537	TA102
		Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
WATER	100 l	26 ± 4 (M)	110 ± 15	15 ± 2	9 ± 5	271 ± 9
Methyl guanidine	8	27 ± 8	110 ± 17	14 ± 8	5 ± 4	275 ± 21
	40	25 ± 2	114 ± 12	10 ± 2	7 ± 1	255 ± 2
	200	29 ± 7	102 ± 4	16 ± 4	8 ± 3	249 ± 2
	1000	26 ± 4	113 ± 20	14 ± 2	9 ± 4	300 ± 20
	5000	27 ± 2	100 ± 36	15 ± 2	5 ± 4	278 ± 26
Positive controls	Compound	AAN	AAN	AAN		
	Dose Level	5 g	5 g	5 g		
	Mean ± SD	950 ± 78	1070 ± 107	155 ± 29		

SD Standard deviation

AAN 2-Aminoanthracene

M : Plate counted manually

Methyl guanidine: summary of mean revertant colonies (-S-9) - Experiment 2

Substance	Dose Level g/plate	TA98	TA100	TA1535	TA1537	TA102
		Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
WATER	100 l	26 ± 4	98 ± 11	13 ± 3	7 ± 3	347 ± 23
Methyl guanidine	51.2	25 ± 5	104 ± 8	14 ± 1	5 ± 4	349 ± 16
	128	33 ± 5	90 ± 3	13 ± 4	7 ± 3	369 ± 20
	320	23 ± 3	79 ± 9	22 ± 5	6 ± 1	344 ± 5
	800	30 ± 3	83 ± 1	16 ± 2	7 ± 2	326 ± 19
	2000	30 ± 1	89 ± 7	11 ± 2	5 ± 4	298 ± 19
	5000	27 ± 5	88 ± 16	17 ± 4	6 ± 1	298 ± 25
Positive controls	Compound	2NF	NaN3	NaN3	AAC	GLU
	Dose Level	5 g	2 g	2 g	50 g	25 g
	Mean ± SD	491 ± 33	533 ± 12	304 ± 9	366 ± 63	601 ± 26

SD Standard deviation

2NF 2-Nitrofluorene
NaN3 Sodium azide
AAC 9-Aminoacridine
GLU Glutaraldehyde

Methyl guanidine: summary of mean revertant colonies (+S-9) - Experiment 2

Substance	Dose Level g/plate	TA98	TA100	TA1535	TA1537	TA102
		Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
WATER	100 l	42 ± 14	147 ± 23	20 ± 5	9 ± 3	475 ± 32 (M)
Methyl guanidine	51.2	43 ± 5	151 ± 7	17 ± 2	9 ± 5	461 ± 47 (M)
	128	41 ± 5	154 ± 14	18 ± 6	8 ± 3	472 ± 42 (M)
	320	32 ± 7	148 ± 18	15 ± 3	7 ± 1	455 ± 54 (M)
	800	46 ± 5	121 ± 21	15 ± 3	8 ± 2	471 ± 67 (M)
	2000	37 ± 4	167 ± 42	16 ± 3	6 ± 2	463 ± 45 (M)
	5000	40 ± 6	151 ± 16	20 ± 6	11 ± 3	485 ± 41 (M)
Positive controls	Compound	AAN	AAN			
	Dose Level	5 . g	5 . g			
	Mean ± SD	839 ± 105	644 ± 99			

SD Standard deviation

AAN 2-Aminoanthracene

M : Plate counted manually

EPA Reviewer: Irving Mauer, Ph.D. Date: 02/20/02
Registration Action Branch 3, HED (7509C)
EPA Secondary Reviewer: Nancy McCarroll Date: 11/19/02
Toxicology Branch, HED (7509C)

TXR NO: 0050321

DATA EVALUATION RECORD

STUDY TYPE: Bacterial system, *e.g.*, *Salmonella*/Mammalian activation gene mutation assay; OPPTS 870.5100 [84-2]

DP BARCODE: D279778
P.C. CODE: 044309

SUBMISSION CODE: S607124
TOX. CHEM NO.: None

TEST MATERIAL (PURITY): BN0230M (94.4% a. i.; Batch No. 30068304)

SYNONYMS: None reported.

CITATION: Dawkes, N. (2000). BN0230M: Reverse Mutation in Five Histidine-Requiring Strains of *Salmonella typhimurium*, performed at Covance Laboratories Ltd., Harrogate, North Yorkshire (ENGLAND), Laboratory Project ID: Covance Report No. 586/224-D5140 (Bayer Corporation Agricultural Division Report No. 110239), dated September 19, 2000. MRID 45422729. Unpublished.

SPONSOR: Takeda Chemical Industries Ltd., Agro Company, Tokyo (JAPAN).

EXECUTIVE SUMMARY: In repeat reverse gene mutation assays in bacteria (MRID 45422729), five histidine-deficient (*his*$^-$) strains of *Salmonella typhimurium* (TA98, TA100, TA1535, TA1537 and TA102) were exposed to BN0230M (94.4% a.i.; Batch No. 30068304) dissolved in sterile anhydrous analytical grade dimethylsulfoxide (DMSO) in the presence and absence of a metabolic activation system (S9), initially in a plate-incorporation procedure at a range of six concentrations from 1.6 to 5000 μg/plate (EXPERIMENT 1), then repeated in a narrower range of six doses from 51.2 to 5000 μg/plate, but including a preincubation step with S9 only (EXPERIMENT 2). After incubation at 37°C in the dark for 3 days, reversions to histidine independence (*his*$^-$ to *his*$^+$) were compared to cultures treated with the solvent. Additional cultures were exposed to strain-specific mutagens, to serve as positive controls.

No evidence of cytotoxicity was found in either experiment in any of the test strains treated up to the limiting dose, 5000 μg/plate, either in the presence or absence of S9. In addition, no increases in revertants were induced in either experiment. By contrast, the positive control chemicals all induced large increases in revertants

Therefore, **BNO230M is considered non-mutagenic in the standard battery of *Salmonella* strains treated up to the limiting dose.**

This study is classified as acceptable/guideline and satisfies the requirement for FIFRA Test Guideline 84-2 for *in vitro* mutagenicity (bacterial reverse gene mutation) data.

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

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Material: BN0230M
   Description: White powder
   Lot/Batch No.: 30068304
   Purity: Initially 97.8% a. i., but degrades to 94.4% in phosphate buffer during time lag, (3-15 minutes), between sample preparation and HPLC analysis.
   Stability of compound: Stated to be the responsibility of the sponsor.
   Solvent used: Sterile anhydrous analytical grade dimethylsulfoxide (DMSO)
   Other comments: Stored refrigerated in the dark

2. Control Materials:
   Negative: None
   Solvent/Final Concentration: DMSO/100μL – plate incorporation assay
   Positive: Non-activated: DMSO/50 μL – preincubation assay

Sodium azide	2.0 μg/plate for TA100 and TA1535
2-Nitrofluorene	5.0 μg/plate for TA98
9-Aminoacridine	50.0 μg/plate for TA1537
Other (list):	
Glutaraldehyde	25.0 μg/plate for TA102

Activated:

2-Aminoanthracene (2-anthramine) 5.0 μg/plate for TA98, TA100, TA1535, TA1537; 20.0 μg/plate for TA102.

Others (list): None.

3. Metabolic Activation: S9 (obtained from Molecular Toxicology, Inc., USA) was derived from male Sprague-Dawley rats.

x	Aroclor 1254	x	induced	x	rat	x	liver
	phenobarbital		non-induced		mouse		lung
	none				hamster		other
	other						other

Describe S9 mix composition:

Ingredient	**Concentration**	**Quantity (mL) in 10% S9 Mix**
Sodium phosphate (buffer, pH 7.4)	500 mM	20.0
Glucose-6-phosphate (disodium)	180 mg/mL	0.845
NADP (disodium)	25 mg/mL	12.6
$MgCl_2$	250 mM	3.2
KCl	150 mM	22.0
L-histidine HCl (in 250 mM $MgCl_2$)	1 mg/mL	4.0
d-biotin	1 mg/mL	4.88
S9	(as detailed)	10.0
Water	-	(To volume)

4. Test Organisms: *Salmonella typhimurium* strains:

	TA97	x	TA98	x	TA100	x	TA102
	TA104	x	TA1535	x	TA1537		TA1538

List any others: None.
Properly maintained? Yes.
Checked for appropriate genetic markers (rfa mutation, R factor)? Yes.

5. Test Compound Concentration Used:

Cytotoxicity Test: (performed in strain TA100 only): 1.6, 8, 40, 200, 1000, 5000 μg/plate

Main Assays	EXPERIMENT 1	EXPERIMENT 2
Non-activated and activated conditions	1.6, 8, 40, 200, 1000, 5000 μg/plate	51.2, 128, 320, 800, 2000, 5000 μg/plate

B. TEST PERFORMANCE:

BNO230M was tested in five strains in two separate experiments at the same concentrations as for the cytotoxicity test using triplicate plates ±S9. Solvent (DMSO) controls (in quintuplicate) and reference mutagen controls (in triplicate) were included in each assay. In EXPERIMENT 1 (plate incorporation), to 2.5 mL molten agar at 46°C were added: 0.1 mL bacterial culture; 0.1 mL test article or control; 0.5 10% S9-mix or buffer solution. The material on the plate was rapidly mixed, and poured onto Minimal Davis agar plates. When set, the plates were inverted and incubated at 37°C in the dark for 3 days. Following incubation, the plates were examined for evidence of toxicity to the background lawn; where possible, revertant colonies were counted. In EXPERIMENT 2 (with a preincubation step), the quantities of the test article or control solution (reduced to 0.05 mL to avoid possible toxic effects if added at the usual 0.1 mL), bacteria and S9-mix were mixed and incubated for 20 minutes at 37°C, with shaking, before the addition to 2.5 mL molten agar at 46°C. Plating then proceeded as for the plate-incorporation procedure. The non-activated portion of EXPERIMENT 2 was performed using the plate incorporation test.

Colony Counting and Analysis of Results:

Colonies were counted electronically using a Seescan Colony Counter, or manually where confounding factors such as split agar prevented accurate automated counts. In each case, the background lawn of representative plates was inspected for signs of cytotoxicity.

Individual plate counts were recorded separately, and the mean and standard deviation of plate counts were determined for each treatment.

Statistical Methods:

The m-statistic was calculated to check that the data were Poisson-distributed. Dunnett's Test was used to compare the counts of each dose with the control. The presence or otherwise of a dose response was checked by linear regression analysis.

Type of *Salmonella* Assay:

_x_ standard plate test (EXPERIMENT 1)
_x_ preincubation (_20_ minutes with S9 only) (EXPERIMENT 2)
____ "Prival" modification
____ spot test
____ other (describe)

Both criteria for assay acceptance and evaluation of results were presented.

II. REPORTED RESULTS

A. SOLUBILITY:

BNO230M was completely soluble in the aqueous assay system at all concentrations treated in both experiments.

B. PRELIMINARY CYTOTOXICITY ASSAY:

There was no evidence of cytotoxicity (manifested as thinning of the background bacterial lawn and/or a marked reduction in revertants) in TA100 treated cultures up to the limit dose applied, 5000 μg/plate.

C. MUTAGENICITY ASSAYS:

In both EXPERIMENT 1 and EXPERIMENT 2, there was also no evidence of toxicity in any test strain either in the presence or absence of S9.

Only in EXPERIMENT 2 treatment of TA98 unstimulated by S9 was a significant increase in revertants noted (when analyzed at the 1% level by Dunnett's Test). However, this increase was very small (1.6-fold increase), occurred only at the maximum treatment concentration (5000 μg/plate), and was not reproducible at a

comparable treatment of TA98 in EXPERIMENT 1. Hence, this increase was considered to have occurred by chance. In no other treatment in any strain in either assay was any clear evidence of BNO230M mutagenic activity when compared to either concurrent controls (MRID 45422729, pp. 26, 27, 39, 40 - ATTACHMENT) or historical background (MRID 45422729, p. 51, APPENDIX 3 - ATTACHMENT). By contrast, the positive control chemicals all induced large increases in revertants in the appropriate strains.

Therefore, the investigator concluded that BNO230M did not induce mutation in five strains of *Salmonella typhimurium* tested under the conditions of this assay up to the limit dose, 5000 μg/plate, both in the presence and absence of metabolic activation (S9).

III. REVIEWER'S DISCUSSION/CONCLUSIONS:

A. The reviewer agrees with the investigator that BNO23M showed no mutagenic activity in the standard battery of *Salmonella typhimurium* strains tested up to minimal cytotoxicity at the limit dose.

B. STUDY CYTODEFICIENCIES:

None.

ATTACHMENT

THE FOLLOWING ATTACHMENT IS NOT AVAILABLE ELECTRONICALLY
SEE THE FILE COPY

BN0230M: summary of mean revertant colonies (-S-9) - Experiment 1

Substance	Dose Level µg/plate	TA98	TA100	TA1535	TA1537	TA102
		Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
DMSO	100 µl	29 ± 6	107 ± 12	14 ± 4	5 ± 2	346 ± 22
BN0230M	1.6	31 ± 4	86 ± 11	13 ± 2	8 ± 2	365 ± 11
	8	22 ± 2	105 ± 9	18 ± 8	6 ± 4	331 ± 45
	40	23 ± 9	102 ± 9	15 ± 4	11 ± 1	356 ± 11
	200	23 ± 2	97 ± 10	21 ± 3	7 ± 4	336 ± 19
	1000	26 ± 11	95 ± 7	15 ± 5	6 ± 3	381 ± 27
	5000	36 ± 5	98 ± 1	18 ± 5	10 ± 6	333 ± 36
Positive Controls	Compound	2NF	NaN3	NaN3	AAC	GLU
	Dose Level	5 µg	2 µg	2 µg	50 µg	2 µg
	Mean ± SD	590 ± 35	707 ± 64	511 ± 7	512 ± 55	614 ± 15

SD Standard deviation

2NF 2-Nitrofluorene
NaN3 Sodium azide
AAC 9-Aminoacridine
GLU Glutaraldehyde

BN0230M: summary of mean revertant colonies (+S-9) - Experiment 1

Substance	Dose Level µg/plate	TA98	TA100	TA1535	TA1537	TA102
		Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
DMSO	100 µl	27 ± 8	112 ± 9	21 ± 4	10 ± 3	383 ± 21
BN0230M	1.6	23 ± 10	120 ± 13	21 ± 2	11 ± 3	433 ± 25
	8	34 ± 2	126 ± 18	18 ± 5	9 ± 1	420 ± 43
	40	31 ± 7	136 ± 4	17 ± 2	9 ± 3	451 ± 18
	200	27 ± 4	112 ± 15	23 ± 6	9 ± 4	463 ± 18
	1000	24 ± 13	110 ± 15	23 ± 5	7 ± 1	462 ± 67
	5000	25 ± 2	100 ± 7	14 ± 4	8 ± 2	435 ± 46
Positive Controls	Compound	AAN	AAN	AAN	AAN	AAN
	Dose Level	5 µg	5 µg	5 µg	5 µg	20 µg
	Mean ± SD	1490 ± 158	2012 ± 112	241 ± 27	296 ± 16	1728 ± 40

SD Standard deviation

AAN 2-Aminoanthracene

BN0230M: summary of mean revertant colonies (-S-9) - Experiment 2

Substance	Dose Level µg/plate	TA98	TA100	TA1535	TA1537	TA102
		Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
DMSO	100 µl	27 ± 2	104 ± 11	11 ± 4	6 ± 2	306 ± 14
BN0230M	51.2	23 ± 6	110 ± 12	14 ± 3	8 ± 1	331 ± 20
	128	29 ± 4	103 ± 4	17 ± 1	5 ± 1	340 ± 16
	320	29 ± 6	100 ± 3	18 ± 1	5 ± 2	340 ± 13
	800	28 ± 3	110 ± 6	15 ± 4	5 ± 2	330 ± 11
	2000	36 ± 6	100 ± 10	13 ± 2	5 ± 1	325 ± 19
	5000	42 ± 1	104 ± 6	17 ± 1	7 ± 1	319 ± 18
Positive controls	Compound	2NF	NaN3	NaN3	AAC	GLU
	Dose Level	5 µg	2 µg	2 µg	50 µg	25 µg
	Mean ± SD	554 ± 45	580 ± 16	421 ± 10	406 ± 127	617 ± 66

SD Standard deviation

2NF 2-Nitrofluorene
NaN3 Sodium azide
AAC 9-Aminoacridine
GLU Glutaraldehyde

BN0230M: summary of mean revertant colonies (+S-9) - Experiment 2

Substance	Dose Level µg/plate	TA98	TA100	TA1535	TA1537	TA102
		Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
DMSO	50 µl	34 ± 7	123 ± 7	15 ± 3	10 ± 3	364 ± 30
BN0230M	51.2	35 ± 5	125 ± 6	19 ± 1	6 ± 1	428 ± 15
	128	31 ± 8	139 ± 10	14 ± 1	8 ± 2	422 ± 38
	320	38 ± 4	115 ± 7	21 ± 4	9 ± 3	405 ± 35
	800	36 ± 5	123 ± 4	17 ± 6	12 ± 2	400 ± 34
	2000	33 ± 12	123 ± 9	21 ± 3	10 ± 2	399 ± 26
	5000	33 ± 3	115 ± 14	18 ± 10	8 ± 2	399 ± 19
Positive controls	Compound	AAN	AAN	AAN	AAN	AAN
	Dose Level	5 µg	5 µg	5 µg	5 µg	20 µg
	Mean ± SD	1612 ± 105	1582 ± 54	202 ± 37	245 ± 31	1804 ± 515

SD Standard deviation

AAN 2-Aminoanthracene

APPENDIX 3

Historical negative (solvent) control values for *S. typhimurium* strains

Strain	S-9	Mean No. of spontaneous revertants	Standard Deviation	Range*	
				Lower	Upper
TA98	-	37.9	9.5	14	62
	+	38.2	9.6	14	63
TA100	-	98.1	16.9	55	142
	+	104.8	22.4	47	163
TA1535	-	15.5	4.1	5	26
	+	18.9	5.2	5	32
TA1537	-	17.5	6.3	1	34
	+	17.1	7.0	1	35
TA102	-	343.0	82.1	131	554
	+	360.8	119.9	52	670

* Ranges are acceptable mean revertant numbers, at or within the 99% confidence limits about the mean

The above ranges are derived from at least 13 data sets over the period 16.10.98 to 14.5.99

APPENDIX 4

Historical positive control values for *S. typhimurium* strains

Strain	S-9	Positive control chemical	Mean No. of induced revertants	Standard Deviation	Range*	
					Lower	Upper
TA98	-	2NF	523.3	227.3	-62	1109
	+	AAN	1338.4	251.0	692	1985
TA100	-	NaN_3	529.3	190.8	38	1021
	+	AAN	1334.2	489.8	72	2596
TA1535	-	NaN_3	489.5	138.9	132	847
	+	AAN	171.3	71.2	-12	355
TA1537	-	AAC	481.2	228.7	-108	1070
	+	AAN	324.2	123.7	6	643
TA102	-	GLU	342.6	190.7	-149	834
	+	AAN	1566.6	763.0	-399	3532

* Ranges are 99% confidence limits about the mean induced revertant numbers

The above ranges are derived from at least 9 data sets over the period 10.2.98 to 14.5.99. All revertant numbers used to derive the above data are induced revertant numbers, i.e. the number of revertant colonies on the positive control plates minus the number of spontaneous revertant colonies on concurrent negative control plates.

2NF 2-nitroflourene
NaN3 Sodium azide
AAC 9-aminoacridine
GLU Glutaraldehyde
AAN 2-Aminoanthracene

CLOTHIANIDIN *SALMONELLA*/MAMMALIAN ACTIVATION; GENE MUTATION (84-2)

EPA Reviewer: Irving Mauer, Ph.D. Date: 06/20/02
Registration Action Branch 3, HED (7509C)
EPA Secondary Reviewer: Nancy McCarroll Date: 11/14/02
Toxicology Branch, HED (7509C)

TXR NO.: 0050321

DATA EVALUATION RECORD

STUDY TYPE: Bacterial system, *e.g.*, *Salmonella*/mammalian activation gene mutation assay; OPPTS 870.5100 [84-2]

DP BARCODE: D279778 SUBMISSION CODE: S607124

P. C. CODE: 044309 TOX. CHEM NO.: None

TEST MATERIAL (PURITY): TMG (96% a.i.; Batch No. 30063517)

SYNONYMS: None

CITATION: Dawkes, N. (1999). TMG: Reverse Mutation in Five Histidine-Requiring Strains of *Salmonella typhimurium*, performed at Covance Laboratories Ltd., Harrogate, North Yorkshire (ENGLAND), Laboratory Project ID: Covance 586/166-D5140 (Bayer Corporation Agricultural Division Report No. 110203), dated June 3, 1999. MRID 45422728. Unpublished.

SPONSOR: Takeda Chemical Industries Ltd., Tokyo (Japan).

EXECUTIVE SUMMARY:

In repeat reverse gene mutation assays (MRID 45422728), five histidine-deficient (*his*$^-$) strains of *Salmonella typhimurium* (TA98, TA100, TA1535, TA1537 and TA102) were exposed in the presence and absence of a metabolic activation system (S9) to TMG (96% a.i.; Batch No. 30063517) dissolved in dimethylformamide (DMF) at five concentrations ranging from 8 to 5000 μg/plate in a standard plate incorporation procedure (EXPERIMENT 1), and repeated at a narrower range of six doses (156.25 to 5000 μg/plate), but including a preincubation step with S9 only (EXPERIMENT 2). After three days incubation at 37°C in the dark, reversion to histidine prototrophy (*his*$^-$ to *his*$^+$) was determined in test plates and compared to solvent-treated (DMF) cultures. Additional cultures were exposed to strain-specific mutagens, to serve as positive controls.

No evidence of cytotoxicity was observed in EXPERIMENT 1 (plate-incorporation assay), but a slight thinning of the background bacterial lawn was apparent at the highest concentration in EXPERIMENT 2

(with TA98, TA100, and TA1537) in the presence of S9. However, in neither experiment were any statistically significant increases in revertants found. The positive control cultures all showed large increases in revertants.

Therefore, TMG is considered not to have mutagenic activity in *Salmonella typhimurium*.

This study is classified as acceptable/guideline and satisfies the requirement for FIFRA Test Guideline 84-2 for *in vitro* mutagenicity (bacterial reverse gene mutation) data.

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

I. MATERIALS AND METHODS

A. MATERIALS:

1. Test Material: TMG
Description: Yellow solid.
Lot/Batch No.: 30063517.
Purity: 96% a.i.
Stability of compound: Reported to be the responsibility of the sponsor.
Solvent used: Dimethylformamide (DMF).
Other comments: Stored at 1-10°C in the dark.

2. Control Materials:
Negative: None.
Solvent/Final concentration: DMF/100 μL - plate incorporation assay.
DMF/50 μL - preincubation assay.
Positive: Nonactivated:

Sodium azide	2.0 μg/plate for TA100, TA1535
2-Nitrofluorene	5.0 μg/plate for TA98
9-Aminoacridine	50.0 μg/plate for TA1537
Other:	
Glutaraldehyde	25.0 μg/plate for TA102

Activated:

2-Aminoanthracene (2-anthramine) 5.0 μg/plate for at least one strain.

3. Metabolic Activation: S9 (obtained from Molecular Toxicology Inc., USA) was derived from male Sprague-Dawley rats.

x	Aroclor 1254	x	induced	x	rat	x	liver
	phenobarbital		non-induced		mouse		lung
	none				hamster		other
	other						other

Describe the S9 composition:

Ingredient	Per 100 mL	mL in 10% S9-Mix
Sodium phosphate (buffer, pH 7.4)	500 mM	20.0
Glucose-6-phosphate (disodium)	180 mg/mL	0.845
NADP (disodium)	25 mg/mL	12.6
$MgCl_2$	250 mM	3.2
KCl	150 mM	22.0
L-histidine HCl (in 250 mM $MgCl_2$)	1 mg/mL	4.0
d-biotin	1 mg/mL	4.0
S9	(as detailed)	10.0
Water	To volume	To volume

4. Test Organisms: *Salmonella typhimurium* strains

	TA97	x	TA98	x	TA100	x	TA102
	TA104	x	TA1535	x	TA1537		TA1538

List any others: None.
Properly maintained? Yes.
Checked for appropriate genetic markers (rfa mutation, R factor)? Yes.

5. Test Compound Concentrations Used:

Cytotoxicity test (performed in TA100 only): 8, 40, 200, 1000, 5000 μg/plate.

Main Assays	EXPERIMENT 1	EXPERIMENT 2
Non-activated and Activated conditions	8, 40, 20, 1000, 5000 μg/plate	156.25, 312.5, 625, 1250, 2500, 5000 μg/plate

B. TEST PERFORMANCE:

In the plate incorporation assay, TMG was tested in five strains in two separate experiments at the same concentrations as for the cytotoxicity test using triplicate plates ± S9. Solvent (DMF) controls (in quintuplicate) and reference mutagen controls (in triplicate) were included in each assay. In EXPERIMENT 1 (plate-incorporation), to 2.5 mL molten agar at 46°C were added: 0.1 mL bactrial culture; 0.1 mL test article or control; 0.5mL 10% S9-mix or buffer solution. The material on the plate was rapidly mixed, and poured onto Minimal Davis agar plates. When set, the plates were inverted and incubated at 37°C in the dark for 3 days. Following incubation, the plates were examined for evidence of toxicity to the background lawn; where possible, revertant colonies were counted. In EXPERIMENT 2 (with a preincubation step), the quantities of the test article or control solution (reduced to 0.05 mL to avoid possible toxic effects if added at the usual 0.1 mL), bacteria and S9-mix were mixed and incubated for 20 minutes at 37°C, with shaking, before the addition to 2.5 mL molten agar at 46°C. Plating then proceeded as for the plate-incorporation procedure. The nonactivated phase of EXPERIMENT 2 was performed as described for the plate incorporation test.

Colony Counting and Analysis of Results:

Colonies were counted electronically using a Seescan Colony Counter, or manually where confounding factors such as split agar prevented accurate automated counts. In each case, the background lawn of representative plates was inspected for signs of toxicity.

Individual plate counts were recorded separately, and the mean and standard deviation of plate counts were determined for each treatment.

Statistical Methods:

The m-statistic was calculated to check that the data were Poisson-distributed. Dunnett's Test was used to compare the counts of each dose with the control. The presence or otherwise of a dose response was checked by linear regression analysis.

Type of *Salmonella* Assay:

x	x Standard plate test (EXPERIMENT 1)
x	x Preincubation (20 minutes) with S9 only (EXPERIMENT 2)
	"Primal" modification
	Spot test
	Other (describe)

Both acceptance and evaluation criteria were presented.

II. REPORTED RESULTS

A. PRELIMINARY CYTOTOXICITY ASSAY:

No evidence of cytotoxicity (as indicated by thinning of the background bacterial lawn and/or a marked reduction in revertant numbers) was observed either in the presence or absence of S9 at concentrations up to the maximum dose, 5000 μg/plate.

B. MUTAGENICITY ASSAYS:

Again, no evidence of cytotoxicity was observed in EXPERIMENT 1, using the same range of doses as above. In EXPERIMENT 2, evidence of cytotoxicity was apparent (slight thinning of background lawn) at the highest treatment concentrations with strains TA98, TA100 and TA1537 in the presence of S9.

TMG treatment of all test strains in both experiments in the presence or absence of a metabolic activation system (S9-mix) resulted in no increases in revertants that were statistically significant when analyzed at the 1% level using Dunnett's Test, when compared to concurrent controls, (MRID 45422728, pp. 27, 28, 40, 41 - ATTACHMENT, or to historical negative background (ATTACHEMNT, APPENDIX 3). The positive control cultures all showed large increases in revertants.

Therefore, the investigator concluded that TMG did not induce mutation in 5 strains of *Salmonella typhimurium* treated up to 5000 μg/plate, both in the presence and absence of a rat metabolic activation system (S9).

III. REVIEWER'S DISCUSSION/CONCLUSIONS:

A. The reviewer agrees with the investigator's conclusion that TMG is not mutagenic in the standard battery of *Salmonella* strains

B. STUDY DEFICIENCIES:

None.

ATTACHMENT

THE FOLLOWING ATTACHMENT IS NOT AVAILABLE ELECTRONICALLY
SEE THE FILE COPY

TMG: summary of mean revertant colonies (-S-9) - Experiment 1

Substance	Dose Level g/plate	TA98	TA100	TA1535	TA1537	TA102
		Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
DMF	100 l	33 ± 9	101 ± 10	16 ± 4	13 ± 5	317 ± 23
TMG	8	28 ± 6	93 ± 10	17 ± 4	15 ± 4	334 ± 3
	40	23 ± 6	101 ± 4	17 ± 6	11 ± 4	303 ± 32
	200	40 ± 5	96 ± 9	17 ± 5	14 ± 5	295 ± 22
	1000	30 ± 4	99 ± 8	16 ± 3	19 ± 5	326 ± 8
	5000	40 ± 5	96 ± 17	20 ± 7	18 ± 0	297 ± 18
Positive controls	Compound	2NF	NaN3	NaN3	AAC	GLU
	Dose Level	5 g	2 g	2 g	50 g	25 g
	Mean ± SD	995 ± 66	583 ± 16	441 ± 19	379 ± 66	616 ± 52

SD Standard deviation

2NF 2-Nitrofluorene
NaN3 Sodium azide
AAC 9-Aminoacridine
GLU Glutaraldehyde

TMG: summary of mean revertant colonies (+S-9) - Experiment 1

Substance	Dose Level g/plate	TA98	TA100	TA1535	TA1537	TA102
		Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
DMF	100 . 1	32 ± 12	105 ± 14	20 ± 4	13 ± 2	345 ± 26
TMG	8	36 ± 12	120 ± 10	18 ± 6	12 ± 2	235 ± 28
	40	33 ± 10	106 ± 1	16 ± 3	11 ± 2	239 ± 23
	200	30 ± 5	110 ± 11	11 ± 1	14 ± 2	299 ± 43
	1000	36 ± 2	96 ± 9	21 ± 4	10 ± 3	292 ± 28
	5000	35 ± 4	100 ± 16	18 ± 3	13 ± 3	255 ± 45
Positive controls	Compound	AAN	AAN	AAN		
	Dose Level	5 g	5 g	5 . g		
	Mean ± SD	1812 ± 141	1742 ± 187	163 ± 53		

SD Standard deviation

AAN 2-Aminoanthracene

TMG: summary of mean revertant colonies (-S-9) - Experiment 2

Substance	Dose Level g/plate	TA98	TA100	TA1535	TA1537	TA102
		Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
DMF	100 . l	40 ± 11	133 ± 4	24 ± 8	13 ± 6	304 ± 20
TMG	156.25	37 ± 8	127 ± 6	22 ± 5	8 ± 3	303 ± 14
	312.5	33 ± 1	119 ± 10	14 ± 2	13 ± 4	301 ± 12
	625	39 ± 3	111 ± 10	25 ± 8	17 ± 5	311 ± 3
	1250	34 ± 4	117 ± 4	13 ± 5	17 ± 2	300 ± 16
	2500	38 ± 4	137 ± 13	19 ± 5	15 ± 3	311 ± 32
	5000	34 ± 8	117 ± 21	21 ± 2	14 ± 3	270 ± 19
Positive controls	Compound	2NF	NaN3	NaN3	AAC	GLU
	Dose Level	5 . g	2 . g	2 . g	50 . g	25 . g
	Mean ± SD	897 ± 33	613 ± 12	566 ± 130	655 ± 72	799 ± 16

SD *Standard deviation*

2NF 2-Nitrofluorene
NaN3 Sodium azide
AAC 9-Aminoacridine
GLU Glutaraldehyde

TMG: summary of mean revertant colonies (+S-9) - Experiment 2

Substance	Dose Level g/plate	TA98	TA100	TA1535	TA1537	TA102
		Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
DMF	50 . l	48 ± 6	149 ± 16	26 ± 6	20 ± 4	395 ± 69
TMG	156.25	47 ± 3	152 ± 9	30 ± 3	14 ± 3	426 ± 50
	312.5	41 ± 3	150 ± 12	24 ± 11	13 ± 2	374 ± 28
	625	40 ± 6	139 ± 16	21 ± 5	12 ± 5	356 ± 64
	1250	41 ± 5	155 ± 5	19 ± 1	10 ± 1	364 ± 52
	2500	47 ± 5	145 ± 16	18 ± 3	12 ± 5	289 ± 29
	5000	36 ± 9 (S)	144 ± 15 (S)	18 ± 6	15 ± 2 (S)	308 ± 51
Positive controls	Compound	AAN	AAN			
	Dose Level	5 . g	5 g			
	Mean ± SD	1615 ± 180	1820 ± 90			

SD Standard deviation

AAN 2-Aminoanthracene

S : Slight thinning of b/g lawn

APPENDIX 3

Historical negative (solvent) control values for *Salmonella* strains

Strain	- or + S-9	Mean Nº of spontaneous revertants	SD	Range* lower	upper
TA98	-	29	7.3	10	48
	+	34	7.3	15	53
TA100	-	112	18.3	65	160
	+	130	16.0	89	171
TA1535	-	17	5.1	4	30
	+	21	5.3	7	34
TA1537	-	13	5.8	1	28
	+	14	6.7	1	32
TA102	-	320	46.1	201	439
	+	374	68.1	198	549

* 99% confidence limits about the mean

The above are pooled data from at least 20 consecutive experiments over the period 23.6.97 to 11.8.97

APPENDIX 4

Historical positive control values for *Salmonella* strains

Strain	- or + S-9	Positive control chemical	Mean Nº of induced revertants*	S.D.	Range 99% confidence limits about mean (min. and max. Mean values of data set) lower	upper
TA98	-	2NF	774.1	234.9	169 (191)	1379 (1176)
	+	AAN	1338.4	251.0	692 (985)	1985 (1858)
TA100	-	NaN_3	459.4	88.7	231 (258)	688 (595)
	+	AAN	1435.9	306.0	648 (479)	2224 (1940)
TA1535	-	NaN_3	372.8	123.9	54 (175)	692 (599)
	+	AAN	205.6	56.0	61 (119)	350 (327)
TA1537	-	AAC	358.4	187.9	126 (108)	842 (795)
	+	N/A	NE	NE	NE	NE
TA102	-	GLU	355.4	89.8	124 (149)	587 (466)
	+	N/A	NE	NE	NE	NE

The above are pooled data from at least 20 consecutive experiments over the period 10.2.98 to 14.5.98.

* All revertant numbers used to derive the above data are **induced** revertant numbers, i.e. the number of revertant colonies on the positive control plates minus the number of spontaneous revertant colonies on concurrent negative control plates.

2NF	2-nitroflourene
NaN_3	Sodium azide
AAC	9-aminoacridine
GLU	Glutaraldehyde
AAN	2-Aminoanthracene
N/A	Not applicable
NE	Not evaluated (insufficient historical data to permit generation of meaningful ranges)

CLOTHIANIDIN

BACTERIAL DNA REPAIR (84-2)

EPA Reviewer: Irving Mauer, Ph.D.
Registration Action Branch 3, (7509C)
EPA Secondary Reviewer: Nancy McCarroll
Toxicology Branch, HED (7509C)

Date: 11/20/02

Date: 11/20/02

TXR NO.: 0050321

DATA EVALUATION RECORD

STUDY TYPE: DNA Repair Test in *Bacillus subtilis*, OPPTS 870.5500 (84-2).

DP BARCODE: D279778

SUBMISSION CODE: S607124

P. C. CODE: 044309

TOX. CHEM. NO.: None.

TEST MATERIAL (% PURITY): TIR-435 (Lot No. 12256321, ≥ 99%).

SYNONYMS: None.

CITATION: Otsuka, M. (1990). DNA Repair Test of TIR-435 in *Bacillus subtillis*, performed at the Hita Research Laboratories, Chemical Biotesting Center, Chemicals Inspection and Testing Institute (JAPAN), Study Code No. KO2-0096 (Bayer Corporation Agricultural Division Report No. 109978), dated April 23, 1990. MRID 45422735. Unpublished.

SPONSOR: Takeda Chemical Industries, Ltd. Agro Company, Tokyo (JAPAN).

EXECUTIVE SUMMARY:

The recombination-proficient H17 (*rec*$^+$) and recombination-deficient M45 (*rec*$^-$) strains of *Bacillus subtilis* were exposed in the presence and absence of a rat liver metabolic activation system to TIR-435 (Lot No. 12256321, ≥ 99% a.i., in dimethylsulfoxide, DMSO) at five doses ranging from 375 to 6000 μg/disc. The S9 fraction was derived from the livers of male Sprague-Dawley rats induced with phenobarbitol/5, 6-benzoflavone. After incubation at 37 ± 5°C for 20 hours, inhibition zones around the treated discs were measured. In addition to treatment with the solvent and a negative control, kanamycin (a protein-synthesis inhibitor), other cultures were exposed to the mutagens, AF-2 and aminoanthracene, to serve as positive controls.

No growth inhibition was found for either strain at any dose both with/without metabolic activation. Also, neither the solvent, DMSO, nor the negative control, kanamycin, inhibited growth of either strain. The positive controls, however, showed clear differences in growth inhibition between strains, reflecting the difference in the ability to repair DNA damage.

Therefore, TIR-435 has no potential for DNA damage under these conditions.

This study is classified as **unacceptable** and does not satisfy the requirement for FIFRA Test Guideline 84-2 for *in vitro* mutagenicity (DNA bacterial repair) data. Only single plates were used for each concentration of the test material, vehicle, negative and positive controls.

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

I. MATERIALS AND METHODS

A. MATERIAL:

1. Test Material: TIR-435, (currently identified as TI-435).

Description: Colorless crystals.
Lot/Batch: 12256321
Purity: ≥ 99% a.i. (HPLC analysis on February 15, 1990, and March 25, 1997 = 99.7%).
Stability: Stable as long as stored at room temperature in the dark.
Solvent: Dimethylsulfoxide (DMSO).

Other comments: Store at room temperature.

2. Control Materials:

Negative: Kanamycin.
Solvent/Final Concentration: DMSO/20 μL/disc.
Kanamycin - 10 μg/disc.
Positive: Non-activated: AF2[1] - 0.001 μg/disc.

Activated: 2-Aminoanthracene (anthramine) - 5 μg/disc.

3. Metabolic Activation: S9 (purchased from Kikkoman Co., Ltd.) was derived from male 7-week-old Sprague-Dawley rats, treated as follows:

	Aroclor 1254	x	induced	x	rat	x	liver
x	30-60 mg/kg/day phenobarbitol (3 days) + 80 mg/kg 5,6-benzoflavone (1day)						

[1]AF-2 = 2-(2-Furyl)-3-(5-nitro-2 furyl) acrylamide.

Composition of Cofactor Solution:

NADP	50.6 μ mol
G-6-P	64.1 μ mol
$MgCl_2$	8.0 μ mol
KCl	33.0 μ mol
NA_2HPO_4	100.0 μ mol
S9	0.3 mL

3. Test Organisms: *B. subtilis* strains.

_x_ H17 (*rec*$^+$) _x_ M45 (*rec*$^-$)
Source: Institute of Environmental Toxicology (JAPAN).
Properly maintained? Yes.

4. Test Compound Concentrations: Single plates containing 375, 750, 1500, 3000, 6000 μg/disc of the test compound.

B. TEST PERFORMANCE:

The test was conducted using the spore method.

Without metabolic activation (-S9), 0.1 mL of a spore solution (2 x 10^7 spores/mL) and 10 mL of Trypticase Soy Agar (agar concentration, 0.8%) heated to 42-43 °C were poured into a culture dish, mixed gently and solidified. A paper disc (8 mm) was added with 20 μL of test solutions, vehicle, negative or positive control. The medium was incubated at 37 ± 0.5 °C for 20 hours, and inhibiting zones around the discs were measured.

With metabolic activation (+S9), the basal medium was made as above, but supplemented with 0.3 mL S9. After 20 μL of cofactor solution was added with 20 μL of the test substance solution, the paper disc was placed on the medium. Plates were incubated at 37 ± 0.5 °C for 20 hours after which the inhibition zones around the discs were measured.

II REPORTED RESULTS:

No growth inhibition was observed for either strain exposed to the test compound at any dose level in the presence or absence of S9-mix. Also, the solvent control, DMSO, caused no growth

inhibition; the negative control, kanamycin, inhibited both strains to the same extent.

The positive control, showed a clear difference of growth inhibition in both strains, reflecting the differential ability to repair DNA damage (MRID 42422735, p. 9 - ATTACHMENT TABLE).

III. REVIEWER's DISCUSSION/CONCLUSIONS:

A. The EPA reviewer agrees with the investigator's conclusion that this limited study demonstrates that TIR-435 has no potential to damage DNA under the conditions of this assay (see below).

B. STUDY DEFICIENCIES:

The number of replicates (1 only) was less than the two independent cultures for each test material, dose, the vehicle, negative and positive controls stipulated by FIFRA Test Guideline 84-2, OPPTS 870.5500.

ATTACHMENT

THE FOLLOWING ATTACHMENT IS NOT AVAILABLE ELECTRONICALLY
SEE THE FILE COPY

13. TABLE

TABLE OF DNA REPAIR TEST

Test substance		Dose (μg/disk)	Inhibitory zone (mm)			
			Without metabolic activation		With metabolic activation	
			M45	H17	M45	H17
Solvent control (DMSO)		0	0	0	0	0
TIR-435		375	0	0	0	0
		750	0	0	0	0
		1,500	0	0	0	0
		3,000	0	0	0	0
		6,000	0	0	0	0
Negative control (KM)		10	7.5	7.0	—	—
Positive control	AF-2	0.001	4.0	0	—	—
	2AA	5	—	—	4.0	0

DMSO: Dimetyl sulfoxide
KM: Kanamycin
AF-2: 2- (2-Furyl) -3- (5-nitro-2-furyl) acrylamide
2AA: 2-Amino anthracene

EPA Reviewer: Irving Mauer, Ph.D. Date: 11/25/02
Registration Action Branch 3, HED (7509C)
EPA Secondary Reviewer: Nancy McCarroll Date: 11/25/02
Toxicology Branch, HED (7509C)

TXR. NO.: 0050321

DATA EVALUATION RECORD

STUDY TYPE: Bacterial system, *e.g.*, *Salmonella*/mammalian activation gene mutation assay; OPPTS 870.5100 [84-2]

DP BARCODE: D279778 SUBMISSION CODE: S607124

P.C. CODE: 044309 TOX. CHEM NO.: None

TEST MATERIAL (PURITY): TI-435 (Lot No. 30034708 and Batch No. NLL 6100-3, 96.0% and 98.6% a.i., respectively).

SYNONYMS: CLOTHIANIDIN (iso).
Chemically: N-[(2-chloro-5-thiazolyl)methyl]-N^{I}-methyl-N^{II} - nitroguanidine.

CITATION: Herbold, B. (1999). TI-435: *Salmonella*/Microsome Test Using *Salmonella typhimurium* TA1535 Plate Incorporation and Preincubation Method, performed at Bayer AG's Department of Toxicology, Wuppertal (GERMANY), Bayer AG Report No. 25739A (Bayer Corporation Agricultural Division Report No. 109989), Bayer Study No. T9053830, dated January 28, 1999. MRID 45422734.

SPONSOR: Takeda Chemical Industries Agro Company, Tokyo (JAPAN).

EXECUTIVE SUMMARY:

In repeat reverse mutation assays in bacteria (MRID 45422734), the histidine-requiring (*his*$^{-}$) mutant TA1535 of *Salmonella typhimurium* was exposed to TI-435 in dimethylsulfoxide (DMSO) in the presence and absence of a mammalian metabolic activation system (± S9), initially by the standard plate-incorporation procedure in cultures with Batch NLL-6100-3 at five concentrations ranging from 1000 to 5000 μg/plate, in cultures with Lot No. 30034708 at 3000, 4000, and 7000 μg/plate; in cultures with both batches in repeat assays including a preincubation step at a range of five concentrations from 1000 to 8000 μg/plate. The S9 fraction was derived from the livers of male Sprague-Dawley rats induced with Aroclor 1254. In addition to cultures treated with the vehicle (DMSO), other cultures were exposed to the mutagens, sodium azide and aminoanthracene, to serve as positive controls.

Weak cytotoxicity was observed at doses above 4000 µg/plate; compound precipitation was seen at 8000 µg/plate. However, at no dose in any assay was evidence of mutagenic activity (increase in revertants, *his*$^-$ to *his*$^+$) seen, compared to solvent controls. Both positive control chemicals induced marked increases in revertants.

Therefore, it appears that neither batch of this compound has mutagenic activity when tested above bacteriotoxic/precipitating/limit dosing in the presence or absence of metabolic activation. This is consistent with the negative results of an earlier assay of different lots of TI-435 (Lot No. 12256321, Report MRID 45422732, 1990; Lot No. 30037120, MRID 45422733) but at variance with the results of a contemporary assay (2000) of one of these batches (Lot No. 30034708) of TI-435, which reported small but significant results in TA1535 cultures at 5000 µg/plate, but no positive results in any other treated culture, even in the more sensitive base-substitution strain, TA-100 and *E. coli* WP2 µvrA$^-$ (MRID 45422731).

This study is classified as acceptable/non-guideline since only TA1535 was assayed. and does not satisfy the requirement for FIFRA Test Guideline 84-2 for *in vitro* mutagenicity (bacterial reverse gene mutation) data.

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

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Material: TI-435
Description: Beige powder.
Lots/Batches Nos.: 30034708 (analyzed February 28, 1996)/NLL 6100-3 (analyzed October 24, 1996).
Purity: 96.0 % a.i./98.6 % a.i., respectively.
Stability of compound: Not stated in this report, but both listed as stable at room temperature for at least 24 hours (see MRID No. 45422731).
CAS No.: 205510-53-8.
Solvent used: Dimethylsulfoxide (DMSO).
Other comments: Store at room temperature. Both batches were dissolved in DMSO to form clear yellow solutions.
Test dates: 10/24/96 to 11/22/96

2. Control Materials:

Negative: None
Solvent/Final Concentration: DMSO/0.1 mL/plate.
Positive: Non-activated:
Sodium azide 10.0 µg/plate for TA1535.
Activated:
2-Aminoanthracene (2-anthramine) 3.0 µg/plate for TA1535.

3. Metabolic Activation: S9 was derived from adult (200-300 g) male Sprague-Dawley rats.

x	Aroclor 1254	x	induced	x	rat	x	liver
	phenobarbital		non-induced		mouse		lung
	none				hamster		other
	other						other

Describe S9 mix composition: 70 mL of cofactor solution contained:

Ingredient	Amount
$MgCl_2$ x 6 H_2O	162.6 mg
KCl	246.0 mg
Glucose-6-phosphate (disodium)	179.1 mg
NADP (disodium)	315.0 mg
Phosphate buffer	100.0 mM

Sufficient S9 homogenate was added to yield a 10% solution.

4. Test Organisms: *Salmonella typhimurium* strain: (obtained from Dr. B. N. Ames, University of California at Berkeley.)

	TA97		TA98		TA100		TA102
	TA104	x	TA1535(only)	x	TA1537		TA1538

List any others: None.
Properly maintained? Yes.
Checked for appropriate genetic markers (rfa mutation, R factor)? Yes.

5. Test Compound Concentrations Used:

Batch NLL 6100-3	Lot 30034708
1000, 2000, 3000, 4000, 5000 µg/plate - by plate incorporation	3000, 5000, 7000 µg/plate - by plate incorporation
1000, 2000, 4000, 6000, 8000 µg/plate with preincubation step	1000, 2000, 4000, 6000, 8000 µg/plate with preincubation step

B. TEST PERFORMANCE:

Standard procedures were employed to perform the assays according to the plate-incorporation method (Ames *et al.*, 1973; Ames *et al.*, 1975; Maron and Ames, 1983)[1,2,3]. Since no mutagenic effects were shown in these initial assays, the repeats were performed with a preincubation step in a water bath at 37°C for 20 minutes. All doses were determined on the basis of the plate incorporation assay.

Type of *Salmonella* Assay:

x	Standard plate test
x	Preincubation (20 minutes)
___	"Prival" modification
___	Spot test
___	Other

[1]Ames, B.N., W.E. Durston, E. Yamasaki and F.D. Lee:
Carcinogens are mutagens: a simple test system combining liver homogenates for activation and bacteria for detection.
Proc. Nat. Acad. Sci. (USA) 70, 2281-2285, 1973a.

[2]Ames, .N., J. McCann and E. Yamasaki:
Methods for detecting carcinogens and mutagens with the *Salmonella*/mammaliann-microsome mutagenicity test.
Mutation Res. 31, 347-364, 1975.

[3]Maron, D.M. and B.N. Ames:
Revised methods for the *Salmonella* mutagenicity test.
Mutation Res. 113, 173-215, 1983.

II REPORTED RESULTS:

A. MUTAGENICITY ASSAYS:

The test material precipitated at 8000 µg/plate.

No evidence of cytotoxicity at test doses up to 4000 µg/plate, with higher concentrations having no clear effect. For both batches, there were no dose-related or biologically relevant increases in mutant counts over those of the solvent control found in either initial or repeat assays (5000, 6000 7000, and/or 8000 µg/plate), in the presence or absence of S9-mix (MRID 45422734, pp. 43, 44, 45, 46, ATTACHMENT Tables 1, 2, 3 and 4). Both positive control chemicals induced large increases in mutant counts. Hence, the investigator concluded that neither batch of TI-435 was mutagenic in the TA1535 strain (base-substitution) of *Salmonella typhimurium.*

III. REVIEWER'S DISCUSSION/CONCLUSIONS

A. The reviewer agrees with the investigator's conclusions based on a very thorough assay of one strain of *Salmonella typhimurium* exposed to two batches of TI-435, but the study does not fully satisfy the requirements of the FIFRA Test Guideline for mutagenicity (bacterial gene mutation) data, since only TA1535 was assayed.

B. STUDY DEFICIENCIES:

None.

ATTACHMENT

THE FOLLOWING ATTACHMENT IS NOT AVAILABLE ELECTRONICALLY
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TI 435
Salmonella/Microsome Test
Study No. T 9053830
BAYER AG

5.2 Tabulated Summary of Data

Summary of Mean Values Without S9 Mix From Tables 1-4

Table and group	Strain TA 1535			
	NLL 6100-3	30034708	NLL 6100-3	30034708
1-4 µg²				
0	13	13	9	11
1000	9		9	11
2000	11		12	8
3000	12	13		
4000	11		10	9
5000	11	11		
6000			8	8
7000		16		
8000			9	9
Na-azide	652	652	626	672

²: per plate or tube

Summary of Mean Values With S9 Mix From Tables 1-4

Table and group	Strain TA 1535			
	NLL 6100-3	30034708	NLL 6100-3	30034708
1-4 µg²				
0	11	11	10	10
1000	10		10	12
2000	13		11	13
3000	9	17		
4000	12		14	10
5000	13	14		
6000			9	11
7000		18		
8000			7	12
2-AA	209	209	302	296

²: per plate or tube

B A Y E R A.G.
DEPARTMENT OF TOXICOLOGY
PHARMA RESEARCH CENTER
WUPPERTAL ELBERFELD
AMES TEST with : TI435, batch NLL 6100-3

Table : 1

Study Number : T 9053830
Study Director : Dr.Herbold
Technician : Bönning
Date : Nov. 18, 1996
Strain: S.typhimurium TA 1535

Dose/Plate (µg/Plate)	REVERTANTS PER PLATE -S9	M	SD	10% +S9	M	SD	TITER Dilution 10^{-6}	TITER per ml 10^{+8}	QUOTIENT -S9	QUOTIENT +S9
DMSO	11 12 15	13	2	9 12 12	11	2	159 127	14.3	1.0	1.0
1000	10 11 7	9	2	10 10 9	10	1	127 132	13.0	0.7	0.9
2000	13 11 8	11	3	16 10 12	13	3	129 140	13.5	0.8	1.2
3000	12 11 14	12	2	8 8 10	9	1	133 134	13.4	1.0	0.8
4000	9 15 10	11	3	11 15 11	12	2	92 168	13.0	0.9	1.1
5000	16 10 7	11	5	16 16 8	13	5	118 114	11.6**	0.9	1.2
Na-azide 10	678 665 614	652	34	%	/	/	137 118	12.8	51.5*	/
2-AA 3	%	/	/	247 206 173	209	37	109 85	9.7**	/	19.0*

%: not tested
*: mutagenic effect
**: bacteriotoxic effect
M: Mean
SD: Standard-Deviation
-S9: without S9 Mix
+S9: with S9 Mix

B A Y E R A.G. Table : 2 Study Number : T 9053830
DEPARTMENT OF TOXICOLOGY Study Director : Dr.Herbold
PHARMA RESEARCH CENTER Technician : Bönning
WUPPERTAL ELBERFELD Date : Nov. 18, 1996
AMES TEST with : TI435, LOT 30034708 Strain: S.typhimurium TA 1535

Dose/Plate (µg/Plate)	REVERTANTS PER PLATE -S9	M	SD	10% +S9	M	SD	TITER Dilution 10^{-6}	TITER per ml 10^{+8}	QUOTIENT -S9	QUOTIENT +S9
DMSO	11 12 15	13	2	9 12 12	11	2	159 127	14.3	1.0	1.0
3000	10 19 10	13	5	14 19 17	17	3	114 150	13.2	1.0	1.5
5000	13 13 7	11	3	16 10 15	14	3	115 112	11.4**	0.9	1.2
7000	11 22 14	16	6	17 20 18	18	2	94 94	9.4**	1.2	1.7
Na-azide 10	678 665 614	652	34	%	/	/	137 118	12.8	51.5*	/
2-AA 3	%	/	/	247 206 173	209	37	109 85	9.7**	/	19.0*

%: not tested
*: mutagenic effect
**: bacteriotoxic effect
M: Mean
SD: Standard-Deviation
-S9: without S9 Mix
+S9: with S9 Mix

B A Y E R A.G.
DEPARTMENT OF TOXICOLOGY
PHARMA RESEARCH CENTER
WUPPERTAL ELBERFELD
PREINCUBATION with : TI435, batch NLL 6100-3

Table : 3

Study Number : T 9053830
Study Director : Dr.Herbold
Technician : Bönning
Date : Nov. 22, 1996
Strain: S.typhimurium TA 1535

Dose/Tube (µg/Tube)	REVERTANTS PER PLATE -S9	M	SD	10% +S9	M	SD	TITER Dilution 10^{-6}	per ml 10^{+8}	QUOTIENT -S9	+S9
DMSO	9 11 6	9	3	12 9 10	10	2	137 175	15.6	1.0	1.0
1000	7 10 9	9	2	11 10 10	10	1	157 177	16.7	1.0	1.0
2000	13 11 11	12	1	12 10 10	11	1	176 175	17.6	1.3	1.0
4000	16 8 6	10	5	12 16 14	14	2	154 155	15.5	1.2	1.4
6000	6 13 6	8	4	8 8 11	9	2	92 110	10.1**	1.0	0.9
8000	12 8 7	9	3	7 P 8 P 6 P	7	1	72 57	6.5**	1.0	0.7
Na-azide 10	577 605 696	626	62	%	/	/	173 186	18.0	72.2*	/
2-AA 3	%	/	/	316 284 305	302	16	154 165	16.0	/	29.2*

*: mutagenic effect
%: not tested
M: Mean
-S9: without S9 Mix
**: bacteriotoxic effect
P: Precipitation
SD: Standard-Deviation
+S9: with S9 Mix

B A Y E R A.G.
DEPARTMENT OF TOXICOLOGY
PHARMA RESEARCH CENTER
WUPPERTAL ELBERFELD
PREINCUBATION with : TI435, LOT 30034708

Table : 4

Study Number : T 9053830
Study Director : Dr.Herbold
Technician : Bönning
Date : Nov. 22, 1996
Strain: S.typhimurium TA 1535

Dose/Tube (µg/Tube)	REVERTANTS PER PLATE -S9	M	SD	10% +S9	M	SD	TITER Dilution 10^{-6}	TITER per ml 10^{+8}	QUOTIENT -S9	QUOTIENT +S9
DMSO	9 16 9	11	4	10 8 12	10	2	146 170	15.8	1.0	1.0
1000	11 9 12	11	2	12 13 12	12	1	155 155	15.5	0.9	1.2
2000	7 12 6	8	3	17 11 10	13	4	163 158	16.1	0.7	1.3
4000	11 8 8	9	2	7 8 14	10	4	150 153	15.2	0.8	1.0
6000	7 9 7	8	1	10 12 10	11	1	63 68	6.6**	0.7	1.1
8000	8 P 14 P 6 P	9	4	12 P 15 P 8 P	12	4	68 40	5.4**	0.8	1.2
Na-azide 10	600 702 714	672	63	%	/	/	172 169	17.1	59.3*	/
2-AA 3	%	/	/	280 292 315	296	18	137 149	14.3	/	29.6*

*: mutagenic effect
%: not tested
M: Mean
-S9: without S9 Mix
**: bacteriotoxic effect
P: Precipitation
SD: Standard-Deviation
+S9: with S9 Mix

CLOTHIANIDIN *SALMONELLA*/MAMMALIAN ACTIVATION; GENE MUTATION (84-2)

EPA Reviewer: Irving Mauer, Ph.D. Date: 10/30/02
Registration Action Branch 3, HED (7509C)
EPA Secondary Reviewer: Nancy McCarroll Date: 11/07/02
Toxicology Branch, HED (7509C)

TXR No.: 0050321

DATA EVALUATION RECORD

STUDY TYPE: Bacterial system, *e.g.*, *Salmonella*/mammalian activation gene mutation assay; OPPTS 870.5100 [84-2]

DP BARCODE: D279778 SUBMISSION CODE: S607124

P.C. CODE: 044309 TOX. CHEM NO.: None

TEST MATERIAL (PURITY): TIR-435 (Lot. No. 12256321, ≥ 99.0 % a.i.)

SYNONYMS: Clothianidin (iso); TI-435 (current designation, see MRID 45422732, p. 28)

CITATION: Otsuka, M. (1990). Bacterial Reverse Mutation Test of TIR-435, performed at Hita Research Laboratories, Chemical Biotesting Center, Chemicals Inspection and Testing Institute (JAPAN), Laboratory Project ID, (Hita) Study Code No. KO1-0820 (Bayer Corporation Agricultural Division Report No. 109975), dated April 23, 1990. MRID 45422732. Unpublished.

SPONSOR: Takeda Chemical Industries Ltd., Agro Company, Tokyo (JAPAN).

EXECUTIVE SUMMARY:

In repeat reverse gene mutation assays using the standard plate-incorporation procedure (EXPERIMENT 1, range-finding test; EXPERIMENT 2, main test) in bacteria (MRID 45422732), four histidine-requiring (*his*$^-$) strains of *Salmonella typhimurium* (TA1535, TA1537, TA98 and TA100) and one tryptophan-requiring (*try*$^-$) strain of *Escherichia coli* (WP2 uvrA) were exposed in the presence and absence of a mammalian metabolic activation system (± S9) to TIR-435 (Lot. No. 12256321, ≥ 99% a.i. dissolved in dimethylsulfoxide, DMSO), initially at five concentrations ranging from 100 to 5000 μg/plate, (EXPERIMENT 1), in the repeat assay (EXPERIMENT 2) at a narrower range of doses (313 to 5000 μg/plate). In addition to cultures exposed to the vehicle, DMSO (negative control), other cultures were treated with strain-specific mutagens, to serve as positive controls. Following incubation at 37°C for 48 hours, reversions to prototrophy (*his*$^-$ to *his*$^+$; *try*$^-$ to *try*$^+$) were compared to vehicle controls.

No evidence of toxicity was apparent in any test culture in either experiment, and **the number of revertant colonies was not appreciably different from solvent controls (< 2x) ± S9.** The positive control chemicals all induced large increases in revertants.

Therefore, TIR-435 is considered to have no mutagenic activity in bacteria (*Salmonella typhimurium* and *Escherichia coli*) under conditions of this assay.

However, in a later study (MRID No. 45422731) using a different batch (30034708, 96% a.i.) the same compound, (TI-435), was considered mutagenic, since small, statistically significant (≤ 0.05, ≤ 0.005) and reproducible (in repeat assays) increased revertants appeared in both stimulated (> 2x, + S9) and unstimulated (< 2x, -S9) cultures.

This study is classified as acceptable/guideline and satisfies the requirement for FIFRA Test Guideline 84-2 for *in vitro* mutagenicity (bacterial reverse gene mutation) data.

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

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Material: TIR-435.
   Description: White crystal
   Lot/Batch No.: 12256321.
   Purity: ≥ 99% a.i.
   Stability of compound: Stable at room temperature in the dark.
   Solvent used: Dimethylsulfoxide (DMSO)
   Other comments: Store at room temperature.

2. Control Materials:

   Negative: None

   Solvent/Final Concentration: DMSO/5 % (w/v)

   Positive: Non-activated:

Sodium azide	0.5 μg/plate for TA1535
AF-2[1]	0.1/0.01 μg/plate for TA98/TA100 and WP2 uvrA, respectively
ICR-191[2]	1.0 μg/plate for TA1537

   Activated: 2-Aminoanthracene (2-anthramine) 0.5/1.0/2.0/20.0 μg/plate for TA98/TA100/TA1535/TA1537/WP uvrA, respectively.

[1]AF-2 = 2-(2-Furyl)-3-(5-nitro-2-furyl)acrylamide.

[2]ICR-191 = 2-Methoxy-6-chloro-9-[3-(2-chloroethyl)-aminopropylamino] acridine.

3. Metabolic Activation: S9 (Lot No. RAA-233, purchased from Kikkoman Company, Ltd.), was derived from 7-week old male Sprague-Dawley rats.

Aroclor 1254	x	induced	x	rat	x	liver
80 mg/kg 5,6 benzflavone x 1 + 30 mg/kg/day phenobarbital by i.p. x 3		non-induced		mouse		lung
none				hamster		other
other						other

Describe S9 mix composition: (Lot No. 99903, purchased from Oriental Yeast Industries, Ltd.).

COMPONENT	mg CONTENTS (1 vial)
$MgCl_2$	16.3
KCl	24.6
G-6-P	17.0
NADPH	36.2
NADH	30.5
Na_2HPO_4	119.6
NaH_2PO_4	24.7

4. Test Organisms: *Salmonella typhimurium* strains: (from the Institute of Environmental Toxicology)

	TA97	x	TA98	x	TA100		TA102
	TA104	x	TA1535	x	TA1537		TA1538

List any others: *Escherichia coli* WP2 uvrA (from the Institute of Medical Science, University of Tokyo)

Properly maintained? Yes.
Checked for appropriate genetic markers (rfa mutation, R factor)? Yes.

5. Test Compound Concentrations Used:

Cytotoxicity Test: (Not performed.).

Main Assays	**EXPERIMENT 1 (Dose Range-Finding)**	**EXPERIMENT 2 (Main Test)**
Non-activated and activated conditions	100, 500, 1000, 2000, 5000 μg/plate	313, 625, 1250, 2500, 5000 μg/plate

B. TEST PERFORMANCE:

The test substance solution in 0.1 mL aliquots, 0.5 mL of 0.1 M sodium phosphate buffer (pH 7.4), or S9-mix, and 0.1 mL of the bacterial culture and 2 mL of soft agar (Bacto-agar, Difco, containing 0.6 % agar and 0.5% NaCl in a ratio of 1:10) were added to test tubes, mixed thoroughly, and poured unto minimal glucose agar plates. Plates were incubated at 37°C for 48 hours, at which time, the number of revertants were counted. (The dose of bacterial contamination was checked by incubating S9-mix and 0.1 M sodium phosphate buffer smeared on a minimal glucose agar plate at 37°C for 48 hours).

The state of revertants (size and number), precipitation of test substance and growth inhibition were examined. Revertant colonies on plates not showing precipitation or growth inhibition were counted (by Toyosokki Company colony analyzer), individual frequencies and means transferred to computer (PC9801) and printed out on data sheets. If precipitation or growth inhibition occurred, the number of revertant colonies was counted manually (simple hand counter) and entered into the PC9801 computer by keyboard. Two plates per test concentration were counted, and means recorded.

1. Type of *Salmonella Assay*:

_x_ Standard plate tests (EXPERIMENT 1 and EXPERIMENT 2)
____ Preincubation (___ minutes)
____ "Prival" modification
____ Spot test
____ Other (describe)

2. Criteria for test validity and evaluation of results were both presented.

3. Statistics:

No statistical tests were performed.

II. REPORTED RESULTS

A. PRELIMINARY CYTOTOXICITY ASSAY:

Not performed.

B. MUTAGENICITY ASSAY:

No toxicity (growth inhibition and/or reduction in revertants) was observed in any test culture at any dose in the presence or absence of S9 in either experiment. Furthermore, **the number of revertants was consistently less than twice the solvent (DMSO) control ± S9 in both experiments (MRID 45422732, pp. 13, 14 - ATTACHMENT 18,19). The positive control chemicals all induced large increases in revertants.**

Therefore, the investigator concluded the TIR-435 has no mutagenic potential under the conditions of this assay in *Salmonella typhimurium* or *Escherichia coli*.

III. REVIEWER'S DISCUSSION/CONCLUSIONS:

A. The reviewer agrees with the conclusion of the investigator that comprehensive testing on April 23, 1990 of TIR-435 as white crystals (Batch No. 12256321, ≥ 99% a.i.) in bacteria gave no evidence of mutagenicity in a battery of *Salmonella typhimurium* strains or *Escherichia coli* WP2 uvrA treated up to the non-toxic limit dose, 5000 μg/plate.

This appears to be at variance with a later study (MRID 45422731, March 8, 2000) using a different batch (30034708, "pale yellow powder") of presumably the same material (TI-435, but 96.0% a.i.) which reported "small statistically significant" (≤ 0.05, ≤ 0.005) and reproducible (in repeat assays) in the frequency of revertants for tester strain TA1535 exposed to 5000 μg/plate in both S9-stimulated (> 2 x solvent control) and non-stimulated (< 2 x control cultures).

B. STUDY DEFICIENCIES:

None.

ATTACHMENT

THE FOLLOWING ATTACHMENT IS NOT AVAILABLE ELECTRONICALLY
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K01-0820

18. Dose range finding test

Test substance: TIR-435

With(+) or without(-) S9 Mix	Test substance concentration (μg/plate)	Number of revertants (number of colonies/plate)				
		Base-pair substitution type			Frameshift type	
		TA 100	TA 1535	WP2 uvrA	TA 98	TA 1537
S9 Mix (-)	solvent control	86 / 75 (81)	7 / 6 (7)	43 / 56 (50)	27 / 25 (26)	4 / 5 (5)
	100	84 / 77 (81)	8 / 4 (6)	44 / 45 (45)	32 / 34 (33)	8 / 8 (8)
	500	97 / 79 (88)	5 / 9 (7)	40 / 36 (38)	28 / 23 (26)	2 / 6 (4)
	1000	80 / 114 (97)	4 / 8 (6)	40 / 38 (40)	28 / 21 (25)	4 / 3 (4)
	2000	72 / 106 (89)	3 / 8 (6)	36 / 41 (38)	36 / 26 (31)	6 / 3 (5)
	5000	86 / 86 (86)	3 / 4 (4)	28 / 43 (36)	24 / 26 (25)	2 / 5 (4)
S9 Mix (+)	solvent control	104 / 103 (104)	6 / 7 (7)	35 / 52 (44)	37 / 25 (31)	6 / 5 (6)
	100	114 / 83 (99)	8 / 5 (7)	49 / 44 (47)	32 / 36 (34)	8 / 3 (6)
	500	94 / 98 (96)	4 / 5 (5)	52 / 58 (55)	28 / 37 (32)	4 / 11 (8)
	1000	94 / 102 (98)	8 / 7 (7)	53 / 60 (57)	37 / 30 (34)	11 / 5 (8)
	2000	110 / 87 (99)	8 / 5 (7)	46 / 53 (50)	27 / 30 (29)	5 / 6 (6)
	5000	94 / 117 (106)	7 / 9 (8)	51 / 45 (48)	28 / 37 (32)	3 / 5 (4)
Positive control not requiring S9 Mix	Name	AF-2	NaN_3	AF-2	AF-2	ICR-191
	Concentration (μg/plate)	0.01	0.5	0.01	0.1	1
	Number of colonies/plate	414 / 383 (399)	310 / 307 (309)	238 / 266 (252)	582 / 532 (557)	803 / 674 (639)
Positive control requiring S9 Mix	Name	2AA	2AA	2AA	2AA	2AA
	Concentration (μg/plate)	1	2	20	0.5	2
	Number of colonies/plate	3384 / 3452 (3408)	483 / 484 (484)	1283 / 1433 (1358)	552 / 557 (555)	816 / 859 (838)

Notes Parenthesis shows the mean of each plate.

·AF-2: 2-(2-Furyl)-3-(5-nitro-2-furyl)acrylamide

·NaN_3: Sodium azide

·ICR-191: 2-Methoxy-6-chloro-9-(3-(2-chloroethyl)-aminopropylamino) acridine·2HCl

·2AA: 2-Aminoanthracene

19. Main test

Test substance: TIR-435

With(+) or without(-) S9 Mix	Test substance concentration (μg/plate)	Number of revertants (number of colonies/plate)				
		Base-pair substitution type			Frameshift type	
		TA 100	TA 1535	WP2*uvrA*	TA 98	TA 1537
S9 Mix (-)	solvent control	83 / 74 (79)	8 / 7 (8)	39 / 33 (36)	29 / 25 (27)	4 / 7 (6)
	313	72 / 75 (74)	4 / 5 (5)	49 / 42 (46)	29 / 20 (25)	4 / 3 (4)
	625	77 / 87 (82)	6 / 7 (7)	37 / 39 (38)	22 / 27 (25)	9 / 7 (8)
	1250	88 / 92 (90)	8 / 6 (7)	39 / 45 (42)	25 / 29 (27)	4 / 4 (4)
	2500	92 / 79 (86)	5 / 2 (4)	39 / 25 (32)	24 / 23 (24)	5 / 3 (4)
	5000	74 / 87 (81)	9 / 2 (6)	29 / 34 (32)	29 / 24 (27)	3 / 5 (4)
S9 Mix (+)	solvent control	70 / 96 (83)	12 / 12 (12)	47 / 48 (48)	28 / 24 (26)	8 / 9 (9)
	313	73 / 72 (73)	11 / 9 (10)	47 / 55 (51)	34 / 23 (29)	9 / 5 (7)
	625	96 / 98 (97)	8 / 6 (7)	46 / 49 (48)	31 / 24 (28)	6 / 14 (10)
	1250	72 / 86 (79)	8 / 11 (10)	59 / 61 (60)	42 / 36 (39)	13 / 4 (9)
	2500	84 / 86 (85)	13 / 9 (11)	33 / 38 (36)	31 / 26 (29)	6 / 6 (6)
	5000	87 / 86 (87)	8 / 7 (8)	50 / 50 (50)	26 / 30 (28)	4 / 9 (7)
Positive control not requiring S9 Mix	Name	AF-2	NaN_3	AF-2	AF-2	ICR-191
	Concentration (μg/plate)	0.01	0.5	0.01	0.1	1
	Number of colonies/plate	254 / 252 (253)	328 / 377 (353)	180 / 187 (184)	372 / 382 (377)	952 / 1060 (1006)
Positive control requiring S9 Mix	Name	2AA	2AA	2AA	2AA	2AA
	Concentration (μg/plate)	1	2	20	0.5	2
	Number of colonies/plate	984 / 924 (954)	408 / 420 (415)	1329 / 1420 (1375)	241 / 261 (251)	151 / 209 (180)

Notes Parenthesis shows the mean of each plate.

- AF-2: 2-(2-Furyl)-3-(5-nitro-2-furyl)acrylamide
- NaN_3: Sodium azide
- ICR-191: 2-Methoxy-6-chloro-9-(3-(2-chloroethyl)-aminopropylamino) acridine·2HCl
- 2AA: 2-Aminoanthracene

CLOTHIANIDIN *SALMONELLA*/MAMMALIAN ACTIVATION; GENE MUTATION (84-2)

EPA Reviewer: Irving Mauer, Ph.D. Date: 11/20/02
Registration Action Branch 3, HED (7509C)
EPA Secondary Reviewer: Nancy McCarroll Date: 11/25/02
Toxicology Branch, HED (7509C)

TXR NO.: 0050321

DATA EVALUATION RECORD

STUDY TYPE: Bacterial system, *e.g.*, *Salmonella*/mammalian activation gene mutation assay; OPPTS 870.5100 [84-2]

DP BARCODE: D279778 SUBMISSION CODE: S607124

P.C. CODE: 044309 TOX. CHEM NO.: None

TEST MATERIAL (PURITY): TI-435 (Batch No. 30034708, 96.0% a.i.)

SYNONYMS: Clothianidin (iso).
Chemically: (E)-1-(2-chloro-5-thiazolylmethyl)-3-methyl-2-nitroguanidine.

CITATION: Thompson, P.W. (2000). TI-435: Reverse Mutation Assay "Ames Test" Using *Salmonella typhimurium* and *Escherichia coli*, performed at the Safepharm Laboratories (SPL) Ltd., Derby (UK), SPL Project No. 178/110 (Bayer Corporation Agricultural Division Report No. 109953), dated March 08, 2000. MRID 45422731. Unpublished.

SPONSOR: Takeda Chemical Industries, Ltd., Agro Company, Tokyo (JAPAN).

EXECUTIVE SUMMARY:

In repeat reverse gene mutation assays using direct plate-incorporation (EXPERIMENT 1 and EXPERIMENT 2) in bacteria (MRID 45422731), four histidine-requiring (*his*$^-$) strains of *Salmonella typhimurium* (TA1535, TA1537, TA98 and TA100) and one tryptophan-requiring (*try*$^-$) strain of *Escherichia coli* (WP2 uvrA$^-$) were exposed in the presence and absence of a mammalian metabolism system (± S9) to TI-435 (Batch No. 30034708, 96.0% a.i. dissolved in dimethylsulfoxide, DMSO) at five concentrations ranging from 50 to 5000 μg/plate. The S9 homogenate was derived from the livers of male Sprague-Dawley rats induced with Aroclor 1254. After 48 hours incubation at 37°C, the frequency of reversion to histidine (*his*$^-$ to *his*$^+$)

and tryptophan (*try*$^-$ to *try*$^+$) independence was determined in test plates and compared to vehicle (DMSO) controls. Additional cultures were treated with strain-specific mutagens, to serve as positive controls.

No toxicity (manifested by thinning of the background bacterial lawn and/or reduction in revertants) was apparent in any culture treated up to the limit dose, 5000 μg/plate. A small, but significant ($p \leq 0.05$ and $p \leq 0.005$) increase in frequency of histidine revertants in tester strain TA1535 treated at 1500 and 5000 μg/plate +/-S9 (more predominant, *i.e.* > 2 x concurrent control in the presence of S9), still present but weaker (*i.e.*, < 2 x concurrent control in its absence). The positive response was only reproducible at 5000 μg/plate +/-S9. Whereas no other treated cultures had comparable increases (even the more sensitive base substitution strains TA100 or *E. coli* WP2 μvrA$^-$), **these consistent positive results recorded in TA1535 treatment renders TI-435 mutagenic under conditions of this test.**

Positive controls all indicated large increases in revertant frequencies.

This study is classified as acceptable/guideline and satisfies the requirement for FIFRA Test Guideline 84-2 for *in vitro* mutagenicity (bacterial reverse gene mutation) data.

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

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Material: TI-435.
Description: Pale, yellow powder.
Lot/Batch No.: 30034708.
Purity: 96% a.i.
Stability of compound: Stable at room temperature for at least 24 hours.
CAS No.: 205510-53-8.
Solvent used: Dimethylsulfoxide (DMSO).
Other comments: Store at 4°C in the dark.
Doses were adjusted to 100% a.i.
Test dates: 8/5/96 - 9/13/96

2. Control Materials:
Negative: None.
Solvent/Final Concentration: DMSO/0.1 mL.
Positive: Non-activated:

ENNG[1]	3/5/2 µg/plate for TA100/TA1535/WP2 uvrA⁻
4NQO[2]	2.0 µg/plate for TA98
9-Aminoacridine	80.0 µg/plate for TA1537
Other (list):	None.

Activated:

2-Aminoanthracene (2-anthramine)	1.0 µg/plate for TA100; 2.0 µg/plate for TA1535 and TA1537: 0.5 µg/plate for TA98; and 10.0 µg/plate for WP2 uvrA⁻

3. Metabolic Activation: S9 was prepared by the performing laboratory from young (7 weeks) male Sprague-Dawley rats.

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[1]ENNG = N-ethyl-N[1]-nitro-N-nitrosoguanidine.

[2]4-NQO = 4-Nitroquinoline-1-oxide.

x	Aroclor 1254	x	induced	x	rat		liver
	phenobarbital		non-induced		mouse		lung
	none				hamster		other
	other						other

Describe S9 mix composition:

S9	5.0 mL
1.65 M KCl/0.4 M $MgCl_2$	1.0 mL
0.1 M Glucose-6-phosphate	2.5 mL
0.1 M NADP	2.0 mL
0.2 M Sodium phosphate buffer (pH 7.4)	25.0 mL
Sterile distilled water	14.5 mL

4. Test Organisms: Salmonella *typhimurium* strains:
(From the University of California, Berkeley)

	TA97	x	TA98	x	TA100		TA102
	TA104	x	TA1535	x	TA1537		TA1538

List any others: *Escherichia coli* WP2 uvraA$^-$ (from BIBRA).

Properly maintained? Yes.

Checked for appropriate genetic markers (rfa mutation, R factor)? Yes.

5. Test Compound Concentration Used:

Cytotoxicity Test: Test performed in strains TA100 and WP2 uvrA$^-$:
0, 50, 150, 500, 1500, 5000 μg/plate

Main Assays	EXPERIMENT 1 (Range-Finding)	EXPERIMENT 2 (Main Study)
Non-activated and activated conditions	0, 50, 150, 500, 1500, 5000 μg/plate	0, 50, 150, 500, 1500,5000 μg/plate

B. TEST PERFORMANCE:

In both experiments 0.1 mL aliquots of bacterial suspensions in triplicate were dispersed in sterile test tubes, followed by 2.0 mL of molten trace histidine/tryptophan supplemented top agar at 45°C, 0.1 mL of diluted test material or vehicle control, and either 0.5 mL of the S9-mix or phosphate buffer. The contents were then mixed thoroughly and equally distributed onto the surface of Vogel-Bonner Minimal agar plates (one tube per plate). This procedure was repeated for each bacterial strain and for each concentration of test material with/without S9-mix in triplicate.

This procedure was also repeated in triplicate for each positive control with/without activation.

1. Incubation and Assessment of Plates:

All plates were incubated at 37°C for 48 hours, and the frequency of revertants determined automatically using a "DOMINO Colony Counter".

2. Interpretation of Results/Statistical Treatment:

To be considered positive, a substance should induce a dose-related and statistically significant increase (according to the *UKEMS Sub-committee on Guidelines for Mutagenicity Testing*, Report, Part III (1989), Cambridge University Press) in mutation rate (at least 2 x the spontaneous reversion rate) in one or more strains ± S9 in both experiments at subtoxic levels. (If a conflict occurs between these two experiments, then a third experiment must be performed to confirm the correct response.) To be considered negative, a substance may induce less than two-fold the spontaneous rate at each dose level, the intervals of which extend to the limits imposed by toxicity, solubility, or up to the recommended limit dose of 5000 μg/plate.

3. Type of *Salmonella Assay*:

_x_ standard plate test (EXPERIMENT 1 and EXPERIMENT 2)
____ preincubation (____ minutes)
____ "Prival" modification
____ spot test
____ others (describe)

4. The mutagenicity data was evaluated for statistical significance ($p \leq 0.05$) in accordance with UKEMS sub-committee guidelines for mutagenicity testing.

II. REPORTED RESULTS

A. ANALYTICAL DETERMINATIONS:

Analytical determinations conducted by the performing laboratory indicated that dosing solutions were stable at room temperature for at least 24 hours and that concentrations were generally within 91% - 118% of nominal levels..

B. PRELIMINARY CYTOTOXICITY ASSAY:

The test material was non-toxic to TA100 and WP2 uvrA$^-$ up to the limit dose, 5000 μg/plate.

C. MUTAGENICITY ASSAYS:

No toxicity was exhibited in any strain of test bacteria up to the limit dose, 5000 μg/plate.

A small, but statistically significant ($p \leq 0.05$) and reproducible increase in the frequency of revertants (*his*$^-$ to *his*$^+$) was recorded for tester strain TA1535 in both experiments, predominantly (> 2x concurrent controls) in the presence of S9 at the highest dose level of 5000 μg/plate, slightly weaker (< 2x concurrent control) in the absence of S9, (MRID 45422731, pp. 29, 30, 31, 32, 44 - ATTACHMENT, Tables 2, 3, 4, 5 - APPENDIX III). All of the positive control chemicals induced marked increases in revertants. There were no other positive responses in any other treated test strains, including TA100.

Hence, because of this persistent increase in revertants the investigator considers TI-435 to be mutagenic under conditions of this test.

Hence, because of this persistent increase in revertants the investigator considers TI-435 to be mutagenic under conditions of this test.

III. REVIEWER'S DISCUSSION/CONCLUSIONS.

A. The reviewer agrees with the investigator's conclusions that the persistent minor, but statistically significant and reproducible increase in revertants in TA1535 test cultures in two assays represents positive mutagenic activity of TI-435, despite the lack of a comparable effect in the more sensitive base-substitution strains, TA100 and *E. coli* WP2 μvrA$^-$.

B. STUDY DEFICIENCIES

None.

ATTACHMENT

THE FOLLOWING ATTACHMENT IS NOT AVAILABLE ELECTRONICALLY
SEE THE FILE COPY

SPL PROJECT NUMBER: 178/110

TI-435 : REVERSE MUTATION ASSAY "AMES TEST"
USING *SALMONELLA TYPHIMURIUM* AND *ESCHERICHIA COLI*
APPENDIX II (continued)
TABLES OF TEST RESULTS

TABLE 1

SPONTANEOUS MUTATION RATES

RANGE-FINDING STUDY

Number of Revertants (Number of Colonies per plate)									
Base-pair Substitution Type						Frameshift Type			
TA100		TA1535		WP2uvrA		TA98		TA1537	
87		21		22		20		12	
83	(88)	21	(20)	27	(26)	26	(21)	9	(11)
94		18		28		16		13	

MAIN STUDY

Number of Revertants (Number of Colonies per plate)									
Base-pair Substitution Type						Frameshift Type			
TA100		TA1535		WP2uvrA		TA98		TA1537	
136		24		24		19		5	
111	(125)	23	(25)	14	(19)	24	(24)	7	(6)
127		27		20		28		5	

SPL PROJECT NUMBER: 178/110

TI-435 : REVERSE MUTATION ASSAY "AMES TEST"
USING *SALMONELLA TYPHIMURIUM* AND *ESCHERICHIA COLI*
APPENDIX II (continued)
TABLES OF TEST RESULTS

TABLE 2

EXPERIMENT 1 - WITHOUT METABOLIC ACTIVATION

With or Without S9-Mix	Test Substance concentration µg/plate	Number of revertants (Number of colonies per plate)				
		Base-pair substitution type			Frameshift type	
		TA 100	TA 1535	WP2uvrA-	TA 98	TA 1537
-	0	108 87 (91) 77 15.8	25 20 (20) 15 5.0	28 27 (24) 16 6.7	21 19 (18) 14 3.6	10 11 (12) 14 2.1
-	50	91 82 (83) 75 8.0	26 28 (27) 28 1.2	22 17 (19) 19 2.5	21 12 (16) 16 4.5	9 9 (11) 16 4.0
-	150	96 95 (94) 90 3.2	23 20 (21) 19 2.1	12 23 (18) 19 5.6	13 14 (16) 22 4.9	17 12 (13) 10 3.6
-	500	94 77 (83) 78 9.5	25 25 (26) 27 1.2	23 23 (22) 20 1.7	25 16 (20) 18 4.7	13 10 (12) 13 1.7
-	1500	94 108 (97) 88 10.3	35 * 20 (30) 36 9.0	15 12 (12) 10 2.5	22 21 (18) 11 6.1	9 8 (11) 16 4.4
-	5000	101 110 (104) 101 5.2	29 * 28 (32) 38 5.5	13 11 (11) 10 1.5	20 22 (20) 18 2.0	12 10 (10) 7 2.5
Positive controls	Name	ENNG	ENNG	ENNG	4NQO	9AA
S9-Mix	Concentration (µg/plate)	3	5	2	0.2	80
-	No. colonies per plate	372 394 (384) 387 11.2	182 198 (190) 190 8.0	583 602 (587) 575 13.9	168 181 (175) 176 6.6	614 608 (605) 593 10.8

ENNG N-ethyl-N'-nitro-N-nitrosoguanidine
4NQO 4-nitroquinoline-1-oxide
9AA 9-aminoacridine
* $p \leq 0.05$

TI-435 : REVERSE MUTATION ASSAY "AMES TEST"
USING *SALMONELLA TYPHIMURIUM* AND *ESCHERICHIA COLI*
APPENDIX II (continued)
TABLES OF TEST RESULTS

TABLE 3

EXPERIMENT 1 - WITH METABOLIC ACTIVATION

With or Without S9-Mix	Test Substance concentration µg/plate	Number of revertants (Number of colonies per plate)				
		Base-pair substitution type			Frameshift type	
		TA 100	TA 1535	WP2uvrA-	TA 98	TA 1537
+	0	113 111 (119) 134 12.7	12 8 (11) 14 3.1	17 18 (18) 20 1.5	35 26 (30) 28 4.7	8 11 (10) 10 1.5
+	50	116 110 (116) 121 5.5	11 9 (12) 17 4.2	22 16 (21) 24 4.2	34 33 (32) 28 3.2	13 12 (13) 13 0.6
+	150	88 101 (96) 98 6.8	18 13 (15) 13 2.9	19 16 (18) 19 1.7	28 29 (30) 32 2.1	10 10 (11) 12 1.2
+	500	126 97 (107) 99 16.2	18 14 (13) 8 5.0	16 23 (19) 17 3.8	27 26 (26) 26 0.6	9 9 (9) 8 0.6
+	1500	111 130 (115) 103 13.9	24 * 16 (20) 20 4.0	14 18 (17) 18 2.3	31 23 (26) 25 4.2	10 9 (11) 13 2.1
+	5000	115 118 (123) 135 10.8	28 $$$ 27 (28) 29 1.0	16 13 (14) 13 1.7	25 26 (28) 33 4.4	9 9 (8) 5 2.3
Positive controls	Name	2AA	2AA	2AA	2AA	2AA
S9-Mix	Concentration (µg/plate)	1	2	10	0.5	2
+	No. colonies per plate	731 809 (771) 772 39.0	181 187 (189) 198 8.6	828 843 (861) 913 45.4	372 420 (407) 428 30.3	278 285 (279) 274 5.6

2AA 2-Aminoanthracene

* $p \leq 0.05$

$$$ $p \leq 0.005$

SPL PROJECT NUMBER: 178/110

TI-435 : REVERSE MUTATION ASSAY "AMES TEST"
USING *SALMONELLA TYPHIMURIUM* AND *ESCHERICHIA COLI*
APPENDIX II (continued)
TABLES OF TEST RESULTS

TABLE 4

EXPERIMENT 2 - WITHOUT METABOLIC ACTIVATION

With or Without S9-Mix	Test Substance concentration µg/plate	Number of revertants (Number of colonies per plate)				
		Base-pair substitution type			Frameshift type	
		TA 100	TA 1535	WP2uvrA-	TA 98	TA 1537
-	0	111 112 (111) 111 0.6	28 14 (19) 16 7.6	23 16 (19) 18 3.6	29 23 (24) 20 4.6	5 10 (9) 11 3.2
-	50	117 95 (105) 104 11.1	22 17 (19) 18 2.6	16 19 (15) 11 4.0	15 24 (20) 21 4.6	7 7 (8) 11 2.3
-	150	109 110 (109) 109 0.6	23 23 (25) 28 2.9	19 11 (14) 11 4.6	23 18 (20) 19 2.6	7 8 (8) 9 1.0
-	500	105 112 (104) 96 8.0	19 26 (24) 27 4.4	11 15 (14) 15 2.3	20 18 (21) 25 3.6	9 6 (6) 4 2.5
-	1500	114 111 (114) 118 3.5	35 32 (29) 21 7.4	15 21 (16) 12 4.6	16 12 (15) 18 3.1	4 6 (6) 8 2.0
-	5000	116 113 (115) 116 1.7	38 $$$ 39 (37) 33 3.2	11 13 (12) 11 1.2	26 24 (23) 18 4.2	5 6 (6) 6 0.6
Positive controls	Name	ENNG	ENNG	ENNG	4NQO	9AA
S9-Mix	Concentration (µg/plate)	3	5	2	0.2	80
-	No. colonies per plate	607 621 (608) 597 12.1	380 359 (385) 417 29.4	1011 1101 (1046) 1027 48.0	166 163 (162) 156 5.1	852 874 (879) 912 30.4

ENNG N-ethyl-N'-nitro-N-nitrosoguanidine
4NQO 4-nitroquinoline-1-oxide
9AA 9-aminoacridine
$$$ $p \leq 0.005$

SPL PROJECT NUMBER: 178/110

TI-435 : REVERSE MUTATION ASSAY "AMES TEST"
USING *SALMONELLA TYPHIMURIUM* AND *ESCHERICHIA COLI*
APPENDIX II (continued)
TABLES OF TEST RESULTS

TABLE 5

EXPERIMENT 2 - WITH METABOLIC ACTIVATION

With or Without S9-Mix	Test Substance concentration µg/plate	Number of revertants (Number of colonies per plate)									
		Base-pair substitution type						Frameshift type			
		TA 100		TA 1535		WP2uvrA-		TA 98		TA 1537	
+	0	110 102 122	(111) 10.1	10 16 10	(12) 3.5	18 16 21	(18) 2.5	23 33 25	(27) 5.3	8 8 13	(10) 2.9
+	50	118 108 115	(114) 5.1	12 15 11	(13) 2.1	18 19 22	(20) 2.1	22 22 22	(22) 0.0	8 12 7	(9) 2.6
+	150	104 90 103	(99) 7.8	13 17 14	(15) 2.1	22 19 21	(21) 1.5	32 20 22	(25) 6.4	8 6 7	(7) 1.0
+	500	89 107 94	(97) 9.3	10 13 12	(12) 1.5	22 16 19	(19) 3.0	17 18 24	(20) 3.8	12 6 6	(8) 3.5
+	1500	97 90 104	(97) 7.0	11 12 13	(12) 1.0	12 18 16	(15) 3.1	17 24 26	(22) 4.7	6 7 10	(8) 2.1
+	5000	111 124 128	(121) 8.9	25 24 28	**$$$** (26) 2.1	12 23 17	(17) 5.5	26 28 28	(27) 1.2	6 10 4	(7) 3.1
Positive controls	Name	2AA		2AA		2AA		2AA		2AA	
S9-Mix	Concentration (µg/plate)	1		2		10		0.5		2	
+	No. colonies per plate	837 876 800	(838) 38.0	211 232 221	(221) 10.5	773 772 673	(739) 57.4	457 449 480	(462) 16.1	266 255 287	(269) 16.3

2AA 2-Aminoanthracene

$$$ $p \leq 0.005$

SPL PROJECT NUMBER: 178/110

TI-435 : REVERSE MUTATION ASSAY "AMES TEST"
USING *SALMONELLA TYPHIMURIUM* AND *ESCHERICHIA COLI*
APPENDIX III (continued)

TABLE OF TEST RESULTS (RELATIVE ACTIVITY)

TEST SUBSTANCE: TI-435

		- S9-Mix		+ S9-Mix	
		Relative activity	Dose Levels used to calculate (mg/plate)	Relative activity	Dose levels used to calculate (mg/plate)
Range-finding Study	TA100	N/A		N/A	
	TA1535	2.3*	5	3.3	5
	WP2uvrA	N/A		N/A	
	TA98	N/A		N/A	
	TA1537	N/A		N/A	
Main Study	TA100	N/A		N/A	
	TA1535	3.5*	5	2.7	5
	WP2uvrA	N/A		N/A	
	TA98	N/A		N/A	
	TA1537	N/A		N/A	

N/A = not applicable as no evidence of mutagenic activity was observed

Relative Activity = number of induced revertant colonies per mg of test material

* Statistically significant increase observed without achieving a two-fold response

CLOTHIANIDIN *SALMONELLA*/MAMMALIAN ACTIVATION; GENE MUTATION (84-2)

EPA Reviewer: Irving Mauer, Ph.D. Date: 06/20/02
Registration Action Branch 3, HED (7509C)
EPA Secondary Reviewer: Nancy McCarroll Date: 11/14/02
Toxicology Branch, HED (7509C)

TXR NO.: 0050321

DATA EVALUATION RECORD

STUDY TYPE: Bacterial system, *e.g., Salmonella*/mammalian activation gene mutation assay; OPPTS 870.5100 [84-2]

DP BARCODE: D279778 SUBMISSION CODE: S607124

P.C. CODE: 044309 TOX. CHEM NO.: None

TEST MATERIAL (PURITY): MA I (99.9% a.i.; Batch No. 30064522)

SYNONYMS: None

CITATION:

Dawkes, N. (1999). MA I: Reverse Mutation in Five Histidine-Requiring Strains of *Salmonella typhimurium*, performed at Covance Laboratories, Ltd., Harrogate, North Yorkshire (ENGLAND). Laboratory Project ID: Covance 586/177-D1540 (Bayer Corporation Agricultural Division Report No. 110205), dated September 06, 1999. MRID 45422730. Unpublished.

SPONSOR: Takeda Chemical Industries, Ltd., Tokyo (JAPAN).

EXECUTIVE SUMMARY:

In repeat reverse gene mutation assays in bacteria (MRID 45422730), five histidine deficient (*his*$^-$) strains of *Salmonella typhimurium* (TA98, TA100, TA1535, TA1537 and TA102) were exposed to MA I (99.9% a.i.; Batch No. 30064522) dissolved in dimethylsulfoxide (DMSO) in the presence and absence of a mammalian metabolic activation system, S9, initially at five concentrations ranging from 8 to 5000 μg/plate in a standard plate-incorporation procedure (EXPERIMENT 1), then repeated in a narrower dose range of six concentrations from 51.2 to 5000 μg/plate, but also including a preincubation step with S9 only (EXPERIMENT 2). After incubation at 37°C for three days in the dark, reversions to prototrophy (from *his*$^-$ to *his*$^+$) were compared to vehicle cultures. Additional culture were treated with strain-specific mutagens, to serve as positive controls.

Test article was completely soluble in the aqueous assay system at all concentrations in both experiments.

Evidence of cytotoxicity (manifested as thinning of background bacterial lawn and/or reduction in revertants) was apparent in EXPERIMENT 1 TA98 test cultures treated at the maximum concentration both ± S9, as well as in EXPERIMENT 2 TA98 test cultures treated at the two highest concentrations in the absence of S9, but only at the highest concentration in its presence. However, no MA I treatment of any test strain in either experiment resulted in an increase in revertants. In contrast, large increases in revertants were induced in all mutagen-treated cultures.

Therefore, MA I is considered to be non-mutagenic in a standard battery of *Salmonella typhimurium* strains assayed up to a limiting/cytotoxic range of concentrations.

This study is classified as acceptable/guideline and satisfies the requirement for FIFRA Test Guideline 84-2 for *in vitro* mutagenicity (bacterial reverse gene mutation) data..

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

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Material: MA I
Description: Pink powder
Lot/Batch No.: 30064522
Purity: 99.9% a.i.
Stability of compound: Stated to be the responsibility of the sponsor.
Solvent used: Anhydrous analytical grade dimethylsulfoxide (DMSO).
Other comments: Stored at 1-10°C in the dark.

2. Control Materials:
Negative: None
Solvent/Final Concentration: DMSO/100μL – plate incorporation assay
Positive: Non-activated: DMSO/50 μL – preincubation assay

Sodium azide	2.0 μG/plate for TA100 and TA 1535
2-Nitrofluorene	5.0 μg/plate for TA98
9-Aminoacridine	50.0 μg/plate for TA1537
Other (list):	
Glutaraldehyde	25.0 μg/plate for TA102

Activated:

2-Aminoanthracene (2-anthramine) 5.0 μg/plate for at least one strain.

3. Metabolic Activation: S9 (obtained from Molecular Toxicology, Inc., USA) was derived from male Sprague-Dawley rats.

x	Aroclor 1254	x	induced	x	rat	x	liver
	phenobarbital		non-induced		mouse		lung
	none				hamster		other
	other						other

Describe S9 mix composition:

Ingredient	Concentration	Quantity (mL) 16% S-9 Mix
Sodium phosphate (buffer, pH 7.4)	500 mM	20.0
Glucose-6-phosphate (disodium)	180 mg/mL	0.845
NADP (disodium)	25 mg/mL	12.6
$MgCl_2$	250 mM	3.2
KCl	150 mM	22.0
L-histidine HCl (in 25 mM $MgCl_2$)	1 mg/mL	4.0
D-biotin	1 mg/mL	4.88
S9	(as detailed)	10.0
Water	-	(To volume)

4. Test Organisms: *Salmonella typhimurium* strains:

	TA97	x	TA98	x	TA100	x	TA102
	TA104	x	TA1535	x	TA1537		TA1538

Properly maintained? Yes.
Checked for appropriate genetic markers (rfa mutation, R factor)? Yes.

5. Test Compound Concentration Used:

Cytotoxicity Test: (performed in strain TA100 only): 8, 40, 200, 1000, 5000 μg/plate

Main Assays	EXPERIMENT 1	EXPERIMENT 2
Non-activated and activated conditions	8, 40, 200, 1000, 5000 μg/plate	51.2, 128, 320, 800, 2000, 5000 μg/plate

B. TEST PERFORMANCE:

MA I was tested in five strains in two separate experiments at the same concentrations as for the cytotoxicity test using triplicate plates ±S9. Solvent (DMSO) controls (in quintuplicate) and reference mutagen controls (in triplicate) were included in each assay. In EXPERIMENT 1 (plate-incorporation), to 2.5 mL, molten agar at 46°C were added: 0.1 mL bacterial culture; 0.1 mL test article or control; 0.5 mL 10% S9-mix or buffer solution. The material on the plate was rapidly mixed, and poured onto Minimal Davis agar plates. When set, the plates were inverted and incubated at 37°C in the dark for three days. Following incubation, the plates were examined for evidence of toxicity to the background lawn, where possible, revertant colonies were counted. In EXPERIMENT 2 (with a preincubation step) for the S9-activated phase of testing, the quantities of the test article or control solution (reduced to 0.05 mL to avoid possible toxic effects if added at the usual 0.1 mL), bacteria and S9-mix were mixed and incubated for 20 minutes at 37°C, with shaking, before the addition to 2.5 mL molten agar at 46°C. Plating then proceeded as for the plate-incorporation procedure. The non-activated portion of EXPERIMENT 2 was performed as described for the plate incorporation test.

Colony Counting and Analysis of Results:

Colonies were counted electronically using a Seescan Colony Counter, or manually where confounding factors such as split agar prevented accurate automated counts. In each case, the background lawn of representative plates was inspected for signs of toxicity.

Individual plate counts were recorded separately, and the mean and standard deviation of plate counts were determined for each treatment.

Statistical Methods:

The m-statistic was calculated to check that the data were Poisson-distributed. Dunnett's Test was used to compare the counts of each dose with the control. The presence or otherwise of a dose response was checked by linear regression analysis.

1. Type of *Salmonella Assay*:

_x_ standard plate test (EXPERIMENT 1)
_x_ preincubation (_20_ minutes) with S9 only (EXPERIMENT 2)
____ "Prival" modification
____ spot test
____ other (describe)

NOTE: The non-activated portion of EXPERIMENT 2 was performed as described for the plate incorporation test.

2. Criteria: Both Acceptance and Evaluation of Results were presented.

II. REPORTED RESULTS

A. SOLUBILITY:

The test article was completely soluble in the aqueous assay system at all concentrations in both experiments.

B. PRELIMINARY CYTOTOXICITY ASSAY:

No evidence of cytotoxicity (thinning of background bacterial lawn and/or reduction in revertants) was observed.

C. MUTAGENICITY ASSAYS:

In EXPERIMENT 1 treatment produced evidence of cytotoxicity (slight thinning of lawn) at 5000 μg/plate but only in TA98 ±S9. Evidence of cytotoxicity was also evident in EXPERIMENT 2 but again only in TA98 treated at the two highest test doses -S9 (2000 and 5000 μg/plate), and only at 5000 μg/plate +S9.

However, **no MA I treatment in any strain in either experiment ± S9 resulted in a statistically significant increase in revertants** (when data was analyzed at the 1% level using Dunnett's Test) compared to concurrent controls, (MRID 45422730, pp. 26, 27, 39, 40 - ATTACHMENT), nor historical control values (p. 51, APPENDIX 3). Positive controls all induced large increases in revertants.

Therefore, the investigator concluded that MA I provided no evidence of mutagenic activity in five strains of *Salmonella typhimurium* treated up to cytotoxic/limiting dose levels in the presence or absence of a rat metabolic activation system.

III. REVIEWER'S DISCUSSION/CONCLUSIONS:

A. The reviewer agrees with the investigator that the MA I did not induce mutation in the battery of *Salmonella typhimurium* strains.

B. STUDY DEFICIENCIES:

None.

ATTACHMENT

THE FOLLOWING ATTACHMENT IS NOT AVAILABLE ELECTRONICALLY
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MA I: summary of mean revertant colonies (-S-9) - Experiment 1

Substance	Dose Level µg/plate	TA98	TA100	TA1535	TA1537	TA102
		Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
DMSO	100 µl	30 ± 9	101 ± 4	15 ± 6	6 ± 2	217 ± 25
MA I	8	36 ± 9	97 ± 5	17 ± 5	5 ± 4	208 ± 14
	40	28 ± 3	91 ± 12	14 ± 3	3 ± 2	183 ± 11
	200	36 ± 3	109 ± 12	13 ± 2	5 ± 1	198 ± 9
	1000	35 ± 3	107 ± 7	17 ± 6	6 ± 2	180 ± 21
	5000	34 ± 3 (S)	105 ± 4	14 ± 1	6 ± 2	205 ± 6
Positive Controls	Compound	2NF	NaN3	NaN3	AAC	GLU
	Dose Level	5 µg	2 µg	2 µg	50 µg	25 µg
	Mean ± SD	608 ± 97	580 ± 17	503 ± 4	119 ± 22	513 ± 17

SD Standard deviation

2NF 2-Nitrofluorene
NaN3 Sodium azide
AAC 9-Aminoacridine
GLU Glutaraldehyde

S : Slight thinning of b/g lawn

MA I: summary of mean revertant colonies (+S-9) - Experiment 1

Substance	Dose Level µg/plate	TA98	TA100	TA1535	TA1537	TA102
		Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
DMSO	100 µl	35 ± 4	97 ± 16	15 ± 6	5 ± 3	297 ± 47
MA I	8	37 ± 10	111 ± 6	13 ± 2	7 ± 3	305 ± 23
	40	36 ± 5	115 ± 18	16 ± 3	7 ± 2	292 ± 9
	200	37 ± 9	112 ± 17	15 ± 5	7 ± 2	324 ± 38
	1000	37 ± 4	113 ± 2	17 ± 5	5 ± 2	359 ± 28
	5000	31 ± 8 (S)	101 ± 7	16 ± 1	6 ± 3	361 ± 23
Positive Controls	Compound	AAN	AAN	AAN	-	-
	Dose Level	5 µg	5 µg	5 µg	-	-
	Mean ± SD	656 ± 104	2280 ± 20	202 ± 27	-	-

SD Standard deviation

AAN 2-Aminoanthracene

S : Slight thinning of b/g lawn

MA I: summary of mean revertant colonies (-S-9) - Experiment 2

Substance	Dose Level µg/plate	TA98	TA100	TA1535	TA1537	TA102
		Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
DMSO	100 µl	32 ± 5	95 ± 15	15 ± 5	8 ± 1	343 ± 48
MA I	51.2	34 ± 5	98 ± 6	17 ± 6	8 ± 1	376 ± 11
	128	37 ± 1	92 ± 8	14 ± 3	5 ± 1	371 ± 11
	320	32 ± 2	109 ± 9	17 ± 1	7 ± 4	384 ± 41
	800	27 ± 6	105 ± 13	20 ± 4	9 ± 1	370 ± 15
	2000	23 ± 13 (S)	111 ± 20	15 ± 3	10 ± 3	363 ± 31
	5000	20 ± 3 (S)	102 ± 7	16 ± 3	11 ± 3	392 ± 32
Positive Controls	Compound	2NF	NaN3	NaN3	AAC	GLU
	Dose Level	5 µg	2 µg	2 µg	50 µg	25 µg
	Mean ± SD	963 ± 165	706 ± 15	631 ± 27	539 ± 104	728 ± 67

SD Standard deviation

2NF 2-Nitrofluorene
NaN3 Sodium azide
AAC 9-Aminoacridine
GLU Glutaraldehyde

S : Slight thinning of b/g lawn

MA 1: summary of mean revertant colonies (+S-9) - Experiment 2

Substance	Dose Level µg/plate	TA98	TA100	TA1535	TA1537	TA102
		Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
DMSO	50 µl	48 ± 9	128 ± 17	18 ± 3	13 ± 2	464 ± 22
MA I	51.2	44 ± 6	114 ± 12	18 ± 6	9 ± 2	472 ± 19
	128	35 ± 11	122 ± 16	13 ± 2	11 ± 3	442 ± 32
	320	49 ± 3	112 ± 9	20 ± 3	13 ± 3	414 ± 9
	800	43 ± 10	131 ± 4	17 ± 4	12 ± 7	515 ± 10
	2000	44 ± 4	116 ± 12	19 ± 4	9 ± 1	524 ± 17
	5000	43 ± 4 (S)	120 ± 7	17 ± 3	11 ± 6	508 ± 50
Positive Controls	Compound	AAN	AAN	-	-	-
	Dose Level	5 µg	5 µg	-	-	-
	Mean ± SD	1735 ± 76	1639 ± 408	-	-	-

SD Standard deviation

AAN 2-Aminoanthracene

S : Slight thinning of b/g lawn

APPENDIX 3

Historical negative (solvent) control values for *S. typhimurium* strains

Strain	– or + S-9	Mean No. of spontaneous revertants	S.D.	Range *	
				Lower	Upper
TA98	–	29	7.3	10	48
	+	34	7.3	15	53
TA100	–	112	18.3	65	160
	+	130	16.0	89	171
TA1535	–	17	5.1	4	30
	+	21	5.3	7	34
TA1537	–	13	5.8	1	28
	+	14	6.7	1	32
TA102	–	320	46.1	201	439
	+	374	68.1	198	549

* 99% confidence limits about the mean

The above are pooled data from at least 20 consecutive experiments over the period 23.6.97 to 11.8.97.

APPENDIX 4

Historical positive control values for *S. typhimurium* strains

Strain	– or + S-9	Positive control chemical	Mean No. of induced revertants #	S.D.	Range * (min. and max. mean values of data set)	
					Lower	Upper
TA98	–	2NF	774.1	234.9	169 (191)	1379 (1176)
	+	AAN	1338.4	251.0	692 (985)	1985 (1858)
TA100	–	NaN_3	459.4	88.7	231 (258)	688 (595)
	+	AAN	1435.9	306.0	648 (479)	2224 (1940)
TA1535	–	NaN_3	372.8	123.9	54 (175)	692 (599)
	+	AAN	205.6	56.0	61 (119)	350 (327)
TA1537	–	AAC	358.4	187.9	–126 (108)	842 (795)
	+	N/A	NE	NE	NE	NE
TA102	–	GLU	355.4	89.8	124 (149)	587 (466)
	+	N/A	NE	NE	NE	NE

The above are pooled data from at least 20 consecutive experiments over the period 10.2.98 to 14.5.98.

\# All revertant numbers used to derive the above data are **induced** revertant numbers, i.e. the number of revertant colonies on the positive control plates minus the number of spontaneous revertant colonies on concurrent negative control plates.

* 99% confidence limits about mean

2NF 2-nitroflourene
NaN_3 Sodium azide
AAC 9-aminoacridine
GLU Glutaraldehyde
AAN 2-Aminoanthracene

N/A Not applicable
NE Not evaluated (insufficient historical data to permit generation of meaningful ranges)

CLOTHIANIDIN SALMONELLA/MAMMALIAN ACTIVATION; GENE MUTATION (84-2)

EPA Reviewer: Irving Mauer, Ph.D. Date: 06/19/02
Registration Action Branch 3, HED (7509C)
EPA Secondary Reviewer: Nancy McCarroll Date: 11/14/02
Toxicology Branch, HED (7509C)

TXR NO. 0050321

DATA EVALUATION RECORD

STUDY TYPE: Bacterial system, *e.g., Salmonella*/mammalian activation gene mutation assay; OPPTS 870.5100 [84-2]

DP BARCODE: D279778 SUBMISSION CODE: S607124

P.C. CODE: 044309 TOX. CHEM NO.: None

TEST MATERIAL (PURITY): TZNG (98.6% a.i.; Batch No. 89066524)

SYNONYMS: None

CITATION: Dawkes, N. (1999). TZNG: Reverse Mutation in Five Histidine-Requiring Strains of *Salmonella typhimurium*, performed at Covance Laboratories Ltd., Harrogate, North Yorkshire (ENGLAND), Laboratory Project ID: Covance 586/165-D5140 (Bayer Corporation Agricultural Division Report No. 110204), dated June 3, 1999. MRID 45422727. Unpublished.

SPONSOR: Takeda Chemical Industries Ltd., Tokyo (Japan).

EXECUTIVE SUMMARY:

In repeat reverse gene mutation assays (MRID 45422727) five histidine-deficient (*his*$^-$) strains of *Salmonella typhimurium* (TA98, TA100, TA1535, TA1537 and TA102) were exposed to TZNG (98.6% a.i., Batch No. 89066524, dissolved in dimethylsulfoxide, DMSO) at five concentrations ranging from 8 to 5000 μg/plate in an initial standard plate-incorporation assay (EXPERIMENT 1), and with a preincubation step (with S9 only) in the repeat assay at a narrower range of six doses of 156.25 to 5000 μg/plate. Reversions to prototrophy (*his*$^-$ to *his*$^+$) were counted after 3 days incubation at 37°C in the dark. Additional strains were exposed to strain-specific mutagens to serve as positive controls.

Evidence of cytotoxicity was observed in EXPERIMENT 1 at 5000 μg/plate in TA98 in the absence of metabolic activation. In EXPERIMENT 2, evidence of cytotoxicity was apparent at the maximum dose in all test strains except TA1535 (no cytotoxicity), both in the presence and absence of the S9-mix. However, in no case was there an increase in revertants in either experiment, while the positive controls showed large increases.

Therefore, TZNG is considered non-mutagenic in the standard battery of *Salmonella typhimurium* strains.

This study is classified as acceptable/guideline and satisfies the requirement for FIFRA Test Guideline 84-2 for *in vitro* mutagenicity (bacterial reverse gene mutation) data.

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

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Material: TZNG
Description: White powder
Lot/Batch No.: 89066524
Purity: 98.6% a.i.
Stability of compound: Said to be the responsibility of the sponsor.
Solvent used: Anhydrous analytical grade dimethylsulfoxide (DMSO)
Other comments: Stored at 1-10°C in the dark.

2. Control Materials:
Negative: None
Solvent/Final Concentration: DMSO/100μL - plate incorporation assay
DMSO/50 μL - preincubation assay

Positive Non-activated	
Sodium azide	2.0 μg/plate for TA100 and TA1535
2-Nitrofluorene	5.0 μg/plate for TA98
9-Aminoacridine	25.0 μg/plate for TA1537
Other (list):	
Glutaraldehyde	25.0 μg/plate for TA102
Activated:	
2-Aminoanthracene	2 or 5 μg/plate

3. Metabolic Activation: S9 (obtained from Molecular Toxicology, Inc., USA) was derived from male Sprague-Dawley rats.

x	Aroclor 1254	x	induced	x	rat	x	liver
	phenobarbital		non-induced		mouse		lung
	none				hamster		other
	other						other

Describe S9 mix composition:

Ingredient	Concentration	Quantity (mL) 16% S-9 Mix
Sodium phosphate (buffer, pH 7.4)	500 mM	20.0
Glucose-6-phosphate (disodium)	180 mg/mL	0.845
NADP (disodium)	25 mg/mL	12.6
$MgCl_2$	250 mM	3.2
KCl	150 mM	22.0
L-histidine HCl (in 25 mM $MgCl_2$)	1 mg/mL	4.0
D-biotin	1 mg/mL	4.88
S9	(as detailed)	10.0
Water	-	(To volume)

4. Test Organisms: *Salmonella typhimurium* strains:

	TA97	x	TA98	x	TA100	x	TA102
	TA104	x	TA1535	x	TA1537		TA1538

Properly maintained? Yes.
Checked for appropriate genetic markers (rfa mutation, R factor)? Yes.

5. Test Compound Concentration Used:
Cytotoxicity Test: (performed in strain TA100 only): 8, 40, 200, 1000, 5000 μg/plate

Main Assays	EXPERIMENT 1	EXPERIMENT 2
Non-activated and activated conditions	8, 40, 200, 1000, 5000 μg/plate	156.25, 312.5, 625, 1250, 2500, 5000 μg/plate

needed), the solution was filter-sterilized (0.2 µM pore size) and further diluted with DMSO. The solutions were protected from light and used within 4.5 hours of initial formulation.

In the range-finding experiment (in TA100 only ± S9), triplicate test article plates were run; solvent and positive controls were included in quintuplicate and triplicate, respectively, with/without S9-mix. Platings were achieved by adding the following to 2.5 mL molten agar at 46°C: 0.1 mL bacterial culture; 0.1 mL test article solution or control; 0.5 mL 10% S9-mix or buffer solution; followed by rapid mixing and pouring onto Minimal Davis agar plates. When set, the plates were inverted and incubated at 37°C in the dark for 3 days. After incubation, plates were examined for evidence of cytotoxicity.

In the initial mutation experiment with all five strains. Test article additions were the same as in the range-finding experiment above; in addition to solvent controls, the bacteria were treated with strain-specific mutagens in triplicate. Platings were achieved as above.

In the repeat mutation experiment, a preincubation step (20 minutes at 37°C) was included in the presence of S9-mix. The nonactivated phase of the repeat assay was performed using the plate incorporation procedure.Plating of these treatments then proceded as for the plate-incorporation procedure. Volume additions for Experiment 2 were reduced to 0.05 mL due to the solvent (DMSO), to minimize or eliminate potential cytotoxic effects of the solvent that may have otherwise occurred.

1. Type of *Salmonella Assay*:

_x_ standard plate test (EXPERIMENT 1)
_x_ preincubation (_20_ minutes with S9 only, EXPERIMENT 2)
____ "Prival" modification
____ spot test
____ other (describe)

2. Colony Counting and Analysis of Results:

Colonies were counted electronically using a Seescan Colony Counter, or manually where confounding factors such as split agar prevented accurate automated counts. In each case, the background lawn of representative plates was inspected for signs of toxicity.

3. Statistical Methods:

The m-statistic was calculated to check that the data were Poisson-distributed; Dunnett's Test was used to compare the counts of each dose with the solvent controls. The presence or otherwise of a dose-response was checked by linear

3. Statistical Methods:

The m-statistic was calculated to check that the data were Poisson-distributed; Dunnett's Test was used to compare the counts of each dose with the solvent controls. The presence or otherwise of a dose-response was checked by linear regression analysis.

4. Both assay acceptance and evaluation criteria were presented.

II. REPORTED RESULTS

A. PRELIMINARY CYTOTOXICITY ASSAY:

No cytotoxicity (as normally indicated by thinning of the background lawn and/or a marked reduction in revertants) was evident in TA100 cultures treated up to 5000 μg/plate. The same range of concentrations was used in the plate-incorporation assay of EXPERIMENT 1.

B. MUTAGENICITY ASSAYS:

Evidence of cytotoxicity (slight thinning of the backgroun lawn) was observed in EXPERIMENT 1 at 5000 μg/plate, but only with strain TA98 in the absence of metabolic activation. In EXPERIMENT 2, evidence of cytotoxicity was also apparent at 5000 μg/plate with all test strains except strain TA 1535 (no toxicity), both in the absence and presence of the S9-mix.

However, in no test article-treated strain was there a consistent increase in revertants, when compared to concurrent controls (MRID 45422727, pp. 26, 27, 39, 40 - ATTACHMENT) or historical background (ATTACHMENT, APPENDIX 3), in contrast to positive controls, all of which showed large increases.

Therefore, the investigator concluded that TZNG was non-mutagenic in five strains of *Salmonella typhimurium* exposed up to the cytotoxic/limit dose levels ± S9.

III. REVIEWER'S DISCUSSION/CONCLUSIONS:

A. The reviewer agrees with the investigator that TZNG shows no evidence of mutagenic activity in the standard battery of *Salmonella typhimurium* strains when tested up to the limit dose in the absence or presence of metabolic activation.

B. STUDY DEFICIENCIES:

None.

ATTACHMENT

THE FOLLOWING ATTACHMENT IS NOT AVAILABLE ELECTRONICALLY
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TZNG: summary of mean revertant colonies (-S-9) - Experiment 1

Substance	Dose Level g/plate	TA98	TA100	TA1535	TA1537	TA102
		Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
DMSO	100 l	34 ± 6	95 ± 15	15 ± 3	13 ± 4	328 ± 21
TZNG	8	37 ± 8	107 ± 14	19 ± 1 (M)	18 ± 6	332 ± 11
	40	30 ± 13	102 ± 17	20 ± 4	13 ± 4	331 ± 13
	200	26 ± 7	95 ± 15	20 ± 5	16 ± 1	313 ± 28
	1000	41 ± 1	99 ± 11	23 ± 8	16 ± 6	326 ± 13
	5000	20 ± 4 (S)	113 ± 8	28 ± 6	13 ± 4	248 ± 12
Positive controls	Compound	2NF	NaN3	NaN3	AAC	GLU
	Dose Level	5 g	2 g	2 g	50 g	25 g
	Mean ± SD	1103 ± 76	574 ± 27	463 ± 4	337 ± 65	658 ± 64

SD Standard deviation

2NF 2-Nitrofluorene
NaN3 Sodium azide
AAC 9-Aminoacridine
GLU Glutaraldehyde

S : Slight thinning of b/g lawn
M : Plate counted manually

TZNG: summary of mean revertant colonies (+S-9) - Experiment 1

Substance	Dose Level g/plate	TA98	TA100	TA1535	TA1537	TA102
		Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
DMSO	100 l	34 ± 8	120 ± 10	21 ± 3	14 ± 4	316 ± 20
TZNG	8	35 ± 8	113 ± 5	20 ± 9	14 ± 2	343 ± 20
	40	35 ± 2	125 ± 4	20 ± 3	12 ± 4	319 ± 8
	200	35 ± 8	113 ± 15	17 ± 4	14 ± 1	339 ± 27
	1000	28 ± 3	125 ± 14	20 ± 2	15 ± 6	262 ± 21
	5000	27 ± 7	135 ± 5	16 ± 5	13 ± 2	214 ± 16
Positive controls	Compound	AAN	AAN	AAN		
	Dose Level	5 g	5 g	5 g		
	Mean ± SD	1912 ± 152	1635 ± 64	163 ± 24		

SD Standard deviation

AAN 2-Aminoanthracene

TZNG: summary of mean revertant colonies (-S-9) - Experiment 2

Substance	Dose Level g/plate	TA98	TA100	TA1535	TA1537	TA102
		Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
DMSO	100 l	37 ± 7	141 ± 13	23 ± 5	16 ± 5	316 ± 20
TZNG	156.25	42 ± 3	144 ± 17	21 ± 2	19 ± 5	308 ± 50
	312.5	36 ± 8	138 ± 11	23 ± 5	15 ± 1	290 ± 67
	625	39 ± 6	145 ± 1	22 ± 4	12 ± 2	282 ± 23
	1250	26 ± 4	138 ± 12	21 ± 9	14 ± 5	268 ± 13
	2500	33 ± 4	145 ± 0	24 ± 6	14 ± 2	274 ± 21
	5000	35 ± 4 (S)	153 ± 3 (S)	24 ± 1	14 ± 4 (S)	225 ± 27 (S)
Positive controls	Compound	2NF	NaN3	NaN3	AAC	GLU
	Dose Level	5 g	2 g	2 g	50 g	25 g
	Mean ± SD	954 ± 229	663 ± 16	450 ± 35	522 ± 119	795 ± 15

SD Standard deviation

2NF 2-Nitrofluorene
NaN3 Sodium azide
AAC 9-Aminoacridine
GLU Glutaraldehyde

S : Slight thinning of b/g lawn

TZNG: summary of mean revertant colonies (+S-9) - Experiment 2

Substance	Dose Level g/plate	TA98	TA100	TA1535	TA1537	TA102
		Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
DMSO	50 l	46 ± 6	157 ± 12	29 ± 4	18 ± 3	354 ± 33
TZNG	156.25	47 ± 5	167 ± 9	30 ± 4	15 ± 5	396 ± 33
	312.5	38 ± 10	182 ± 11	22 ± 4	17 ± 2	307 ± 31
	625	35 ± 10	165 ± 13	26 ± 3	16 ± 2	339 ± 40
	1250	38 ± 8	158 ± 14	34 ± 6	19 ± 3	283 ± 22
	2500	32 ± 6	163 ± 8	18 ± 6	15 ± 5	267 ± 13
	5000	27 ± 2 (S)	145 ± 19 (S)	23 ± 6	10 ± 1 (S)	170 ± 40 (S)
Positive controls	Compound	AAN	AAN			
	Dose Level	5 g	5 g			
	Mean ± SD	1466 ± 44	1582 ± 91			

SD Standard deviation

AAN 2-Aminoanthracene

S : Slight thinning of b/g lawn

HED Records Center Series 361 Science Reviews - File R107689 - Page 147 of 721

APPENDIX 3

Historical negative (solvent) control values for *Salmonella* strains

Strain	- or + S-9	Mean Nº of spontaneous revertants	SD	Range* lower	upper
TA98	-	29	7.3	10	48
	+	34	7.3	15	53
TA100	-	112	18.3	65	160
	+	130	16.0	89	171
TA1535	-	17	5.1	4	30
	+	21	5.3	7	34
TA1537	-	13	5.8	1	28
	+	14	6.7	1	32
TA102	-	320	46.1	201	439
	+	374	68.1	198	549

* 99% confidence limits about the mean

The above are pooled data from at least 20 consecutive experiments over the period 23.6.97 to 11.8.97

APPENDIX 4

Historical positive control values for *Salmonella* strains

Strain	- or + S-9	Positive control chemical	Mean Nº of induced revertants*	S.D.	Range 99% confidence limits about mean (min. and max. Mean values of data set) lower	upper
TA98	-	2NF	774.1	234.9	169 (191)	1379 (1176)
	+	AAN	1338.4	251.0	692 (985)	1985 (1858)
TA100	-	NaN_3	459.4	88.7	231 (258)	688 (595)
	+	AAN	1435.9	306.0	648 (479)	2224 (1940)
TA1535	-	NaN_3	372.8	123.9	54 (175)	692 (599)
	+	AAN	205.6	56.0	61 (119)	350 (327)
TA1537	-	AAC	358.4	187.9	126 (108)	842 (795)
	+	N/A	NE	NE	NE	NE
TA102	-	GLU	355.4	89.8	124 (149)	587 (466)
	+	N/A	NE	NE	NE	NE

The above are pooled data from at least 20 consecutive experiments over the period 10.2.98 to 14.5.98.

* All revertant numbers used to derive the above data are **induced** revertant numbers, i.e. the number of revertant colonies on the positive control plates minus the number of spontaneous revertant colonies on concurrent negative control plates.

2NF	2-nitroflourene
NaN_3	Sodium azide
AAC	9-aminoacridine
GLU	Glutaraldehyde
AAN	2-Aminoanthracene
N/A	Not applicable
NE	Not evaluated (insufficient historical data to permit generation of meaningful ranges)

CLOTHIANIDIN *SALMONELLA*/MAMMALIAN ACTIVATION; GENE MUTATION (84-2)

EPA Reviewer: Irving Mauer, Ph.D. Date: 06/13/02
Registration Action Branch 3, HED (7509C)
EPA Secondary Reviewer: Nancy McCarroll Date: 11/14/02
Toxicology Branch, HED (7509C)

TXR No.: 0050321

DATA EVALUATION RECORD

STUDY TYPE: Bacterial system, *e.g.*, *Salmonella*/mammalian activation gene mutation assay; OPPTS 870.5100 [84-2]

DP BARCODE: D279778 SUBMISSION CODE: S607124
P.C. CODE: 044309 TOX. CHEM. NO.: None.

TEST MATERIAL (PURITY): BN0335E2 (99% a.i.; Batch No. 30070904)

SYNONYMS: None.

CITATION: Dawkes, N. (2000). BN0335E2 Reverse Mutation in Five Histidine-Requiring Strains of *Salmonella typhymurium*, performed at Covance Laboratories, Ltd., Harrogate, North Yorkshire (ENG), Covance Report No. 586/225-D5140 (Bayer Corporation Agricultural Division Report No. 110238), dated September 19, 2000, MRID 45422724. Unpublished.

SPONSOR: Takeda Chemical Industries, Ltd., Tokyo, Japan.

EXECUTIVE SUMMARY:

In repeat reverse gene mutation assays in bacteria (MRID 45422724), five histidine-deficient (*his*$^-$) mutants of *Salmonella typhimurium* (TA98, TA100, TA1535, TA1537 and TA102) were exposed to BN0335E2 (99% a.i.; Batch No. 30070904) in dimethylsulfoxide (DMSO) at concentrations of 1.6, 8, 40, 200, 1000 and 5000 μg/plate in a plate incorporation assay (EXPERIMENT 1) and 51.2, 128, 320, 800, 2000 and 5000 μg/plate (EXPERIMENT 2), both in the absence and presence of metabolic activation provided by Aroclor 1254-induced rat liver post-mitochrondrial fraction (S9). A preincubation step was included in the S9-activated phase of EXPERIMENT 2. Reversion to prototrophy (*his*$^-$ to *his*$^+$) was determined after 3 days incubation in the dark at 37° C. Additional cultures were exposed to the vehicle (DMSO) and strain-specific mutagens, the latter to serve as positive controls.

No evidence of toxicity was found in either experiment, and no treatment with BNO335E2 resulted in any statistically significant increase in revertant numbers (at the 1% level using Dunnett's Test). Mutagen-treated cultures showed statistically significant increases in revertants.

Therefore, this study has provided no evidence of the ability of BN0335E2 to induce bacterial mutation.

This study is classified as acceptable/guideline and satisfies the requirement for FIFRA Test Guideline 84-2 for *in vitro* mutagenicity (bacterial reverse gene mutation) data.

COMPLIANCE:

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

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Material: BN0335E2
Description: Pale yellow crystalline powder.
Lot Batch No.: 30070904
Purity: 99% a.i.
Stability of compound: Not reported (responsibility of the sponsor).
CAS No.: Not reported.
Solvent used: Nitrogen-purged anhydrous dimethylsulfoxide (DMSO).
Other comments: Stored refrigerated in the dark, under nitrogen.

2. Control Materials:
Negative: None
Solvent/Final concentration: DMSO/100 μL. (plate incorporation assay)
DMSO/50 μL (preincubation assay)
Positive: Nonactivated:

Sodium azide	2.0 μg/plate for TA100, TA1535.
2-Nitrofluorene	5.0 μg/plate for TA98
9-Aminoacridine	50.0 μg/plate for TA1537
Other (list):	
Glutaraldehyde	25.0 μg/plate for TA102

Activated:
2-Aminoanthracene (2-anthramine) 5.0 μg/plate for TA98, TA100, TA1535, TA1537; 20 μg/plate for TA102.
Other (list): None.

3. Metabolic Activation: S9 was prepared by Molecular Toxicology, Inc., and derived from male Sprague-Dawley rats:

x	Aroclor 1254	x	induced	x	rat	x	liver
	phenobarbital		non-induced		mouse		lung
	none				hamster		other
	other						other

Describe S9 mix composition (if purchased, give details):

The S9-mix contained the following components:

Per 100 mL	**10% S9 Mix**
Sodium phosphate buffer	20
Glucose-6-phosphate buffer, disodium	0.845
NADP, disodium	12.6
$MgCl_2$	3.2
KCl	22.0
L-Histidine	4.0
D-Biotin	4.88
S9	10.0

4. Test organisms:

	TA97	x	TA98	x	TA100	x	TA102
	TA104	x	TA1535	x	TA1537		TA1538

List any others: None.
Properly maintained? Yes.
Checked for appropriate genetic markers (rfa mutation, R factor)? Yes.

5. Test compound concentrations used

Cytotoxicity test: 1.6, 8, 40, 200, 1000, 5000 μg/plate.

The initial range-finding experiment was carried out in strain TA100 only, plus negative (solvent) and positive controls.

Main Assays:

	Experiment 1	**Experiment 2**
Non-activated/Activated Conditions	1.6, 8, 40, 200, 1000, 5000 μg/plate	51.2, 128, 320, 800, 2000, 5000 μg/plate

B. TEST PERFORMANCE

Experiment 1 treatments of the remaining strains were performed as a standard plate assay at the same dose range as in the range-finder test. Experiment 2 retained 5000 µg/plate as the highest dose but employed a narrower range of doses in order to more thoroughly investigate concentrations of BN0335E2 more likely to induce a mutagenic response. In addition, a preincubation step was included in the presence of S9, in order to increase the range of mutagenic chemicals detectable in the assay system. Negative and positive controls were included in quintriplicate and triplicate, respectively with/without S9 mix.

At the end of the 3-day incubation period, colonies were counted electronically (Seescan Colony Counter) or manually where confounding factors, such as split agar or the presence of spreading colonies prevented accurate automated counts. All plates had their background bacterial lawn inspected for signs of toxicity.

1. Type of *Salmonella* assay:

x	standard plate test (Experiment 1)
x	preincubation (20 minutes) (Experiment 2) with S9 only
	"Prival" modification
	spot test
	other (describe)

Note: The nonactivated portion of Experiment 2 was performed as described for the plate incorporation test.

2. Statistical Analysis:

The m-statistic was calculated to check that the data were Poisson-distributed. Dunnett's Test was used to compare colony counts at each dose with the control (solvent). The presence or otherwise of a dose response was checked by linear regression analysis.

3. Criteria:
Acceptance criteria and evaluation of results were presented.

II REPORTED RESULTS

A. PRELIMINARY CYTOTOXICITY ASSAY

No evidence of toxicity (thinning of background bacterial lawn, and/or a marked reduction in revertant numbers) was found in the preliminary range-finder experiment using TA100.

B. MUTAGENICITY ASSAYS

In neither Experiment 1 nor Experiment 2 was there any evidence of toxicity in test-article exposed cultures treated up to 5000 µg/plate, nor increases in revertant number over concurrent or background negative control values (MRID 45422724, pp. 26, 27, 39, 40 - ATTACHMENT, EXPERIMENT 1, EXPERIMENT 2). By contrast, all positive controls induced large increases in revertants.

Therefore the investigator concluded the BN0335E2 did not induce mutation in five strains of *Salmonella typhimurium* under conditions employed in this study, either in the absence or presence of metabolic activation (S9).

III. REVIEWER'S DISCUSSION/CONCLUSIONS

A. The reviewer agrees with the investigator that BN0335E2 did not induce increased revertants in five strains of *Salmonella typhimurium* treated up to cytotoxic limits.

B. STUDY DEFICIENCIES

None that would affect the conclusions of this study.

ATTACHMENT

THE FOLLOWING ATTACHMENT IS NOT AVAILABLE ELECTRONICALLY
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BN0335E2: summary of mean revertant colonies (-S-9) - Experiment 1

Substance	Dose Level µg/plate	TA98	TA100	TA1535	TA1537	TA102
		Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
DMSO (N2 Purged)	100 µl	27 ± 6	110 ± 13	18 ± 3	6 ± 1	387 ± 39
BN0335E2	1.6	22 ± 1	111 ± 16	15 ± 3	6 ± 2	405 ± 11
	8	30 ± 1	108 ± 4	16 ± 4	7 ± 2	408 ± 20
	40	23 ± 5	96 ± 7	16 ± 6	9 ± 2	411 ± 6
	200	23 ± 3	111 ± 18	19 ± 5	9 ± 3	390 ± 10
	1000	29 ± 4	106 ± 22	17 ± 2	7 ± 2	365 ± 9
	5000	28 ± 5	99 ± 9	22 ± 6	9 ± 3	368 ± 14
Positive Controls	Compound	2NF	NaN3	NaN3	AAC	GLU
	Dose Level	5 µg	2 µg	2 µg	50 µg	25 µg
	Mean ± SD	575 ± 120	724 ± 28	515 ± 13	461 ± 176	667 ± 26

SD Standard deviation

2NF 2-Nitrofluorene
NaN3 Sodium azide
AAC 9-Aminoacridine
GLU Glutaraldehyde

BN0335E2: summary of mean revertant colonies (+S-9) - Experiment 1

Substance	Dose Level µg/plate	TA98	TA100	TA1535	TA1537	TA102
		Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
DMSO (N2 Purged)	100 µl	28 ± 4	127 ± 8	22 ± 4	8 ± 4	481 ± 58
BN0335E2	1.6	20 ± 8	122 ± 6	21 ± 1	12 ± 5	507 ± 83
	8	24 ± 2	126 ± 6	21 ± 2	9 ± 2	429 ± 37
	40	25 ± 4	131 ± 6	21 ± 5	8 ± 3	419 ± 19
	200	26 ± 5	136 ± 14	26 ± 3	8 ± 2	501 ± 35
	1000	29 ± 3	137 ± 18	19 ± 1	9 ± 3	497 ± 31
	5000	25 ± 7	141 ± 8	21 ± 1	7 ± 3	427 ± 44
Positive Controls	Compound	AAN	AAN	AAN	AAN	AAN
	Dose Level	5 µg	5 µg	5 µg	5 µg	20 µg
	Mean ± SD	1349 ± 36	1904 ± 108	178 ± 59	308 ± 21	2045 ± 112

SD Standard deviation

AAN 2-Aminoanthracene

BN0335E2: summary of mean revertant colonies (-S-9) - Experiment 2

Substance	Dose Level µg/plate	TA98	TA100	TA1535	TA1537	TA102
		Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
DMSO (N2 Purged)	100 µl	25 ± 5	99 ± 6	17 ± 3	6 ± 2	303 ± 23
BN0335E2	51.2	29 ± 2	104 ± 10	16 ± 1	9 ± 3	285 ± 10
	128	35 ± 4	98 ± 5	16 ± 1	5 ± 3	306 ± 18
	320	24 ± 1	91 ± 15	14 ± 3	7 ± 4	307 ± 30
	800	42 ± 19	91 ± 5	18 ± 3	4 ± 3	294 ± 41
	2000	33 ± 7	88 ± 10	18 ± 6	9 ± 3	315 ± 13
	5000	20 ± 1	96 ± 14	14 ± 3	5 ± 1	337 ± 19
Positive Controls	Compound	2NF	NaN3	NaN3	AAC	GLU
	Dose Level	5 µg	2 µg	2 µg	50 µg	25 µg
	Mean ± SD	477 ± 69	597 ± 19	467 ± 21	379 ± 87	656 ± 28

SD Standard deviation

2NF 2-Nitrofluorene
NaN3 Sodium azide
AAC 9-Aminoacridine
GLU Glutaraldehyde

BN0335E2: summary of mean revertant colonies (+S-9) - Experiment 2

Substance	Dose Level µg/plate	TA98	TA100	TA1535	TA1537	TA102
		Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
DMSO (N2 Purged)	50 µl	30 ± 9	129 ± 10	21 ± 6	7 ± 3	288 ± 22 (M)
BN0335E2	51.2	31 ± 4	120 ± 13	18 ± 4	3 ± 2	332 ± 36 (M)
	128	26 ± 2	131 ± 20	16 ± 2	7 ± 1	303 ± 39 (M)
	320	27 ± 5	118 ± 8	21 ± 8	5 ± 1	281 ± 15 (M)
	800	29 ± 5	123 ± 22	18 ± 3	9 ± 6	275 ± 41 (M)
	2000	35 ± 11	129 ± 15	14 ± 1	7 ± 2	283 ± 60 (M)
	5000	30 ± 5	111 ± 6	16 ± 3	9 ± 4	282 ± 25 (M)
- Positive Controls	Compound	AAN	AAN	AAN	AAN	AAN
	Dose Level	5 µg	5 µg	5 µg	5 µg	20 µg
	Mean ± SD	1178 ± 92	1392 ± 124	173 ± 38	220 ± 9	1424 ± 456

SD Standard deviation

AAN 2-Aminoanthracene

M : Plate counted manually

CLOTHIANIDIN

MICRONUCLEUS (84-2)

EPA Reviewer: Irving Mauer, Ph.D.
Registration Action Branch 3, HED (7509C)
EPA Secondary Reviewer: Nancy McCarroll
Toxicology Branch, HED (7509C)

Date: 11/25/02

Date: 11/25/02

TXR. No.: 0050321

DATA EVALUATION RECORD

STUDY TYPE: *In vivo* mammalian cytogenetics - micronucleus assay in the mouse; OPPTS 870.5395 [84-2].

DP BARCODE: D279778

SUBMISSION CODE: S607124

P.C. CODE: 044309

TOX. CHEM. NO.: None.

TEST MATERIAL (PURITY): TI-435 (Batch No. 30034708, 96.0% a.i.)

SYNONYMS: Clothianidin (iso)
Chemically: (E)-1-(2-chloro-5-thiazolylmethyl)-3-methyl nitroguanidine.

CITATION: Durwood, R. (2000). TI-435: Micronucleus Test in the Mouse, performed at Safepharm Laboratories, Ltd. (SPL), Derby (UK). SPL Project No. 178/113 (Bayer Corporation Agricultural Division Report No. 109969), dated March 8, 2000. MRID 45422740. Unpublished.

SPONSOR: Takeda Chemical Industries, Ltd., Agro Company, Tokyo (JAPAN).

EXECUTIVE SUMMARY: In a micronucleus test (MRID 45422740), groups of mice (5M:5F) were administered TI-435 (Batch No. 30034708, 96.0% a.i., dissolved in arachis oil) in a single oral dose of 25, 50, or 100 mg/kg, and sacrificed 24, 48 or 72 hours later, at which times bone marrow was extracted and smear preparations made and stained. Polychromatic and normochromatic erythrocytes (PCE/NCE) were scored for the presence of micronuclei. Additional animals were given single oral doses of the vehicle, arachis oil, or the clastogen, cyclophosphamide, the latter to serve as a positive control.

Three unscheduled deaths occurred at 100 mg/kg; other clinical signs of toxicity in all treatment groups included hunched posture, decreased respiratory rate, labored respiration, ptosis and lethargy. There was, however, no evidence of an increase in incidence of micronucleated polychromatic erythrocytes (MPCE), and no statistically significant decreases in PCE/NCE ratios at 24, 48 or 72 hours when compared with concurrent control groups. Vehicle controls had frequencies of MPCE within the normal

range, and the positive chemical induced a marked increase in the frequency of MPCE. **Therefore, TI-435 is considered to be neither clastogenic or aneugenic under these test conditions.**

This study is classified as acceptable/guideline and satisfies the requirement for FIFRA Test Guideline for *in vivo* cytogenetic mutagenicity data.

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

I. MATERIALS AND METHODS

A. MATERIALS:

1. Test Material: TI-435
   Description: Pale, yellow powder.
   Lot/Batch No.: 30034708.
   Purity: 96.0% a.i. [Same as reported in MRID 45422734].
   Stability of compound: Stable for at least 24 hours.
   CAS No. 205510-53-8
   Solvent used: Arachis oil.
   Other comments: Store at 4°C in the dark.

2. Control Materials:

   Negative/Route of Administration: None.
   Vehicle/Final Volume/Route of Administration: Arachis oil/10 mL/kg /oral.
   Positive/Final Dose(s)/Route of Administration: Cyclosphosphamide/50 mg/kg/oral.

3. Test Compound Administration: Range-Finding Toxicity Study/Main Assay:

   Volume: 10 mL/kg.
   Route of Administration: Oral: 100, 200, 300, 400 mg/mL.
   Micronucleus Assay: 25, 50, 100 mg/kg

4. Test Animals:

   a. Species: Mice; Strain CD-1; Age 5-7 weeks; Weight: male; 21-26 g; female; 20-25 g.
      Source: Charles River, Ltd., Margate, Kent (UK).

   b. Number of animals used per dose: 5 male; 5 females.

   c. Properly maintained? Yes.

B. TEST PERFORMANCE:

1. Treatment and Sampling Times:

   a. Test compound and vehicle control:

Dosing: _x_ once; ____ twice (24 hours apart); ___ other (describe):
Sampling (after last dose): ___6 hr. ___ 12 hr. _x_ 24 hr. _x_ 48 hr. _x_ 72 hr (mark all that are appropriate), ____ other (describe):

b. Positive control:

Dosing: _x_ once; ___ twice (24 hours apart); ___ other (describe):

Sampling (after last dose): ___ 6 hr.; ___ 12 hr.; _x_ 24 hr.; ___ 48 hr.; ____ 72 hr.; (mark all that are appropriate); ____ other (describe):

2. Tissues and Cells Examined:

_x_ bone marrow; ____ other (list):

Number of polychromatic erythrocytes (PCE) examined per animal: 1000.

Number of normochromatic erythrocytes (NCE; more mature RBCs) examined per animal: per 1000 erythrocytes.

Other (if other cell types were examined, describe): None.

3. Details of Slide Preparation:

Smears were air-dried, fixed in methanol and stained in May-Grünwald/Giemsa.

4. Statistical Methods:

All data were analyzed using appropriate methods "as recommended by the UK-EMS Sub-Committee on Guidelines for Mutagenicity Testing Report, Part III (1989)" (but not referenced in this Report). The data were analyzed following a $\sqrt{(x + 1)}$ transformation using Student's t-test (two-tailed), and any significant results were confirmed using the one-way analysis of variance.

5. Evaluation Criteria:

Criteria for assay acceptance and response were presented.

II. REPORTED RESULTS:

A. ANALYTICAL DETERMINATIONS:

Despite technical difficulties in determining analytical concentrations and homogeneity, dosing formulations were assumed to be adequately prepared since clear signs of toxicity were seen at 100 mg/kg.

B. PRELIMINARY TOXICITY ASSAY:

There was clinical toxicity (including hunched posture, decreased repiratory rate, labored respiration, ptosis and lethargy) at all doses (100-400 mg/kg), and premature deaths at and above 200 mg/kg were observed. It was concluded that 100 mg/kg was the maximum tolerated dose (MTD) for this assay.

C. MICRONUCLEUS ASSAY:

There were three premature deaths in the micronucleus assay, as given in the summary of results (MRID 45422740, p. 23 -ATTACHMENT Table 1); and clinical toxicity similar to that seen in the preliminary toxicity assay (*i.e.*, hunched posture, decreased respiratory rate, ptosis, labored respiration and lethargy) were recorded at all doses. There were no statistically significant increases in the frequency of MPCE in any dose group compared to concurrent controls. Also, there were no statistically significant decreases in the PCE/NCE ratio in any of the test groups.

The vehicle control animals had frequencies of MPCE within the normal range for the historical control groups (MRID 45422740, p. 44 - APPENDIX II). The positive chemical induced a marked increase in the incidence of MPCE.

Therefore, the investigator concluded that TI-435 was found <u>not</u> to produce a significant increase in the frequency of micronuclei in PCE of mice under conditions of this assay.

III. REVIEWER'S DISCUSSION/CONCLUSIONS:

A. The EPA reviewer agrees with the investigator that TI-435 was not a clastogen, *i.e.*, did not produce an increase in micronuclei in polychromatic erythrocytes of mice treated up to clinically toxic doses.

B. DEFICIENCIES:

None.

ATTACHMENT

THE FOLLOWING ATTACHMENT IS NOT AVAILABLE ELECTRONICALLY
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TI-435 : MICRONUCLEUS TEST IN THE MOUSE

TABLE 1

MICRONUCLEUS STUDY - SUMMARY OF GROUP MEAN DATA

TREATMENT GROUP	NUMBER OF PCE WITH MICRONUCLEI PER 1000 PCE		PCE/NCE RATIO	
	GROUP MEAN	SD	GROUP MEAN	SD
1. VEHICLE CONTROL 72-hour sampling time	0.7	1.2	1.31	0.26
2. VEHICLE CONTROL 48-hour sampling time	0.2	0.4	1.49	0.51
3. VEHICLE CONTROL 24-hour sampling time	0.7	1.5	1.74	0.59
4. POSITIVE CONTROL 24-hour sampling time	35.4***	9.3	1.27*	0.31
5. TI-435 100 mg/kg 72-hour sampling time	0.2	0.4	1.18	0.24
6. TI-435 50 mg/kg 72-hour sampling time	0.6	0.7	1.44	0.34
7. TI-435 25 mg/kg 72-hour sampling time	0.3	0.5	1.13	0.37
8. TI-435[a] 100 mg/kg 48-hour sampling time	0.3	0.5	1.46	0.38
9. TI-435 50 mg/kg 48-hour sampling time	0.3	0.5	1.43	0.59
10. TI-435 25 mg/kg 48-hour sampling time	0.5	0.8	1.31	0.62
11. TI-435 100 mg/kg 24-hour sampling time	0.2	0.4	1.66	0.58
12. TI-435 50 mg/kg 24-hour sampling time	0.2	0.4	1.56	0.45
13. TI-435 25 mg/kg 24-hour sampling time	0.7	1.1	2.05	0.70

Key: PCE = polychromatic erythrocytes
NCE = normochromatic erythrocytes
SD = standard deviation
*** = $p < 0.001$
* = $p < 0.05$
a = data from only seven animals

CLOTHIANIDIN *SALMONELLA*/MAMMALIAN ACTIVATION; GENE MUTATION (84-2)

EPA Reviewer: Irving Mauer, Ph.D.
Registration Action Branch 3, HED (7509C)
EPA Secondary Reviewer: Nancy McCarroll
Toxicology Branch, HED (7509C)

Date: 11/19/02

Date: 11/19/02

TXR NO. 005321

DATA EVALUATION RECORD

STUDY TYPE: Bacterial system: *Salmonella*/mammalian activation gene mutation assay; OPPTS 870.5100 [84-2]

DP BARCODE: D279778 SUBMISSION CODE: S607124

P.C. CODE: 044309 TOX. CHEM. NO.: None.

TEST MATERIAL (PURITY): TI 435-Triazan (95.6% a.i.; Batch No. VE97040 Pt.17003/99)

SYNONYMS: Intermediate for TI 435; $C_{11}H_{15}N_5O_2$

CITATION: Herbold, B. (2000). TI 435 Triazan. *Salmonella*/Microsome Test. Plate Incorporation and Preincubation Method, performed at Bayer AG's Department of Toxicology, Wuppertal (Germany), August 18, 2000, Bayer AG Study No. T4069135, Bayer AG Report No. PH 30172/Bayer Corporation Agriculture Division Report No. 110241. MRID 45422822. Unpublished.

SPONSOR: Takeda Chemical Industries, Ltd., Tokyo (Japan).

EXECUTIVE SUMMARY: In repeat reverse gene mutation assays in bacteria (MRID 45422822), five histidine-deficient mutant strains (*his*$^-$) of *Salmonella typhimurium* (TA1535, TA100, TA1537, TA98, TA102) were exposed in the presence and absence of metabolic activation (S9) to TI 435-Triazan (95.6% a.i.; Batch No. VE 97040 Pt.17003/99) in dimethylsulfoxide (DMSO) by plate incorporation (at five concentrations ranging from 50 to 5000 μg/plate), as well as preincubation (at seven concentrations ranging from 600 to 4800 μg/plate), and reversion to prototrophy (*his*$^-$ to *his*$^+$) determined after 48 hours incubation at 37°C. The S9-activated portion of the preincubation assay was repeated for strains TA100, TA98 and TA102 with doses ranging from 600 to 4800 μg/plate. Additional cultures were exposed to the vehicle (DMSO), and to strain-specific mutagens, the latter to serve as positive controls.

Weak, strain-specific bacteriotoxicity and cytotoxicity were observed at higher concentrations (2400-5000 μg/plate), but **at no concentration in any treated strain of either type of assay (plate incorporation or preincubation) was an increase over DMSO vehicle controls in *his*$^+$ revertants found.** All positive control cultures manifested substantial increases in revertant colonies.

Therefore, TI 435-Triazan is considered to be non-mutagenic when tested in these *Salmonella* strains.

This study is classified as acceptable/guideline and satisfies the requirement for FIFRA Test. Guideline 84-2 for *in vitro* mutagenicity (bacterial reverse gene mutation) data.

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

I. MATERIALS AND METHODS

A. MATERIALS:

1. Test Material: TI 435-Triazan.

Description: Fine white powder.
Lot/Batch No.: VE 97040 Pt. 17003/99.
Purity: 95.6% a.i. (Analysis: March 7, 2000).
Stability of compound: Not provided.
CAS No.: 141856-57-7.
Solvent used: Dimethylsulfoxide (DMSO).
Other comments: Stored at room temperature. Intermediate for TI 435.

2. Control Materials:

Negative: None.
Solvent/Final concentration: DMSO/0.1 mL/plate.
Positive: Non-activated:

Sodium azide	10 µg/plate for TA1535.
Nitrofurantoin	0.2 µg/plate for TA100.
4-NPDA[1]	10 µg/plate for TA1537.
Other (list):	
4-NPDA	0.5 µg/plate for TA98.
Cumene hydroperoxide	50 µg/plate for TA100.

Activated: 2-Aminoanthracene (2-anthramine) 3 µg/plate for all strains.

3. Metabolic Activation: S9 derived from adult (200-300 g) male Sprague-Dawley rats.

[1]4-NPDA = 4-nitrophenylene diamine.

x	Aroclor 1254	x	induced	x	rat	x	liver
	phenobarbital		non-induced		mouse		lung
	none				hamster		other
	other						other

Describe S9 mix composition:
Seventy mL of cofactor solution contained:

$MgCl_2$ x 6 H_2O	162.6 mg
KCl	246.0 mg
Glucose-6-phosphate,disodium salt	179.1 mg
NADP, disodium salt	315.0 mg
Phosphate buffer	100 mM

4. Test Organisms: *Salmonella typhimurium* strains.

	TA97	x	TA98	x	TA100	x	TA102
	TA104	x	TA1535	x	TA1537		TA1538

Properly maintained. Yes.
Checked for appropriate genetic markers (rfa mutation, R factor)? Yes.

5. Test Compound Concentrations Used:

Cytotoxicity Test: (Not performed.)
Main Assays:
Non-activated conditions: 50, 158, 500, 1581, 5000 μg/plate.
Activated conditions: 50, 158, 500, 1581, 5000 μg/plate.
Repeat Activated Conditions: 600, 800, 1200, 1600, 2400, 3200, 4800 μg/plate.

B. TEST PERFORMANCE

The initial plate incorporation assay followed the directions of Ames *et al.* (1973a, 1975)[2] and Maron and Ames (1983)[3].

Triplicate plates were used at each concentration of test and control cultures in both types of assay.

1. Type of *Salmonella* Assay:

x	standard plate test
x	preincubation (_20_ minutes)
	"Prival" modification
	spot test
	other (describe)

2. Criteria for assay acceptance and assessment of results were presented.

II REPORTED RESULTS

A. PRELIMINARY CYTOTOXICITY ASSAY: (None was conducted.)

B. MUTAGENICITY ASSAYS:

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[2]Ames, B.N., W.E. Yamasaki and F.D. Lee:
Carcinogens are mutagens: A simple test system combining liver homogenates for activation and bacteria for detection.
Proc. Nat. Acad. Sci. (USA) 70, 2281-2285, 1973a.

Ames, B.N., J. McCann and E. Yamasaki:
Methods for detecting carcinogens and mutagens with *Salmonella*/mammalian-microsome mutagenicity test.
Mutation Res. 31, 347-364, 1975.

[3]Maron, D.M. and B.N. Ames:
Revised methods for the *Salmonella* mutagenicity test.
Mutation Res. 113, 173-215, 1983.

Cytotoxicity reduction in background lawn in a few preincubation S9-supplemented cultures at 5000 μg/plate (MRID 45422822, pp. 46-49, ATTACHMENT Tables 6, 7, 8, 9); and a bacteriotoxic effect above 1200 μg/plate in treated preincubation cultures was observed. However, none of the five strains showed a dose-related and biologically relevant increase in revertants over that of the vehicle control in either type of assay in the presence or absence of metabolic inhibition (ATTACHMENT, Tables 1-13).

The positive controls consistently had pronounced increases in revertants.

Therefore, the investigator concluded that TI 435-Triazan was non-mutagenic in these bacterial assays.

III REVIEWER'S DISCUSSION/CONCLUSIONS

A. The reviewer agrees with the investigator's conclusions that comprehensive testing of TI 435 Triazan showed it to be non-mutagenic in *Salmonella typhimurium.*

B. STUDY DEFICIENCIES

None.

ATTACHMENT

THE FOLLOWING ATTACHMENT IS NOT AVAILABLE ELECTRONICALLY
SEE THE FILE COPY

5. Results

5.1. Description of Results

The colony number of each plate and mean values are listed for each dose in Tables 1 to 13. As may be seen, there was no indication of a bacteriotoxic effect of TI 435-Triazan at doses of up to and including 1200 µg per plate. The total bacteria counts consistently produced results comparable to the negative controls, or differed only insignificantly. No inhibition of growth was noted as well. Higher doses had only a weak, strain-specific bacteriotoxic effect. Therefore they could nevertheless be used for assessment purposes up to and including 5000 µg per plate, however, partly only to a limited degree.

None of the five strains concerned showed in the plate incorporation test a dose-related and biologically relevant increase in mutant counts over those of the negative controls. This applied both to the tests with and without S9 mix (Tables 1 to 5) and was confirmed by the results of the preincubation trials (Tables 6 to 13).

Summary of the Results with TI 435-Triazan
in the Salmonella/Microsome Test

S9 mix	TA 1535	TA 100	TA 1537	TA 98	TA 102
without	-ve	-ve	-ve	-ve	-ve
with	-ve	-ve	-ve	-ve	-ve

-ve = negative

The positive controls sodium azide, nitrofurantoin, 4-nitro-1,2-phenylene diamine, cumene hydroperoxide and 2-aminoanthracene increased mutant counts to well over those of the negative controls, and thus demonstrated the system's sensitivity and the activity of the S9 mix.

5.2. Tabulated Summary of Data

Summary of Mean Values Without S9 Mix

Table and Group	Strain				
	TA 1535	TA 100	TA 1537	TA 98	TA 102
1-5 µg/Plate					
0	8	74	7	17	149
50	8	70	7	23	175
158	8	66	8	21	150
500	8	64	6	21	162
1581	8	62	7	23	146
5000	10	89	6	20	147
Na-azide	678				
NF		214			
4-NPDA			119	175	
Cumene					307
6-10 µg/Tube					
0	7	56	9	22	223
50	6	61	6	24	228
158	8	57	8	27	232
500	6	68	8	26	269
1581	6	75	4	18	236
5000	-	38	-	7	214
Na-azide	692				
NF		342			
4-NPDA			121	188	
Cumene					557

Summary of Mean Values With S9 Mix

Table and Group	Strain				
	TA 1535	TA 100	TA 1537	TA 98	TA 102
1-5 µg/Plate					
0	12	94	8	28	224
50	13	72	9	24	228
158	10	89	9	25	241
500	13	87	8	21	247
1581	13	91	7	26	180
5000	12	119	8	32	205
2-AA	278	1662	401	1553	474
6-10 µg/Tube					
0	10	80	11	26	311
50	9	79	9	28	239
158	6	73	10	30	313
500	8	87	8	31	312
1581	8	103	7	38	335
5000	5	139	4	29	371
2-AA	145	1086	238	1098	496
11-13 µg/Tube					
0		72		26	299
600				29	
800		75		30	322
1200		77		30	336
1600		96		27	359
2400		73		42	319
3200		65		26	337
4800		56			328
2-AA		1007		1143	430

9. Abbreviations used in the Tables

M	mean
SD	standard deviation
-S9	without S9 mix
+S9	with S9 mix
%	not tested
P	precipitate
B	background lawn reduced
C	contaminated
*	mutagenic effect
**	bacteriotoxic effect

10. Tables 1-13

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WUPPERTAL ELBERFELD
AMES TEST with : TI 435-Triazan

Table : 1

Study Number : T 4069135
Study Director : Dr.Herbold
Technician : Bönning
Date : June 8, 2000
Strain: S.typhimurium TA 1535

Dose/Plate (µg/Plate)	REVERTANTS PER PLATE -S9	M	SD	10% +S9	M	SD	TITER Dilution 10^{-6}	TITER per ml 10^{+8}	QUOTIENT -S9	QUOTIENT +S9
DMSO	6 10 7	8	2	10 12 14	12	2	187 182	18.5	1.0	1.0
50	10 9 6	8	2	15 10 14	13	3	%	/	1.1	1.1
158	10 7 8	8	2	7 13 11	10	3	%	/	1.1	0.9
500	9 8 7	8	1	13 15 12	13	2	%	/	1.0	1.1
1581	6 9 8	8	2	14 10 15	13	3	%	/	1.0	1.1
5000	10 9 10	10	1	13 16 8	12	4	%	/	1.3	1.0
Na-azide 10	701 674 660	678	21	%	/	/	%	/	88.5*	/
2-AA 3	%	/	/	289 262 284	278	14	%	/	/	23.2*

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AMES TEST with : TI 435-Triazan

Table : 2

Study Number : T 4069135
Study Director : Dr.Herbold
Technician : Bönning
Date : June 8, 2000
Strain: S.typhimurium TA 100

Dose/Plate (µg/Plate)	REVERTANTS PER PLATE -S9	M	SD	10% +S9	M	SD	TITER Dilution 10^{-6}	TITER per ml 10^{+8}	QUOTIENT -S9	QUOTIENT +S9
DMSO	65 86 72	74	11	91 91 99	94	5	73 74	7.4	1.0	1.0
50	60 78 73	70	9	67 80 69	72	7	%	/	0.9	0.8
158	67 58 72	66	7	90 89 88	89	1	%	/	0.9	1.0
500	69 66 58	64	6	102 70 88	87	16	%	/	0.9	0.9
1581	80 59 47	62	17	94 79 99	91	10	%	/	0.8	1.0
5000	89 106 72	89	17	145 99 112	119	24	%	/	1.2	1.3
NF 0.2	219 212 211	214	4	%	/	/	%	/	2.9*	/
2-AA 3	%	/	/	1610 1750 1626	1662	77	%	/	/	17.7*

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AMES TEST with : TI 435-Triazan

Table : 3

Study Number : T 4069135
Study Director : Dr.Herbold
Technician : Bönning
Date : June 8, 2000
Strain: S.typhimurium TA 1537

Dose/Plate (µg/Plate)	REVERTANTS PER PLATE -S9	M	SD	10% +S9	M	SD	TITER Dilution 10^{-6}	TITER per ml 10^{+8}	QUOTIENT -S9	QUOTIENT +S9
DMSO	8 7 5	7	2	7 9 7	8	1	92 130	11.1	1.0	1.0
50	7 9 6	7	2	9 10 9	9	1	%	/	1.1	1.2
158	5 11 8	8	3	6 9 11	9	3	%	/	1.2	1.1
500	6 8 3	6	3	7 9 8	8	1	%	/	0.9	1.0
1581	8 9 5	7	2	8 3 11	7	4	%	/	1.1	1.0
5000	4 8 7	6	2	9 8 8	8	1	%	/	1.0	1.1
4-NPDA 10	122 107 129	119	11	%	/	/	%	/	17.9*	/
2-AA 3	%	/	/	463 412 329	401	68	%	/	/	52.3*

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AMES TEST with : TI 435-Triazan

Table : 4

Study Number : T 4069135
Study Director : Dr.Herbold
Technician : Bönning
Date : June 8, 2000
Strain: S.typhimurium TA 98

Dose/Plate (µg/Plate)	REVERTANTS PER PLATE -S9	M	SD	10% +S9	M	SD	TITER Dilution 10^{-6}	per ml 10^{-8}	QUOTIENT -S9	+S9
DMSO	13 20 17	17	4	31 26 28	28	3	152 158	15.5	1.0	1.0
50	26 22 20	23	3	28 22 23	24	3	%	/	1.4	0.9
158	20 26 18	21	4	17 34 24	25	9	%	/	1.3	0.9
500	24 20 19	21	3	15 18 29	21	7	%	/	1.3	0.7
1581	19 24 25	23	3	22 26 31	26	5	%	/	1.4	0.9
5000	22 23 16	20	4	37 26 34	32	6	%	/	1.2	1.1
4-NPDA 0.5	176 171 178	175	4	%	/	/	%	/	10.5*	/
2-AA 3	%	/	/	1491 1592 1577	1553	55	%	/	/	54.8*

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AMES TEST with : TI 435-Triazan

Table : 5

Study Number : T 4069135
Study Director : Dr.Herbold
Technician : Bönning
Date : June 8, 2000
Strain: S.typhimurium TA 102

Dose/Plate (µg/Plate)	REVERTANTS PER PLATE -S9	M	SD	10% +S9	M	SD	TITER Dilution 10^{-6}	TITER per ml 10^{+8}	QUOTIENT -S9	QUOTIENT +S9
DMSO	150 139 157	149	9	197 232 243	224	24	80 72	7.6	1.0	1.0
50	189 193 144	175	27	205 249 231	228	22	%	/	1.2	1.0
158	169 132 148	150	19	252 241 229	241	12	%	/	1.0	1.1
500	168 144 175	162	16	249 252 241	247	6	%	/	1.1	1.1
1581	163 122 152	146	21	184 148 207	180	30	%	/	1.0	0.8
5000	134 122 186	147	34	209 196 210	205	8	%	/	1.0	0.9
Cumene 50	267 296 357	307	46	%	/	/	%	/	2.1*	/
2-AA 3	%	/	/	494 444 483	474	26	%	/	/	2.1*

B A Y E R A.G. Table : 6 Study Number : T 4069135
DEPARTMENT OF TOXICOLOGY Study Director : Dr.Herbold
PHARMA RESEARCH CENTER Technician : Bönning
WUPPERTAL ELBERFELD Date : June 23, 2000
PREINCUBATION with : TI 435-Triazan Strain: S.typhimurium TA 1535

Dose/Tube (µg/Tube)	REVERTANTS PER PLATE -S9	M	SD	10% +S9	M	SD	TITER Dilution 10^{-6}	TITER per ml 10^{+8}	QUOTIENT -S9	QUOTIENT +S9
DMSO	6 8 6	7	1	12 10 8	10	2	30 35	3.3	1.0	1.0
50	6 8 4	6	2	9 9 9	9	0	%	/	0.9	0.9
158	8 8 8	8	0	8 6 4	6	2	%	/	1.2	0.6
500	5 6 7	6	1	11 7 6	8	3	%	/	0.9	0.8
1581	7 B 6 B 4 B	6	2	10 8 5	8	3	%	/	0.9	0.8
5000	B	/	/	6 B 5 B 4 B	5	1	%	/	/	0.5
Na-azide 10	685 697 693	692	6	%	/	/	%	/	103.8*	/
2-AA 3	%	/	/	129 160 145	145	16	%	/	/	14.5*

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PREINCUBATION with : TI 435-Triazan

Table : 7

Study Number : T 4069135
Study Director : Dr.Herbold
Technician : Bönning
Date : June 23, 2000
Strain: S.typhimurium TA 100

Dose/Tube (µg/Tube)	REVERTANTS PER PLATE -S9	M	SD	10% +S9	M	SD	TITER Dilution 10^{-6}	TITER per ml 10^{+8}	QUOTIENT -S9	QUOTIENT +S9
DMSO	52 58 57	56	3	82 79 80	80	2	44 44	4.4	1.0	1.0
50	61 71 50	61	11	92 70 75	79	12	%	/	1.1	1.0
158	58 59 53	57	3	70 63 86	73	12	%	/	1.0	0.9
500	56 69 79	68	12	91 86 84	87	4	%	/	1.2	1.1
1581	64 85 75	75	11	94 103 111	103	9	%	/	1.3	1.3
5000	33 B 23 B 58 B	38	18	120 B 138 B 159 B	139	20	%	/	0.7	1.7
NF 0.2	330 349 347	342	10	%	/	/	%	/	6.1*	/
2-AA 3	%	/	/	1122 1106 1029	1086	50	%	/	/	13.5*

B A Y E R A.G. Table : 8 Study Number : T 4069135
DEPARTMENT OF TOXICOLOGY Study Director : Dr.Herbold
PHARMA RESEARCH CENTER Technician : Bönning
WUPPERTAL ELBERFELD Date : June 23, 2000
PREINCUBATION with : TI 435-Triazan Strain: S.typhimurium TA 1537

Dose/Tube (µg/Tube)	REVERTANTS PER PLATE -S9	M	SD	10% +S9	M	SD	TITER Dilution 10^{-6}	TITER per ml 10^{+8}	QUOTIENT -S9	QUOTIENT +S9
DMSO	11 8 7	9	2	13 9 12	11	2	53 63	5.8	1.0	1.0
50	9 6 4	6	3	6 13 9	9	4	%	/	0.7	0.8
158	8 5 11	8	3	11 7 12	10	3	%	/	0.9	0.9
500	7 10 8	8	2	7 12 5	8	4	%	/	1.0	0.7
1581	4 B 3 B 6 B	4	2	6 11 5	7	3	%	/	0.5	0.6
5000	B	/	/	5 B 2 B 5 B	4	2	%	/	/	0.4
4-NPDA 10	121 122 121	121	1	%	/	/	%	/	14.0*	/
2-AA 3	%	/	/	246 232 235	238	7	%	/	/	21.0*

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PREINCUBATION with : TI 435-Triazan

Table : 9

Study Number : T 4069135
Study Director : Dr.Herbold
Technician : Bönning
Date : June 23, 2000
Strain: S.typhimurium TA 98

Dose/Tube (µg/Tube)	REVERTANTS PER PLATE -S9	M	SD	10% +S9	M	SD	TITER Dilution 10^{-6}	TITER per ml 10^{+8}	QUOTIENT -S9	QUOTIENT +S9
DMSO	14 26 27	22	7	23 28 26	26	3	182 167	17.5	1.0	1.0
50	23 26 23	24	2	26 31 27	28	3	%	/	1.1	1.1
158	30 19 31	27	7	37 31 23	30	7	%	/	1.2	1.2
500	27 20 30	26	5	33 35 25	31	5	%	/	1.1	1.2
1581	15 26 12	18	7	44 44 27	38	10	%	/	0.8	1.5
5000	6 B 4 B 12 B	7	4	23 B 29 B 34 B	29	6	%	/	0.3	1.1
4-NPDA 0.5	183 202 178	188	13	%	/	/	%	/	8.4*	/
2-AA 3	%	/	/	1100 1162 1031	1098	66	%	/	/	42.8*

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PREINCUBATION with : TI 435-Triazan

Table : 10

Study Number : T 4069135
Study Director : Dr.Herbold
Technician : Bönning
Date : June 23, 2000
Strain: S.typhimurium TA 102

Dose/Tube (µg/Tube)	REVERTANTS PER PLATE -S9	M	SD	10% +S9	M	SD	TITER Dilution 10^{-6}	per ml 10^{+6}	QUOTIENT -S9	+S9
DMSO	208 243 218	223	18	315 316 303	311	7	396 343	37.0	1.0	1.0
50	223 246 216	228	16	247 224 245	239	13	%	/	1.0	0.8
158	224 232 241	232	9	353 279 307	313	37	%	/	1.0	1.0
500	277 259 271	269	9	280 300 357	312	40	%	/	1.2	1.0
1581	235 241 233	236	4	339 305 360	335	28	%	/	1.1	1.1
5000	173 248 220	214	38	389 357 367	371	16	%	/	1.0	1.2
Cumene 50	588 542 541	557	27	%	/	/	%	/	2.5*	/
2-AA 3	%	/	/	508 483 497	496	13	%	/	/	1.6*

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PREINCUBATION with : TI 435-Triazan

Table : 11

Study Number : T 4069135
Study Director : Dr.Herbold
Technician : Bönning
Date : July 3, 2000
Strain: S.typhimurium TA 100

Dose/Tube (µg/Tube)	REVERTANTS PER PLATE -S9	M	SD	10% +S9	M	SD	TITER Dilution 10^{-6}	TITER per ml 10^{+8}	QUOTIENT -S9	QUOTIENT +S9
DMSO				70 79 68	72	6	100 89	9.5		1.0
800				72 85 69	75	9	%	/		1.0
1200				91 71 70	77	12	%	/		1.1
1600				101 93 95	96	4	%	/		1.3
2400				74 B 75 B 70 B	73	3	%	/		1.0
3200				73 B 67 B 56 B	65	9	%	/		0.9
4800				63 B 25 B 79 B	56	28	%	/		0.8
2-AA 3				1034 937 1050	1007	61	%	/		13.9*

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PREINCUBATION with : TI 435-Triazan

Table : 12

Study Number : T 4069135
Study Director : Dr.Herbold
Technician : Bönning
Date : July 3, 2000
Strain: S.typhimurium TA 98

Dose/Tube (µg/Tube)	REVERTANTS PER PLATE -S9	M	SD	10% +S9	M	SD	TITER Dilution 10^{-6}	TITER Dilution 10^{-8}	QUOTIENT -S9	QUOTIENT +S9
DMSO				34 22 21	26	7	188 215	20.2		1.0
600				17 31 39	29	11	%	/		1.1
800				29 28 33	30	3	%	/		1.2
1200				31 37 22	30	8	%	/		1.2
1600				23 31 27	27	4	%	/		1.1
2400				41 40 44	42	2	%	/		1.6
3200				25 28 26	26	2	%	/		1.0
2-AA 3				1223 1108 1097	1143	70	%	/		44.5*

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PREINCUBATION with : TI 435-Triazan

Table : 13

Study Number : T 4069135
Study Director : Dr.Herbold
Technician : Bönning
Date : July 3, 2000
Strain: S.typhimurium TA 102

Dose/Tube (µg/Tube)	REVERTANTS PER PLATE -S9	M	SD	10% +S9	M	SD	TITER Dilution 10^{-5}	TITER per ml 10^{+8}	QUOTIENT -S9	QUOTIENT +S9
DMSO				301 306 290	299	8	319 341	33.0		1.0
800				328 325 313	322	8	%	/		1.1
1200				327 319 362	336	23	%	/		1.1
1600				384 366 326	359	30	%	/		1.2
2400				318 308 332	319	12	%	/		1.1
3200				356 353 302	337	30	%	/		1.1
4800				325 328 330	328	3	%	/		1.1
2-AA 3				436 440 414	430	14	%	/		1.4*

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WUPPERTAL ELBERFELD
AMES TEST with : TI 435-Triazan

Table : 3

Study Number : T 4069135
Study Director : Dr.Herbold
Technician : Bönning
Date : June 8, 2000
Strain: S.typhimurium TA 1537

Dose/Plate (µg/Plate)	REVERTANTS PER PLATE -S9	M	SD	10% +S9	M	SD	TITER Dilution 10^{-6}	per ml 10^{+8}	QUOTIENT -S9	+S9
DMSO	8 7 5	7	2	7 9 7	8	1	92 130	11.1	1.0	1.0
50	7 9 6	7	2	9 10 9	9	1	%	/	1.1	1.2
158	5 11 8	8	3	6 9 11	9	3	%	/	1.2	1.1
500	6 8 3	6	3	7 9 8	8	1	%	/	0.9	1.0
1581	8 9 5	7	2	8 3 11	7	4	%	/	1.1	1.0
5000	4 8 7	6	2	9 8 8	8	1	%	/	1.0	1.1
4-NPDA 10	122 107 129	119	11	%	/	/	%	/	17.9*	/
2-AA 3	%	/	/	463 412 329	401	68	%	/	/	52.3*

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AMES TEST with : TI 435-Triazan

Table : 4

Study Number : T 4069135
Study Director : Dr.Herbold
Technician : Bönning
Date : June 8, 2000
Strain: S.typhimurium TA 98

Dose/Plate (µg/Plate)	REVERTANTS PER PLATE -S9	M	SD	10% +S9	M	SD	TITER Dilution 10^{-6}	per ml 10^{+8}	QUOTIENT -S9	+S9
DMSO	13 20 17	17	4	31 26 28	28	3	152 158	15.5	1.0	1.0
50	26 22 20	23	3	28 22 23	24	3	%	/	1.4	0.9
158	20 26 18	21	4	17 34 24	25	9	%	/	1.3	0.9
500	24 20 19	21	3	15 18 29	21	7	%	/	1.3	0.7
1581	19 24 25	23	3	22 26 31	26	5	%	/	1.4	0.9
5000	22 23 16	20	4	37 26 34	32	6	%	/	1.2	1.1
4-NPDA 0.5	176 171 178	175	4	%	/	/	%	/	10.5*	/
2-AA 3	%	/	/	1491 1592 1577	1553	55	%	/	/	54.8*

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AMES TEST with : TI 435-Triazan

Table : 5

Study Number : T 4069135
Study Director : Dr.Herbold
Technician : Bönning
Date : June 8, 2000
Strain: S.typhimurium TA 102

Dose/Plate (µg/Plate)	REVERTANTS PER PLATE -S9	M	SD	10% +S9	M	SD	TITER Dilution 10^{-6}	per ml 10^{+8}	QUOTIENT -S9	+S9
DMSO	150 139 157	149	9	197 232 243	224	24	80 72	7.6	1.0	1.0
50	189 193 144	175	27	205 249 231	228	22	%	/	1.2	1.0
158	169 132 148	150	19	252 241 229	241	12	%	/	1.0	1.1
500	168 144 175	162	16	249 252 241	247	6	%	/	1.1	1.1
1581	163 122 152	146	21	184 148 207	180	30	%	/	1.0	0.8
5000	134 122 186	147	34	209 196 210	205	8	%	/	1.0	0.9
Cumene 50	267 296 357	307	46	%	/	/	%	/	2.1*	/
2-AA 3	%	/	/	494 444 483	474	26	%	/	/	2.1*

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PHARMA RESEARCH CENTER
WUPPERTAL ELBERFELD
PREINCUBATION with : TI 435-Triazan

Table : 6

Study Number : T 4069135
Study Director : Dr.Herbold
Technician : Bönning
Date : June 23, 2000
Strain: S.typhimurium TA 1535

Dose/Tube (µg/Tube)	REVERTANTS PER PLATE -S9	M	SD	10% +S9	M	SD	TITER Dilution 10^{-6}	TITER per ml 10^{-8}	QUOTIENT -S9	QUOTIENT +S9
DMSO	6 8 6	7	1	12 10 8	10	2	30 35	3.3	1.0	1.0
50	6 8 4	6	2	9 9 9	9	0	%	/	0.9	0.9
158	8 8 8	8	0	8 6 4	6	2	%	/	1.2	0.6
500	5 6 7	6	1	11 7 6	8	3	%	/	0.9	0.8
1581	7 B 6 B 4 B	6	2	10 8 5	8	3	%	/	0.9	0.8
5000	B	/	/	6 B 5 B 4 B	5	1	%	/	/	0.5
Na-azide 10	685 697 693	692	6	%	/	/	%	/	103.8*	/
2-AA 3	%	/	/	129 160 145	145	16	%	/	/	14.5*

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DEPARTMENT OF TOXICOLOGY
PHARMA RESEARCH CENTER
WUPPERTAL ELBERFELD
PREINCUBATION with : TI 435-Triazan

Table : 7

Study Number : T 4069135
Study Director : Dr.Herbold
Technician : Bönning
Date : June 23, 2000
Strain: S.typhimurium TA 100

Dose/Tube (µg/Tube)	REVERTANTS PER PLATE -S9	M	SD	10% +S9	M	SD	TITER Dilution 10^{-6}	TITER per ml 10^{+8}	QUOTIENT -S9	QUOTIENT +S9
DMSO	52 58 57	56	3	82 79 80	80	2	44 44	4.4	1.0	1.0
50	61 71 50	61	11	92 70 75	79	12	%	/	1.1	1.0
158	58 59 53	57	3	70 63 86	73	12	%	/	1.0	0.9
500	56 69 79	68	12	91 86 84	87	4	%	/	1.2	1.1
1581	64 85 75	75	11	94 103 111	103	9	%	/	1.3	1.3
5000	33 B 23 B 58 B	38	18	120 B 138 B 159 B	139	20	%	/	0.7	1.7
NF 0.2	330 349 347	342	10	%	/	/	%	/	6.1*	/
2-AA 3	%	/	/	1122 1106 1029	1086	50	%	/	/	13.5*

B A Y E R A.G. Table : 8 Study Number : T 4069135
DEPARTMENT OF TOXICOLOGY Study Director : Dr.Herbold
PHARMA RESEARCH CENTER Technician : Bönning
WUPPERTAL ELBERFELD Date : June 23, 2000
PREINCUBATION with : TI 435-Triazan Strain: S.typhimurium TA 1537

Dose/Tube (µg/Tube)	REVERTANTS PER PLATE -S9	M	SD	10% +S9	M	SD	TITER Dilution 10^{-6}	TITER per ml 10^{+8}	QUOTIENT -S9	QUOTIENT +S9
DMSO	11 8 7	9	2	13 9 12	11	2	53 63	5.8	1.0	1.0
50	9 6 4	6	3	6 13 9	9	4	%	/	0.7	0.8
158	8 5 11	8	3	11 7 12	10	3	%	/	0.9	0.9
500	7 10 8	8	2	7 12 5	8	4	%	/	1.0	0.7
1581	4 B 3 B 6 B	4	2	6 11 5	7	3	%	/	0.5	0.6
5000	B	/	/	5 B 2 B 5 B	4	2	%	/	/	0.4
4-NPDA 10	121 122 121	121	1	%	/	/	%	/	14.0*	/
2-AA 3	%	/	/	246 232 235	238	7	%	/	/	21.0*

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PHARMA RESEARCH CENTER
WUPPERTAL ELBERFELD
PREINCUBATION with : TI 435-Triazan

Table : 9

Study Number : T 4069135
Study Director : Dr.Herbold
Technician : Bönning
Date : June 23, 2000
Strain: S.typhimurium TA 98

Dose/Tube (µg/Tube)	REVERTANTS PER PLATE -S9	M	SD	10% +S9	M	SD	TITER Dilution 10^{-6}	TITER per ml 10^{+8}	QUOTIENT -S9	QUOTIENT +S9
DMSO	14 26 27	22	7	23 28 26	26	3	182 167	17.5	1.0	1.0
50	23 26 23	24	2	26 31 27	28	3	%	/	1.1	1.1
158	30 19 31	27	7	37 31 23	30	7	%	/	1.2	1.2
500	27 20 30	26	5	33 35 25	31	5	%	/	1.1	1.2
1581	15 26 12	18	7	44 44 27	38	10	%	/	0.8	1.5
5000	6 B 4 B 12 B	7	4	23 B 29 B 34 B	29	6	%	/	0.3	1.1
4-NPDA 0.5	183 202 178	188	13	%	/	/	%	/	8.4*	/
2-AA 3	%	/	/	1100 1162 1031	1098	66	%	/	/	42.8*

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PHARMA RESEARCH CENTER
WUPPERTAL ELBERFELD
PREINCUBATION with : TI 435-Triazan

Table : 10

Study Number : T 4069135
Study Director : Dr.Herbold
Technician : Bönning
Date : June 23, 2000
Strain: S.typhimurium TA 102

Dose/Tube (µg/Tube)	REVERTANTS PER PLATE -S9	M	SD	10% +S9	M	SD	TITER Dilution 10^{-6}	TITER per ml 10^{+8}	QUOTIENT -S9	QUOTIENT +S9
DMSO	208 243 218	223	18	315 316 303	311	7	396 343	37.0	1.0	1.0
50	223 246 216	228	16	247 224 245	239	13	%	/	1.0	0.8
158	224 232 241	232	9	353 279 307	313	37	%	/	1.0	1.0
500	277 259 271	269	9	280 300 357	312	40	%	/	1.2	1.0
1581	235 241 233	236	4	339 305 360	335	28	%	/	1.1	1.1
5000	173 248 220	214	38	389 357 367	371	16	%	/	1.0	1.2
Cumene 50	588 542 541	557	27	%	/	/	%	/	2.5*	/
2-AA 3	%	/	/	508 483 497	496	13	%	/	/	1.6*

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PHARMA RESEARCH CENTER
WUPPERTAL ELBERFELD
PREINCUBATION with : TI 435-Triazan

Table : 11

Study Number : T 4069135
Study Director : Dr.Herbold
Technician : Bönning
Date : July 3, 2000
Strain: S.typhimurium TA 100

Dose/Tube (µg/Tube)	REVERTANTS PER PLATE -S9	M	SD	10% +S9	M	SD	TITER Dilution 10^{-6}	TITER per ml 10^{+8}	QUOTIENT -S9	QUOTIENT +S9
DMSO				70 79 68	72	6	100 89	9.5		1.0
800				72 85 69	75	9	%	/		1.0
1200				91 71 70	77	12	%	/		1.1
1600				101 93 95	96	4	%	/		1.3
2400				74 B 75 B 70 B	73	3	%	/		1.0
3200				73 B 67 B 56 B	65	9	%	/		0.9
4800				63 B 25 B 79 B	56	28	%	/		0.8
2-AA 3				1034 937 1050	1007	61	%	/		13.9*

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WUPPERTAL ELBERFELD
PREINCUBATION with : TI 435-Triazan

Table : 12

Study Number : T 4069135
Study Director : Dr.Herbold
Technician : Bönning
Date : July 3, 2000
Strain: S.typhimurium TA 98

Dose/Tube (µg/Tube)	REVERTANTS PER PLATE -S9	M	SD	10% +S9	M	SD	TITER Dilution 10^{-6}	TITER per ml 10^{+8}	QUOTIENT -S9	QUOTIENT +S9
DMSO				34 22 21	26	7	188 215	20.2		1.0
600				17 31 39	29	11	%	/		1.1
800				29 28 33	30	3	%	/		1.2
1200				31 37 22	30	8	%	/		1.2
1600				23 31 27	27	4	%	/		1.1
2400				41 40 44	42	2	%	/		1.6
3200				25 28 26	26	2	%	/		1.0
2-AA 3				1223 1108 1097	1143	70	%	/		44.5*

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PHARMA RESEARCH CENTER
WUPPERTAL ELBERFELD
PREINCUBATION with : TI 435-Triazan

Table : 13

Study Number : T 4069135
Study Director : Dr.Herbold
Technician : Bönning
Date : July 3, 2000
Strain: S.typhimurium TA 102

Dose/Tube (µg/Tube)	REVERTANTS PER PLATE -S9	M	SD	10% +S9	M	SD	TITER Dilution 10^{-5}	TITER per ml 10^{+8}	QUOTIENT -S9	QUOTIENT +S9
DMSO				301 306 290	299	8	319 341	33.0		1.0
800				328 325 313	322	8	%	/		1.1
1200				327 319 362	336	23	%	/		1.1
1600				384 366 326	359	30	%	/		1.2
2400				318 308 332	319	12	%	/		1.1
3200				356 353 302	337	30	%	/		1.1
4800				325 328 330	328	3	%	/		1.1
2-AA 3				436 440 414	430	14	%	/		1.4*

CLOTHIANIDIN *Salmonella*/Mammalian Activation: Gene Mutation (84-2)

EPA Reviewer: Irving Mauer, Ph.D. Date: 6/13/02
Registration Action Branch 3, HED (7509C)
EPA Secondary Reviewer: Nancy McCarroll Date: 11/19/02
Toxicology Branch 1, HED (7509C)

TXR NO. 0050321

DATA EVALUATION RECORD

STUDY TYPE: Bacterial system: *Salmonella*/mammalian activation, gene mutation assay; OPPTS 870.5100 [84-2]

DP BARCODE: D279778 SUBMISSION CODE: S607124
P.C. CODE: 044309 TOX. CHEM. NO.: None.

TEST MATERIAL (PURITY): TI 435-Hexahydropyrimidine (99.5% a.i.; Batch No. GAW 2641)

SYNONYMS: Chemically: 1, 3-Dibenzyl-5-ethylhexahydro-5-pyrimidinecarbonitrile

CITATION: Herbold, B. (2001). TI-435-Hexahydropyrimidine. *Salmonella*/Microsome Test. Plate Incorporation Method, performed at Bayer AG's Department of Toxicology, Wuppertal, (Germany), 12 February 2001. Bayer AG Study No. T2069890, Bayer AG Report No. PH 30734/Bayer Corporation Agriculture Division Report No. 110243. MRID 45422818. Unpublished.

SPONSOR: Takeda Chemical Industries Ltd., Tokyo (Japan).

EXECUTIVE SUMMARY:

In repeat reverse gene mutation assays in bacteria (MRID 45422818), five histidine-deficient (*his*$^-$) mutant strains of *Salmonella typhimurium* (TA1535, TA100, TA1537, TA98, TA102) were exposed to six concentrations (ranging from 16 to 5000 μg/plate) of TI 435-Hexahydropyrimidine (99.5% a.i., Batch No. GAW 2641) in dimethylsulfoxide (DMSO), by plate incorporation (initial assay), and following 20 minutes incubation in a preincubation assay (repeat assay), and reversion to prototrophy (*his*$^-$ to *his*$^+$) determined after 48 hours incubation at 37°C. Test material-treated cultures were treated in the presence (S9) and absence of metabolic activation provided by hepatic microsomes from rats pretreated with Aroclor 1254. Additional cultures were exposed to the vehicle (DMSO), and to strain-specific mutagens, the latter to serve as positive controls.

Only the highest dose of 5000 μg/plate TI-435-Hexahydropyrimidine caused a weak

bacteriotoxic effect, as well as precipitation. However, **no mutagenic activity (*i.e.*, increased frequency of *his*$^+$ revertants) was evident in any test material-treated cultures, either in the presence or absence of S9.** Substantial increases in revertants were observed in all mutagen-treated cultures.

Therefore, TI-435 Hexahydropyrimidine is considered to be non-mutagenic in repeat assays in *Salmonella*/microsome tests.

This study is classified as acceptable/guideline and satisfies the requirement for FIFRA Test Guideline 84-2 for *in vitro* mutagenicity (bacterial reverse gene mutation) data.

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

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Material: TI 435-Hexahydropyrimidine

Description: Fine white powder.
Lot/Batch No.: GAW2641
Purity: 99.5% a.i. (by analysis dated 7 September 2000)
Stability of compound: Not provided.
CAS No.: Not provided.
Solvent used: Dimethylsulfoxide (DMSO).
Other comments: Stored at room temperature. Use not indicated by sponsor.

2. Control Materials:

Negative: None.

Solvent/Final concentration: DMSO/0.1 mL/plate.

Positive: Non-activated

Sodium azide	10 µg/plate for TA1535.
Nitrofurantoin	0.2 µg/plate for TA100.
4-NPDA[1]	10 µg/plate for TA1537.
Other (list):	
4-NPDA	0.5 µg/plate for TA98.
Cumene hydroperoxide	50 µg/plate for TA102.

Activated: 2-Aminoanthracene (2-anthramine) 3 µg/plate for all strains.

[1]4-NDPA = 4-nitro-phenylene diamine.

3. Metabolic Activation: S9 derived from adult (200 - 300g) male Sprague-Dawley rats.

x	Aroclor 1254	x	induced	x	rat	x	liver
	phenobarital		non-induced		mouse		lung
	none				hamster		other
	other						other

Describe S9 mix composition:

Seventy mL of cofactor solution contained:

$MgCl_2$ x 6 H_2O	162.6 mg
KCl	246.0 mg
Glucose-6-phosphate, disodium salt	179.1 mg
NADP, disodium salt	315.0 mg
Phosphate buffer	100.0 mM

4. Test Organisms: *Salmonella typhimurium* strains:

	TA97	x	TA98	x	TA100	x	TA102
	TA104	x	TA1535	x	TA1537		TA1538

List any others: None.

Properly maintained? Yes.

Checked for appropriate genetic markers (rfa mutation, R factor)? Yes.

5. Test Compound Concentrations Used:

Cytotoxicity Test: Not performed.

Main Assays:

Non-activated conditions: 16, 50, 158, 500, 1581, 5000 μg/plate.
Activated conditions: 16, 50, 158, 500, 1581, 5000 μg/plate.

B. TEST PERFORMANCE

The initial plate incorporation assay followed the directions of Ames *et al.* (1973a, 1975)[2] and Maron and Ames (1983)[3]. Triplicate cultures were used for each concentration in each culture of both assays.

1. Type of *Salmonella* Assay:

x	standard plate test
x	preincubation (20 minutes)
	"Prival" modification
	spot test
	other (describe)

2. Criteria for assay acceptance and assessment of results were presented.

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[2]Ames, B.N., W.E. Durston, E. Yamasaki and F.D. Lee:
Carcinogens are mutagens: a simple test system combining liver homogenates for activation and bacteria for detection.
Proc. Nat. Acad. Sci. (USA) 70, 2281-2285, 197f3a.

Ames, B.N., J. McCann and E. Yamasaki:
Methods for detecting carcinogens and mutgens with the *Salmonella*/mammalian-microsome mutagenicity test.
Mutation Res. 31, 347-364, 1975.

[3]Maron, D.M. and B.N. Ames:
Revised methods for the *Salmonella* mutagenicity test.
Mutation Res. 113, 173-215, 1983.

II REPORTED RESULTS

A. PRELIMINARY CYTOTOXICITY ASSAY: (No preliminary assay performed).

B. MUTAGENICITY ASSAYS:

A weak strain-specific bacteriostatic effect was seen at the highest dose, 5000 μg/plate, at which precipitation also occurred. However, at no concentration in either the initial or repeat assay was there a dose-related or biologically relevant increase in revertants, with or without the presence of S9 (MRID 45422818, pp. 25-27; pp. 41-51, ATTACHMENT Tables 1-10). Frequencies of revertants were substantially increased in all positive controls.

Therefore, the investigator concluded that TI 435-hexahydropyrimidine was non-mutagenic in these *Salmonella* assays.

III. REVIEWER'S DISCUSSION/CONCLUSIONS

A. The reviewer agrees with the conclusion expressed by the investigator that comprehensive testing in *Salmonella typhimurium* demonstrated TI 435-hexahydropyrimidine to be non-mutagenic.

B. STUDY DEFICIENCIES:

None.

ATTACHMENT

THE FOLLOWING ATTACHMENT IS NOT AVAILABLE ELECTRONICALLY
SEE THE FILE COPY

5. Results

5.1. Description of Results

The colony number of each plate and mean values are listed for each dose in Tables 1 to 10. As may be seen, there was no indication of a bacteriotoxic effect of TI 435-Hexahydropyrimidine at doses of up to and including 1581 µg per plate. The total bacteria counts consistently produced results comparable to the negative controls, or differed only insignificantly. No inhibition of growth was noted as well. 5000 µg per plate had only a weak, strain-specific bacteriotoxic effect. Therefore this dose could nevertheless be used for assessment purposes. Substance precipitation occurred at 5000 µg per plate.

None of the five strains concerned showed in the plate incorporation test a dose-related and biologically relevant increase in mutant counts over those of the negative controls. This applied both to the tests with and without S9 mix (Tables 1 to 5) and was confirmed by the results of the preincubation trials (Tables 6 to 10).

Summary of the Results with TI 435-Hexahydropyrimidine
in the Salmonella/Microsome Test

S9 mix	TA 1535	TA 100	TA 1537	TA 98	TA 102
without	-ve	-ve	-ve	-ve	-ve
with	-ve	-ve	-ve	-ve	-ve

-ve = negative

The positive controls sodium azide, nitrofurantoin, 4-nitro-1,2-phenylene diamine, cumene hydroperoxide and 2-aminoanthracene increased mutant counts to well over those of the negative controls, and thus demonstrated the system's sensitivity and the activity of the S9 mix.

5.2. Tabulated Summary of Data

Summary of Mean Values Without S9 Mix

Table and Group	Strain				
	TA 1535	TA 100	TA 1537	TA 98	TA 102
1-5 µg/Plate					
0	12	72	5	40	185
16	11	72	5	31	161
50	10	69	5	40	163
158	10	66	6	39	150
500	8	71	5	34	164
1581	12	67	5	33	160
5000	11	64	6	45	118
Na-azide	521				
NF		230			
4-NPDA			133	243	
Cumene					338
6-10 µg/Tube					
0	7	116	6	37	233
16	6	96	6	43	242
50	7	91	4	27	241
158	6	87	6	31	253
500	8	90	7	30	215
1581	8	81	6	31	206
5000	7	73	5	28	190
Na-azide	496				
NF		499			
4-NPDA			159	196	
Cumene					496

Summary of Mean Values With S9 Mix

Table and Group	Strain				
	TA 1535	TA 100	TA 1537	TA 98	TA 102
1-5 µg/Plate					
0	11	96	9	52	225
16	10	84	6	51	222
50	11	85	6	56	229
158	8	74	7	35	218
500	12	75	8	63	198
1581	8	55	10	48	211
5000	9	77	5	44	179
2-AA	161	1597	159	1885	470
6-10 µg/Tube					
0	9	137	7	54	250
16	10	137	7	37	252
50	13	139	7	42	229
158	10	134	9	39	246
500	7	124	9	50	260
1581	9	133	4	43	283
5000	8	130	7	50	242
2-AA	154	1838	329	1820	533

10. Abbreviations used in the Tables

M	mean
SD	standard deviation
-S9	without S9 mix
+S9	with S9 mix
%	not tested
P	precipitate
B	background lawn reduced
C	contaminated
*	mutagenic effect
**	bacteriotoxic effect

11. Tables 1-10

B A Y E R A.G. Table : 1 Study Number : T 2069890
DEPARTMENT OF TOXICOLOGY Study Director : Dr.Herbold
PHARMA RESEARCH CENTER Technician : Düver
WUPPERTAL ELBERFELD Date : Nov. 24, 2000
AMES TEST with : TI 435-Hexahydropyrimidin Strain: S.typhimuriu TA 1535

Dose/Plate (µg/Plate)	REVERTANTS PER PLATE -S9	M	SD	10% +S9	M	SD	TITER Dilution 10^{-6}	TITER per ml 10^{*8}	QUOTIENT -S9	QUOTIENT +S9
DMSO	10 12 13	12	2	11 11 10	11	1	208 141	17.5	1.0	1.0
16	9 12 11	11	2	8 10 12	10	2	166 149	15.8	0.9	0.9
50	10 11 8	10	2	10 12 11	11	1	144 128	13.6	0.8	1.0
158	8 12 10	10	2	5 11 9	8	3	148 155	15.2	0.9	0.8
500	11 7 5	8	3	13 10 14	12	2	112 140	12.6	0.7	1.2
1581	13 12 10	12	2	8 5 11	8	3	95 148	12.2	1.0	0.8
5000	12 P 7 P 15 P	11	4	10 P 7 P 9 P	9	2	137 155	14.6	1.0	0.8
Na-azide 10	513 515 534	521	12	%	/	/	169 136	15.3	44.6*	/
2-AA 3	%	/	/	154 164 165	161	6	97 113	10.5	/	15.1*

198

194

UB A Y E R A.G. Table : 2 Study Number : T 2069890
DEPARTMENT OF TOXICOLOGY Study Director : Dr.Herbold
PHARMA RESEARCH CENTER Technician : Düver
WUPPERTAL ELBERFELD Date : Nov. 24, 2000
AMES TEST with : TI 435-Hexahydropyrimidin Strain: S.typhimurium TA 100

Dose/Plate (µg/Plate)	REVERTANTS PER PLATE -S9	M	SD	10% +S9	M	SD	TITER Dilution 10^{-6}	TITER per ml 10^{+8}	QUOTIENT -S9	QUOTIENT +S9
DMSO	67 81 69	72	8	81 104 104	96	13	95 106	10.1	1.0	1.0
16	70 77 69	72	4	78 95 78	84	10	92 93	9.3	1.0	0.9
50	90 61 55	69	19	95 80 81	85	8	92 108	10.0	0.9	0.9
158	59 73 66	66	7	67 59 95	74	19	106 67	8.7	0.9	0.8
500	69 71 74	71	3	68 80 78	75	6	89 99	9.4	1.0	0.8
1581	66 67 69	67	2	57 41 66	55	13	82 71	7.7	0.9	0.6
5000	55 P 61 P 75 P	64	10	84 P 71 P 77 P	77	7	66 66	6.6	0.9	0.8
NF 0.2	249 211 229	230	19	%	/	/	112 99	10.6	3.2*	/
2-AA 3	%	/	/	1710 1612 1469	1597	121	89 82	8.6	/	16.6*

199

B A Y E R A.G. Table : 3 Study Number : T 2069890
DEPARTMENT OF TOXICOLOGY Study Director : Dr.Herbold
PHARMA RESEARCH CENTER Technician : Düver
WUPPERTAL ELBERFELD Date : Nov. 24, 2000
AMES TEST with : TI 435-Hexahydropyrimidin Strain: S.typhimurium TA 1537

Dose/Plate (µg/Plate)	REVERTANTS PER PLATE -S9	M	SD	10% +S9	M	SD	TITER Dilution 10^{-6}	TITER per ml 10^{+8}	QUOTIENT -S9	QUOTIENT +S9
DMSO	6 5 5	5	1	8 11 8	9	2	47 42	4.5	1.0	1.0
16	5 5 5	5	0	7 7 3	6	2	33 36	3.5	0.9	0.6
50	4 6 6	5	1	4 8 5	6	2	28 35	3.2	1.0	0.6
158	6 6 6	6	0	6 6 9	7	2	49 45	4.7	1.1	0.8
500	8 4 4	5	2	7 11 6	8	3	47 33	4.0	1.0	0.9
1581	6 6 3	5	2	11 8 10	10	2	40 30	3.5	0.9	1.1
5000	8 P 3 P 6 P	6	3	3 P 6 P 6 P	5	2	55 47	5.1	1.1	0.6
4-NPDA 10	145 131 123	133	11	%	/	/	36 52	4.4	24.9*	/
2-AA 3	%	/	/	179 153 144	159	18	23 29	2.6	/	17.6*

200

197

B A Y E R A.G. Table : 4 Study Number : T 2069890
DEPARTMENT OF TOXICOLOGY Study Director : Dr.Herbold
PHARMA RESEARCH CENTER Technician : Düver
WUPPERTAL ELBERFELD Date : Nov. 24, 2000
AMES TEST with : TI 435-Hexahydropyrimidin Strain: S.typhimurium TA 98

Dose/Plate (µg/Plate)	REVERTANTS PER PLATE -S9	M	SD	10% +S9	M	SD	TITER Dilution 10^{-6}	per ml 10^{-8}	QUOTIENT -S9	+S9
DMSO	41 40 40	40	1	55 52 49	52	3	212 211	21.2	1.0	1.0
16	31 36 27	31	5	61 60 31	51	17	168 231	20.0	0.8	1.0
50	42 38 41	40	2	63 48 57	56	8	240 212	22.6	1.0	1.1
158	49 41 28	39	11	37 30 38	35	4	185 201	19.3	1.0	0.7
500	32 36 33	34	2	68 53 67	63	8	205 165	18.5	0.8	1.2
1581	32 33 33	33	1	48 41 54	48	7	193 165	17.9	0.8	0.9
5000	48 P 44 P 44 P	45	2	38 P 44 P 49 P	44	6	151 164	15.8	1.1	0.8
4-NPDA 0.5	222 228 278	243	31	%	/	/	205 174	19.0	6.0*	/
2-AA 3	%	/	/	2084 1837 1735	1885	179	170 141	15.6	/	36.3*

201

B A Y E R A.G. Table : 5 Study Number : T 2069890
DEPARTMENT OF TOXICOLOGY Study Director : Dr.Herbold
PHARMA RESEARCH CENTER Technician : Düver
WUPPERTAL ELBERFELD Date : Nov. 24, 2000
AMES TEST with : TI 435-Hexahydropyrimidin Strain: S.typhimurium TA 102

Dose/Plate (µg/Plate)	REVERTANTS PER PLATE -S9	M	SD	10% +S9	M	SD	TITER Dilution 10^{-6}	per ml 10^{-8}	QUOTIENT -S9	+S9
DMSO	188 184 183	185	3	223 221 230	225	5	261 298	28.0	1.0	1.0
16	147 151 184	161	20	251 212 202	222	26	236 275	25.6	0.9	1.0
50	153 176 161	163	12	227 227 232	229	3	371 302	33.7	0.9	1.0
158	155 144 152	150	6	214 215 224	218	6	240 285	26.3	0.8	1.0
500	151 176 164	164	13	220 184 191	198	19	220 195	20.8	0.9	0.9
1581	137 167 176	160	20	207 210 215	211	4	264 202	23.3	0.9	0.9
5000	141 P 98 P 116 P	118	22	208 P 161 P 167 P	179	26	63 111	8.7**	0.6	0.8
Cumene 50	350 351 314	338	21	%	/	/	249 191	22.0	1.8*	/
2-AA 3	%	/	/	446 529 434	470	52	144 159	15.2**	/	2.1*

202

198

B A Y E R A.G. Table : 6 Study Number : T 2069890
DEPARTMENT OF TOXICOLOGY Study Director : Dr.Herbold
PHARMA RESEARCH CENTER Technician : Düver
WUPPERTAL ELBERFELD Date : Dec. 7, 2000
PREINCUBATION with : TI 435-Hexahydropyrimidin Strain: S.typhimurium TA 1535

Dose/Tube (µg/Tube)	REVERTANTS PER PLATE -S9	M	SD	10% +S9	M	SD	TITER Dilution 10^{-6}	TITER per ml 10^{+8}	QUOTIENT -S9	QUOTIENT +S9
DMSO	7 7 6	7	1	8 8 10	9	1	89 106	9.8	1.0	1.0
16	6 6 6	6	0	11 7 13	10	3	92 119	10.6	0.9	1.2
50	6 9 6	7	2	13 13 14	13	1	116 110	11.3	1.1	1.5
158	4 5 10	6	3	10 10 11	10	1	98 76	8.7	1.0	1.2
500	9 9 5	8	2	8 7 7	7	1	83 112	9.8	1.2	0.8
1581	10 11 4	8	4	13 7 8	9	3	85 112	9.9	1.3	1.1
5000	8 P 6 P 6 P	7	1	7 P 11 P 6 P	8	3	106 98	10.2	1.0	0.9
Na-azide 10	551 477 461	496	48	%	/	/	83 79	8.1	74.5*	/
2-AA 3	%	/	/	154 150 159	154	5	61 63	6.2**	/	17.8*

203

B A Y E R A.G. Table : 7 Study Number : T 2069890
DEPARTMENT OF TOXICOLOGY Study Director : Dr.Herbold
PHARMA RESEARCH CENTER Technician : Düver
WUPPERTAL ELBERFELD Date : Dec. 7, 2000
PREINCUBATION with : TI 435-Hexahydropyrimidin Strain: S.typhimurium TA 100

Dose/Tube (µg/Tube)	REVERTANTS PER PLATE -S9	M	SD	10% +S9	M	SD	TITER Dilution 10^{-6}	TITER per ml 10^{-8}	QUOTIENT -S9	QUOTIENT +S9
DMSO	120 116 113	116	4	152 114 144	137	20	172 195	18.4	1.0	1.0
16	97 97 94	96	2	131 133 146	137	8	183 166	17.5	0.8	1.0
50	84 96 92	91	6	133 147 137	139	7	218 181	20.0	0.8	1.0
158	95 73 93	87	12	126 133 142	134	8	173 157	16.5	0.7	1.0
500	85 99 86	90	8	114 117 141	124	15	185 166	17.6	0.8	0.9
1581	84 68 91	81	12	147 123 128	133	13	160 155	15.8	0.7	1.0
5000	68 P 85 P 66 P	73	10	139 P 127 P 125 P	130	8	169 157	16.3	0.6	1.0
NF 0.2	540 493 463	499	39	%	/	/	194 169	18.2	4.3*	/
2-AA 3	%	/	/	1832 1824 1858	1838	18	172 194	18.3	/	13.4

204

B A Y E R A.G.
DEPARTMENT OF TOXICOLOGY
PHARMA RESEARCH CENTER
WUPPERTAL ELBERFELD
PREINCUBATION with : TI 435-Hexahydropyrimidin

Table : 8

Study Number : T 2069890
Study Director : Dr.Herbold
Technician : Düver
Date : Dec. 7, 2000
Strain: S.typhimurium TA 1537

Dose/Tube (µg/Tube)	REVERTANTS PER PLATE -S9	M	SD	10% +S9	M	SD	TITER Dilution 10^{-6}	TITER per ml 10^{+8}	QUOTIENT -S9	QUOTIENT +S9
DMSO	7 5 6	6	1	7 9 6	7	2	39 50	4.5	1.0	1.0
16	4 7 8	6	2	10 5 6	7	3	50 39	4.5	1.1	1.0
50	6 3 2	4	2	5 9 6	7	2	57 44	5.1	0.6	0.9
158	5 6 6	6	1	8 9 9	9	1	40 49	4.5	0.9	1.2
500	6 8 6	7	1	9 8 10	9	1	45 36	4.1	1.1	1.2
1581	6 9 4	6	3	4 4 3	4	1	38 35	3.7	1.1	0.5
5000	4 P 7 P 5 P	5	2	6 P 8 P 6 P	7	1	39 40	4.0	0.9	0.9
4-NPDA 10	168 153 156	159	8	%	/	/	49 48	4.9	26.5*	/
2-AA 3	%	/	/	308 353 327	329	23	37 41	3.9	/	44.9*

205

B A Y E R A.G. Table : 9 Study Number : T 2069890
DEPARTMENT OF TOXICOLOGY Study Director : Dr.Herbold
PHARMA RESEARCH CENTER Technician : Düver
WUPPERTAL ELBERFELD Date : Dec. 7, 2000
PREINCUBATION with : TI 435-Hexahydropyrimidin Strain: S.typhimurium TA 98

Dose/Tube (µg/Tube)	REVERTANTS PER PLATE -S9	M	SD	10% +S9	M	SD	TITER Dilution 10^{-6}	TITER per ml 10^{+8}	QUOTIENT -S9	QUOTIENT +S9
DMSO	32 36 44	37	6	53 45 63	54	9	272 272	27.2	1.0	1.0
16	51 41 38	43	7	39 40 32	37	4	291 295	29.3	1.2	0.7
50	32 29 21	27	6	51 32 42	42	10	281 285	28.3	0.7	0.8
158	26 29 38	31	6	30 36 52	39	11	257 235	24.6	0.8	0.7
500	38 28 24	30	7	49 49 52	50	2	247 241	24.4	0.8	0.9
1581	17 38 37	31	12	37 44 48	43	6	289 279	28.4	0.8	0.8
5000	36 P 31 P 18 P	28	9	40 P 50 P 61 P	50	11	281 296	28.9	0.8	0.9
4-NPDA 0.5	194 175 220	196	23	%	/	/	271 284	27.8	5.3*	/
2-AA 3	%	/	/	1831 1862 1766	1820	49	255 245	25.0	/	33.9*

206

B A Y E R A.G. Table : 10 Study Number : T 2069890
DEPARTMENT OF TOXICOLOGY Study Director : Dr.Herbold
PHARMA RESEARCH CENTER Technician : Düver
WUPPERTAL ELBERFELD Date : Dec. 7, 2000
PREINCUBATION with : TI 435-Hexahydropyrimidin Strain: S.typhimurium TA 102

Dose/Tube (µg/Tube)	REVERTANTS PER PLATE -S9	M	SD	10% +S9	M	SD	TITER Dilution 10^{-6}	TITER per ml 10^{+8}	QUOTIENT -S9	QUOTIENT +S9
DMSO	241 223 236	233	9	251 251 248	250	2	377 340	35.9	1.0	1.0
16	246 232 249	242	9	268 252 237	252	16	335 355	34.5	1.0	1.0
50	246 251 227	241	13	238 235 214	229	13	323 319	32.1	1.0	0.9
158	250 249 261	253	7	251 237 251	246	8	314 330	32.2	1.1	1.0
500	203 220 222	215	10	210 267 302	260	46	308 240	27.4	0.9	1.0
1581	216 201 201	206	9	272 302 275	283	17	382 320	35.1	0.9	1.1
5000	188 P 215 P 166 P	190	25	271 P 232 P 222 P	242	26	300 302	30.1	0.8	1.0
Cumene 50	486 499 504	496	9	%	/	/	333 300	31.7	2.1*	/
2-AA 3	%	/	/	558 516 525	533	22	389 347	36.8	/	2.1*

207

EPA Reviewer: Irving Mauer, Ph.D. Date: 06/13/02
Registration Action Branch 3, HED (7509C)
EPA Secondary Reviewer: Nancy McCarroll Date: 11/14/02
Toxicology Branch 1, HED (7509C)

TRX NO. 0050321

DATA EVALUATION RECORD

STUDY TYPE: Bacterial system *e.g.*, *Salmonella*/mammalian activation gene mutation assay; OPPTS 870.5100 [84-2]

DP BARCODE: D279778 SUBMISSION CODE: S607124
P.C. CODE: 044309 TOX. CHEM. NO.: None.

TEST MATERIAL (PURITY): TI 435-Benzyltriazinone (98.3% a.i.; Batch No. KTS 9475-11-2)

SYNONYMS: None.

CITATION: Herbold, B. (2000). TI 435-Benzyltriazinone; *Salmonella*/Microsome Test; Plate Incorporation and Preincubation Method, performed at Bayer AG's Department of Toxicology, Wuppertal (Germany). Bayer AG Study No. T6068886, dated July 24, 2000 (Bayer AG Report No. PH30065/Bayer Corporation Agricultural Division Report No. 1102-15). MRID 45422817. Unpublished.

SPONSOR: Takeda Chemical Industries, Ltd., Tokyo (Japan).

EXECUTIVE SUMMARY: In repeat reverse gene mutation assays (MRID 45422817), five histidine-deficient mutants (*his*$^-$) of *Salmonella typhimurium* (TA 1535, TA100, TA1537, TA98, TA102) were exposed to six concentrations (ranging from 16 to 5000 μg/plate) of TI 435-Benzyltriazinone (98.3% a.i.; Batch No. KTS 9475-11-2) in dimethylsulfoxide (DMSO), by plate incorporation as well as preincubation methods, and reversion to histidine prototrophy (*his*$^-$ to *his*$^+$) determined after 48 hours incubation at 37°C. Test article-treated strains were cultured in the presence and absence of metabolic activation provided by Aroclor 1254-activated rat liver homogenates (S9). Additional cultures were exposed to the DMSO vehicle as well as to strain-specific mutagens, the latter serving as positive controls.

TI 435-Benzyltriazinone did not cause any cytotoxicity up to the limit dose (5000 μg/plate), and **no increases in revertants (*his*$^+$) compared to DMSO negative controls was observed in any strain in the presence or absence of S9**. The positive controls all showed substantial increases in the frequency of revertants.

This study is classified as acceptable/guideline and satisfies the requirement for FIFRA Test Guideline 84-2 for *in vitro* mutagenicity (bacterial reverse gene mutation) data.

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

I. MATTERIALS AND METHODS

A. MATERIALS

1. Test Material: TI 435-Benzyltriazinone
   Description: White crystalline solid
   Lot/Batch No. KTS 947-5-11-2.
   Purity: 98.3% a.i. (Latest analytical result, dated May 5, 2000)
   Stability of compound: Not reported.
   CAS No.: Not reported.
   Solvent used: Dimethylsulfoxide (DMSO).
   Other comments: Stored at room temperature. It was not indicated by the sponsor what was the use of this compound.

2. Control Materials:

   Negative: None.
   Solvent/Final concentration: DMSO/0.1 mL/plate.

Positive: Nonactivated:	
Sodium azide	10 μg/plate for TA1535
Nitrofurantoin	0.2 μg/plate for TA100
4-NPDA[1]	10 μg/plate for TA1537
Cumene hydroperoxide	50 μg/plate for TA102
Other (list):	
4-NPNA	0.5 μg/plate for TA98
Activated:	
2-Aminoanthracene (2-anthramine)	3 μg/plate for all strains

[1]4-NPDA = 4-nitro-phenylene diamine

3. Metabolic Activation: S9 derived from adult (200-300g) Sprague-Dawley rats.

x	Aroclor 1254	x	induced	x	rat	x	liver
	phenobarbital		non-induced		mouse		lung
	none				hamster		other
	other						other

Describe S9 mix composition:

Seventy mL of cofactor solution contained:

$MgCl_2$ x 6 H_2O	162.6 mg
KCl	246.0 mg
Glucose-6-phosphate, disodium salt	179.1 mg
NADP, disodium salt	315.0 mg
Phosphate buffer	100.0 mM

4. Test Organisms: *Salmonella typhimurium* strains:

	TA97	x	TA98	x	TA100	x	TA102
	TA104	x	TA1535	x	TA1537		TA1538

List any others: None.

Properly maintained? Yes.

Checked for appropriate genetic markers (rfa mutation, R factor)? Yes.

5. Test Compound Concentrations Used:

Cytotoxicity test: (Not performed).

Main Assays:

Non-activated conditions	16, 50, 158, 500, 1581, 5000 μg/plate
Activated conditions:	16, 50, 158, 500, 1581, 5000 μg/plate

B. TEST PERFORMANCE

The initial plate incorporation test followed the directions of Ames *et al.* (1973a, 1975)[2] and Maron and Ames (1983)[3]. Triplicate cultures were prepared for each concentration of each strain for both assays.

1. Type of *Salmonella* assay:

x	standard plate test
x	preincubation (20 minutes)
	"Prival" modification
	spot test
	other (describe)

2. Criteria for both assay acceptance and assessment of results were presented.

---

[2]Ames, B.N., W.E. Durston, E. Yamasaki and F.D. Lee:
Carcinogens are mutagens: a simple test system combining liver homogenates for activation and bacteria for detection.
Proc. Nat. Acad. Sci. (USA) 70, 2281-2285, 1973a.

Ames, B.N., J. McCann and E. Yamasaki:
Methods for detecting carcinogens and mutagens with the *Salmonella*/mammalian-microsome mutagenicity test.
Mutation Res. 31, 347-364, 1975.

[3]Maron, D.M. and B.N. Ames: Revised methods for the *Salmonella* mutagenicity test.
Mutation Res. 113, 173-215, 1983.

II REPORTED RESULTS

A. PRELIMINARY CYTOTOXICITY ASSAY

No preliminary cytotoxicity assay was performed.

B. MUTAGENICITY ASSAY

There was no indication of cytotoxicity (bacteriostatisis and growth inhibition) at any dose in any test article-treated strain in the presence or absence of metabolic activation. None of the treated strains showed a dose-related increase in revertant counts in either the initial (plate incorporation) or repeat (preincubation) assays (MRID 45422817, pp. 25-27; pp. 42-51 - ATTACHMENT Tables 1-10). The positive control cultures all exhibited substantial increases in revertant frequencies. Therefore, the investigator concluded that TI 435-Benzyltriazinone was not mutagenic in these mutagenic assays in *Salmonella typhimurium.*

III. REVIEWER'S DISCUSSION/CONCLUSIONS

A. The reviewer agrees with the investigator's conclusion that TI 435-Benzyltriazinone was non-mutagenic when tested in *Salmonella typhimurium* mutagenicity assays by conventionally acceptable procedures.

B. STUDY DEFICIENCIES

None.

ATTACHMENT

THE FOLLOWING ATTACHMENT IS NOT AVAILABLE ELECTRONICALLY
SEE THE FILE COPY

5. Results

5.1. Description of Results

The colony number of each plate and mean values are listed for each dose in Tables 1 to 10. As may be seen, there was no indication of a bacteriotoxic effect of TI 435-Benzyltriazinone at doses of up to and including 5000 µg per plate. The total bacteria counts consistently produced results comparable to the negative controls, or differed only insignificantly. No inhibition of growth was noted as well.

None of the five strains concerned showed in the plate incorporation test a dose-related and biologically relevant increase in mutant counts over those of the negative controls. This applied both to the tests with and without S9 mix (Tables 1 to 5) and was confirmed by the results of the preincubation trials (Tables 6 to 10).

Summary of the Results with TI 435-Benzyltriazinone
in the Salmonella/Microsome Test

S9 mix	TA 1535	TA 100	TA 1537	TA 98	TA 102
without	-ve	-ve	-ve	-ve	-ve
with	-ve	-ve	-ve	-ve	-ve

-ve = negative

The positive controls sodium azide, nitrofurantoin, 4-nitro-1,2-phenylene diamine, cumene hydroperoxide and 2-aminoanthracene increased mutant counts to well over those of the negative controls, and thus demonstrated the system's sensitivity and the activity of the S9 mix.

215

5.2. Tabulated Summary of Data

Summary of Mean Values Without S9 Mix

Table and Group	Strain				
	TA 1535	TA 100	TA 1537	TA 98	TA 102
1-5 µg/Plate					
0	6	55	6	19	124
16	5	63	6	18	118
50	5	55	6	16	124
158	7	52	6	15	112
500	6	62	7	15	104
1581	6	69	4	17	101
5000	5	58	6	13	73
Na-azide	549				
NF		193			
4-NPDA			99	170	
Cumene					270
6-10 µg/Tube					
0	8	62	7	13	237
16	5	68	11	14	241
50	4	62	7	10	233
158	7	57	6	13	233
500	6	64	5	16	228
1581	5	62	7	15	237
5000	8	67	8	14	223
Na-azide	532				
NF		206			
4-NPDA			127	142	
Cumene					589

Summary of Mean Values With S9 Mix

Table and Group	Strain				
	TA 1535	TA 100	TA 1537	TA 98	TA 102
1-5 µg/Plate					
0	9	73	9	26	207
16	10	73	5	23	198
50	10	76	6	22	179
158	8	77	9	24	195
500	14	76	7	23	198
1581	7	78	8	25	194
5000	10	72	8	27	188
2-AA	221	1663	120	1476	414
6-10 µg/Tube					
0	9	69	9	14	309
16	10	71	9	18	345
50	10	79	10	19	343
158	9	76	12	16	345
500	8	88	7	21	301
1581	9	81	5	19	293
5000	5	91	6	18	289
2-AA	123	1036	149	1147	481

10. Abbreviations used in the Tables

M	mean
SD	standard deviation
-S9	without S9 mix
+S9	with S9 mix
%	not tested
P	precipitate
B	background lawn reduced
C	contaminated
*	mutagenic effect
**	bacteriotoxic effect

11. Tables 1-10

B A Y E R A.G. Table : 1 Study Number : T 6068886
DEPARTMENT OF TOXICOLOGY Study Director : Dr. Herbold
PHARMA RESEARCH CENTER Technician : Düver
WUPPERTAL ELBERFELD Date : June 8, 2000
AMES TEST with : TI 435-Benzyltriazanone Strain: S.typhimurium TA 1535

Dose/Plate (µg/Plate)	REVERTANTS PER PLATE -S9	M	SD	10% +S9	M	SD	TITER Dilution 10^{-5}	TITER per ml 10^{-8}	QUOTIENT -S9	QUOTIENT +S9
DMSO	5 6 6	6	1	9 11 8	9	2	139 154	14.7	1.0	1.0
16	5 5 6	5	1	9 9 11	10	1	173 188	18.1	0.9	1.0
50	7 4 3	5	2	9 9 13	10	2	172 190	18.1	0.8	1.1
158	6 6 9	7	2	8 9 8	8	1	165 187	17.6	1.2	0.9
500	5 5 7	6	1	13 17 11	14	3	150 175	16.3	1.0	1.5
1581	7 7 5	6	1	6 6 10	7	2	160 178	16.9	1.1	0.8
5000	3 5 8	5	3	14 11 6	10	4	164 169	16.7	0.9	1.1
Na-azide 10	574 542 532	549	22	%	/	/	174 181	17.8	96.9*	/
2-AA 3	%	/	/	226 246 190	221	28	87 79	8.3	/	23.6*

219

B A Y E R A.G. Table : 2 Study Number : T 6068886
DEPARTMENT OF TOXICOLOGY Study Director : Dr.Herbold
PHARMA RESEARCH CENTER Technician : Düver
WUPPERTAL ELBERFELD Date : June 8, 2000
AMES TEST with : TI 435-Benzyltriazanone Strain: S.typhimurium TA 100

Dose/Plate (µg/Plate)	REVERTANTS PER PLATE -S9	M	SD	10% +S9	M	SD	TITER Dilution 10^{-6}	TITER per ml 10^{+8}	QUOTIENT -S9	QUOTIENT +S9
DMSO	51 57 58	55	4	64 79 77	73	8	81 89	8.5	1.0	1.0
16	68 72 50	63	12	74 84 60	73	12	83 101	9.2	1.1	1.0
50	45 47 74	55	16	72 83 74	76	6	90 80	8.5	1.0	1.0
158	53 53 51	52	1	72 93 67	77	14	80 89	8.5	0.9	1.1
500	63 50 74	62	12	70 78 81	76	6	91 118	10.5	1.1	1.0
1581	62 79 66	69	9	77 75 82	78	4	106 103	10.5	1.2	1.1
5000	52 61 60	58	5	64 74 79	72	8	69 102	8.6	1.0	1.0
NF 0.2	196 152 230	193	39	%	/	/	101 78	9.0	3.5*	/
2-AA 3	%	/	/	1835 1473 1682	1663	182	66 67	6.7	/	22.7*

220

B A Y E R A.G. Table : 3 Study Number : T 6068886
DEPARTMENT OF TOXICOLOGY Study Director : Dr.Herbold
PHARMA RESEARCH CENTER Technician : Düver
WUPPERTAL ELBERFELD Date : June 8, 2000
AMES TEST with : TI 435-Benzyltriazanone Strain: S.typhimurium TA 1537

Dose/Plate (µg/Plate)	REVERTANTS PER PLATE -S9	M	SD	10% +S9	M	SD	TITER Dilution 10^{-6}	TITER per ml 10^{-8}	QUOTIENT -S9	QUOTIENT +S9
DMSO	7 6 4	6	2	12 8 7	9	3	77 118	9.8	1.0	1.0
16	6 5 6	6	1	5 7 4	5	2	117 132	12.5	1.0	0.6
50	9 5 5	6	2	4 7 8	6	2	107 126	11.7	1.1	0.7
158	5 9 5	6	2	9 11 7	9	2	100 100	10.0	1.1	1.0
500	5 7 8	7	2	6 9 7	7	2	107 128	11.8	1.2	0.8
1581	5 3 5	4	1	11 6 7	8	3	125 101	11.3	0.8	0.9
5000	7 5 7	6	1	6 11 6	8	3	130 124	12.7	1.1	0.9
4-NPDA 10	102 104 91	99	7	%	/	/	118 129	12.4	17.5*	/
2-AA 3	%	/	/	123 108 130	120	11	107 92	10.0	/	13.4*

2-21

B A Y E R A.G. Table : 4 Study Number : T 6068886
DEPARTMENT OF TOXICOLOGY Study Director : Dr.Herbold
PHARMA RESEARCH CENTER Technician : Düver
WUPPERTAL ELBERFELD Date : June 8, 2000
AMES TEST with : TI 435-Benzyltriazanone Strain: S.typhimurium TA 98

	REVERTANTS PER PLATE						TITER		QUOTIENT	
Dose/Plate (µg/Plate)	-S9	M	SD	10% +S9	M	SD	Dilution 10^{-6}	per ml 10^{-8}	-S9	+S9
DMSO	19 17 20	19	2	25 24 30	26	3	220 192	20.6	1.0	1.0
16	19 17 19	18	1	26 21 21	23	3	206 218	21.2	1.0	0.9
50	15 20 13	16	4	26 21 19	22	4	196 204	20.0	0.9	0.8
158	17 18 9	15	5	27 24 21	24	3	217 208	21.3	0.8	0.9
500	18 12 14	15	3	28 21 21	23	4	165 189	17.7	0.8	0.9
1581	17 20 13	17	4	26 27 21	25	3	176 181	17.9	0.9	0.9
5000	13 13 13	13	0	26 27 29	27	2	184 180	18.2	0.7	1.0
4-NPDA 0.5	164 149 196	170	24	%	/	/	169 206	18.8	9.1*	/
2-AA 3	%	/	/	1470 1377 1580	1476	102	175 138	15.7	/	56.0*

222

B A Y E R A.G. Table : 5 Study Number : T 6068886
DEPARTMENT OF TOXICOLOGY Study Director : Dr.Herbold
PHARMA RESEARCH CENTER Technician : Düver
WUPPERTAL ELBERFELD Date : June 8, 2000
AMES TEST with : TI 435-Benzyltriazanone Strain: S.typhimurium TA 102

Dose/Plate (µg/Plate)	REVERTANTS PER PLATE -S9	M	SD	10% +S9	M	SD	TITER Dilution 10^{-6}	TITER per ml 10^{+8}	QUOTIENT -S9	QUOTIENT +S9
DMSO	117 125 131	124	7	203 197 222	207	13	60 117	8.9	1.0	1.0
16	113 101 141	118	21	200 192 203	198	6	73 84	7.9	1.0	1.0
50	130 126 116	124	7	156 211 171	179	28	65 45	5.5	1.0	0.9
158	123 104 109	112	10	208 186 190	195	12	45 89	6.7	0.9	0.9
500	84 113 115	104	17	210 171 214	198	24	44 87	6.6	0.8	1.0
1581	97 96 109	101	7	180 200 203	194	13	41 63	5.2	0.8	0.9
5000	67 64 89	73	14	203 176 185	188	14	55 52	5.4	0.6	0.9
Cumene 50	248 276 287	270	20	%	/	/	68 44	5.6	2.2*	/
2-AA 3	%	/	/	425 405 411	414	10	31 33	3.2**	/	2.0*

223

B A Y E R A.G. Table : 6 Study Number : T 6068886
DEPARTMENT OF TOXICOLOGY Study Director : Dr.Herbold
PHARMA RESEARCH CENTER Technician : Düver
WUPPERTAL ELBERFELD Date : June 16, 2000
PREINCUBATION with : TI 435-Benzyltriazanone Strain: S.typhimurium TA 1535

Dose/Tube (µg/Tube)	REVERTANTS PER PLATE -S9	M	SD	10% +S9	M	SD	TITER Dilution 10^{-6}	TITER per ml 10^{-8}	QUOTIENT -S9	QUOTIENT +S9
DMSO	10 6 8	8	2	10 10 7	9	2	51 51	5.1	1.0	1.0
16	5 6 4	5	1	11 9 11	10	1	59 48	5.4	0.6	1.1
50	5 3 4	4	1	11 9 9	10	1	45 58	5.2	0.5	1.1
158	8 6 7	7	1	12 7 8	9	3	64 69	6.7	0.9	1.0
500	5 5 8	6	2	11 6 7	8	3	57 58	5.8	0.8	0.9
1581	4 6 4	5	1	8 10 8	9	1	39 43	4.1	0.6	1.0
5000	11 6 6	8	3	3 3 9	5	3	37 39	3.8	1.0	0.6
Na-azide 10	561 537 497	532	32	%	/	/	61 45	5.3	66.5*	/
2-AA 3	%	/	/	122 122 126	123	2	54 55	5.5	/	13.7*

224

B A Y E R A.G. Table : 7 Study Number : T 6068886
DEPARTMENT OF TOXICOLOGY Study Director : Dr.Herbold
PHARMA RESEARCH CENTER Technician : Düver
WUPPERTAL ELBERFELD Date : June 16, 2000
PREINCUBATION with : TI 435-Benzyltriazanone Strain: S.typhimurium TA 100

Dose/Tube (µg/Tube)	REVERTANTS PER PLATE -S9	M	SD	10% +S9	M	SD	TITER Dilution 10^{-6}	TITER per ml 10^{-8}	QUOTIENT -S9	QUOTIENT +S9
DMSO	59 68 58	62	6	63 69 74	69	6	30 39	3.5	1.0	1.0
16	74 62 69	68	6	69 77 66	71	6	61 62	6.2	1.1	1.0
50	66 68 53	62	8	81 71 86	79	8	41 58	5.0	1.0	1.2
158	55 52 63	57	6	78 74 75	76	2	62 64	6.3	0.9	1.1
500	60 59 73	64	8	86 82 96	88	7	41 48	4.5	1.0	1.3
1581	67 68 52	62	9	92 62 88	81	16	48 44	4.6	1.0	1.2
5000	80 60 61	67	11	79 101 92	91	11	48 46	4.7	1.1	1.3
NF 0.2	213 212 194	206	11	%	/	/	47 48	4.8	3.3*	/
2-AA 3	%	/	/	968 1063 1076	1036	59	49 52	5.1	/	15.1*

225

B A Y E R A.G. Table : 8 Study Number : T 6068886
DEPARTMENT OF TOXICOLOGY Study Director : Dr.Herbold
PHARMA RESEARCH CENTER Technician : Düver
WUPPERTAL ELBERFELD Date : June 16, 2000
PREINCUBATION with : TI 435-Benzyltriazanone Strain: S.typhimurium TA 1537

Dose/Tube (µg/Tube)	REVERTANTS PER PLATE -S9	M	SD	10% +S9	M	SD	TITER Dilution 10^{-6}	TITER per ml 10^{-8}	QUOTIENT -S9	QUOTIENT +S9
DMSO	7 8 6	7	1	8 9 11	9	2	63 60	6.2	1.0	1.0
16	11 9 12	11	2	8 9 11	9	2	74 48	6.1	1.5	1.0
50	8 7 5	7	2	6 11 12	10	3	86 59	7.3	1.0	1.0
158	5 5 8	6	2	15 9 11	12	3	71 69	7.0	0.9	1.3
500	3 5 6	5	2	9 4 9	7	3	66 62	6.4	0.7	0.8
1581	9 4 8	7	3	7 4 5	5	2	82 64	7.3	1.0	0.6
5000	11 5 7	8	3	8 5 6	6	2	68 55	6.2	1.1	0.7
4-NPDA 10	118 122 141	127	12	%	/	/	75 61	6.8	18.1*	/
2-AA 3	%	/	/	155 154 139	149	9	74 74	7.4	/	16.0*

224

B A Y E R A.G. Table : 9 Study Number : T 6068886
DEPARTMENT OF TOXICOLOGY Study Director : Dr.Herbold
PHARMA RESEARCH CENTER Technician : Düver
WUPPERTAL ELBERFELD Date : June 16, 2000
PREINCUBATION with : TI 435-Benzyltriazanone Strain: S.typhimurium TA 98

Dose/Tube (µg/Tube)	REVERTANTS PER PLATE -S9	M	SD	10% +S9	M	SD	TITER Dilution 10^{-6}	TITER per ml 10^{+8}	QUOTIENT -S9	QUOTIENT +S9
DMSO	13 13 12	13	1	12 15 16	14	2	129 112	12.1	1.0	1.0
16	14 15 14	14	1	16 16 21	18	3	173 165	16.9	1.1	1.2
50	9 9 13	10	2	21 18 17	19	2	158 161	16.0	0.8	1.3
158	16 16 8	13	5	13 16 20	16	4	159 146	15.3	1.1	1.1
500	15 17 16	16	1	21 24 17	21	4	157 135	14.6	1.3	1.4
1581	16 14 14	15	1	24 18 16	19	4	157 145	15.1	1.2	1.3
5000	9 16 16	14	4	20 19 15	18	3	146 124	13.5	1.1	1.3
4-NPDA 0.5	141 144 141	142	2	%	/	/	160 144	15.2	11.2*	/
2-AA 3	%	/	/	1053 1209 1179	1147	83	147 132	14.0	/	80.0*

227

B A Y E R A.G. Table : 10 Study Number : T 6068886
DEPARTMENT OF TOXICOLOGY Study Director : Dr.Herbold
PHARMA RESEARCH CENTER Technician : Düver
WUPPERTAL ELBERFELD Date : June 16, 2000
PREINCUBATION with : TI 435-Benzyltriazanone Strain: S.typhimurium TA 102

Dose/Tube (µg/Tube)	REVERTANTS PER PLATE -S9	M	SD	10% +S9	M	SD	TITER Dilution 10^{-6}	TITER per ml 10^{-8}	QUOTIENT -S9	QUOTIENT +S9
DMSO	228 233 250	237	12	334 297 297	309	21	399 418	40.9	1.0	1.0
16	260 252 212	241	26	312 339 384	345	36	412 386	39.9	1.0	1.1
50	238 228 232	233	5	331 339 360	343	15	421 432	42.7	1.0	1.1
158	251 239 210	233	21	339 323 372	345	25	442 429	43.6	1.0	1.1
500	249 221 213	228	19	309 301 294	301	8	389 369	37.9	1.0	1.0
1581	227 228 255	237	16	261 320 298	293	30	344 400	37.2	1.0	0.9
5000	221 228 219	223	5	291 288 288	289	2	410 387	39.9	0.9	0.9
Cumene 50	568 577 621	589	28	%	/	/	363 326	34.5	2.5*	/
2-AA 3	%	/	/	494 483 466	481	14	355 360	35.8	/	1.6*

228

CLOTHIANIDIN *SALMONELLA*/MAMMALIAN ACTIVATION; GENE MUTATION (84-2)

EPA Reviewer: Irving Mauer, Ph.D. Date: 06/13/02
Registration Action Branch 3, HED (7509C)
EPA Secondary Reviewer: Nancy McCarroll Date: 11/14/02
Toxicology Branch 1, HED (7509C)

TXR NO. 0050321

DATA EVALUATION RECORD

STUDY TYPE: Bacterial system: *Salmonella*/mammalian activation gene mutation assay; OPPTS 870.5100 [84-2].

DP BARCODE: D279778 SUBMISSION CODE: S607124
P.C. CODE: 044309 TOX. CHEM. CODE: None.

TEST MATERIAL (PURITY): TI 435-CCMT-Adduct (99.5% a.i.; Batch No. 17007/99)

SYNONYMS: Chemically: 1-(2-chlorothiazol-5-ylmethyl)-2-nitro-imino-5-benzyl-3-methyl-1, 3, 5-triazacyclohexane; $C_{15}H_{17}ClN_6O_2S$.

CITATION: Herbold, B. (2000). TI 435-CCMT-Adduct. *Salmonella*/Microsome Test. Plate Incorporation and Preincubation Method, performed at Bayer AG's Department of Toxicology, Wuppertal (Germany), August 8, 2000. Laboratory Study No. T8068860 (Bayer AG Report No. PH 30144; Bayer Corporation Agricultural Division Report No. 110216). MRID 45422816. Unpublished.

SPONSOR: Takeda Chemical Industries, Ltd., Agro Company, Tokyo (Japan).

EXECUTIVE SUMMARY: In repeat reverse gene mutation assays (MRID 45422816), five histidine-deficient (*his*$^-$) mutant strains of *Salmonella typhimurium* were exposed to a range of six concentrations (from 16 to 5000 μg/plate) of TI 435-CCMT-Adduct (99.5% a.i.; Batch No. 17007/99) in dimethylsulfoxide (DMSO) by both plate incorporation as well as preincubation methods, and revertant colonies to prototrophy (*his*$^-$ to *his*$^+$) counted after 48 hours incubation at 37°C. Additional cultures were exposed to the vehicle (DMSO) and to strain-specific mutagens, the latter to serve as positive controls. Assays were conducted in the presence and absence of mammalian activation (S9).

The test article was assayed up to the dose limit (5000 μg/plate), and showed a weak cytotoxic effect at doses > 500 μg/plate, and substance precipitation at 5000 μg/plate. However, **no evidence of a mutagenic effect (increase in *his*$^+$ revertants compared to vehicle controls) was observed in any culture, either in the presence or absence of metabolic activation in either the initial or repeat assay.**

Therefore, TI 435-CCMT-Adduct is considered to be non-mutagenic in these bacterial assays with *Salmonella*.

This study is classified as acceptable/guideline and satisfies the requirement for FIFRA Test Guideline 84-2 for *in vitro* mutagenicity (bacterial reverse gene mutation) data.

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

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Materials: TI 435-CCMT-Adduct

Description: Light brown powder
Lot/Batch No.: 17007/99
Purity: 99.5% a.i. (latest analytical result, May 5, 2000)
Stability of compound: Not reported.
CAS No.: Not reported.
Solvent used: Dimethylsulfoxide (DMSO)
Other comments: Stored at room temperature. Use not indicated by sponsor.

2. Control Materials:

Negative: None.
Solvent/Final concentration: DMSO/0.1 mL/plate
Positive: Non-activated:

Positive: Non-activated:	
Sodium azide	10 µg/plate for TA1535
Nitrofurantoin	0.2 µg/plate for TA100
4-NDPA[1]	10 µg/plate for TA1537
Cumene hydroperoxide	50 µg/plate for TA102
Other (list):	
4-NDPA	0.5 µg/plate for TA98

Activated:	
2-Aminoanthracene (2-anthramine)	3 µg/plate for all strains

[1]4-NDPA = 4-nitro-1,2-phenylene diamine.

3. Metabolic Activation: S9 derived from adult (200-300 g) male Sprague-Dawley rats.

x	Aroclor 1254	x	innduced	x	rat	x	liver
	phenobarbital		non-induced		mouse		lung
	none				hamster		other
	other						other

Describe S9 mix composition:

Seventy mL of co-factor solution contained:

$MgCl_2$ x 6 H_2O	162.6
KCl	246.0
Glucose-6-phosphate, disodium salt	179.1
NADP, disodium salt	315.0
Phosphate buffer	100 mM

4. Test Organisms: *Salmonella typhimurium* strains:

	TA97	x	TA98	x	TA100	x	TA102
	TA104	x	TA1535	x	TA1537		TA1538

Properly maintained? Yes.

Checked for appropriate genetic markers (rfa mutation, R factor)? Yes.

5. Test Compound Concentrations used:

Cytotoxicity Test: Not performed.

Main Assays:

Non-activated conditions: 16, 50, 158, 500, 1581, 5000 μg/plate.
Activated conditions: 16, 50, 158, 500, 1581, 5000 μg/plate.

B. TEST PERFORMANCE

The initial plate incorporation assay followed the directions of Ames *et al.* (1973a, 1975)[2] and Maron and Ames (1983)[3]. Triplicate plates for each strain and concentration of test article and both control cultures were provided.

1. Type of *Salmonella* Assay:

x	standard plate test
x	pre-incubation (20 minutes)
	"Prival" modification
	spot test
	other (describe)

2. Criteria for both assay acceptance and assesment of results were provided.

II REPORTED RESULTS

A. PRELIMINARY CYTOTOXICITY ASSAY

Not performed.

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[2]Ames, B.N., W.E. Durston, E. Yamasaki and F.D. Lee:
Carcinogens are mutagens: A simple test system combining liver homogenates for activation and bacteria for detection .
Proc. Nat. Acad. Sci. (USA) 70, 2281-2285, 1973a.

Ames, B.N., J. McCann and E. Yamasaki:
Methods for detecting carcinogens and mutagens with the *Salmonella*/mammalian-microsome mutgenicity test.
Mutation Res. 31, 347-364, 1975.

[3]Maron, D.M. and B.N. Ames:
Revised methods for the *Salmonella* mutagenicity test.
Mutation Res. 113, 173-215, 1983.

B. MUTAGENICITY ASSAY

Weak cytotoxicity was observed at concentrations greater than 500 μg/plate, and precipitation at 5000 μg/plate, **but at no concentration in any test article-treated strain (±S9) was there a biologically relevant increase in revertant (*his*$^+$) colonies compared to the DMSO control.** (MRID 45422816, pp. 25-26; 42-51 - ATTACHMENT Tables 1-10). Positive control cultures showed the expected large increases in revertants.

Therefore, the investigator concluded that TI 435-CCMT-Adduct is non-mutagenic in these assays.

III. REVIEWER'S DISCUSSION/CONCLUSIONS:

A. The reviewer agrees with the investigator that this comprehensive series of assays in *Salmonella* demonstrates no mutagenic activity.

B. STUDY DEFICIENCIES

None.

ATTACHMENT

THE FOLLOWING ATTACHMENT IS NOT AVAILABLE ELECTRONICALLY
SEE THE FILE COPY

5. Results

5.1. Description of Results

The colony number of each plate and mean values are listed for each dose in Tables 1 to 10. As may be seen, there was no indication of a bacteriotoxic effect of TI 435-CCMT-Adduct at doses of up to and including 500 µg per plate. The total bacteria counts consistently produced results comparable to the negative controls, or differed only insignificantly. No inhibition of growth was noted as well. Higher doses had only a weak, strain-specific bacteriotoxic effect. Therefore they could nevertheless be used for assessment purposes up to and including 5000 µg per plate. At 5000 µg per plate, the substance precipitated.

None of the five strains concerned showed in the plate incorporation test a dose-related and biologically relevant increase in mutant counts over those of the negative controls. This applied both to the tests with and without S9 mix (Tables 1 to 5) and was confirmed by the results of the preincubation trials (Tables 6 to 10).

Summary of the Results with TI 435-CCMT-Adduct
in the Salmonella/Microsome Test

S9 mix	TA 1535	TA 100	TA 1537	TA 98	TA 102
without	-ve	-ve	-ve	-ve	-ve
with	-ve	-ve	-ve	-ve	-ve

-ve = negative

The positive controls sodium azide, nitrofurantoin, 4-nitro-1,2-phenylene diamine, cumene hydroperoxide and 2-aminoanthracene increased mutant counts to well over those of the negative controls, and thus demonstrated the system's sensitivity and the activity of the S9 mix.

Summary of Mean Values With S9 Mix

Table and Group	Strain				
	TA 1535	TA 100	TA 1537	TA 98	TA 102
1-5 µg/Plate					
0	11	88	10	23	310
16	10	100	11	21	328
50	9	100	7	19	310
158	10	101	11	24	326
500	9	100	10	22	305
1581	8	100	9	23	303
5000	10	77	8	28	246
2-AA	223	1552	253	1599	525
6-10 µg/Tube					
0	9	86	12	22	317
16	10	76	11	23	340
50	9	88	9	20	332
158	10	81	10	26	321
500	11	80	9	26	316
1581	8	81	7	19	297
5000	5	62	4	21	258
2-AA	184	1161	205	1297	426

238

10. Abbreviations used in the Tables

M	mean
SD	standard deviation
-S9	without S9 mix
+S9	with S9 mix
%	not tested
P	precipitate
B	background lawn reduced
C	contaminated
*	mutagenic effect
**	bacteriotoxic effect

11. Tables 1-10

B A Y E R A.G.
DEPARTMENT OF TOXICOLOGY
PHARMA RESEARCH CENTER
WUPPERTAL ELBERFELD
AMES TEST with : TI 435-CCMT-Adduct

Table : 1

Study Number : T 8068860
Study Director : Dr.Herbold
Technician : Bönning
Date : June 5, 2000
Strain: S.typhimurium TA 1535

Dose/Plate (µg/Plate)	REVERTANTS PER PLATE -S9	M	SD	10% +S9	M	SD	TITER Dilution 10^{-6}	TITER per ml 10^{-8}	QUOTIENT -S9	QUOTIENT +S9
DMSO	11 10 8	10	2	9 10 13	11	2	124 144	13.4	1.0	1.0
16	8 13 9	10	3	12 6 13	10	4	123 114	11.9	1.0	1.0
50	7 6 7	7	1	7 11 8	9	2	137 134	13.6	0.7	0.8
158	10 6 5	7	3	7 14 10	10	4	122 126	12.4	0.7	1.0
500	7 8 7	7	1	6 8 14	9	4	117 145	13.1	0.8	0.9
1581	7 11 11	10	2	7 6 12	8	3	141 121	13.1	1.0	0.8
5000	13 10 8	10	3	9 10 12	10	2	141 96	11.9	1.1	1.0
Na-azide 10	763 709 716	729	29	%	/	/	119 123	12.1	75.4*	/
2-AA 3	%	/	/	271 195 202	223	42	77 84	8.1**	/	20.9*

B A Y E R A.G.
DEPARTMENT OF TOXICOLOGY
PHARMA RESEARCH CENTER
WUPPERTAL ELBERFELD
AMES TEST with : TI 435-CCMT-Adduct

Table : 2

Study Number : T 8068860
Study Director : Dr.Herbold
Technician : Bönning
Date : June 5, 2000
Strain: S.typhimurium TA 100

Dose/Plate (µg/Plate)	REVERTANTS PER PLATE -S9	M	SD	10% +S9	M	SD	TITER Dilution 10^{-6}	TITER per ml 10^{+8}	QUOTIENT -S9	QUOTIENT +S9
DMSO	84 82 85	84	2	78 88 97	88	10	63 73	6.8	1.0	1.0
16	77 83 84	81	4	102 85 112	100	14	57 82	7.0	1.0	1.1
50	81 84 75	80	5	86 111 104	100	13	90 77	8.4	1.0	1.1
158	74 83 85	81	6	95 114 93	101	12	88 91	9.0	1.0	1.1
500	77 94 75	82	10	94 95 112	100	10	91 83	8.7	1.0	1.1
1581	88 70 60	73	14	102 101 97	100	3	45 68	5.7	0.9	1.1
5000	66 B 68 B 56 B	63	6	55 B 89 B 86 B	77	19	61 50	5.6	0.8	0.9
NF 0.2	275 237 231	248	24	%	/	/	96 82	8.9	3.0*	/
2-AA 3	%	/	/	1527 1573 1555	1552	23	64 55	6.0	/	17.7*

B A Y E R A.G. Table : 3 Study Number : T 8068860
DEPARTMENT OF TOXICOLOGY Study Director : Dr.Herbold
PHARMA RESEARCH CENTER Technician : Bönning
WUPPERTAL ELBERFELD Date : June 5, 2000
AMES TEST with : TI 435-CCMT-Adduct Strain: S.typhimurium TA 1537

Dose/Plate (µg/Plate)	REVERTANTS PER PLATE -S9	M	SD	10% +S9	M	SD	TITER Dilution 10^{-6}	TITER per ml 10^{-8}	QUOTIENT -S9	QUOTIENT +S9
DMSO	9 11 6	9	3	14 7 9	10	4	95 140	11.8	1.0	1.0
16	8 6 5	6	2	9 12 11	11	2	114 113	11.4	0.7	1.1
50	6 9 4	6	3	6 9 6	7	2	107 115	11.1	0.7	0.7
158	4 5 6	5	1	8 13 12	11	3	114 116	11.5	0.6	1.1
500	6 6 5	6	1	13 7 9	10	3	118 125	12.2	0.7	1.0
1581	5 8 6	6	2	8 13 6	9	4	118 117	11.8	0.7	0.9
5000	6 B 3 B 4 B	4	2	3 13 8	8	5	119 107	11.3	0.5	0.8
4-NPDA 10	92 105 95	97	7	%	/	/	121 136	12.9	11.2*	/
2-AA 3	%	/	/	289 266 203	253	45	117 119	11.8	/	25.3*

242

B A Y E R A.G.
DEPARTMENT OF TOXICOLOGY
PHARMA RESEARCH CENTER
WUPPERTAL ELBERFELD
AMES TEST with : TI 435-CCMT-Adduct

Table : 4

Study Number : T 8068860
Study Director : Dr.Herbold
Technician : Bönning
Date : June 5, 2000
Strain: S.typhimurium TA 98

Dose/Plate (µg/Plate)	REVERTANTS PER PLATE -S9	M	SD	10% +S9	M	SD	TITER Dilution 10^{-6}	TITER per ml 10^{-8}	QUOTIENT -S9	QUOTIENT +S9
DMSO	10 20 23	18	7	24 22 23	23	1	201 223	21.2	1.0	1.0
16	13 26 18	19	7	19 22 23	21	2	240 224	23.2	1.1	0.9
50	19 14 15	16	3	14 20 22	19	4	215 203	20.9	0.9	0.8
158	17 9 13	13	4	17 27 27	24	6	208 189	19.9	0.7	1.0
500	18 19 22	20	2	16 24 25	22	5	201 197	19.9	1.1	0.9
1581	19 7 11	12	6	18 27 25	23	5	210 223	21.7	0.7	1.0
5000	22 16 17	18	3	30 27 26	28	2	189 190	19.0	1.0	1.2
4-NPDA 0.5	164 181 167	171	9	%	/	/	242 203	22.3	9.7*	/
2-AA 3	%	/	/	1596 1643 1558	1599	43	203 169	18.6	/	69.5*

243

B A Y E R A.G. Table : 5 Study Number : T 8068860
DEPARTMENT OF TOXICOLOGY Study Director : Dr.Herbold
PHARMA RESEARCH CENTER Technician : Bönning
WUPPERTAL ELBERFELD Date : June 5, 2000
AMES TEST with : TI 435-CCMT-Adduct Strain: S.typhimurium TA 102

Dose/Plate (µg/Plate)	REVERTANTS PER PLATE -S9	M	SD	10% +S9	M	SD	TITER Dilution 10^{-6}	TITER per ml 10^{+8}	QUOTIENT -S9	QUOTIENT +S9
DMSO	248 233 270	250	19	314 312 303	310	6	117 99	10.8	1.0	1.0
16	266 241 269	259	15	326 328 330	328	2	117 109	11.3	1.0	1.1
50	270 241 279	263	20	313 291 325	310	17	147 126	13.7	1.1	1.0
158	282 294 307	294	13	329 344 305	326	20	123 112	11.8	1.2	1.1
500	221 280 271	257	32	284 292 339	305	30	122 124	12.3	1.0	1.0
1581	272 253 242	256	15	303 305 300	303	3	68 104	8.6	1.0	1.0
5000	201 198 189	196	6	258 236 245	246	11	101 108	10.5	0.8	0.8
Cumene 50	438 397 366	400	36	%	/	/	113 97	10.5	1.6*	/
2-AA 3	%	/	/	522 510 544	525	17	56 113	8.5	/	1.7*

B A Y E R A.G. Table : 6 Study Number : T 8068860
DEPARTMENT OF TOXICOLOGY Study Director : Dr.Herbold
PHARMA RESEARCH CENTER Technician : Bönning
WUPPERTAL ELBERFELD Date : June 16, 2000
PREINCUBATION with : TI 435-CCMT-Adduct Strain: S.typhimurium TA 1535

Dose/Tube (µg/Tube)	REVERTANTS PER PLATE -S9	M	SD	10% +S9	M	SD	TITER Dilution 10^{-6}	TITER per ml 10^{-8}	QUOTIENT -S9	QUOTIENT +S9
DMSO	9 6 7	7	2	7 9 11	9	2	35 52	4.4	1.0	1.0
16	6 8 9	8	2	9 12 8	10	2	64 65	6.5	1.0	1.1
50	6 8 10	8	2	6 8 13	9	4	40 62	5.1	1.1	1.0
158	7 8 5	7	2	9 12 10	10	2	49 40	4.5	0.9	1.1
500	6 7 6	6	1	7 13 12	11	3	65 50	5.8	0.9	1.2
1581	8 9 7	8	1	8 10 6	8	2	34 29	3.2	1.1	0.9
5000	B P	/	/	6 B 5 B 4 P	5	1	12 15	1.4**	/	0.6
Na-azide 10	603 558 554	572	27	%	/	/	59 55	5.7	78.0	/
2-AA 3	%	/	/	193 203 156	184	25	43 37	4.0	/	20.4

B A Y E R A.G. Table : 7 Study Number : T 8068860
DEPARTMENT OF TOXICOLOGY Study Director : Dr.Herbold
PHARMA RESEARCH CENTER Technician : Bönning
WUPPERTAL ELBERFELD Date : June 16, 2000
PREINCUBATION with : TI 435-CCMT-Adduct Strain: S.typhimurium TA 100

Dose/Tube (µg/Tube)	REVERTANTS PER PLATE -S9	M	SD	10% +S9	M	SD	TITER Dilution 10^{-6}	per ml 10^{-8}	QUOTIENT -S9	+S9
DMSO	59 79 59	66	12	68 85 104	86	18	26 42	3.4	1.0	1.0
16	60 62 70	64	5	80 79 69	76	6	40 47	4.4	1.0	0.9
50	70 77 51	66	13	103 78 84	88	13	37 40	3.9	1.0	1.0
158	70 75 82	76	6	77 77 88	81	6	46 53	5.0	1.2	0.9
500	69 83 81	78	8	91 62 86	80	16	34 27	3.1	1.2	0.9
1581	56 50 58	55	4	63 73 106	81	23	25 40	3.3	0.8	0.9
5000	52 B 38 B 33 P	41	10	66 B 60 B 61 P	62	3	6 3	0.5**	0.6	0.7
NF 0.2	230 236 195	220	22	%	/	/	79 47	6.3	3.4*	/
2-AA 3	%	/	/	1166 1192 1125	1161	34	41 46	4.4	/	13.6*

246

B A Y E R A.G. Table : 8 Study Number : T 8068860
DEPARTMENT OF TOXICOLOGY Study Director : Dr.Herbold
PHARMA RESEARCH CENTER Technician : Bönning
WUPPERTAL ELBERFELD Date : June 16, 2000
PREINCUBATION with : TI 435-CCMT-Adduct Strain: S.typhimurium TA 1537

Dose/Tube (µg/Tube)	REVERTANTS PER PLATE -S9	M	SD	10% +S9	M	SD	TITER Dilution 10^{-6}	TITER per ml 10^{+8}	QUOTIENT -S9	QUOTIENT +S9
DMSO	12 8 8	9	2	13 14 9	12	3	80 79	8.0	1.0	1.0
16	8 8 10	9	1	9 13 11	11	2	95 84	9.0	0.9	0.9
50	10 11 9	10	1	7 12 8	9	3	122 93	10.8	1.1	0.8
158	8 12 9	10	2	14 8 9	10	3	86 83	8.5	1.0	0.9
500	12 8 9	10	2	8 11 7	9	2	114 99	10.7	1.0	0.7
1581	8 11 8	9	2	7 9 6	7	2	56 49	5.3**	1.0	0.6
5000	7 B 0 B 5 P	4	4	4 B 2 B 7 P	4	3	35 50	4.3**	0.4	0.4
4-NPDA 10	166 164 176	169	6	%	/	/	114 108	11.1	18.1*	/
2-AA 3	%	/	/	232 183 201	205	25	103 97	10.0	/	17.1*

297

B A Y E R A.G. Table : 9 Study Number : T 8068860
DEPARTMENT OF TOXICOLOGY Study Director : Dr.Herbold
PHARMA RESEARCH CENTER Technician : Bönning
WUPPERTAL ELBERFELD Date : June 16, 2000
PREINCUBATION with : TI 435-CCMT-Adduct Strain: S.typhimurium TA 98

Dose/Tube (µg/Tube)	REVERTANTS PER PLATE -S9	M	SD	10% +S9	M	SD	TITER Dilution 10^{-6}	TITER per ml 10^{-8}	QUOTIENT -S9	QUOTIENT +S9
DMSO	12 23 29	21	9	23 25 17	22	4	167 168	16.8	1.0	1.0
16	27 23 19	23	4	22 27 20	23	4	204 158	18.1	1.1	1.1
50	17 17 18	17	1	17 24 20	20	4	221 202	21.2	0.8	0.9
158	19 24 15	19	5	27 26 26	26	1	191 256	22.4	0.9	1.2
500	20 25 27	24	4	27 24 28	26	2	193 219	20.6	1.1	1.2
1581	20 17 13	17	4	22 21 15	19	4	172 221	19.7	0.8	0.9
5000	10 B 11 B 18 P	13	4	14 B 33 B 17 P	21	10	153 133	14.3	0.6	1.0
4-NPDA 0.5	156 182 177	172	14	%	/	/	225 198	21.2	8.0*	/
2-AA 3	%	/	/	1389 1261 1240	1297	81	208 189	19.9	/	59.8*

B A Y E R A.G. Table : 10 Study Number : T 8068860
DEPARTMENT OF TOXICOLOGY Study Director : Dr.Herbold
PHARMA RESEARCH CENTER Technician : Bönning
WUPPERTAL ELBERFELD Date : June 16, 2000
PREINCUBATION with : TI 435-CCMT-Adduct Strain: S.typhimurium TA 102

Dose/Tube (µg/Tube)	REVERTANTS PER PLATE -S9	M	SD	10% +S9	M	SD	TITER Dilution 10^{-6}	TITER per ml 10^{-8}	QUOTIENT -S9	QUOTIENT +S9
DMSO	222 245 256	241	17	309 318 324	317	8	332 358	34.5	1.0	1.0
16	283 277 274	278	5	316 312 393	340	46	358 364	36.1	1.2	1.1
50	224 284 262	257	30	301 334 360	332	30	342 329	33.6	1.1	1.0
158	241 271 254	255	15	315 323 324	321	5	337 352	34.5	1.1	1.0
500	247 273 232	251	21	319 329 301	316	14	369 333	35.1	1.0	1.0
1581	262 224 219	235	24	287 302 301	297	8	306 289	29.8	1.0	0.9
5000	109 B 135 B 153 P	132	22	264 B 284 B 225 P	258	30	303 355	32.9	0.5	0.8
Cumene 50	554 485 495	511	37	%	/	/	333 339	33.6	2.1*	/
2-AA 3	%	/	/	434 412 431	426	12	367 313	34.0	/	1.3*

CLOTHIANIDIN *SALMONELLA*/MAMMALIAN ACTIVATION; GENE MUTATION (84-2)

EPA Reviewer: Irving Mauer, Ph.D. Date: 06/13/02
Registration Action Branch 3, HED (7509C)
EPA Secondary Reviewer: Nancy McCarroll Date: 11/14/02
Toxicology Branch 1, HED (7509C)

TXR NO. 0050321

DATA EVALUATION RECORD

STUDY TYPE: Bacterial system, *e.g.*, *Salmonella*/mammalian activation gene mutation assay; OPPTS 870.5100 [84-2]

DP BARCODE: D279778 SUBMISSION CODE: S607124
P.C. CODE: 044309 TOX. CHEM. NO. None

TEST MATERIAL (PURITY): N-Methylnitroguanidin (97.3% a.i.; Batch No. VE96026Pt.17004/98 - Intermediate for TI 435)

SYNONYMS: $C_2H_6N_4O_2$

CITATION: Herbold, B. (2001). N-Methylnitroguanidin. *Salmonella*/Microsome Test. Plate Incorporation and Preincubation Method, performed at Bayer AG Department of Toxicology, Wuppertal (Germany). Bayer AG Study No. T0069528 (Bayer Corporation Agricultural Division Report No. 110246), Bayer AG Report No. PH 30755, dated February 12, 2001. MRID 45422812. Unpublished.

SPONSOR: Bayer Corporation, Agricultural Division.

EXECUTIVE SUMMARY: In repeat reverse gene mutation assays in bacteria (MRID 45422812), five histidine-requiring mutant strains (*his*$^-$) of *Salmonella typhimurium* (TA1535, TA100, TA1537, TA98 and TA102) were exposed in an initial plate-incorporation assay, as well as following a 20-minute preincubation period in the independent repeat assay, to a range of five concentrations (50 to 5000 μg/plate) of N-methylnitroguanidin technical (97.3% a.i., VE96026Pt.17004/98) in dimethylsulfoxide (DMSO), and histidine-proficient revertant colonies (*his*$^+$) counted after 48 hours incubation at 37°C. All tests were conducted both in the presence and absence of metabolic activation supplied by Aroclor 1254-induced liver homogenates (S9) supplemented with an active cofactor solution (S9-mix). Additional cultures were exposed to the DMSO vehicle and to strain-specific mutagens, the latter to serve as positive controls.

Although tested up to the dose limit (5000 μg/plate), none of the cultures showed growth inhibition, or a significant increase in *his*$^+$ colonies, either in the presence or absence of S9. In mutagen-treated cultures, there were significant increases in revertants.

N-methylnitroguanidin is considered non-mutagenic in this *Salmonella* reverse mutation assay.

This study is classified as acceptable/guideline, and satisfies the requirement for FIFRA Test Guideline 84-2 for *in vitro* mutagenicty (bacterial reverse gene mutation) data.

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

I. MATERIALS AND METHODS

A. MATERIALS:

1. Test Material: N-Methylnitroguanidine

Description: White powder
Lot/Batch No.: VE 96026 Pt. 17004/98
Purity: 97.3% a.i.
Stability of compound: Not reported.
CAS No.: 4245-76-5.
Vehicle used: Dimethylsulfoxide (DMSO).
Other comments: Stored at room temperatuure, protected from light. It was indicated this was for use as an intermediate for TI 435.

2. Control Materials:

Negative: None.

Solvent/Final concentration: DMSO/0.1 mL/plate.

Positive: Non-activated:

Sodium azide: 10 μg/plate for TA1535
Nitrofurantoin: 0.2 μg/plate for TA100
4-NDPA[1] 10 μg/plate for TA1537
Others (list):
4-NDPA[1] 0.5 μg/plate for TA98
Cumene hydroperoxide: 50 μg/plate for TA102

Activated:
2-Aminoanthracene (2-anthramine) 3 μg/plate for all strains.

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[1]4-NDPA = 4-nitro-1,2-phenylene diamine.

3. Metabolic Activation: S9 derived from adult (200-300g) male Sprague-Dawley rats.

x	Aroclor 1254	x	induced	x	rat	x	liver
	phenobarbital		non-induced		mouse		lung
	none				hamster		other
	other						other

Describe S9 mix composition (give details):

Seventy mL of cofactor solution were composed of:

$MgCl_2$ x 6 H_2O	162.6 mg
KCl	246.0 mg
Glucose-6-phosphate, disodium salt	179.1 mg
NADP, disodium salt	315.0 mg
Phosphate buffer	100.0 mM

4. Test organisms: *Salmonella typhimurium* strains.

	TA97	x	TA98	x	TA100	x	TA102		TA104	x	TA1535	x	TA1537		TA1538

Properly maintained? Yes.
Checked for appropriate genetic markers (rfa mutation, R factor, etc.)? Yes.

5. Test compound concentrations used:

Cytotoxicity test (not performed):

Main assay:

Non-activated conditions: 50, 158, 500, 1581, 5000 μg/plate.
Activated conditions: 50, 158, 500, 1581, 5000 μg/plate.

B. TEST PERFORMANCE

The initial plate incorporation assay followed the directions given by Ames *et al.* (1973a,1975)[2] and Maron and Ames (1983)[3]. Triplicate plates were used in both initial and preincubation assays.

1. Type of *Salmonella* assay:

x	standard plate test
x	pre-incubation (20 minutes)
	"Prival" modification
	spot test
	other (describe)

2. Criteria:

Criteria were presented for both assay acceptance and assessment of results.

II. REPORTED RESULTS

A. PRELIMINARY CYTOTOXICITY ASSAY (not performed)

B. MUTAGENICITY ASSAYS

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[2]Ames, B.N., W.E. Durston, E. Yamasaki and F. D. Lee:
Carcinogens are mutagens: A simple test system combining liver homogenates for activation and bacteria for detection.
Proc. Nat. Acad. Sci. (USA) 70, 2281-2285, 1973a.

Ames, B.N., J. McCann and E. Yamasaki:
Methods for detecting carcinogens and mutagens with the *Salmonella*/mammalian-microsome mutagenicity test.
Mutation Res. 31, 347-364, 1975.

[3]Maron, D.M. and B.N. Ames:
Revised methods for the *Salmonella* mutagenicity test.
Mutation Res. 113, 173-215, 1983.

At no concentration up to the limit dose (5000 μg/plate) was there any cytotoxicity in N-methylnitroguanidin-treated cultures, and **no differences in frequency of revertant colonies from vehicle (DMSO) control counts in either the standard plate (initial) or the preincubation (repeat) assays** (MRID 45422812, pp. 24-26, 40-49 - ATTACHMENT, Tables 1 to 10). The positive control cultures showed the expected increases in revertant colonies.

Therefore the investigator concluded that N-methylnitroguanidin was not mutagenic in this *Salmonella* assay.

III. REVIEWER'S COMMENT/CONCLUSIONS

A. The reviewer agrees with the investigator that this comprehensive assay of N-methylnitroguanidin performed by conventionally acceptable criteria and practice resulted in a demonstrated result that this compound is not a bacterial mutagen in *Salmonella typhimurium*.

B. STUDY DEICIENCIES

None.

ATTACHMENT

THE FOLLOWING ATTACHMENT IS NOT AVAILABLE ELECTRONICALLY
SEE THE FILE COPY

5. Results

5.1. Description of Results

The colony number of each plate and mean values are listed for each dose in Tables 1 to 10. As may be seen, there was no indication of a bacteriotoxic effect of N-Methylnitroguanidin at doses of up to and including 5000 µg per plate. The total bacteria counts consistently produced results comparable to the negative controls, or differed only insignificantly. No inhibition of growth was noted as well.

None of the five strains concerned showed in the plate incorporation test a dose-related and biologically relevant increase in mutant counts over those of the negative controls. This applied both to the tests with and without S9 mix (Tables 1 to 5) and was confirmed by the results of the preincubation trials (Tables 6 to 10).

Summary of the Results with N-Methylnitroguanidin
in the Salmonella/Microsome Test

S9 mix	TA 1535	TA 100	TA 1537	TA 98	TA 102
without	-ve	-ve	-ve	-ve	-ve
with	-ve	-ve	-ve	-ve	-ve

-ve = negative

The positive controls sodium azide, nitrofurantoin, 4-nitro-1,2-phenylene diamine, cumene hydroperoxide and 2-aminoanthracene increased mutant counts to well over those of the negative controls, and thus demonstrated the system's sensitivity and the activity of the S9 mix.

257

5.2. Tabulated Summary of Data

Summary of Mean Values Without S9 Mix

Table and Group	Strain				
	TA 1535	TA 100	TA 1537	TA 98	TA 102
1-5 µg/Plate					
0	8	107	5	38	179
50	8	97	5	40	168
158	7	100	6	40	161
500	9	84	6	37	153
1581	6	103	6	44	160
5000	6	101	6	44	172
Na-azide	574				
NF		248			
4-NPDA			87	155	
Cumene					309
6-10 µg/Tube					
0	10	88	6	33	219
50	10	91	6	31	235
158	10	78	6	30	229
500	9	83	8	29	224
1581	8	82	7	26	227
5000	12	76	6	33	218
Na-azide	701				
NF		300			
4-NPDA			98	175	
Cumene					438

Summary of Mean Values With S9 Mix

Table and Group	Strain				
	TA 1535	TA 100	TA 1537	TA 98	TA 102
1-5 µg/Plate					
0	10	140	8	43	226
50	8	122	6	37	244
158	9	129	6	41	257
500	9	115	8	43	264
1581	8	137	8	40	239
5000	7	120	7	41	210
2-AA	235	1436	291	1322	425
6-10 µg/Tube					
0	12	98	9	36	293
50	11	125	9	47	261
158	10	100	8	41	272
500	14	110	10	40	261
1581	13	101	10	49	271
5000	9	93	8	37	234
2-AA	292	1681	210	1462	404

9. Abbreviations used in the Tables

M	mean
SD	standard deviation
-S9	without S9 mix
+S9	with S9 mix
%	not tested
P	precipitate
B	background lawn reduced
C	contaminated
*	mutagenic effect
**	bacteriotoxic effect

10. Tables 1-10

B A Y E R A.G. Table : 1 Study Number : T 0069528
DEPARTMENT OF TOXICOLOGY Study Director : Dr.Herbold
PHARMA RESEARCH CENTER Technician : Bönning
WUPPERTAL ELBERFELD Date : Dec. 14, 2000
AMES TEST with : N-Methylnitroguanidin Strain: S.typhimurium TA 1535

Dose/Plate (µg/Plate)	REVERTANTS PER PLATE -S9	M	SD	10% +S9	M	SD	TITER Dilution 10^{-6}	TITER per ml 10^{+8}	QUOTIENT -S9	QUOTIENT +S9
DMSO	8 6 9	8	2	9 12 8	10	2	187 172	18.0	1.0	1.0
50	6 11 7	8	3	6 9 8	8	2	%	/	1.0	0.8
158	7 6 9	7	2	8 13 6	9	4	%	/	1.0	0.9
500	9 7 12	9	3	11 8 7	9	2	%	/	1.2	0.9
1581	8 5 6	6	2	6 11 8	8	3	%	/	0.8	0.9
5000	5 8 5	6	2	6 11 4	7	4	%	/	0.8	0.7
Na-azide 10	585 581 557	574	15	%	/	/	%	/	74.9*	/
2-AA 3	%	/	/	221 246 237	235	13	%	/	/	24.3*

261

B A Y E R A.G. Table : 2 Study Number : T 0069528
DEPARTMENT OF TOXICOLOGY Study Director : Dr.Herbold
PHARMA RESEARCH CENTER Technician : Bönning
WUPPERTAL ELBERFELD Date : Dec. 14, 2000
AMES TEST with : N-Methylnitroguanidin Strain: S.typhimurium TA 100

Dose/Plate (µg/Plate)	REVERTANTS PER PLATE -S9	M	SD	10% +S9	M	SD	TITER Dilution 10^{-6}	per ml 10^{-8}	QUOTIENT -S9	+S9
DMSO	107 107 108	107	1	114 146 161	140	24	180 182	18.1	1.0	1.0
50	99 99 94	97	3	140 108 119	122	16	%	/	0.9	0.9
158	89 124 86	100	21	150 112 125	129	19	%	/	0.9	0.9
500	70 77 106	84	19	133 94 119	115	20	%	/	0.8	0.8
1581	95 106 107	103	7	141 157 114	137	22	%	/	1.0	1.0
5000	90 112 102	101	11	128 117 114	120	7	%	/	0.9	0.9
NF 0.2	252 230 262	248	16	%	/	/	%	/	2.3*	/
2-AA 3	%	/	/	1511 1426 1370	1436	71	%	/	/	10.2*

B A Y E R A.G. Table : 3 Study Number : T 0069528
DEPARTMENT OF TOXICOLOGY Study Director : Dr.Herbold
PHARMA RESEARCH CENTER Technician : Bönning
WUPPERTAL ELBERFELD Date : Dec. 14, 2000
AMES TEST with : N-Methylnitroguanidin Strain: S.typhimurium TA 1537

Dose/Plate (µg/Plate)	REVERTANTS PER PLATE -S9	M	SD	10% +S9	M	SD	TITER Dilution 10^{-6}	TITER per ml 10^{*8}	QUOTIENT -S9	QUOTIENT +S9
DMSO	5 6 5	5	1	8 9 7	8	1	104 66	8.5	1.0	1.0
50	6 5 5	5	1	4 6 7	6	2	%	/	1.0	0.7
158	6 5 7	6	1	6 6 5	6	1	%	/	1.1	0.7
500	4 8 6	6	2	12 7 6	8	3	%	/	1.1	1.0
1581	7 5 6	6	1	7 8 8	8	1	%	/	1.1	1.0
5000	4 9 4	6	3	8 6 8	7	1	%	/	1.1	0.9
4-NPDA 10	90 106 64	87	21	%	/	/	%	/	16.3*	/
2-AA 3	%	/	/	275 343 255	291	46	%	/	/	36.4*

263

B A Y E R A.G. Table : 4 Study Number : T 0069528
DEPARTMENT OF TOXICOLOGY Study Director : Dr.Herbold
PHARMA RESEARCH CENTER Technician : Bönning
WUPPERTAL ELBERFELD Date : Dec. 14, 2000
AMES TEST with : N-Methylnitroguanidin Strain: S.typhimurium TA 98

Dose/Plate (µg/Plate)	REVERTANTS PER PLATE -S9	M	SD	10% +S9	M	SD	TITER Dilution 10^{-6}	TITER per ml 10^{+8}	QUOTIENT -S9	QUOTIENT +S9
DMSO	30 42 42	38	7	40 46 42	43	3	220 244	23.2	1.0	1.0
50	44 31 46	40	8	45 29 37	37	8	%	/	1.1	0.9
158	51 30 40	40	11	41 39 42	41	2	%	/	1.1	1.0
500	37 46 28	37	9	44 38 46	43	4	%	/	1.0	1.0
1581	44 47 41	44	3	40 44 37	40	4	%	/	1.2	0.9
5000	41 48 44	44	4	45 41 37	41	4	%	/	1.2	1.0
4-NPDA 0.5	169 155 142	155	14	%	/	/	%	/	4.1*	/
2-AA 3	%	/	/	1339 1353 1274	1322	42	%	/	/	31.0*

B A Y E R A.G. Table : 5 Study Number : T 0069528
DEPARTMENT OF TOXICOLOGY Study Director : Dr.Herbold
PHARMA RESEARCH CENTER Technician : Bönning
WUPPERTAL ELBERFELD Date : Dec. 14, 2000
AMES TEST with : N-Methylnitroguanidin Strain: S.typhimurium TA 102

Dose/Plate (µg/Plate)	REVERTANTS PER PLATE -S9	M	SD	10% +S9	M	SD	TITER Dilution 10^{-6}	per ml 10^{*8}	QUOTIENT -S9	+S9
DMSO	189 189 160	179	17	225 220 233	226	7	237 163	20.0	1.0	1.0
50	161 176 168	168	8	248 220 264	244	22	%	/	0.9	1.1
158	148 174 162	161	13	264 266 241	257	14	%	/	0.9	1.1
500	152 165 141	153	12	241 269 282	264	21	%	/	0.9	1.2
1581	162 161 157	160	3	224 235 258	239	17	%	/	0.9	1.1
5000	160 181 175	172	11	200 229 201	210	16	%	/	1.0	0.9
Cumene 50	316 316 295	309	12	%	/	/	%	/	1.7*	/
2-AA 3	%	/	/	425 441 408	425	17	%	/	/	1.9*

265

B A Y E R A.G. Table : 6 Study Number : T 0069528
DEPARTMENT OF TOXICOLOGY Study Director : Dr.Herbold
PHARMA RESEARCH CENTER Technician : Bönning
WUPPERTAL ELBERFELD Date : Jan. 8, 2001
PREINCUBATION with : N-Methylnitroguanidin Strain: S.typhimurium TA 1535

Dose/Tube (µg/Tube)	REVERTANTS PER PLATE -S9	M	SD	10% +S9	M	SD	TITER Dilution 10^{-6}	per ml 10^{-8}	QUOTIENT -S9	+S9
DMSO	13 8 8	10	3	13 15 7	12	4	154 166	16.0	1.0	1.0
50	11 8 11	10	2	10 12 12	11	1	%	/	1.0	1.0
158	7 10 12	10	3	10 12 9	10	2	%	/	1.0	0.9
500	9 10 8	9	1	12 18 11	14	4	%	/	0.9	1.2
1581	11 8 5	8	3	11 15 12	13	2	%	/	0.8	1.1
5000	15 9 11	12	3	5 8 15	9	5	%	/	1.2	0.8
Na-azide 10	716 728 660	701	36	%	/	/	%	/	72.6*	/
2-AA 3	%	/	/	294 295 286	292	5	%	/	/	25.0*

B A Y E R A.G. Table : 7 Study Number : T 0069528
DEPARTMENT OF TOXICOLOGY Study Director : Dr.Herbold
PHARMA RESEARCH CENTER Technician : Bönning
WUPPERTAL ELBERFELD Date : Jan. 8, 2001
PREINCUBATION with : N-Methylnitroguanidin Strain: S.typhimurium TA 100

Dose/Tube (µg/Tube)	REVERTANTS PER PLATE -S9	M	SD	10% +S9	M	SD	TITER Dilution 10^{-6}	per ml 10^{+8}	QUOTIENT -S9	+S9
DMSO	100	88	13	99	98	2	119	12.3	1.0	1.0
	74			100			126			
	89			96						
50	91	91	5	123	125	2	%	/	1.0	1.3
	95			125						
	86			127						
158	85	78	7	114	100	13	%	/	0.9	1.0
	78			99						
	72			88						
500	69	83	12	113	110	6	%	/	1.0	1.1
	90			114						
	91			103						
1581	85	82	3	100	101	5	%	/	0.9	1.0
	82			106						
	80			97						
5000	79	76	3	93	93	5	%	/	0.9	0.9
	73			88						
	77			97						
NF 0.2	316	300	20	%	/	/	%	/	3.4*	/
	278									
	305									
2-AA 3	%	/	/	1715	1681	43	%	/	/	17.1*
				1696						
				1633						

B A Y E R A.G. Table : 8 Study Number : T 0069528
DEPARTMENT OF TOXICOLOGY Study Director : Dr.Herbold
PHARMA RESEARCH CENTER Technician : Bönning
WUPPERTAL ELBERFELD Date : Jan. 8, 2001
PREINCUBATION with : N-Methylnitroguanidin Strain: S.typhimurium TA 1537

Dose/Tube (µg/Tube)	REVERTANTS PER PLATE -S9	M	SD	10% +S9	M	SD	TITER Dilution 10^{-6}	per ml 10^{-8}	QUOTIENT -S9	+S9
DMSO	5 5 8	6	2	8 7 11	9	2	31 39	3.5	1.0	1.0
50	8 5 4	6	2	13 7 8	9	3	%	/	0.9	1.1
158	4 6 7	6	2	11 5 8	8	3	%	/	0.9	0.9
500	5 8 10	8	3	6 11 13	10	4	%	/	1.3	1.2
1581	8 6 6	7	1	11 9 10	10	1	%	/	1.1	1.2
5000	5 7 6	6	1	9 6 8	8	2	%	/	1.0	0.9
4-NPDA 10	107 96 91	98	8	%	/	/	%	/	16.3*	/
2-AA 3	%	/	/	205 228 196	210	17	%	/	/	24.2*

B A Y E R A.G. Table : 9 Study Number : T 0069528
DEPARTMENT OF TOXICOLOGY Study Director : Dr.Herbold
PHARMA RESEARCH CENTER Technician : Bönning
WUPPERTAL ELBERFELD Date : Jan. 8, 2001
PREINCUBATION with : N-Methylnitroguanidin Strain: S.typhimurium TA 98

Dose/Tube (µg/Tube)	REVERTANTS PER PLATE -S9	M	SD	10% +S9	M	SD	TITER Dilution 10^{-6}	per ml 10^{-8}	QUOTIENT -S9	+S9
DMSO	35 29 35	33	3	50 30 28	36	12	207 214	21.1	1.0	1.0
50	25 33 35	31	5	45 42 53	47	6	%	/	0.9	1.3
158	31 20 38	30	9	42 42 39	41	2	%	/	0.9	1.1
500	23 26 37	29	7	40 38 41	40	2	%	/	0.9	1.1
1581	20 30 27	26	5	49 51 47	49	2	%	/	0.8	1.4
5000	33 39 28	33	6	39 37 35	37	2	%	/	1.0	1.0
4-NPDA 0.5	200 150 174	175	25	%	/	/	%	/	5.3*	/
2-AA 3	%	/	/	1415 1525 1447	1462	57	%	/	/	40.6*

269

B A Y E R A.G.
DEPARTMENT OF TOXICOLOGY
PHARMA RESEARCH CENTER
WUPPERTAL ELBERFELD
PREINCUBATION with : N-Methylnitroguanidin

Table : 10

Study Number : T 0069528
Study Director : Dr.Herbold
Technician : Bönning
Date : Jan. 8, 2001
Strain: S.typhimurium TA 102

Dose/Tube (µg/Tube)	REVERTANTS PER PLATE -S9	M	SD	10% +S9	M	SD	TITER Dilution 10^{-6}	per ml 10^{+8}	QUOTIENT -S9	+S9
DMSO	221 219 218	219	2	280 307 291	293	14	244 222	23.3	1.0	1.0
50	225 249 232	235	12	248 267 268	261	11	%	/	1.1	0.9
158	230 235 222	229	7	277 258 282	272	13	%	/	1.0	0.9
500	232 240 201	224	21	264 278 241	261	19	%	/	1.0	0.9
1581	224 221 236	227	8	278 246 288	271	22	%	/	1.0	0.9
5000	237 233 185	218	29	253 247 201	234	28	%	/	1.0	0.8
Cumene 50	468 445 401	438	34	%	/	/	%	/	2.0*	/
2-AA 3	%	/	/	421 424 368	404	32	%	/	/	1.4*

DATA EVALUATION RECORD

TI 435 (CLOTHIANIDIN)

Study Type: §82-1a, Subchronic Oral Toxicity Study in Rats

Work Assignment No. 4-02-167A (Formerly 4-01-167A) (MRIDs 45422809 and 45422708)

Prepared for
Health Effects Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
1921 Jefferson Davis Highway
Arlington, VA 22202

Prepared by
Pesticides Health Effects Group
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Dynamac Corporation
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Date: 7/22/02

Quality Assurance
Steve Brecher, Ph.D.

Signature: Mary Menetrez for Steve Brecher
Date: 7/22/02

Disclaimer

This Data Evaluation Record may have been altered by the Health Effects Division subsequent to signing by Dynamac Corporation personnel.

PMRA/EPA Reviewers: Scott Hancock and Pamela Hurley Signature: Pamela M Hurley
EPA Work Assignment Manager:Ghazi Dannan, Ph.D. Signature: Ghazi Dannan
Registration Action Branch 3, Health Effects Division (7509C) Date 12/10/04

Template version 11/01

TXR#: 0050321

DATA EVALUATION RECORD

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

PC CODE: 044309 **DP BARCODE:** D279596, D276450, D277188
SUBMISSION NO.: S607124, S600587

TEST MATERIAL (PURITY): TI 435 (Clothianidin; >95.3% a.i.)

SYNONYMS: (E)-1-(2-chloro-5-thiazolylmethyl)-3-methyl-2-nitroguanidine

CITATION: Wahle, B.S. (2000). Technical Grade TI 435: A Subchronic Toxicity Testing Study in the Rat. Bayer Corp. Agricultural Division, Toxicology, Stilwell, KS. Laboratory Project Study Id: 98-172-QO, February 22, 2000. MRID 45422809. Unpublished.

Chambers, P.R. (1997). TI 435: Toxicity to Rats by Dietary Administration for 4 Weeks. Huntingdon Life Sciences Ltd., Cambridgeshire, UK. Laboratory Project Id: TDA 179/960496, February 19, 1997. MRID 45422708. Unpublished.

SPONSOR: Takeda Chemical Industries Ltd., 2-13-10, Nihonbashi, Chuo-ku, Tokyo, Japan

EXECUTIVE SUMMARY: In a subchronic oral toxicity study (MRIDs 45422809 and 45422708), TI 435 (clothianidin; >95.3% a.i., Lot/batch #: 30037120) was administered to 15 Sprague-Dawley rats/sex/group in the diet at dose levels of 0, 150, 500, or 3000 ppm (equivalent to 9.0/10.9, 27.9/34.0, and 202.0/254.2 mg/kg/day in males and females respectively) for 90 days. Additionally, 15 rats/sex were assigned to the control and 3000 ppm groups and were allowed to recover for approximately seven weeks following dosing termination.

Mortality, clinical signs, ophthalmoscopy, hematology, urinalysis, organ weight, and gross and microscopic pathology were unaffected by treatment.

At 3000 ppm, body weights were decreased ($p <= 0.05$) in both sexes throughout the main study (decr 7-14%) and in the recovery animals (decr 6-16%). In addition, overall (Days 0 through 91) body weight gains (calculated by the reviewers) were decreased in both sexes during the main study (decr 22-32%). Furthermore, overall body weight gains were decreased following the recovery period (Days 0 through 140; decr 10-19%). Body weight gains during the recovery period (Days 91-140) were increased above controls (incr 34 g in males and 16 g in females).

The LOAEL for this study is 3000 ppm (equivalent to 202.0/254.2 mg/kg/day [M/F]) based on decreased body weight and body weight gains in both sexes. The NOAEL is 500 ppm (equivalent to 27.9/34.0 mg/kg/day [M/F]).

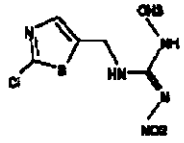
This study is classified as **acceptable/guideline** and satisfies the guideline requirements (OPPTS 870.3100a; OECD 408) for a subchronic oral toxicity study in the rat.

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

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Description:	Pale yellow powder
Lot/Batch #:	300371200
Purity:	>95.3% a.i.
Compound Stability:	The test material was stable in the diet for up to 7 days at room temperature and 28 days refrigerated.
CAS # of TGAI:	210880-92-5
Structure:	

2. Vehicle: Diet

3. Test animals:

Species:	Rat	
Strain:	Sprague-Dawley	
Age at study initiation:	Approximately 8 weeks old	
Wt. at study initiation:	278.5-282.0 g males and 188.6-193.9 g females	
Source:	Charles River Laboratories, Inc., Portage, MI	
Housing:	Individually in suspended, stainless steel, wire-mesh cages.	
Diet:	Purina Mills Rodent Lab Chow 5001-4 "etts" form (PMI, St. Louis, MO), *ad libitum*; except overnight prior to blood collection.	
Water:	Municipal tap water *ad libitum*	
Environmental conditions:	**Temperature:**	18-26 °C
	Humidity:	40-70 %
	Air changes:	not reported
	Photoperiod:	12 hrs dark/12 hrs light
Acclimation period:	2 weeks	

B. STUDY DESIGN

1. In life dates: Start: May 21, 1998 End: November 14, 1998 (estimated by reviewers)

2. Animal assignment: Animals were randomly assigned, stratified by body weight, to the test groups presented in Table 1.

Table 1. Study design. [a]

Test Group	Dose (ppm)	Mean Chemical Intake (mg/kg bw/day) [M/F]	Main Study		Recovery Group [b]	
			Males	Females	Males	Females
Control	0	0/0	15	15	15	15
Low	150	9.0/10.9	15	15	0	0
Mid	500	27.9/34.0	15	15	0	0
High	3000	202.0/254.2	15	15	15	15

a Data were obtained from MRID 45422809, pages 15 and 97.
b Animals in the recovery groups were not treated for approximately seven weeks following termination of dosing.

3. Dose selection rationale: A summary of a 4-week range finding study in the rat (MRID 45422708) is presented in an Appendix to this DER.

4. Treatment preparation, administration, and analysis: The appropriate amount of test substance was suspended in an acetone/corn oil mixture (ratio not reported) and then combined with the diet to form a premix. The premix was diluted with additional diet to achieve the desired concentrations. Test diets were prepared weekly and stored refrigerated until used. Homogeneity (top, middle, bottom) was determined for the 50 and 5000 ppm formulations. Stability was determined for the 50 and 5000 ppm formulations after 7 days refrigerated, followed by storage at room temperature for 0, 1, 4, or 7 days. Additionally, stability was determined after refrigerated storage for 14 (50 ppm), 16 (5000 ppm), and 28 days (50 and 5000 ppm). Samples of all test formulations (150, 500, or 3000 ppm) were taken during study weeks 1, 5, 9, and 14 and were analyzed to confirm targeted concentrations of the test substance.

Results

Homogeneity Analysis (range as % CV): 6.4-7.0

Stability Analysis (range as % of Day 0, calculated by reviewers)
After refrigerated storage for 7 days, followed by subsequent storage at room temperature for up to 7 days: 97.8-108.5
After refrigerated storage for up to 28 days: 104.5-113.9

Concentration Analysis (range as % of nominal): 94-115

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

5. Statistics: All continuous data were evaluated for homogeneity of variance using Bartlett's test. Group means were then analyzed using an ANOVA (non-parametric analysis, as appropriate) followed by Dunnett's or Mann-Whitney-U test, respectively. Frequency data were examined for trends, then further evaluated using chi-square, Fisher exact, or both if there was

evidence of a potential effect. The data from the 7-pentoxyresorufin depenthylation (PROD) and 7-ethoxyresorufin deethylation (EROD) tests were analyzed using Student t-test. Statistical significance was denoted at $p \leq 0.001$ (Bartlett's) or 0.05 (all others).

C. METHODS

1. Observations

a. Cageside observations: Animals were checked for mortality, moribundity, and clinical signs of toxicity once daily.

b. Clinical examinations: Detailed physical examinations were conducted weekly. In addition, the estrous cycle of each female was determined by examining daily vaginal smears and charted over a three-week period prior to termination.

c. Neurological evaluations: Neurological evaluations were not performed.

2. Body weight: All animals were weighed prior to treatment, weekly throughout the study, and at termination. Overall (Weeks 0-13 or 0-20) body weight gains were calculated by the reviewers from the group mean body weight data.

3. Food consumption and food efficiency: Food consumption (g) was recorded weekly for each animal. Absolute (g/animal/day) and relative (g/kg bw/day) food consumption were calculated for each group. Food efficiency was not reported.

4. Ophthalmoscopic examination: Ophthalmoscopic examinations were performed on all animals prior to initiation of treatment and on all surviving animals just prior to termination.

5. Hematology & clinical chemistry: Blood was collected from the orbital sinus following an overnight fast of each surviving rat prior to necropsy. An additional blood sample for prothrombin time determination was collected via cardiac puncture immediately prior to necropsy. The following CHECKED (X) parameters were examined.

a. Hematology

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

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

b. Clinical Chemistry

	ELECTROLYTES		OTHER
X	Calcium	X	Albumin*
X	Chloride	X	Creatinine*
X	Magnesium	X	Urea nitrogen*
X	Phosphorus	X	Total Cholesterol*
X	Potassium*	X	Globulins
X	Sodium*	X	Glucose-fasting*
		X	Total and direct bilirubin
	ENZYMES	X	Total protein (TP)*
X	Alkaline phosphatase (ALK)*	X	Triglycerides
	Cholinesterase (ChE)		Serum protein electrophores
X	Creatine phosphokinase	X	Uric acid
X	Lactic acid dehydrogenase (LDH)	X	Thyroxine (T4)
X	Alanine aminotransferase (ALT/also SGPT)*	X	Triiodothyronine (T3)
X	Aspartate aminotransferase (AST/also SGOT)*	X	Thyroid stimulating hormone (TSH)
	Sorbitol dehydrogenase*		
X	*Gamma glutamyl transferase (GGT)**		
	Glutamate dehydrogenase		

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

In addition, the following hepatic enzymes were measured: cytochrome P-450, N-demethylase, O-demethylase, ethoxyresorufin O-deethylase, and pentoxyresorufin O-dealkylase.

6. Urinalysis: Urine was collected overnight from non-fasted animals one week prior to blood collection. The CHECKED (X) parameters were examined.

X	Appearance (clarity/color)*	X	Glucose
X	Volume*	X	Ketones
X	Specific gravity*	X	Bilirubin
X	pH*	X	Blood*
X	Sediment (microscopic)	X	Nitrite
X	Protein*	X	Urobilinogen
		X	Leukocytes

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

8. Sacrifice and pathology: Upon study termination, all animals were sacrificed by CO_2 asphyxiation followed by exsanguination, and subjected to gross pathological examination. When possible, females in the same estrous cycle stage were sacrificed together to alleviate possible effects of non-synchronous estrus cycle stage effects on measured parameters (uterine weight, follicular population, etc.). The CHECKED (X) tissues were collected and preserved in 10% neutral buffered formalin. Additionally, the (XX) organs were weighed.

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

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

With the exception of the vagina and the preputial, clitoral, and exorbital lacrimal glands, microscopic examination was performed on histological sections (stained with hematoxylin and eosin) prepared from tissues collected from animals in the control and 3000 ppm groups (main study and recovery). Additionally, all gross lesions were examined in the intermediate dose groups as well.

II. RESULTS

A. OBSERVATIONS

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

2. Mortality: One 500 ppm male was found dead on Day 17. The cause of death was undetermined. All other animals survived to scheduled sacrifice.

B. BODY WEIGHT AND WEIGHT GAIN: Selected body weights and body weight gains are presented in Tables 2a and 2b. At 3000 ppm, body weights were decreased ($p \leq 0.05$) compared to controls, throughout the main study (Days 7-91; ↓7-11%, males and 7-14%, females) and in the recovery animals from Days 7-112 in the males (↓6-11%) and Days 7-140 in the females (↓8-16%). Overall (Days 0 through 91) body weight gains (calculated by the reviewers) were decreased during the main study at 3000 ppm (↓22%, males and 32%, females). Furthermore, overall body weight gains remained decreased at 3000 ppm following the recovery period (Days 0 through 140; ↓10%, males and 19%, females); however, body weight gains during the recovery period (Days 91-140) were increased above controls (↑34 g in males and 16 g in females). Body weights and overall body weight gains were comparable between treated groups and controls throughout the study at ≤500 ppm.

Table 2a. Mean (± SE) body weights and overall body weight gains (g) in main study rats treated with clothianidin in the diet for up to 13 weeks. [a]

Study Day	Dose (ppm)			
	0	150	500	3000
Males				
0	280.1±2.9	281.4±3.1	278.5±2.8	280.2±2.9
7	330.5±3.9	337.2±2.9	337.3±3.5	307.8±4.5* (↓7)
56	518.0±8.8	537.2±6.2	522.3±7.7	464.9±9.5* (↓10)
91	560.1±9.6	585.3±9.2	565.9±7.6	499.8±12.6* (↓11)
Overall (0-91) gain[b]	280.0	303.9	287.4	219.6 (↓22)
Females				
0	192.6±1.9	190.3±2.2	188.6±2.3	192.5±2.4
7	212.9±2.2	211.7±3.2	209.3±3.6	197.9±3.6* (↓7)
63	302.3±6.3	299.7±8.4	291.5±4.5	261.0±4.1* (↓14)
91	308.3±6.5	303.1±6.7	298.2±4.8	270.8±5.1* (↓12)
Overall (0-91) gain[b]	115.7	112.8	109.6	78.3 (↓32)

a Data were obtained from MRID 45422809, pages 47-49 and 55-57; n=15; except for the 500 ppm males (n=14). Percent difference from controls, calculated by the reviewers, is included in parentheses.

b Calculated by the reviewers from the group mean body weight data presented in this table.

* Significantly different from the control group at $p \leq 0.05$.

Table 2b. Mean (± SE) body weights and overall body weight gains (g) in recovery group rats treated with Clothianidin in the diet for up to 13 weeks followed by 7 weeks on untreated diet. [a]

Study Day	Dose (ppm)	
	0	3000
Males		
0	280.2±3.0	282.0±3.2
14	373.0±3.8	350.8±4.3* (↓6)
91	593.1±10.1	529.3±13.0* (↓11)
112	620.8±11.8	576.5±14.6* (↓7)
140	606.2±13.4	576.5±16.0
Overall (0-140) gain[b]	326.0	294.5 (↓10)
Overall recovery (91-140) gain[b]	13.1	47.2
Females		

(table continues next page)

280

Study Day	Dose (ppm)	
	0	3000
0	193.9±2.4	188.6±2.2
7	218.3±3.2	200.2±2.7* (↓8)
56	304.5±5.0	257.2±4.4* (↓16)
91	329.7±6.3	282.4±5.2* (↓14)
140	331.0±6.4	299.8±5.1* (↓9)
Overall (0-140) gain [b]	137.1	111.2 (↓19)
Overall recovery (91-140) gain [b]	1.3	17.4

a Data were obtained from MRID 45422809, pages 50-54 and 58-62; n=15. Percent difference from controls, calculated by the reviewers, is included in parentheses.
b Calculated by the reviewers from the group mean body weight data presented in this table.
* Significantly different from the control group at $p \leq 0.05$.

C. FOOD CONSUMPTION

1. Food consumption: At 3000 ppm, transient decreases ($p \leq 0.05$) in absolute food consumption (g/animal/day) were observed in the main study on Day 7 in the males (↓19%) and females (↓21%). In the recovery group (Table 3), decreases in absolute food consumption were observed sporadically in the males on Days 7 through 49 (↓7-19%), and in the females on Days 7 through 98 (↓6-29%). These findings are considered unrelated to treatment as they were minor, transient in nature, and/or not consistently noted in either the main or recovery groups during treatment. Relative food consumption (g/kg bw/day) was sporadically increased ($p \leq 0.05$) in the main study (↑6-9% males and 12-30% females) and recovery group (↑7-20% males and 8-14% females); however, this finding was likely due to the decreased body weights observed. Absolute and relative food consumption were comparable between controls and the 150 and 500 ppm groups throughout the study.

Table 3. Mean (±SE) food consumption (g/animal/day) in recovery group rats exposed to Clothianidin in the diet for up to 13 weeks followed by 7 weeks on untreated diet.[a]

Study Day	Dose (ppm)	
	0	3000
Male		
7	28.72±0.44	23.32±0.48* (↓19)
28	30.68±0.54	28.62±0.81* (↓7)
42	30.32±0.48	28.19±0.79* (↓7)
49	30.24±0.72	27.78±0.73* (↓8)
Female		
7	21.20±0.32	15.04±0.36* (↓29)
56	21.17±0.37	19.83±0.44* (↓6)
77	21.11±0.63	19.21±0.46* (↓9)
98	22.07±0.59	19.40±0.20* (↓12)

a Data obtained from MRID 45422809, pages 73-77 and 82-86; n=15. Percent difference from controls, calculated by the reviewers, is included in parentheses.
* Significantly different from the control group at $p \leq 0.05$.

2. Compound consumption: Group mean compound intake values (mg/kg/day) are reported in Table 1.

D. OPHTHALMOSCOPIC EXAMINATION: No treatment-related ophthalmic effects were observed at any dose.

E. BLOOD ANALYSES

1. Hematology: No treatment-related changes in hematology parameters were observed. Minor increases ($p \leq 0.05$) in red blood cells (↑6% males), hemoglobin (↑6% males), hematocrit (↑5% males), prothrombin time (↑9% females), and percent lymphocytes (↑7% males) were noted at 3000 ppm during treatment. In the recovery animals, differences ($p \leq 0.05$) in white blood cells (↓18%), mean corpuscular hemoglobin (↑3%), mean corpuscular hemoglobin concentration (↑1%), and percent reticulocytes (↓31%) were noted in the 3000 ppm males, and percent large unstained cells were decreased (↓30%) in the 3000 ppm females. All of these findings were considered unrelated to treatment because they differed minimally from controls in magnitude and/or percent difference, and/or they were only observed in the recovery animals. All other hematological parameters examined in the treated animals were similar to controls.

2. Clinical chemistry: In the main study 3000 ppm males, significant increases were observed in N-demethylase (↑32%), o-demethylase (↑50%), ethoxyresorufin o-deethylase (↑127%), and pentoxyresorufin o-dealkylase (↑211%) activities (Table 4). Additionally, in the main study males, increases ($p \leq 0.05$) in creatinine (↑25%), total protein (↑7%), and phosphorus (↑11%) were observed at 3000 ppm; however, these findings were considered unrelated to treatment because they were minor and/or lacked corroborative histopathological data. In the main study females, decreases ($p \leq 0.05$) were observed in creatine phosphokinase at 150 (↓36%) and 3000 (↓46%) ppm and lactate dehydrogenase at 150 (↓54%) and 3000 (↓61%) ppm; however, these findings were considered unrelated to treatment, as they were not dose-dependent. During the recovery period, numerous differences were observed at 3000 ppm; however, they were considered unrelated to treatment as they were not observed in the main study animals. All other clinical chemistry changes were not dose-dependent and considered unrelated to treatment.

Table 4. Selected clinical chemistry parameters in main study male rats treated with Clothianidin in the diet for up to 13 weeks. [a]

Parameter	Dose (ppm)			
	0	150	500	3000
n-demethylase (nM/mi/mgpr)	2.43±0.74	2.23±0.58	2.51±0.70	3.21±1.08* (↑32)
o-demethylase (nM/mi/mgpr)	0.10±0.04	0.10±0.05	0.13±0.05	0.15±0.07* (↑50)
ethoxyresorufin o-deethylase [b]	0.15±0.09	0.18±0.07	0.23±0.07	0.34±0.09* (↑127)
pentoxyresorufin o-dealkylase [b]	0.036±0.024	0.042±0.019	0.045±0.028	0.112±0.061* (↑211)

a Data obtained from MRID 45422809, pages 165 and 993; n=5. Percent difference from controls, calculated by the reviewers, is included in parentheses.
b Units not reported.
* Significantly different from the control group at $p \leq 0.05$.

F. URINALYSIS: There were no treatment-related effects on urinalysis parameters. The significant ($p \leq 0.05$) increases in ketones, urobilinogen, and specific gravity observed in the recovery group females were considered incidental and unrelated to treatment. There were no other significant differences in any urinalysis parameter.

G. SACRIFICE AND PATHOLOGY

1. Organ weight: At 3000 ppm, terminal body weights were slightly decreased ($p \leq 0.05$) in the main study males and females (↓11% each) and in the recovery females (↓10%). The following significant differences were observed at 3000 ppm: (i) main study males, absolute heart (↓13%), absolute liver (↓9%), relative (to body) brain (↑11%), relative kidney (↑9%), and relative testes (↑18%); (ii) main study females, relative brain (↑12%) and relative heart (↑10%); and (iii) recovery females, absolute kidney (↓7%), absolute liver (↓6%), relative (to body) brain (↑11%), relative lung (↑8%), relative ovary (↑17%), and relative spleen (↑10%). These findings were

considered unrelated to treatment and likely due to decreased terminal body weights, because they were minor and/or lacked corroborative gross or histopathological data. All absolute and relative (to body) organ weights in the recovery males were similar to controls. In addition, absolute and relative (to body) kidney weights were increased ($p \leq 0.05$) compared to controls in the 150 ppm females; however, as these findings were not dose-dependent, they were considered unrelated to treatment.

2. **Gross pathology:** No treatment-related gross pathological findings were noted.

3. **Microscopic pathology:** No treatment-related histopathological findings were noted. In the 3000 ppm main study males, increased ($p \leq 0.05$) pigmentation of the spleen was observed (13/15 treated vs 6/15 controls); however, because this finding was minimal to slight in severity and was observed at a similar frequency in all females it was considered unrelated to treatment (Table 5). The pathology report indicated that the pigmentation was due to deposition of hemosiderin. All recovery group animals were free of microscopic findings.

Table 5. Minimal to slight spleen pigmentation (# of animals affected/15 treated) in main study rats exposed to Clothianidin in the diet for up to 13 weeks. [a]

Dose (ppm)							
Males				Females			
0	150	500	3000	0	150	500	3000
6	6	8	13* (↑9)	14	13	14	15

a Data obtained from MRID 45422809, page 277; n=15. Percent difference from controls, calculated by the reviewers, is included in parentheses.

* Significantly different from the control group at $p \leq 0.05$.

III. DISCUSSION and CONCLUSIONS

A. **INVESTIGATORS' CONCLUSIONS:** It was concluded that the NOAEL was 500 ppm based on decreased body weight gains observed at 3000 ppm in both sexes. In addition, minor differences in liver enzymes and pigmentation of the spleen were observed in these animals.

B. **REVIEWER COMMENTS:** No treatment-related effects on mortality, clinical signs, ophthalmoscopy, hematology, urinalysis, organ weight, and gross and microscopic pathology were observed.

At 3000 ppm, body weights were decreased ($p \leq 0.05$) compared to controls in both sexes throughout the main study (↓7-14%) and in the recovery animals (↓6-16%). In addition, overall (Days 0 through 91) body weight gains (calculated by the reviewers) were decreased in both sexes during the main study (↓22-32%). Furthermore, overall body weight gains were decreased following the recovery period (Days 0 through 140; ↓10-19%). Body weight gains during the recovery period (Days 91-140) were increased above controls (↑34 g in males and 16 g in

HED Records Center Series 361 Science Reviews - File R107689 - Page 300 of 721

females).

The LOAEL for this study is 3000 ppm (equivalent to 202.0/254.2 mg/kg/day [M/F]) based on decreased body weight and body weight gains in both sexes. The NOAEL is 500 ppm (equivalent to 27.9/34.0 mg/kg/day [M/F]).

This study is classified as **acceptable/guideline** and satisfies the guideline requirements (OPPTS 870.3100a; OECD 408) for a subchronic oral toxicity study in the rat.

C. STUDY DEFICIENCIES: None

DATA FOR ENTRY INTO ISIS

90-Day Study - rats (870.3100a)

PC code	MRID #	Study type	Species	Duration	Route	Dosing method	Dose range mg/kg	Doses tested mg/kg	NOAEL mg/kg	LOAEL mg/kg	Target organ(s)	Comments
044309	45422809	subchronic	rat	90 days	oral	diet	9.0-254.2	0/0, 9.0/10.9, 27.9/34.0, 202.0/254.2 [M/F]	27.9	202.0	BW, BWG	

HED Records Center Series 361 Science Reviews - File R107689 - Page 301 of 721

APPENDIX

28-day Dietary Range-Finding Study in the Rat

This range-finding study was performed to determine adequate dose levels for subsequent studies. Therefore, a summary is provided in order to confirm the adequacy of the dose rationale in later studies.

In this oral toxicity study (MRID 45422708), TI 435 (clothianidin; 97.5% a.i.; Batch/Lot# 30033623) was administered in the diet to 5 Crl:CD BR rats/sex/group at doses of 0, 1250, 2500, 5000, or 7500 ppm (equivalent to 120/137, 249/228, 475/454, 602/689 [M/F], respectively) for 28 days.

All animals survived to scheduled sacrifice. Clinical signs observed included half-closed eyes in both sexes at ≥5000 ppm beginning at Week 2, and brown nasal staining in both sexes at 7500 ppm beginning in Week 3.

At ≥2500 ppm, overall (Weeks 1-4) body weight gains were dose-dependently decreased ($p \leq 0.05$) in both sexes (↓25-114%). Related decreases were also observed in food consumption (↓12-51%, no statistics performed). Additionally, food efficiency was reduced (indicated by increased food conversion ratios) in males and females (5.8-16.7 treated vs 4.9-7.5 controls; note: the treated values are for 2500-5000 ppm, as there were mean body weight losses [↓5-22 g] at 7500 ppm, and a meaningful ratio cannot be calculated).

In the thymus, relative (to body) organ weight was decreased ($p \leq 0.05$) compared to controls at 5000 (↓27% in females) and 7500 (↓64% in males and ↓55% in females) ppm. Grossly, small thymus was noted in both sexes at 5000 and 7500 ppm. These findings corresponded with the reduced medullary cellularity observed microscopically in 4/5 males and 2/3 females at 5000 ppm (vs 0 controls) and involution observed in 4/5 males and 4/5 females at 7500 ppm (vs 0 controls). Other decreases ($p \leq 0.05$) observed in absolute and relative (to body) organ weights were considered unrelated to treatment and likely due to decreased terminal body weights (↓9-53%), based on a lack of corroborative histopathological data.

In the males at ≥5000 ppm, decreases ($p \leq 0.05$) in total white blood cell count (↓33-68%) and neutrophils (↓74-91%) were observed. Additionally, lymphocytes, basophils, monocytes, eosinophils, and leucocytes were decreased (↓57-92%, $p \leq 0.01$) in the 7500 ppm males. Reticulocytes were decreased at 5000 ppm (↓59% in males, $p \leq 0.05$) and at 7500 ppm (↓67-84% in both sexes, $p \leq 0.05$). Significant increases ($p \leq 0.05$) over controls were observed in red blood cell parameters (hematocrit, hemoglobin, and RBC counts) at ≥2500 ppm (↑12-20%); however, it was stated in the pathology report that in the absence of any corresponding increase in white blood cell counts or reticulocytes, these finding were considered to be secondary to decreased body weights and impaired nutrition in the treated animals. Increases ($p \leq 0.05$) compared to controls were observed in the following clinical chemistry parameters: (i) blood urea nitrogen, at 5000 ppm in the males (↑50%) and at 7500 ppm in the males (↑200%) and females (↑71%); (ii) alkaline phosphatase, in the females at 5000 (↑57%) and 7500 (↑97%) ppm; and (iii) aspartate aminotransferase, at 7500 ppm in the males (↑34%) and females (↑25%). All other differences

in hematological and clinical chemistry parameters were considered unrelated to treatment, because they differed minimally from controls in magnitude and/or percent difference, and/or were not dose-dependent. Urinalysis revealed increased ($p \leq 0.01$) pH in the ≥5000 ppm males (↑0.4-0.8) and in the 7500 ppm females (↑0.6). Additionally, protein levels were decreased ($p \leq 0.01$) in ≥5000 ppm males (↓69-71%) and in the 7500 ppm females (↓45%).

During gross pathological examinations, small size was noted in the following organs at ≥5000 ppm: (i) prostate, (ii) seminal vesicles, and (iii) uterus. Additionally, small testes and spleen were noted in the 7500 ppm males. The following corresponding microscopic observations were noted: (i) colloid absent in the prostate, (ii) reduced seminal colloid, (iii) reduced uterine wall thickness, (iv) atrophy of the testes, and (v) reduced cellularity of the white pulp in the spleen.

The LOAEL for this study is 2500 ppm (equivalent to 249/228 mg/kg/day [M/F]) based on decreased body weight gains and food consumption in both sexes. The NOAEL is 1250 ppm (equivalent to 120/137 mg/kg/day [M/F]).

Based upon the results of this 28-day range-finding study, the doses summarized in Table 1 were selected for the 90-day study.

The submitted study is classified as **acceptable/guideline** and satisfies the purpose for which is was intended, as a range-finding study.

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

CLOTHIANIDIN MAMMALIAN CELLS IN CULTURE, GENE MUTATION (84-2)

EPA Reviewer: Irving Mauer, Ph.D.
Registration Action Branch 3, HED (7509C)
EPA Secondary Reviewer: Nancy McCarroll
Toxicology Branch, HED (7509C)

Date: 10/30/02

Date: 11/07/02

TXR No.: 0050321

DATA EVALUATION RECORD

STUDY TYPE: Mammalian cells in culture, gene mutation assay in V79 cells; OPPTS 870.5300 [84-2].

DP BARCODE: D279778 SUBMISSION CODE: S607124

P.C. CODE: 044309 TOX. CHEM NO.: None

TEST MATERIAL (PURITY): TI-435 (Batch No. 30034708, 95.2% a.i.)

SYNONYMS: Clothianidin (iso). Chemically: N-[(2-chloro-5-thiozolyl) methyl] N′-methyl]-N″-nitroguanidine (from MRID 45422734).

CITATION: Brendler-Schwaab, S. (1999). TI-435: Mutagenicity Study for the Detection of Induced Forward Mutations in the V79-HPRT Assay *in Vitro*, performed at Bayer's AG Department of Toxicology, Wuppertal (GERMANY), Bayer AG Report No. 28851, Study No. TI053841 (Bayer Corporation Agricultural Division Report No. 109992) dated June 8, 1999. MRID 45422738. Unpublished.

SPONSOR: Takeda Chemical Industries, Ltd., Agro Company, Tokyo (JAPAN).

EXECUTIVE SUMMARY:

In repeat mammalian cell gene mutation assays (MRID 45422738), cultures of V79 cells were exposed to TI-435 (Batch No. 30034708, 95.2% a.i. dissolved in dimethylsulfoxide, DMSO) at 6 concentrations ranging from 156 to 5000 μg/mL, both in the presence and absence of metabolic activation (± S9). The S9 fraction was derived from the livers of male Sprague-Dawley rats induced with Arochlor 1254.. Thereafter, cultures were incubated for 7 days (expression period), followed by incubation for 4 to 7 days in selection medium (6-thioguanine) for determining mutant colonies. In addition to solvent controls, additional cultures were exposed to the mutagens, ethylmethane sulfonate (EMS) and dimethylbenzanthracene (DMBA), to serve as positive controls.

The test material was insoluble at ≥ 2500 μg/mL +/- S9 and no cytotoxicity was observed at any dose level. **No significant dose-related or reproducible increase in mutant frequency above that of the negative contrrol was observed.** In contrast, the positive chemicals induced clear mutagenic effects.

Therefore, TI-435 is considered to be non-mutagenic in this V79-HPRT Forward Mutation Assay, both in the presence and absence of metabolic activation. This negative result is at variance with a positive recorded in another laboratory at cytotoxic doses in a different mammalian cell system, L5178Y TK +/- (MRID 45422737).

This study is classified as acceptable/guideline, and satisfies the requirement for the FIFRA Test Guideline for *in vitro* mutagenicity (mammalian cell forward mutation) data.

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

I. MATERIALS AND METHODS

A. MATERIALS:

1. Test Material: TI-435.

Description: Beige powder.
Lot/Batch No.: 30034708.
Purity: 95.2% a.i. (Analyzed HPLC on December 4, 1996).
Stability of compound: Stable in the solvent at room temperature for at least 5 hrs.
CAS No.: 205510-53-8.
Solvent used: Dimethylsulfoxide (DMSO).
Other comments: Storage: Room temperature in the dark.

2. Control Materials:

Negative: Culture medium.
Solvent/Final Concentration: DMSO/1.0 %.
Positive: Non-activated (concentrations, solvent): EMS (900 μg/mL); solvent not identified.
Activated (concentrations, solvent): DMBA (20 μg/mL); solvent not identified.

3. Metabolic Activation: S9 (purchased from ICN Biomedicals, Inc.) was derived from male Sprague-Dawley rats.

x	Aroclor 1254	x	induced	x	rat	x	liver
	phenobarbital		non-induced		mouse		lung
	none				hamster		other
	other						other

If other, describe below: None.

Describe S9-mix composition (final concentrations):

Ingredient	Concentration
$MgCl_2$ x 6 H.20	8 mM
KCl	33 mM
G-6-P	5 mM
NADP	1 mM
S9 fraction (v/v)	40%
Buffer (in 25 mM $MgCl_2$)	60%

4. Test Cells: Mammalian cells in culture.

	Mouse lymphoma L5178Y cells
	Chinese hamster ovary (CHO) cells
x	V79 cells (Chinese hamster lung fibroblasts)
	Other (list):

Properly maintained? Yes.
Periodically checked for Mycoplasma contamination? Yes.
Periodically checked for karyotype stability? Yes.
Periodically "cleansed" against high spontaneous background? Yes.
Media: Hypoxanthine-free Eagle's Minimal Essential Medium (MEM, Earle), supplemented with amino acids, glutamine, vitamins, $NaHCO_3$ penicillin, streptomycin, and heat inactivated fetal cell serum.

5. Locus Examined:

_____ thymidine kinase (TK)
Selection agent: _____ bromodeoxyuridine (BrdU)
(give concentration) _____ fluorodeoxyuridine (FdU)
_____ trifluorothymidine (TFT)

__x__ hypoxanthine-guantine-phosphoribosyl transferase (HPRT)
Selecion agent: _____ 8-azaguanine (8-AG)
(give concentration) 10 μg/mL 6-thioguanine (6-TG)

293

_____ Na^+/K^+ ATPase
Selection agent: _____ ouabain
(give concentration):

_____ Other (locus and/or selection agent; give details):

6. Test Compound Concentrations Used:

Preliminary cytotoxicity test: Non-activated and activated conditions: 62.5, 125, 250, 500, 1000, 1250, 2000, 2500 μg/mL.

Mutation Assays:
Non-activated and activated conditions: 156, 313, 625, 1250, 2500, 5000 μg/mL (both trials).

B. TEST PERFORMANCE:

1. Cell Treatment:

a. Cells exposed to test compound, negative/solvent or positive controls for: 5 hours (non-activated) 5 hours (activated).

b. After washing, cells cultured for 7 days (expression period) before cell selection:

c. After expression, 3 x 10^5 cells/dish (8 dishes/group) were cultured for 6-7 days in selection medium to determine numbers of mutants and 200 cells/dish (3 dishes/group) were cultured for 6-7 days without selective agent to determine cloning efficiency.

2. Statistical Methods:

Statistical analysis relies on the mutation frequency (MF) rather than individual plate counts, which are submitted to a weighted analysis of variance, as well as to a weighted recursive regression, both with Poisson derived weights (Arlett *et al.*, 1989)[1].

Mutation frequencies based on less than 5 plate counts are very uncertain estimates of the true mutation frequencies and provide no basis for a rational

[1]Arlett, C.F.; Smith, D.M.; Green, M.H.L.; McGregor, D.B; Clarke, G.M.; Cole, J. and Asquith, J.C. (1989). In: Kirkland, D.J. (Ed.), Statistical evaluation of mutagenicity test data. UKEMS subcommittee on guidelines for mutagenicity testing. Report Part III, Cambridge University Press, Cambridge, 66-101.

estimation of the variance. Therefore, such values are not included in the statistical analysis. If the relative population growth in an experiment falls below 10%, the corresponding mutation frequency is discarded.

The two mutation frequency values obtained per group in the standard HPRT assay are, although somewhat related, considered as independent measurements, thus, increasing the power of the statistical tests applied. Since the protocol of the HPRT assay requires at least two replicates, the overall analysis per type of metabolic activation is the most important one for classifying substances into mutagens and non-mutagens. However, separate analyses will be run for each assay in order to examine the consistency of the results.

All groups are included in the weighted analysis of variance followed by pairwise comparisons to the vehicle control on a nominal significance level of $\propto = 0.05$ using the Dunnett test (Dunnett, 1955). The regression analysis part is performed on the basis of the actual dose levels thereby omitting the positive, negative and vehicle controls. If there is a significant increase of the mutation frequency with dose ($\propto = 0.05$) in the main analysis, the highest dose group will be dropped and the analysis will be repeated until $p > 0.05$. Dose levels eliminated in that way are flagged correspondingly.

The following determinations were made:

Relative Survival (or Relative Cloning Efficiency):

$$(\%) = \frac{\text{Average No. of Colonies per Treated Culture}}{\text{Average No. of Colonies per Vehicle Control}} \times 100$$

Absolute Population Growth (APG) = Cell No. Day 4 x Cell No. Day 7 for each Culture

$$\text{Relative Population Growth (\%)} = \frac{\text{APS Population Growth of Treated Cultures}}{\text{ABS Population Growth of Corresponding Vehicle Control Culture}} \times 100$$

Absolute Cloning Efficiency (CE) (%) = Average No. Viable Colonies per Dish/200 x 100

$$\text{Mutation Frequency (MF)} = \frac{\text{Total No. Mutant Colonies x 100}}{\text{No. of Evaluated Dishes x 3 x } 10^5 \text{ x C.E.}}$$

3. Evaluation Criteria:

Both assay acceptance and analysis of results criteria were presented.

ATTACHMENT

THE FOLLOWING ATTACHMENT IS NOT AVAILABLE ELECTRONICALLY
SEE THE FILE COPY

Table : 3

V79/HPRT MUTAGENESIS ASSAY RESULTS: NONACTIVATION

B A Y E R A G

Study Number: T1053841 | Batch Number: LOT30034708 | Test Date: February 18, 1997

Test Substance: TI 435 | Vehicle: DMSO | Selective Agent: 10 µg/ml 6-thioguanine

Cells seeded for analysis: 200 Cells / Dish for C.E.; 3 X 10E5 / Dish for mutant selection

Concentration[1] Concentration[2]	SURVIVAL TO TREATMENT MEAN COLONY NUMBER	SD	PERCENT VEH.CONTROL	POPULATION ABSOLUTE X 10E12	GROWTH REL.(% VEH. CONTROL)	MUTANT COLONIES DISH NUMBER 1	2	3	4	5	6	7	8	TOTAL MUTANT Colonies	ABSOLUTE C.E. (%)	SD	MUTANT FREQUENCY X 10E-6 [a]
Negative Control [b]	C	--		728.50	71.7	1	1	1	0	0	0	0	0	3	68.2	+/- 1.6	1.8
Negative Control [b]	C	--		1117.19	127.6	1	0	0	0	0	0	C	C	1	71.3	+/- 3.0	0.8
Vehicle Control [c]	157.0	--	100.0	1015.88	100.0	2	0	0	0	0	0	0	0	2	66.7	+/- 3.1	1.3
Vehicle Control [c]	147.0	--	100.0	875.75	100.0	1	1	0	0	0	0	0	0	2	66.3	+/- 2.0	1.3
Positive Control EMS 900 µg/ml	139.0	--	88.5	291.38	28.7	75	68	64	55	71	72	48	49	502	59.2	+/- 3.7	353.5
Positive Control EMS 900 µg/ml	C	--		217.00	24.8	76	79	52	83	55	43	53	C	441	59.5	+/- 1.0	352.9
TEST SUBSTANCE																	
156 µg/ml	172.0	--	109.6	735.75	72.4	1	2	3	3	2	0	0	0	11	66.5	+/- 0.5	6.9
156 µg/ml	184.0	+/- 7.1	125.2	770.00	87.9	1	0	0	0	0	0	0	0	1	73.2	+/- 4.3	0.6
313 µg/ml	133.5	+/- 2.1	85.0	907.63	89.3	3	0	0	0	0	0	0	C	3	61.5	+/- 4.0	2.3
313 µg/ml	156.0	--	106.1	1213.63	138.6	1	1	0	0	0	0	C	C	2	54.8	+/- 7.2	2.0
625 µg/ml	143.0	--	91.1	1225.13	120.6	1	0	0	0	0	0	0	0	1	66.0	+/- 0.9	0.6
625 µg/ml	142.0	--	96.6	1013.38	115.7	1	1	1	2	0	0	0	0	5	61.8	+/- 1.2	3.4
1250 µg/ml	146.5	+/- 7.8	93.3	754.69	74.3	2	1	1	0	0	0	0	0	4	70.0	+/- 5.2	2.4
1250 µg/ml	161.0	+/- 4.4	109.5	686.00	78.3	4	0	0	0	0	C	C	C	4	74.5	+/- 7.8	3.6
2500 µg/ml P	119.7	+/- 10.1	76.2	474.00	46.7	1	0	0	0	0	C	C	C	1	78.2	+/- 8.5	0.9
2500 µg/ml P	160.0	+/- 11.5	108.8	693.75	79.2	1	1	0	0	0	C	C	C	2	79.7	+/- 5.7	1.7
5000 µg/ml P	123.3	+/- 5.8	78.6	604.75	59.5	2	0	0	0	0	0	0	C	2	73.8	+/- 1.5	1.3
5000 µg/ml P	141.5	+/- 16.3	96.3	730.13	83.4	1	0	0	C	C	C	C	C	1	61.0	+/- 1.3	---

For explanation of table see page: 29

HED Records Center Series 361 Science Reviews - File R107689 - Page 312 of 721

AC Report No. 109992
Page 32 of 37

Table : 4

V79/HPRT MUTAGENESIS ASSAY RESULTS: NONACTIVATION

B A Y E R A G

Study Number: T1053841 | Batch Number: LOT30034708 | Test Date: February 28, 1997

Test Substance: TI 435 | Vehicle: DMSO | Selective Agent: 10 µg/ml 6-thioguanine

Cells seeded for analysis: 200 Cells / Dish for C.E.; 3 X 10E5 / Dish for mutant selection

Concentration[1] Concentration[2]	SURVIVAL TO TREATMENT MEAN COLONY NUMBER	SD	PERCENT VEH.CONTROL	POPULATION ABSOLUTE X 10E12	GROWTH REL.(% VEH. CONTROL)	MUTANT COLONIES DISH NUMBER 1	2	3	4	5	6	7	8	TOTAL MUTANT Colonies	ABSOLUTE C.E. (%)	SD	MUTANT FREQUENCY X 10E-6 [a]
Negative Control[b]	183.3	+/- 12.0	102.4	983.13	158.9	1	1	0	0	0	0	0	C	2	97.5	+/- 9.3	1.0
Negative Control[b]	198.3	+/- 30.9	98.3	905.63	91.9	2	3	0	0	0	0	0	0	5	92.8	+/- 4.0	2.2
Vehicle Control[c]	179.0	+/- 2.0	100.0	618.63	100.0	2	1	3	2	1	1	3	2	15	104.5	+/- 11.0	6.0
Vehicle Control[c]	201.7	+/- 8.5	100.0	985.13	100.0	1	0	0	0	0	0	0	0	1	100.8	+/- 6.0	0.4
Positive Control EMS 900 µg/ml	58.0	+/- 5.0	32.4	228.56	36.9	172	178	82	119	95	77	73	C	796	45.0	+/- 4.9	842.3
Positive Control EMS 900 µg/ml	60.3	+/- 5.7	29.9	185.63	18.8	133	143	168	146	140	139	160	139	1168	54.2	+/- 6.5	898.5
TEST SUBSTANCE																	
156 µg/ml	159.7	+/- 17.6	89.2	818.63	132.3	1	2	4	5	1	1	2	3	19	79.3	+/- 5.4	10.0
156 µg/ml	211.0	+/- 15.6	104.6	839.94	85.3	1	1	2	0	2	0	2	2	10	110.3	+/- 6.8	3.8
313 µg/ml	147.3	+/- 20.5	82.3	610.94	98.8	1	1	2	1	2	2	1	1	11	84.7	+/- 1.3	5.4
313 µg/ml	195.5	+/- 36.1	96.9	872.00	88.5	0	1	0	0	0	1	0	1	3	99.0	+/- 7.0	1.3
625 µg/ml	161.3	+/- 8.0	90.1	754.88	122.0	1	0	0	1	0	0	1	C	3	86.3	+/- 6.0	1.7
625 µg/ml	153.0	+/- 11.3	75.9	796.88	80.9	1	0	4	1	2	2	0	3	13	99.8	+/- 7.5	5.4
1250 µg/ml	156.0	+/- 15.7	87.2	541.13	87.5	2	0	0	0	0	0	0	0	2	104.0	+/- 10.6	0.8
1250 µg/ml	147.7	+/- 4.2	73.2	1013.44	102.9	2	2	0	0	0	0	0	0	4	93.2	+/- 6.4	1.8
2500 µg/ml P	77.3	+/- 12.1	43.2	331.25	53.5	2	0	1	5	0	3	3	1	15	89.8	+/- 12.3	7.0
2500 µg/ml P	95.7	+/- 6.7	47.4	756.00	76.7	1	1	2	0	1	0	0	1	6	72.8	+/- 10.8	3.4
5000 µg/ml P	119.3	+/- 8.1	66.7	511.50	82.7	3	2	5	4	2	3	2	3	24	93.0	+/- 1.0	10.8
5000 µg/ml P	117.7	+/- 17.6	58.3	973.00	98.8	1	0	0	1	0	1	2	0	5	80.2	+/- 2.8	2.6

For explanation of table see page: 29

AC Report No. 109992
Page 33 of 37

HED Records Center Series 361 Science Reviews - File R107689 - Page 313 of 721

Table : 5

V79/HPRT MUTAGENESIS ASSAY RESULTS: ACTIVATION

B A Y E R A G

Study Number: T1053841 | Batch Number: LOT30034708 | Test Date: March 14, 1997

Test Substance: TI 435 | Vehicle: DMSO | Selective Agent: 10 µg/ml 6-thioguanine

Cells seeded for analysis: 200 Cells / Dish for C.E.; 3 X 10E5 / Dish for mutant selection

Concentration[1] Concentration[2]	SURVIVAL TO TREATMENT MEAN COLONY NUMBER	SD	PERCENT VEH.CONTROL	POPULATION ABSOLUTE X 10E12	GROWTH REL.(% VEH. CONTROL)	MUTANT COLONIES DISH NUMBER 1	2	3	4	5	6	7	8	TOTAL MUTANT Colonies	ABSOLUTE C.E. (%)	SD	MUTANT FREQUENCY X 10E-6 [a]
Negative Control [b]	189.3	+/- 18.1	99.5	804.75	115.6	1	1	0	0	0	0	0	0	2	84.0	+/- 3.3	1.0
Negative Control [b]	190.3	+/- 3.5	98.4	618.00	86.6	2	1	0	0	0	0	0	0	3	86.8	+/- 4.6	1.4
Vehicle Control [c]	190.3	+/- 5.9	100.0	696.00	100.0	4	1	0	0	0	0	0	0	5	92.5	+/- 4.8	2.3
Vehicle Control [c]	193.3	+/- 6.4	100.0	714.00	100.0	2	1	2	1	1	1	0	0	8	75.2	+/- 7.0	4.4
Positive Control DMBA 20 µg/ml	178.7	+/- 9.3	93.9	394.63	56.7	8	6	8	8	9	6	11	19	75	73.8	+/- 12.0	42.3
Positive Control DMBA 20 µg/ml	181.0	+/- 7.5	93.6	587.25	82.2	14	7	11	12	10	13	19	15	101	74.3	+/- 6.5	56.6
TEST SUBSTANCE																	
156 µg/ml	193.3	+/- 6.4	101.6	805.13	115.7	1	2	0	0	0	0	0	0	3	91.2	+/- 6.4	1.4
156 µg/ml	188.7	+/- 5.0	97.6	675.75	94.6	2	1	0	0	0	0	0	0	3	90.2	+/- 8.6	1.4
313 µg/ml	197.0	+/- 7.0	103.5	574.44	82.5	1	1	1	0	0	0	0	0	3	90.8	+/- 4.6	1.4
313 µg/ml	181.7	+/- 4.5	94.0	733.88	102.8	1	1	0	0	0	0	0	0	2	90.3	+/- 4.5	0.9
625 µg/ml	163.3	+/- 14.7	85.8	618.75	88.9	2	1	1	0	0	0	0	0	4	94.7	+/- 0.8	1.8
625 µg/ml	185.7	+/- 12.7	96.0	714.00	100.0	2	1	2	0	0	0	0	0	5	80.2	+/- 7.6	2.6
1250 µg/ml	185.7	+/- 4.5	97.5	456.75	65.6	1	1	1	0	0	0	0	0	3	93.3	+/- 9.8	1.3
1250 µg/ml	174.3	+/- 8.7	90.2	643.50	90.1	2	2	2	1	1	0	0	0	8	84.0	+/- 5.8	4.0
2500 µg/ml P	135.7	+/- 30.2	71.3	915.00	131.5	3	1	3	1	2	0	0	0	10	86.8	+/- 8.0	4.8
2500 µg/ml P	155.0	+/- 3.6	80.2	869.69	121.8	2	1	1	1	0	0	0	0	5	91.2	+/- 7.3	2.3
5000 µg/ml P	161.0	+/- 7.5	84.6	725.94	104.3	1	0	0	0	0	0	0	0	1	68.2	+/- 2.0	0.6
5000 µg/ml P	155.3	+/- 0.6	80.3	751.75	105.3	1	1	0	0	0	0	0	0	2	73.0	+/- 2.2	1.1

For explanation of table see page: 29

AC Report No. 109992
Page 34 of 37

HED Records Center Series 361 Science Reviews - File R107689 - Page 314 of 721

Table : 6

V79/HPRT MUTAGENESIS ASSAY RESULTS: ACTIVATION

B A Y E R A G

Study Number: T1053841 | Batch Number: LOT30034708 | Test Date: March 21, 1997

Test Substance: TI 435 | Vehicle: DMSO | Selective Agent: 10 µg/ml 6-thioguanine

Cells seeded for analysis: 200 Cells / Dish for C.E.; 3 X 10E5 / Dish for mutant selection

Concentration[1] Concentration[2]	SURVIVAL TO TREATMENT MEAN COLONY NUMBER	SD	PERCENT VEH.CONTROL	POPULATION ABSOLUTE X 10E12	GROWTH REL.(% VEH. CONTROL)	MUTANT COLONIES DISH NUMBER 1	2	3	4	5	6	7	8	TOTAL MUTANT Colonies	ABSOLUTE C.E. (%)	SD	MUTANT FREQUENCY X 10E-6 [a]
Negative Control [b]	214.3	+/- 14.5	128.3	1216.25	149.7	2	0	1	0	0	0	0	0	3	78.7	+/- 7.1	1.6
Negative Control [b]	167.3	+/- 19.1	86.9	926.25	98.1	0	0	0	0	1	C	C	C	1	89.7	+/- 4.5	0.7
Vehicle Control [c]	167.0	+/- 7.9	100.0	812.25	100.0	1	1	0	0	0	1	0	0	3	94.3	+/- 7.6	1.3
Vehicle Control [c]	192.7	+/- 18.0	100.0	944.56	100.0	1	0	0	0	0	1	0	C	2	89.0	+/- 5.1	1.1
Positive Control DMBA 20 µg/ml	190.5	+/- 12.0	114.1	795.00	97.9	18	20	15	13	5	12	13	4	100	72.0	+/- 3.8	57.9
Positive Control DMBA 20 µg/ml	153.7	+/- 3.1	79.8	603.75	63.9	18	11	11	4	5	4	4	C	57	84.5	+/- 2.8	32.1
TEST SUBSTANCE																	
156 µg/ml	154.3	+/- 15.0	92.4	1110.00	136.7	0	1	1	1	1	2	0	0	6	68.0	+/- 4.8	3.7
156 µg/ml	138.3	+/- 23.2	71.8	606.25	64.2	0	0	0	0	0	1	C	C	1	101.7	+/- 2.8	0.5
313 µg/ml	159.3	+/- 12.9	95.4	1188.00	146.3	2	0	0	0	0	0	C	C	2	78.2	+/- 3.3	1.4
313 µg/ml	217.3	+/- 16.7	112.8	1129.88	119.6	1	1	1	0	0	0	0	0	3	84.3	+/- 2.8	1.5
625 µg/ml	159.3	+/- 14.4	95.4	1253.06	154.3	0	0	0	1	0	0	0	0	1	68.7	+/- 4.9	0.6
625 µg/ml	152.3	+/- 5.1	79.1	749.25	79.3	1	0	0	0	0	0	0	C	1	94.2	+/- 6.7	0.5
1250 µg/ml	166.0	+/- 14.7	99.4	975.00	120.0	0	1	0	1	0	1	0	0	3	66.0	+/- 1.8	1.9
1250 µg/ml	178.3	+/- 14.5	92.6	935.25	99.0	0	0	0	0	1	1	0	0	2	74.5	+/- 5.4	1.1
2500 µg/ml P	145.3	+/- 3.8	87.0	793.13	97.6	1	2	0	0	3	0	1	0	7	86.8	+/- 3.2	3.4
2500 µg/ml P	153.3	+/- 10.4	79.6	871.88	92.3	2	4	2	1	0	2	1	3	15	68.8	+/- 6.3	9.1
5000 µg/ml P	190.7	+/- 25.7	114.2	789.25	97.2	0	1	0	0	1	0	0	2	4	82.3	+/- 3.5	2.0
5000 µg/ml P	164.7	+/- 3.8	85.5	642.69	68.0	0	0	1	0	0	0	0	1	2	124.3	+/- 4.5	0.7

For explanation of table see page: 29

AC Report No. 109992
Page 35 of 37

HED Records Center Series 361 Science Reviews - File R107689 - Page 315 of 721

TI 435
V79-HPRT Assay
Study No. T 1053841
BAYER AG

Table 7: Statistical Analysis: Weighted ANOVA and regression results[1)]

Test Substance: TI 435

Batch Number : LOT30034708

Vehicle : DMSO

S9	p Values Weighted ANOVA	Regression	PC	Concentration (µg/ml) 156	313	625	1250	2500	5000
-	0.924	0.552	*	-	-	-	-	-	-
+	0.074	0.807	*	-	-	-	-	*	-

1) Description of statistical method see page 18

* = Significant (α = 0.05) increase relative to vehicle control using the Dunnett test
PC = Positive Control

CLOTHIANIDIN MAMMALIAN CELLS IN CULTURE; GENE MUTATION (84-2)

EPA Reviewer: Irving Mauer, Ph.D. Date: 11/20/02
Registration Action Branch 3, HED (7509C)
EPA Secondary Reviewer: Nancy McCarroll Date: 11/25/02
Toxicology Branch, HED (7509C)

TXR No.: 0050321

DATA EVALUATION RECORD

STUDY TYPE: Mammalian cells in cultures; gene mutation assay in mouse lymphoma (L5178Y) cells; OPPTS 870.5300 [84-2].

DP BARCODE: D279778 SUBMISSION CODE: S607124

P. C. CODE: 044309 TOX. CHEM. NO.: None.

TEST MATERIAL (PURITY): TI-435 (Batch No. 30034708, 96.0% a.i., see MRID 45422736)

SYNONYMS: Clothianidin (iso)
Chemically: (E)-1-(2-chloro-5-thiazolylmethyl)-3-methyl-2-nitroguanidine

CITATION: Durwood, R. (2000). L5178Y TK +/- Mouse Lymphoma Assay, performed at Safepharm Laboratories, Ltd. (SPL), Derby (UK), SPL Project No. 178/112 (Bayer Corporation Agricultural Division Report No. 109830), dated March 8, 2000. MRID 45422737. Unpublished.

SPONSOR: Takeda Chemical Industries, Ltd., Agro Company, Tokyo (JAPAN).

EXECUTIVE SUMMARY: In repeat microsuspension mammalian cell gene mutations assays (MRID 45422737), cultures of heterozygous L5178Y TK +/- mouse lymphoma cells were exposed to TI-435 (Batch No. 30034708, 96.0% a.i. in dimethylsulfoxide DMSO) in the presence and absence of metabolic activation (± S9), initially at a dose range of 312.5 to 2500 μg/mL (EXPERIMENT 1), and repeated at dose ranges of 300 to 2000 μg/mL -S9 and 600 to 2400 μg/mL +S9 (EXPERIMENT 2). The S9 liver homogenate was derived from Sprague-Dawley male rats induced with Aroclor 1254. All cultures were then incubated for 3 hours at 37°C, and forward mutation (TK-/-) determined in resultant colonies after treatment with trifluorothymidine. In addition to vehicle (DMSO) control, additional cultures were exposed to the mutagens ethylmethane sulfonate (EMS) and cyclophosphamide (CP), to serve as positive controls.

TI-435 induced statistically significant and dose-related increases in mutant frequency (MF), both in the presence and absence of metabolic activation, but these increases were only observed at dose levels that were cytotoxic. In EXPERIMENT 1, slight increases in the MF (1.6-fold -S9; 1.5-fold +S9) were seen at cytotoxic levels (*i.e.*, < 10% survival). In EXPERIMENT 2, the relative total growth (RTG) at 1667 μg/mL -S9 was 0.3 and the MF was 272.04/10^6 viable cells which was significant ($p < 0.05$) and ~3-fold higher than the control (93.50/10^6 viable cells). Higher concentrations (> 2000 μg/mL) were severely cytotoxic (RTG = 0.02). With S9 activation; RTG was 0.67 and 0.26 at 2000 and 2400 μg/mL, respectively. MFs at these levels (217.03/10^6 viable cells at 2000 and 304.28/10^6 viable cells at 2400 μg/mL) were significant ($p < 0.05$) and 2.3 to 3.3-fold higher than the control (94.09/10^6 viable cells). Although the response was limited to high concentrations, the degree of cytotoxicity in EXPERIMENT 2 was less than the level (≥ 80%) considered to produce false positive results. **It was, therefore, concluded that TI-435 Batch No. 30034708 was positive in this mammalian system. The observed response was primarily due to small colony formation, indicating clastogenic activity**, which is consistent with the positive response at similar cytotoxic levels found for treated CHL cells (MRID 45422736).

This study is classified as acceptable/guideline, and satisfies the FIFRA Test Guideline for *in vitro* mutagenicity (mammalian forward mutation) data.

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

302

I. MATERIALS AND METHODS:

A. MATERIALS:

1. Test Material: TI-435.
Description: Pale yellow powder.
Lot/Batch No.: 30034708.
Purity: 96% a.i. [Same batch as in MRID 45422734 and 45422731].
Stability of compound: Stable at room temperature for at least 24 hours (see MRID No. 45422731).
CAS No. 205510-53-8
Solvent used: Dimethylsulfoxide (DMSO).
Other comments: Store at room temperature in the dark.

2. Control Materials:
Negative: None.
Solvent/Final Concentration: DMSO/Final concentration not given.
Positive: Non-activated (concentration, solvent): Ethylmethane sulfonate (EMS, 800 μg/mL); solvent not identified.
Activated (concentration, solvent): Cyclophosphamide (CP, 7.5 μg/mL); solvent not identified..

3. Metabolic Activation: S9 derived "in-house" from male Sprague-Dawley rats (weighing 200 g).

x	Aroclor 1254	x	induced	x	rat	x	liver
	phenobarbital		non-induced		mouse		lung
	none				hamster		other
	other				other		

If other, describe below.

Describe S9-mix composition (prepared "in-house") by "mixing S9, NADP (25mM) and G-6-P (25 mM) in RPMI 1640 culture medium without horse serum..

4. Test Cells: Mammalian cells in culture.

__x__ Mouse lymphoma L5178Y cells.
_____ Chinese hamster ovary (CHO) cells.
_____ V79 cells (Chinese hamster lung fibroblasts).
_____ Other (list):
Source: Dr. D. Clive, Burroughs-Welcome, Glaxo (UK).

Properly maintained? Yes.
Periodically checked for Mycoplasma contamination? Not stated.
Periodically checked for karyotype stability? Not stated.
Periodically "cleansed" against high spontaneous background? Yes.
Media: RPMI 1640.

5. Locus Examined:

__x__ thymidine kinase (TK)
Selection agent: _____ bromodeoxyuridine (BrdU)
(give concentration): 4 μg/mL__ 5-trifluorothymidine (TFT)

_____ hypoxanthine-guanine-phosphoribosyl transferase (HPRT)
Selection agent: _____ 8-azaguanine (8-AG)
(give concentration): _____6-thioguanine (6-TG)

_____ NA^+/K^+ ATPase
Selection agent: _____ ouabain
(give concentration):

_____ other (locus and/or selection agent; give details):

6. Test Compound Concentrations Used:

Preliminary Cytotoxicity Test: Non-activated and Activated Conditions:
39, 78.1, 156.25, 312.5, 625, 1250, 2500 μg/mL.

Main Assay: Non-activated and Activated Conditions:

EXPERIMENT 1: 312.5, 625, 1250, 1667, 2500 μg/mL.

#304

EXPERIMENT 2: 300, 600, 1200, 1600, 2000 μg/mL -S9
600, 1200, 1600, 2000, 2400 μg/mL +S9

B. TEST PERFORMANCE:

1. Cell Treatment:

a. Cells exposed to test compound, negative/solvent or positive controls for:
__3__ hours (non-activated) __3__ hours (activated)

b. After washing, cells cultured for __2__ days (expression period) before cell selection:

c. After expression, __2000__ cells/well (96 wells/group) were cultured for __10-14__ days in selection medium to determine numbers of mutants and __2__ cells/well (96 wells/plate) were cultured for __10-14__ days without selective agent to determine cloning efficieny.

2. Statistical Methods:

Since the distribution of colony-forming units over the wells is described by the Poisson distribution, the plating efficiencey (P.E.) was calculated using the zero term of the Poisson distribution method [P(O)].

3. Evaluation Criteria:

$$P(O) = \frac{\text{Number of negative wells}}{\text{Total wells plated.}}$$

$$P.E.\% = \frac{-1n\,P(O) \times 100}{\text{Number of cells/well}}$$

$$\text{M.F. per survivor} = \frac{[1\ P(O)\ \text{selective medium/cells per well in selec. medium}]}{\text{Surviving fraction in non-selective medium}}$$

4. Criteria for assay acceptability as well as mutagenic response were provided.

A. ANALYTICAL DETERMINATIONS:

Analytical determinations conducted by the performing laboratory indicated that the test material was stable at ambient temperatures for at least 24 hours, uniformly dispersed throughout the formulation and contained 97-104% of the nominal concentrations.

B. PRELIMINARY CYTOTOXICITY ASSAY:

A reduction in P.E.% (19% of control -S9 and 67% of control +S9) at the maximum dose (2500 µg/mL) compared to solvent was seen.

The test material was not cytotoxic for the remaining concentrations with or without S9 (39 - 1250 µg/mL). Accordingly, doses ranging from 312.5 to 2500 µg/mL were evaluated in EXPERIMENT 1.

C. MUTAGENICITY ASSAY:

For EXPERIMENT 1, TI-435 induced a reduction in relative total growth (RTG), particularly at the maximum dose level, 2500 µg/mL, in both the absence (0.01) and presence (0.05) of S9 (MRID 45422737, pp. 19, 20, 21, 22 - ATTACHMENT Tables 1, 2). Although the test material did not induce any statistically significant or dose-related (linear trend) increase in mutant frequency (MF x 10^6 per viable cell) ± S9, in both treatment conditions there was evidence of an increase in MF above the control value at the 1667 µg/mL test material dose level (MF = 179.65 x 10^6 -S9 and 133.77 x 10^6 +S9), that were greater than the normal upper value of 125 x 10^6 per viable cells for control cultures (ATTACHMENT, Tables 3, 4, 13). Fold increases at this concentration were 1.6-fold (-S9) and 1.5-fold (+S9). The frequency of colony sizes and their analysis demonstrated that, in the presence and absence of metabolic activation, the increases in MF observed were due to small colony formation, indicative of clastogenic (chromosomal aberration) activity rather than point (gene mutation) activity (ATTACHMENT Tables 5, 6).

For EXPERIMENT 2, doses evaluated were 300, 600, 1200, 1600 and 2000 µg/mL -S9 and 600, 1200, 1600, 2000 and 2400 µg/mL +S9. RTG at 2000 µg/mL -S9 was 0.02 indicating severe cytotoxicity (<10% survival). RTG was 0.3 and the MF (272.04/10^6 viable cells) was ~3-fold higher than the control (93.50/10^6 viable cells) at 1600 µg/mL; the increased MF was significant ($p < 0.05$). With S9 activation, RTG was 0.67 and 0.26 at 2000 and 2400 µg/mL, respectively. MFs at these levels (217.03/10^6 viable cells at 2000 µg/mL and 307.28 at 2400 µg/mL) were 2.3 and 3.3-fold higher than the control (94.09/10^6

viable cells). Both of these values were significant ($p < 0.05$). In agreement with the colony size analysis for EXPERIMENT 1, the increased MFs were associated with an increase in small colony formation (ATTACHMENT Tables 11, 12).

Both positive controls produced marked increases in MF.

The investigator concluded that TI-435 was mutagenic under conditions of this assay. However, significant increases in MF were only observed at dose levels that were cytotoxic.

III. REVIEWER'S DISCUSSION/CONCLUSIONS:

A. The EPA reviewer agrees with the investigator that TI-435 appears to be mutagenic in L5178Y cells assayed up to cytotoxic levels, a response observed primarily due to small colony formation, indicating clastogenic activity rather than point mutations. Although the response was limited to high concentrations, the degree of cytotoxicity was less than the levels (≥ 80%) considered to produce false positive results (Galloway SM 2000)[1]. This positive result appears to be consistent with positive results reported in the *in vitro* cytogenetic assay (MRID 45422736) in CHL cells.

B. STUDY DEFICIENCIES:

None.

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[1]Galloway, S.M. (2000). Cytotoxicity and chromosome aberrations *in vitro*; experience in industry and the case for an upper level on toxicity in the aberration assay. ENVIRON. MOL. MUTAGEN. 35: 191-220.

307

ATTACHMENT

THE FOLLOWING ATTACHMENT IS NOT AVAILABLE ELECTRONICALLY
SEE THE FILE COPY

SPL PROJECT NUMBER: 178/112

TI-435 : L5178Y TK +/- MOUSE LYMPHOMA ASSAY

TABLE 1

96-WELL PLATE COUNTS

Experiment 1 (Without S9)

Replicates

Treatment (μg/ml)		Cell Counts $ 24h	48h	Survival § at day 0		Viability § after day 2		Resistant mutants § after day 2	
0	A	5.61	5.92	73 66	70 59	75 76	73 72	7 15	12 14
	B	5.52	5.54	74 66	64 62	71 70	70 73	7 20	15 19
312.5	A	5.42	6.70	77	71	79	76	15	13
	B	5.83	5.44	69	67	65	73	20	19
625	A	5.44	5.31	75	64	80	80	12	7
	B	5.30	5.86	72	65	74	78	9	16
1250	A	4.53	5.66	56	57	74	74	15	19
	B	4.45	5.94	61	66	76	67	27	7
1667	A	2.65	3.72(2.65)	26	26	61	55	18	13
	B	2.21	3.95(2.65)	25	26	54	57	14	12
2500	A	1.65	1.66(1.65)	3	1	8	6	4	2
	B	1.68	2.25(1.68)	3	2	18	14	0	0

Positive control EMS (μg/ml)

800	A	4.30	5.42	51	43	56	47	53	64
	B	5.38	5.59	47	52	49	50	56	51

$ = Cell counts ($x10^5$), set up on previous day to 2×10^{-5} unless otherwise stated

§ = Positive wells per tray, 96 wells plated unless otherwise stated

EMS = Ethylmethanesulphonate

SPL PROJECT NUMBER: 178/112

TI-435 : L5178Y TK +/- MOUSE LYMPHOMA ASSAY

TABLE 2

96-WELL PLATE COUNTS

Experiment 1 (With S9)

Replicates

Treatment (μg/ml)		Cell Counts $ 24h	Cell Counts $ 48h	Survival § at day 0	Viability § after day 2	Resistant mutants § after day 2
0	A	6.36	6.12	70 76 71 67	66 69 78 60	10 10 11 9
	B	6.69	5.36	68 70 76 73	64 72 72 76	13 9 9 9
312.5	A	6.15	5.89	74 77	63 65	16 7
	B	6.11	5.64	64 54	68 75	6 5
625	A	6.52	6.18	77 70	78 80	12 8
	B	6.23	6.36	66 76	72 70	12 6
1250	A	6.42	5.72	76 67	79 83	10 3
	B	6.11	6.16	69 70	68 65	7 7
1667	A	6.75	5.88	67 70	77 80	22 12
	B	3.79	5.48	54 48	62 69	19 12
2500	A	2.22	2.94	25 16	42 28	28 28
	B	2.31	2.54	10 11	23 29	8 2

Positive control CP (μg/ml)

7.5	A	6.07	5.70	46	55	43	56	40	37
	B	5.99	6.12	52	61	46	47	25	24

$ = Cell counts ($x10^5$), set up on previous day to 2×10^{-5} unless otherwise stated

§ = Positive wells per tray, 96 wells plated unless otherwise stated

CP = Cyclophosphamide

SPL PROJECT NUMBER: 178/112

TI-435 : L5178Y TK +/- MOUSE LYMPHOMA ASSAY

TABLE 3

DATA ANALYSIS

Experiment 1 (Without S9)

Summary of results

Treatment (µg/ml)	%S	%RS	%V	RTG	MF§
0	59.42	100.00	70.37	1.00	108.76
312.5	67.27	97.72	71.99	1.09	133.17
625	63.43	94.42	83.70	1.12	72.70
1250	49.04	72.88	70.90	0.83	137.44
1667	15.61	22.46	44.72	0.14	179.65
2500	1.19	1.43	6.38	0.01	123.42

Positive control (EMS)

Treatment (µg/ml)	%S	%RS	%V	RTG	MF§
800	34.92	52.28	37.33	0.44	1172.55

Comparison with solvent control

Treatment	LMF	W	V	D_i	Var(D)	D^2/Var(D)
0	-9.126	3.32E+09	0.0255			
312.5	-8.924	1.30E+09	0.0432	0.202	0.0687	0.597
625	-9.529	3.12E+09	0.0607	-0.403	0.0862	-----
1250	-8.892	6.97E+08	0.0760	0.234	0.1014	0.540
1667	-8.624	6.12E+08	0.0506	0.502	0.0761	3.310
2500	-9.000	1.54E+08	0.4265	0.126	0.4519	0.035

Test for linear trend

Slope	1.81E-08
Variance	3.62E-16
b^2/S_b	0.909

%RS = Relative survival
%S = Survival day 0
%V = Viability day 2
RTG = Relative total growth
MF§ = TFT resistant mutants/10^6 viable cells 2 days after treatment
EMS = Ethylmethanesulphonate

311

SPL PROJECT NUMBER: 178/112

TI-435 : L5178Y TK +/- MOUSE LYMPHOMA ASSAY

TABLE 4

DATA ANALYSIS

Experiment 1 (With S9)

Summary of results

Treatment (µg/ml)		%S	%RS	%V	RTG	MF§
0		68.03	100.00	64.60	1.00	85.14
312.5		60.29	89.56	61.16	0.89	75.79
625		69.84	99.34	75.99	1.26	68.56
1250		66.28	91.97	73.10	1.13	49.87
1667		48.70	56.78	69.31	0.87	133.77
2500	$$	8.80	8.77	19.11	0.05	(493.31)

Positive control (CP)

Treatment (µg/ml)	%S	%RS	%V	RTG	MF§
7.5	40.74	53.86	34.66	0.51	573.74

Comparison with solvent control

Treatment	LMF	W	V	D_i	Var(D)	D^2/Var(D)
0	-9.371	4.56E+09	0.0303			
312.5	-9.488	2.50E+09	0.0696	-0.116	0.0998	-----
625	-9.588	3.38E+09	0.0630	-0.217	0.0932	-----
1250	-9.906	4.76E+09	0.0846	-0.535	0.1148	-----
1667	-8.919	1.36E+09	0.0411	0.452	0.0714	2.860

Test for linear trend

Slope	-3.82E-09
Variance	1.88E-16
b^2/S_b	0.078

%RS = Relative survival
%S = Survival day 0
%V = Viability day 2
RTG = Relative total growth
$$ = Treatment excluded from analysis due to excessive heterogeneity
MF§ = TFT resistant mutants/10^6 viable cells 2 days after treatment
CP = Cyclophosphamide

SPL PROJECT NUMBER: 178/112

TI-435 : L5178Y TK +/- MOUSE LYMPHOMA ASSAY

TABLE 5

PLATE COUNTS AND MUTATION FREQUENCIES FOR LARGE AND SMALL COLONIES - EXPERIMENT 1 IN THE ABSENCE OF S9

Plate Counts

Treatment (μg/ml)		Small colonies # after day 2				Large colonies # after day 2			
0	A	3	9	10	10	4	3	5	4
	B	2	8	16	9	5	7	4	10
312.5	A	8	7			7	6		
	B	16	10			4	9		
625	A	7	4			5	3		
	B	7	12			2	4		
1250	A	11	14			4	5		
	B	21	2			6	5		
1667	A	16	11			2	2		
	B	11	11			3	1		
2500	A	4	2			0	0		
	B	0	0			0	0		
800 EMS	A	22	28			31	36		
	B	20	13			36	38		

Mutation frequencies

Treatment (μg/ml)	Viable		Small colonies			Large Colonies			Proportion small colony mutants
			Mutants			Mutants			
	Yv	Nv	Ym	Nm	MF§	Ym	Nm	MF§	
0	188	768	701	768	64.9	726	768	40.0	0.61
312.5	91	384	343	384	78.4	358	384	48.7	0.61
625	72	384	354	384	48.6	370	384	22.2	0.68
1250	93	384	170	192	98.4	183	192	33.9	0.74
1667	157	384	335	384	152.6	376	384	23.5	0.86
2500	338	384	378	384	123.4	384	384	0.0	1.00
800 EMS	182	384	301	384	326.2	243	384	612.9	0.37

= Positive wells per tray. Total number of wells scored is 96 unless otherwise stated
MF§ = TFT resistant mutants/10^6 viable cells 2 days after treatment
EMS = Ethylmethanesulphonate
Yv = Number of wells without colonies, day 2 viability plates
Nv = Number of wells scored, day 2 viability plates
Ym = Number of wells without colonies, mutation plates
Nm = Number of wells scored, mutation plates

SPL PROJECT NUMBER: 178/112

TI-435 : L5178Y TK +/- MOUSE LYMPHOMA ASSAY

TABLE 6

PLATE COUNTS AND MUTATION FREQUENCIES FOR LARGE AND SMALL COLONIES - EXPERIMENT 1 IN THE PRESENCE OF S9

Plate Counts

Treatment (µg/ml)		Small colonies # after day 2				Large colonies # after day 2			
0	A	4	8	5	5	6	3	5	4
	B	6	4	3	6	7	5	6	3
312.5	A	11	2			5	5		
	B	3	1			3	4		
625	A	7	2			5	6		
	B	5	1			7	5		
1250	A	2	1			8	2		
	B	5	3			2	4		
1667	A	17	6			5	6		
	B	13	10			6	2		
2500	A	21	22			7	6		
	B	5	1			3	1		
7.5 CP	A	28	30			12	7		
	B	12	21			13	3		

Mutation frequencies

Treatment (µg/ml)		Viable		Small colonies			Large Colonies			Proportion small colony mutants
				Mutants			Mutants			
		Yv	Nv	Ym	Nm	MF§	Ym	Nm	MF§	
0		211	768	727	768	42.5	729	768	40.3	0.51
312.5		113	384	367	384	37.0	367	384	37.0	0.50
625		84	384	369	384	26.2	361	384	40.6	0.39
1250		89	384	373	384	19.9	368	384	29.1	0.41
1667		96	384	338	384	92.0	365	384	36.6	0.71
2500	$$	262	384	335	384	357.1	367	384	118.4	0.74
7.5 CP		192	384	293	384	390.2	349	384	137.9	0.72

= Positive wells per tray. Total number of wells scored is 96 unless otherwise stated
MF§ = TFT resistant mutants/10^6 viable cells 2 days after treatment
CP = Cyclophosphamide
Yv = Number of wells without colonies, day 2 viability plates
Nv = Number of wells scored, day 2 viability plates
Ym = Number of wells without colonies, mutation plates
Nm = Number of wells scored, mutation plates
$$ = Heterogeneity between cultures in well totals

SPL PROJECT NUMBER: 178/112

TI-435 : L5178Y TK +/- MOUSE LYMPHOMA ASSAY

TABLE 7

96 WELL PLATE COUNTS

Experiment 2 (Without S9)

Replicates

Treatment (μg/ml)		Cell Counts $ 24h	48h	Survival § at day 0		Viability § after day 2		Resistant mutants § after day 2	
0	A	6.85	7.31	75 62	76 68	63 72	64 70	11 16	5 6
	B	7.03	7.52	65 78	65 74	56 58	62 55	9 10	8 7
300	A	6.74	7.04	80	82	70	66	10	14
	B	7.04	6.69	83	78	71	72	6	11
600	A	6.73	7.15	74	77	73	72	14	13
	B	6.11	7.22	70	79	65	48	9	8
1200	A	5.58	6.23	65	60	79	73	18	11
	B	5.94	6.82	65	79	66	68	17	11
1600	A	3.31	4.39	44	26	64	66	24	29
	B	3.61	4.34	42	32	62	62	26	19
2000	A	2.24	2.22	8	3	11	18	2	5
	B	2.18	2.08	5	4	21	17	4	2

Positive control EMS (μg/ml)

800	A	4.97	6.73	59	67	52	47	46	47
	B	4.97	6.40	57	68	57	46	59	62

$ = Cell counts (x 10^5), set up on previous day to 2 x 10^5 unless otherwise stated

§ = Positive wells per tray, 96 wells plated unless otherwise stated

EMS = Ethylmethanesulphonate

SPL PROJECT NUMBER: 178/112

TI-435 : L5178Y TK +/- MOUSE LYMPHOMA ASSAY

TABLE 8

96 WELL PLATE COUNTS

Experiment 2 (With S9)

Replicates

Treatment (μg/ml)		Cell Counts $ 24h	48h	Survival § at day 0		Viability § after day 2		Resistant mutants § after day 2	
0	A	7.17	5.78	80 78	74 77	76 83	87 76	19 15	14 14
	B	7.23	6.35	81 81	81 81	64 71	63 68	5 11	6 14
600	A	6.35	6.40	72	67	75	73	12	21
	B	7.20	5.73	80	75	72	77	12	12
1200	A	6.44	7.16	76	76	77	79	17	18
	B	5.55	7.62	78	74	71	66	10	14
1600	A	6.50	6.76	74	79	74	72	12	13
	B	5.72	7.90	72	73	70	65	9	11
2000	A	5.78	6.38	70	68	69	71	23	25
	B	4.47	6.61	57	59	72	65	26	19
2400	A	3.90	5.48	43	33	64	57	23	23
	B	3.18	4.59	34	28	51	58	25	23

Positive control CP (μg/ml)

7.5	A	5.60	7.28	69	62	67	61	PL	31
	B	5.66	6.17	67	52	54	58	35	25

$ = Cell counts (x 10^5), set up on previous day to 2 x 10^5 unless otherwise stated

§ = Positive wells per tray, 96 wells plated unless otherwise stated

CP = Cyclophosphamide

PL = Plate lost

SPL PROJECT NUMBER: 178/112

TI-435 : L5178Y TK +/- MOUSE LYMPHOMA ASSAY

TABLE 9

DATA ANALYSIS

Experiment 2 (Without S9)

Summary of results

Treatment (μg/ml)	%S	%RS	%V	RTG	MF§
0	66.04	100.00	52.64	1.00	93.50
300	91.99	126.91	64.83	1.13	87.08
600	75.99	101.84	55.72	0.95	109.21
1200	60.29	79.01	68.28	0.95	117.66
1600	23.50	31.28	54.16	0.30	272.04
2000	2.67	3.42	9.59	0.02	179.62

Positive control (EMS)

Treatment (μg/ml)	%S	%RS	%V	RTG	MF§
800	53.01	80.89	37.33	0.45	1091.35

Comparison with solvent control

Treatment	LMF	W	V	D_i	Var(D)	D^2/Var(D)
0	-9.278	3.21E+09	0.0357			
300	-9.349	2.08E+09	0.0633	-0.071	0.0990	-----
600	-9.122	1.39E+09	0.0601	0.155	0.0958	0.252
1200	-9.048	1.50E+09	0.0483	0.230	0.0840	0.629
1600	-8.210	4.09E+08	0.0330	1.068	0.0687	16.602*
2000	-8.625	1.52E+08	0.2044	0.653	0.2400	1.776

Test for linear trend

Slope	5.37E-08
Variance	4.06E-16
b^2/S_b	7.114**

%RS = Relative survival
%S = Survival day 0
%V = Viability day 2
RTG = Relative total growth
MF§ = TFT resistant mutants/10^6 viable cells 2 days after treatment
EMS = Ethylmethanesulphonate
* = $p<0.05$
** = $p<0.01$

SPL PROJECT NUMBER: 178/112

TI-435 : L5178Y TK +/- MOUSE LYMPHOMA ASSAY
T A B L E 10
DATA ANALYSIS

Experiment 2 (With S9)

Summary of results

Treatment (µg/ml)	%S	%RS	%V	RTG	MF§
0	86.93	100.00	72.54	1.00	94.09
600	72.54	83.22	74.24	0.96	108.22
1200	78.43	88.25	71.99	1.00	115.86
1600	74.81	79.24	65.80	0.93	94.72
2000	54.16	57.81	63.89	0.67	217.03
2400	22.27	19.03	45.68	0.26	307.28

Positive control (CP)

Treatment (µg/ml)	%S	%RS	%V	RTG	MF§
7.5	52.64	58.65	49.04	0.59	387.18

Comparison with solvent control

Treatment	LMF	W	V	D_i	Var(D)	D^2/Var(D)
0	-9.271	4.33E+09	0.0261			
600	-9.131	1.84E+09	0.0463	0.140	0.0724	0.270
1200	-9.063	1.65E+09	0.0451	0.208	0.0712	0.608
1600	-9.265	1.99E+09	0.0561	0.007	0.0822	0.001
2000	-8.435	6.53E+08	0.0325	0.836	0.0586	11.911*
2400	-8.088	3.15E+08	0.0336	1.183	0.0597	23.460*

Test for linear trend

Slope	3.59E-08
Variance	1.63E-16
b^2/S_b	7.893**

%RS = Relative survival
%S = Survival day 0
%V = Viability day 2
RTG = Relative total growth
MF§ = TFT resistant mutants/10^6 viable cells 2 days after treatment
CP = Cyclophosphamide
* = $p<0.05$
** = $p<0.01$

318

SPL PROJECT NUMBER: 178/112

TI-435 : L5178Y TK +/- MOUSE LYMPHOMA ASSAY

TABLE 11

PLATE COUNTS AND MUTATION FREQUENCIES FOR LARGE AND SMALL COLONIES - EXPERIMENT 2 IN THE ABSENCE OF S9

Plate Counts

Treatment (µg/ml)		Small colonies # after day 2				Large colonies # after day 2			
0	A	2	2	6	1	9	3	10	5
	B	1	3	4	3	8	5	6	4
300	A	6	5			4	9		
	B	1	3			5	8		
600	A	7	5			7	8		
	B	1	2			8	6		
1200	A	16	2			2	9		
	B	8	6			9	5		
1600	A	13	17			11	12		
	B	10	11			16	8		
2000	A	2	5			0	0		
	B	2	0			2	2		
800 EMS	A	9	11			37	36		
	B	18	24			41	38		

Mutation frequencies

Treatment (µg/ml)	Viable		Small colonies			Large Colonies			Proportion small colony mutants
			Mutants			Mutants			
	Yv	Nv	Ym	Nm	MF§	Ym	Nm	MF§	
0	268	768	746	768	27.6	718	768	63.9	0.31
300	105	384	369	384	30.7	358	384	54.1	0.37
600	126	384	369	384	35.8	355	384	70.5	0.34
1200	98	384	352	384	63.7	359	384	49.3	0.56
1600	130	384	334	384	131.6	337	384	120.5	0.52
2000	317	384	375	384	123.7	380	384	54.6	0.69
800 EMS	182	384	322	384	235.8	232	384	674.9	0.29

= Positive wells per tray. Total number of wells scored is 96 unless otherwise stated

MF§ = TFT resistant mutants/10^6 viable cells 2 days after treatment

EMS = Ethylmethanesulphonate

Yv = Number of wells without colonies, day 2 viability plates

Nv = Number of wells scored, day 2 viability plates

Ym = Number of wells without colonies, mutation plates

Nm = Number of wells scored, mutation plates

SPL PROJECT NUMBER: 178/112

TI-435 : L5178Y TK +/- MOUSE LYMPHOMA ASSAY

TABLE 12

PLATE COUNTS AND MUTATION FREQUENCIES FOR LARGE AND SMALL COLONIES - EXPERIMENT 2 IN THE PRESENCE OF S9

Plate Counts

Treatment (µg/ml)		Small colonies # after day 2				Large colonies # after day 2			
0	A	8	4	3	7	11	10	12	7
	B	0	3	1	2	5	3	10	12
600	A	5	6			7	15		
	B	4	4			8	8		
1200	A	7	7			10	11		
	B	1	3			9	11		
1600	A	5	3			7	10		
	B	1	6			8	5		
2000	A	10	18			13	7		
	B	14	9			12	10		
2400	A	12	17			11	6		
	B	14	11			11	12		
7.5 CP	A	PL	18			PL	31		
	B	20	13			15	12		

Mutation frequencies

Treatment (µg/ml)	Viable		Small colonies			Large Colonies			Proportion small colony mutants
			Mutants			Mutants			
	Yv	Nv	Ym	Nm	MF§	Ym	Nm	MF§	
0	180	768	740	768	25.6	698	768	65.9	0.29
600	87	384	365	384	34.2	346	384	70.2	0.33
1200	91	384	366	384	33.3	343	384	78.4	0.31
1600	103	384	369	384	30.3	354	384	61.8	0.33
2000	107	384	333	384	111.5	342	384	90.6	0.55
2400	154	384	330	384	165.9	344	384	120.4	0.57
7.5 CP	144	384	237	288	198.7	230	288	229.3	0.47

= Positive wells per tray. Total number of wells scored is 96 unless otherwise stated
MF§ = TFT resistant mutants/10^6 viable cells 2 days after treatment
CP = Cyclophosphamide
Yv = Number of wells without colonies, day 2 viability plates
Nv = Number of wells scored, day 2 viability plates
Ym = Number of wells without colonies, mutation plates
Nm = Number of wells scored, mutation plates
PL = plate lost

SPL PROJECT NUMBER: 178/112

TI-435 : L5178Y TK +/- MOUSE LYMPHOMA ASSAY

TABLE 13

SUMMARY OF RESULTS

Experiment 1

Treatment	-S-9				Treatment	+S-9			
(µg/ml)	%RS	RTG	MF§		(µg/ml)	%RS	RTG	MF§	
0	100.00	1.00	108.76		0	100.00	1.00	85.14	
312.5	97.72	1.09	133.17	NS	312.5	89.56	0.89	75.79	NS
625	94.42	1.12	72.70	NS	625	99.34	1.26	68.56	NS
1250	72.88	0.82	137.44	NS	1250	91.97	1.13	49.87	NS
1667	22.46	0.14	179.65	NS	1667	56.78	0.87	133.77	NS
2500	1.43	0.01	123.42	NS	2500 $$	8.77	0.05	(493.31)	
Linear trend				NS	Linear trend				NS
EMS					CP				
800	52.28	0.44	1172.55		7.5	53.86	0.51	573.74	

Experiment 2

Treatment	-S-9				Treatment	+S-9			
(µg/ml)	%RS	RTG	MF§		(µg/ml)	%RS	RTG	MF§	
0	100.00	1.00	93.50		0	100.00	1.00	94.09	
300	126.91	1.13	87.08	NS	600	83.22	0.96	108.22	NS
600	101.84	0.95	109.21	NS	1200	88.25	1.00	115.86	NS
1200	79.01	0.95	117.66	NS	1600	79.24	0.93	94.72	NS
1600	31.28	0.30	272.04	*	2000	57.81	0.67	217.03	*
2000	3.42	0.02	179.62	NS	2400	19.03	0.26	307.28	*
Linear trend				**	Linear trend				**
EMS					CP				
800	80.89	0.45	1091.35		7.5	58.65	0.59	387.18	

$$ = Treatment excluded from analysis because of excessive heterogeneity

%RS = Relative survival

RTG = Relative total growth

MF§ = TFT resistant mutants/10^6 viable cells 2 days after treatment

EMS = Ethylmethanesulphonate

CP = Cyclophosphamide

NS = not significant

* = $p<0.05$

** = $p<0.01$

SPL PROJECT NUMBER: 178/112

APPENDIX II

HISTORICAL CONTROL DATA FOR NEGATIVE AND POSITIVE CONTROLS IN THE L5178Y TK +/- MOUSE LYMPHOMA ASSAY AT SAFEPHARM LABORATORIES (1996)

Archive name	Experiments -S9		Experiments +S9	
	Negative control	Positive control	Negative control	Positive control
	MF	MF	MF	MF
178/1121	108.76	1172.55	85.14	573.74
178/1122	93.50	1091.35	94.09	387.18
813/4291	105.01	703.88	184.04	1880.80
813/3111	90.16	1081.85	77.45	565.96
813/3112	30.33	678.67	62.41	245.77
813/3131	51.18	1177.49	124.24	650.26
0813/3041	93.23	1043.98	81.07	300.74
813/2821	151.81	1221.23	65.71	931.61
719/191	103.74	971.46	71.96	427.82
813/3091	132.17	1584.77	64.30	640.34
813/3092	82.64	833.35	69.64	701.03
445/1701	78.81	1099.67	83.73	442.61
445/1702	81.14	965.22	50.54	450.82
445/1701	138.93	799.82	121.82	346.56
445/1702	131.82	1287.57	83.90	984.66
813/3101	89.32	1334.03	108.37	363.42
813/3102	119.47	985.18	131.99	600.39
813/3191	115.59	1291.72	67.39	490.90
813/3192	126.09	985.18	105.07	382.79
Min	30.33	678.67	50.54	245.77
Max	151.81	1584.77	184.04	1880.80
Mean	101.25	1068.89	91.20	598.28
SD	30.07	227.25	32.06	367.49

SD = standard deviation

EPA Reviewer: Irving Mauer, Ph.D. Date: 11/25/02
Registration Action Branch 3, HED (7509C)
EPA Secondary Reviewer: Nancy McCarroll Date: 11/25/02
Toxicology Branch, HED, (7509C)

TXR No.: 0050321

DATA EVALUATION RECORD

STUDY TYPE: *In vitro* mammalian chromosome aberrations, OPPTS 870.5375 [84-2] in Chinese hamster lung (CHL) cells.

DP BARCODE: D279778 SUBMISSION CODE: S607124

PC CODE: 044309 TOX. CHEM. NO.: None.

TEST MATERIAL (PURITY): TI-435 (Batch No. 30034708, 96.0% a.i.)

SYNONYM(S): Clothianidin (iso).
Chemically: (E)-1-(2-chloro-5-thiazolylmethyl)-3-methyl-2-nitroguanidine

CITATION: Wright, N.P. (2000). TI-435: Chromosome Aberration Test in CHL Cells *In Vitro*, performed at the Safepharm Laboratories, Ltd. (SPL), Derby (UK), SPL Project No. 178/111 (Bayer Corporation Agricultural Division Report No. 110232), dated March 8, 2000. MRID 45422736. Unpublished.

SPONSOR: Takeda Chemical Industries Ltd., Agro Company, Tokyo (JAPAN).

EXECUTIVE SUMMARY:

In repeat cytogenetic (chromosome aberration) assays (MRID 45422736), duplicate cultures of Chinese hamster lung (CHL) cells were exposed to TI-435 (Batch No. 30034708, 96.0% a.i., in dimethylsulfoxide, DMSO) at concentrations ranging from 156.25 to 2500 μg/mL in the presence (and absence) of a metabolic activation system (± S9) derived from the livers of Sprague-Dawley rats induced with Aroclor 1254. Cultures exposed under non-activation conditions were harvested after 12 hours; cultures in S9-activated phase of testing were exposed for 4 hours and harvested after an 8-hour recovery (EXPERIMENT 1).

Subsequently, CHL cells were exposed continuously for 6, 24 or 48 hours to concentrations ranging from 78-1250 μg/mL -S9, or exposed for 6 or 4 hours to concentrations of 312.5 - 1875 μg/mL +S9, followed by an 18- or 8-hour recovery prior to cell harvest (EXPERIMENT 2).

In addition to cultures exposed to the vehicle (DMSO), others were treated with the clastogens, mytomycin C and cyclophosphamide, to serve as positive controls for the non-activation and activation test series, respectively.

TI-435 produced mild to moderate cytotoxicity at the intermediate dose level (625-937.5 μg/mL -S9), increasing to severe dose-related cytotoxicity at higher concentrations (1875 and 1250 μg/mL). The test material also induced significant increases in the frequency of cells with structural aberrations at 1250 μg/mL (6-hr treatment), and at 625 μg/mL (24- and 48-hour treatments). The predominant types of induced aberration were chromatid breaks and exchanges. With S9, the test material was cytotoxic to CHL cells and induced significant increases in chromatid aberrations at 1875 μg/mL following a 6-hour exposure and an 18-hour recovery. Significant effects were also seen at 1562 μg/mL (4-hour treatment, 8-hour recovery); however, damage to chromosomes at this level was accompanied by moderate cytotoxicity (MI was reduced to 39% of control). The most frequently observed aberrations were chromatid exchanges and chromatid breaks; there was, however, no clear indication of a dose-related response in either the presence or absence of S9 activation.

Therefore, TI-435 was shown to be clastogenic to CHL cells *in vitro* in both the presence and absence of S9 activation.

This study is classified as **acceptable/guideline** and satisfies the requirement for FIFRA Test Guideline (*in vitro* cytogenetic) data.

COMPLIANCE:

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

I. MATERIALS AND METHODS:

A. MATERIALS:

1. Test Material: TI-435.

Description: Pale yellow powder.
Lot/Batch No.: 30034708.
Purity: 96.0% a.i.
Stability of compound: Stable at room temperature for 24 hours (see MRID 45422731).
Solvent used: Dimethylsulfoxide (DMSO).
Other comments: Store at 4°C in the dark.
Doses were adjusted to 100% a.i.

2. Control Materials:

Negative: None.

Solvent: DMSO/Final concentration not provided.
Positive: **Non-activated** (concentrations, solvent): Mitomycin C (MMC: 0.025, 0.05, 0.075 μg/mL; solvent not stated).

Activated (concentrations, solvent): Cyclophosphamide (10 μg/mL): solvent not stated.

3. Metabolic Activation: S9 was prepared "in-house" from the livers of male Sprague-Dawley rats weighing about 200 g induced with Aroclor 1254.

x	Aroclor 1254	x	induced	x	rat	x	liver
	phenobaribital		non-induced		mouse		lung
	none				hamster		other
	other						other

If, other, describe below: None.

Describe S9-mix composition (prepared in-house, see MRID 45422736, p.36, Appendix 1):

Constituent	Amount (in 1 mL S9-Mix)
S9	0.5 mL
$MgCl_2$	8.0 μ mol
KCl	33.0 μ mol
G-6-P	5.0 μ mol
NADP	5.0 μ mol
Buffer (phosphate)	30.0 μ mol

5. Test Compound Concentrations Used: Preliminary Cytotoxicity Test:

Non-Activated Conditions: 9.77, 19.53, 39.06, 78.13, 156.25, 312.5, 625, 1250, 2500 μg/mL for 12, 24 and 48 hours.

Activated Conditions: 9.77, 19.53, 39.06, 78.13, 156.25, 312.5, 625, 1250, 2500 μg/mL for 6 and 4 hours followed by 18 and 8 hours in treatment-free medium.

Main Assay:

Non-Activation: 156.25, 312.5, 625, 937.5, 1250, 1875 μg/mL for 12, 24 and 48 hours.

Activation: 156.25, 312.5, 625, 937.5, 1250, 1875 μg for 4- and 6-hour treatment plus 8 and 18 hours recovery.

6. Test Cells: Mammalian Cells in Culture: Chinese hamster lung (CHL) cells, grown in Eagle's Minimum Essential medium with Earle's salts, supplemennted with 10% fetal bovine serum; average generation time = 11 hours.
Properly maintained? Yes.
Cell line or strain periodically checked for Mycoplasma contamination? Not stated.

Cell line or strain periodically checked for karyotype stability? Not stated.

B. TEST PERFORMANCE:

1. Preliminary Cytotoxicity Assay: (See A.6, above). Dose range = 9.77 to 2500 μg/mL; precipitation formed at 2500 μg/mL. Moderate cytotoxicity at 625 and 1250 μg/mL, increased at 2500 μg/mL.

2. Cytogenetic Assay:

a. **Cell Treatment**: Cells exposed to test compound, solvent, or positive control for 12, 24, 48 hours (non-activated), 4,6 hours (activated), followed by 8 or 18 hours recovery in treatment-free medium.

b. **Spindle Inhibition**: Inhibition used/concentration: Colcemid (demelcolcine)/0.1 μg/mL.
Administration time: 2 hours (before cell harvest).

c. **Cell Harvest**:
Cells exposed to test material, solvent or positive control were harvested 12, 24, 48 hours after termination of treatment (non-activated), 12, 24 hours after termination of treatment and recovery (activated).

d. ***Details of Slide Preparation***: Standard cytological preparation onto wet microscope slides; dried; and stained with Gurr's Giemsa R66, then covered with Depex mountant.

e. **Metaphase analysis**:

Number of cells examined per dose: 100.
Scored for structural? Yes.
Scored for numerical? (polyploid, endoreduplicated cells)? Yes.
Coded prior to analysis: Yes.

f. **Statistical analysis**: Data were not evaluated for statistical significance.

g. **Mitotic Index (MI):** 1000 cells examined; expressed as the percentage of the control.

II REPORTED RESULTS:

A. PRELIMINARY CYTOTOXICITY ASSAY:
Precipitation occurred at 2500 μg/mL and dose-related increased cytotoxicity at 625 μg/mL (62% of control) and 2500 μg/mL (35% of control) in the absence of S9.

With S9, compound insolubility was also seen at 2500 μg/mL; but there was no clear evidence of cytotoxicity.

B. CYTOGENETIC ASSAYS:

EXPERIMENT 1:

Due to cytotoxicity, no scorable metaphases were available following 12-hours of treatment with 1250 or 1875 μg/mL -S9, and the MI at 937.5 μg/mL -S9 was only 30% of control. There was, however, no increase in structural chromosome aberrations at 156.25 to 937.5 μg/mL -S9. Similar results were achieved in the presence of S9 activation [*i.e.*, reduced MI (27% of control)] at the highest tested dose (HTD), 1875 μg/mL, but with no evidence of a clastogen response at 937.5 to 1875 μg/mL +S9.

EXPERIMENT 2:

In the non-activated phase of testing, no scorable metaphases was seen at 1250 μg/mL (12 and 24 hours of treatment) and at 937.5 μg/mL (48-hour treatment) but not at 937.5 μg/mL (24-hour treatment). Significant increases in structural chromosome aberrations were seen at the HDT (1250 μg/mL 6-hour treatment), at 625 μg/mL (24- and 48-hour treatments) but not at 937.5 μg/mL (24-hour treatment). The predominant type of induced aberrations were chromatid breaks and chromatid exchanges. The test material did not induce a significant increase in the numbers of polyploid cells at any dose level in any of the treatment cases.

In the presence of S9, the test material induced significant increases in the frequency of cells with structural aberrations at the maximum dose level, 1875 μg/mL in the 6-hour treatment/18-hour recovery, as well as at 1562 μg/mL in the 12-hour treatment/8-hr recovery (MRID 45422726, pp. 43, 44, 45 - ATTACHMENT, Table 6). The most frequently observed aberrations were

chromatid exchanges and breaks. It was noted, however, that damage to chromosomes was accompanied by cytotoxicity (MI reduced to 39% of control) at 1562.5 μg/mL. There was, further, no clear indication of a concentration-dependent response either with or without S9.

The positive controls induced significant increases in the frequency of cells with chromosome aberrations in the presence and absence of metabolic activation.

III. REVIEWER'S DISCUSSION/CONCLUSIONS:

A. The Agency reviewer agrees with the investigator that TI-435 increases the frequency of structural (but not numerical) aberrations in treated CHL cells at doses producing mild to moderate toxicity, *i.e.,* TI-435 may be considered clastogenic at these levels.

B. STUDY DEFICIENCIES:

None.

ATTACHMENT

THE FOLLOWING ATTACHMENT IS NOT AVAILABLE ELECTRONICALLY
SEE THE FILE COPY

SPL PROJECT NUMBER: 178/111

TI-435 : CHROMOSOME ABERRATION TEST IN CHL CELLS *IN VITRO*

TABLE 2

RESULTS OF CHROMOSOME ABERRATION TEST (EXPERIMENT 1) - CELL COUNTS

12-HOUR HARVEST

(4-HOUR TREATMENT, 8-HOUR RECOVERY +S9; 12-HOUR TREATMENT -S9)

CONCENTRATION (μg/ml)	WITHOUT S9				MEAN %
	CULTURE A		CULTURE B		
	NUMBER OF CELLS x 10^5/ml	% OF CONTROL	NUMBER OF CELLS x 10^5/ml	% OF CONTROL	
0	1.71	100	1.94	100	100
156.25	1.32	77	1.68	86	82
312.5	1.24	72	1.60	82	77
625	1.10	65	1.62	83	74
937.5	0.80	47	0.86	44	46
1250	1.07	63 NM	1.31	68 NM	66
1875	1.62	95 NM	1.63	84 NM	90
MMC 0.075 μg/ml	0.94	55	0.96	49	58

CONCENTRATION (μg/ml)	WITH S9				MEAN %
	CULTURE A		CULTURE B		
	NUMBER OF CELLS x 10^5/ml	% OF CONTROL	NUMBER OF CELLS x 10^5/ml	% OF CONTROL	
0	0.87	100	1.04	100	100
156.25	0.87	100	0.99	95	98
312.5	0.85	98	0.98	94	96
625	0.85	97	1.38	132	115
937.5	1.00	115	0.85	81	98
1250	0.90	104	0.83	80	92
1875	0.84	97	1.07	103	100
CP 10 μg/ml	0.59	68	0.65	62	65

NM = no scorable metaphases

331

SPL PROJECT NUMBER: 178/111

TI-435 : CHROMOSOME ABERRATION TEST IN CHL CELLS *IN VITRO*

TABLE 3

RESULTS OF CHROMOSOME ABERRATION TEST (EXPERIMENT 1) - MITOTIC INDEX

12-HOUR HARVEST

(4-HOUR TREATMENT, 8-HOUR RECOVERY +S9; 12-HOUR TREATMENT -S9)

WITHOUT S9

CONCENTRATION (μg/ml)	CULTURE A MITOTIC INDEX	CULTURE B MITOTIC INDEX	MEAN MITOTIC INDEX	% OF CONTROL
0	5.3	11.1	8.2	100
156.25	4.4	5.9	5.2	63
312.5	4.3	4.3	4.3	52
625	2.1	5.9	4.0	49
937.5	2.0	3.0	2.5	30
1250	NM	NM	-	-
MMC 0.075 μg/ml	3.9	3.3	3.6	44

WITH S9

CONCENTRATION (μg/ml)	CULTURE A MITOTIC INDEX	CULTURE B MITOTIC INDEX	MEAN MITOTIC INDEX	% OF CONTROL
0	8.6	6.8	7.7	100
937.5	8.2	8.3	8.3	107
1250	8.9	5.5	7.2	94
1875	0.9	3.2	2.1	27
CP 10 μg/ml	5.4	4.1	4.8	62

NM = no scorable metaphases - = not assessed for mitotic index

SPL PROJECT NUMBER: 178/111

TI-435 : CHROMOSOME ABERRATION TEST IN CHL CELLS *IN VITRO*

TABLE 4

RESULTS OF CHROMOSOME ABERRATION TEST (EXPERIMENT 2) - CELL COUNTS

12-HOUR HARVEST

(4-HOUR TREATMENT, 8-HOUR RECOVERY +S9; 12-HOUR TREATMENT -S9)

CONCENTRATION (μg/ml)	WITHOUT S9				MEAN %
	CULTURE A		CULTURE B		
	NUMBER OF CELLS x 10^5/ml	% OF CONTROL	NUMBER OF CELLS x 10^5/ml	% OF CONTROL	
0	1.75	100	1.72	100	100
78.13	1.59	91	1.49	86	89
156.25	1.37	78	1.47	86	82
312.5	1.29	74	1.25	73	74
625	1.08	62	1.15	67	65
937.5	0.92	53	0.99	57	55
1250	1.46	84 NM	1.17	68 NM	76
MMC 0.075 μg/ml	1.16	66	1.04	60	63

CONCENTRATION (μg/ml)	WITH S9				MEAN %
	CULTURE A		CULTURE B		
	NUMBER OF CELLS x 10^5/ml	% OF CONTROL	NUMBER OF CELLS x 10^5/ml	% OF CONTROL	
0	1.28	100	1.19	100	100
312.5	1.27	99	1.18	99	99
625	1.21	95	1.09	91	93
937.5	1.13	88	1.11	93	91
1250	0.99	77	1.03	87	82
1562.5	1.17	91	0.92	77	84
1875	1.39	109	0.91	77	93
CP 10 μg/ml	0.80	63	0.75	63	63

NM = no scorable metaphases

SPL PROJECT NUMBER: 178/111

TI-435 : CHROMOSOME ABERRATION TEST IN CHL CELLS *IN VITRO*

T A B L E 4 (continued)

RESULTS OF CHROMOSOME ABERRATION TEST (EXPERIMENT 2) - CELL COUNTS

24-HOUR HARVEST

(6-HOUR TREATMENT, 18-HOUR RECOVERY +S9; 6-HOUR TREATMENT -S9)

CONCENTRATION (μg/ml)	WITHOUT S9				MEAN %
	CULTURE A		CULTURE B		
	NUMBER OF CELLS x 10^5/ml	% OF CONTROL	NUMBER OF CELLS x 10^5/ml	% OF CONTROL	
0	1.01	100	0.96	100	100
312.5	1.00	100	1.13	118	109
625	0.77	77	0.97	101	89
937.5	0.69	69	0.72	75	72
1250	0.52	52	0.44	46	49
CP 10 μg/ml	1.07	106	1.27	133	120

CONCENTRATION (μg/ml)	WITH S9				MEAN %
	CULTURE A		CULTURE B		
	NUMBER OF CELLS x 10^5/ml	% OF CONTROL	NUMBER OF CELLS x 10^5/ml	% OF CONTROL	
0	0.80	100	0.79	100	100
312.5	0.81	101	0.70	88	95
625	0.86	106	0.73	92	99
1250	0.59	74	0.58	73	74
1875	0.54	67	0.41	52	60
CP 10 μg/ml	0.46	57	0.47	60	59

334

SPL PROJECT NUMBER: 178/111

TI-435 : CHROMOSOME ABERRATION TEST IN CHL CELLS *IN VITRO*

TABLE 4 (continued)

RESULTS OF CHROMOSOME ABERRATION TEST (EXPERIMENT 2) - CELL COUNTS

24 AND 48-HOUR HARVESTS (CONTINUOUS TREATMENT)

CONCENTRATION (µg/ml)	24-HOUR TREATMENT				MEAN %
	CULTURE A		CULTURE B		
	NUMBER OF CELLS x 10^5/ml	% OF CONTROL	NUMBER OF CELLS x 10^5/ml	% OF CONTROL	
0	1.63	100	1.66	100	100
78.13	1.43	88	1.31	79	84
156.25	1.29	79	1.56	94	87
312.5	1.27	78	1.39	84	81
625	0.97	59	0.80	48	54
937.5	0.64	39	0.64	38	39
1250	1.04	64 NM	1.10	66 NM	65
MMC 0.05 µg/ml	0.66	40	0.60	36	38

CONCENTRATION (µg/ml)	48-HOUR TREATMENT				MEAN %
	CULTURE A		CULTURE B		
	NUMBER OF CELLS x 10^5/ml	% OF CONTROL	NUMBER OF CELLS x 10^5/ml	% OF CONTROL	
0	2.13	100	1.37	100	100
39	1.63	77	1.14	84	81
78.13	1.36	64	1.12	82	73
156.25	1.11	52	0.98	72	62
312.5	0.68	32	0.65	47	40
625	0.37	17	0.38	28	23
937.5	0.41	19 NM	0.41	30 NM	25
MMC 0.025 µg/ml	0.84	39	0.89	65	52

NM = no scorable metaphases

335

SPL PROJECT NUMBER: 178/111

TI-435 : CHROMOSOME ABERRATION TEST IN CHL CELLS *IN VITRO*

TABLE 5

RESULTS OF CHROMOSOME ABERRATION TEST (EXPERIMENT 2) - MITOTIC INDEX

12-HOUR HARVEST

(4-HOUR TREATMENT, 8-HOUR RECOVERY +S9; 12-HOUR TREATMENT -S9)

WITHOUT S9

CONCENTRATION (μg/ml)	CULTURE A MITOTIC INDEX	CULTURE B MITOTIC INDEX	MEAN MITOTIC INDEX	% OF CONTROL
0	10.9	11.6	11.25	100
156.25	11.3	7.2	9.25	82
312.5	9.1	7.4	8.25	73
625	7.3	8.1	7.70	68
937.5	5.2	4.1	4.65	41
1250	NM	NM	-	-
MMC 0.075 μg/ml	5.5	6.9	6.20	55

WITH S9

CONCENTRATION (μg/ml)	CULTURE A MITOTIC INDEX	CULTURE B MITOTIC INDEX	MEAN MITOTIC INDEX	% OF CONTROL
0	15.1	13.6	14.35	100
937.5	10.7	10.3	10.50	73
1250	11.4	11.1	11.25	78
1562.5	5.6	5.7	5.65	39
1875	0.8	1.3	1.05	7
CP 10 μg/ml	4.4	4.2	4.30	30

NM = no scorable metaphases - = not assessed for mitotic index

336

SPL PROJECT NUMBER: 178/111

TI-435 : CHROMOSOME ABERRATION TEST IN CHL CELLS *IN VITRO*

TABLE 5 (continued)

RESULTS OF CHROMOSOME ABERRATION TEST (EXPERIMENT 2) - MITOTIC INDEX

24-HOUR HARVEST (6-HOUR TREATMENT, 18-HOUR RECOVERY)

WITHOUT S9

CONCENTRATION (μg/ml)	CULTURE A MITOTIC INDEX	CULTURE B MITOTIC INDEX	MEAN MITOTIC INDEX	% OF CONTROL
0	4.6	4.6	4.60	100
312.5	5.8	2.6	4.20	91
625	4.9	4.6	4.75	103
937.5	5.7	3.5	4.60	100
1250	3.7	3.9	3.80	83
CP 10 μg/ml	5.2	2.8	4.00	87

WITH S9

CONCENTRATION (μg/ml)	CULTURE A MITOTIC INDEX	CULTURE B MITOTIC INDEX	MEAN MITOTIC INDEX	% OF CONTROL
0	6.7	6.0	6.35	100
312.5	5.4	5.6	5.50	87
625	4.7	3.0	3.85	61
1250	8.1	11.0	9.55	150
1875	3.2	1.7	2.45	39
CP 10 μg/ml	1.9	1.2	1.55	24

SPL PROJECT NUMBER: 178/111

TI-435 : CHROMOSOME ABERRATION TEST IN CHL CELLS *IN VITRO*

T A B L E 5 (continued)

RESULTS OF CHROMOSOME ABERRATION TEST (EXPERIMENT 2) - MITOTIC INDEX

24 AND 48-HOUR HARVEST (CONTINUOUS TREATMENT)

24-HOUR

CONCENTRATION (μg/ml)	CULTURE A MITOTIC INDEX	CULTURE B MITOTIC INDEX	MEAN MITOTIC INDEX	% OF CONTROL
0	9.1	6.4	7.75	100
156.25	7.4	3.6	5.50	71
312.5	6.0	2.9	4.45	57
625	9.6	8.8	9.20	119
937.5	4.4	4.1	4.25	55
1250	NM	NM	-	-
MMC 0.05 μg/ml	7.0	5.1	6.05	78

48-HOUR

CONCENTRATION (μg/ml)	CULTURE A MITOTIC INDEX	CULTURE B MITOTIC INDEX	MEAN MITOTIC INDEX	% OF CONTROL
0	9.8	8.6	9.20	100
78.13	8.6	7.5	8.05	88
156.25	7.5	7.8	7.65	83
312.5	5.1	6.7	5.90	64
625	2.6	2.0	2.30	25
937.5	NM	NM	-	-
CP 10 μg/ml	11.6	11.8	11.70	127

- = not assessed for mitotic index NM = no scorable metaphases

TI-435 : CHROMOSOME ABERRATION TEST IN CHL CELLS *IN VITRO*
APPENDIX I (continued)
EXPERIMENT 1: RESULTS OF CHROMOSOME ABERRATION TEST WITH AND WITHOUT S9
6. RESULTS

TREATMENT	WITH (+) OR WITHOUT (-) S9-MIX	CONCENTRATION (μg/ml)	NUMBER OF CELLS OBSERVED	NUMBER OF POLYPLOIDS	JUDGEMENT	NUMBER AND PERCENTAGES (%) OF CELLS SHOWING STRUCTURAL CHROMOSOMAL ABERRATIONS								
						GAP	CHROMATID-TYPE		CHROMOSOME-TYPE		OTHERS	TOTAL		JUDGEMENT
						g	ctb	cte	csb	cse	x	-g	+g	
SOLVENT (DMSO)	-	0	100	2		1	0	0	0	0	0	0	1	
			100	0		0	0	0	0	0	0	0	0	
			200	2 (1.0)		1 (0.5)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0	1 (0.5)	
	+	0	100	0		0	1	0	0	0	0	1	1	
			100	2		1	0	0	0	0	0	0	1	
			200	2 (1.0)		1 (0.5)	1 (0.5)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.5)	2 (1.0)	
12 hr	-	156.25	100	1	-	0	0	0	0	0	0	0	0	
			100	0		0	0	1	0	0	0	1	1	
			200	1 (0.5)		0 (0.0)	0 (0.0)	1 (0.5)	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.5)	1 (0.5)	
		312.5	100	2	-	0	0	0	0	0	0	0	0	
			100	0		0	0	0	0	0	0	0	0	
			200	2 (1.0)		0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	
		625	100	1	-	1	0	0	0	0	0	0	1	
			100	1		0	0	0	0	0	0	0	0	
			200	2 (1.0)		1 (0.5)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.5)	
TEST SUBSTANCE TI-435		937.5	100	0	-	1	0	0	0	0	0	0	1	
			100	0		0	0	0	0	0	0	0	0	
			200	0 (0.0)		1 (0.5)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.5)	
		937.5	100	0	-	0	0	0	0	0	0	0	0	
			100	0		1	0	0	0	0	0	0	1	
			200	0 (0.0)		1 (0.5)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.5)	
12 hr	+	1250	100	0	-	0	0	0	0	0	0	0	0	
			100	3		0	0	0	0	0	0	0	0	
			200	3 (1.5)		0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	
		1875	100	0	-	0	3	0	0	0	0	3	3	
			100	1		0	0	0	0	0	0	0	0	
			200	1 (0.5)		0 (0.0)	3 (1.5)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	3 (1.5)	3 (1.5)	
POSITIVE CONTROL (MMC)	-	0.075	100	0	-	1	0	0	0	0	0	0	1	
			100	3		0	0	1	0	0	0	1	1	
			200	3 (1.5)		1 (0.5)	0 (0.0)	1 (0.5)	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.5)	2 (1.0)	
POSITIVE CONTROL (CP)	+	10	100	0	-	0	3	3	0	1	0	4	4	
			100	0		1	0	0	0	0	0	0	1	
			200	0 (0.0)		1 (0.5)	1 (0.5)	3 (1.5)	0 (0.0)	1 (0.5)	0 (0.0)	4 (2.0)	5 (2.5)	

TI-435 : CHROMOSOME ABERRATION TEST IN CHL CELLS *IN VITRO*
APPENDIX I (continued)
EXPERIMENT 2: RESULTS OF CHROMOSOME ABERRATION TEST WITHOUT S9

6. RESULTS

TREATMENT	WITHOUT S9-MIX	CONCEN-TRATION (μg/ml)	NUMBER OF CELLS OBSERVED	NUMBER OF POLYPLOIDS	JUDGE-MENT	NUMBER AND PERCENTAGES (%) OF CELLS SHOWING STRUCTURAL CHROMOSOMAL ABERRATIONS								
						GAP	CHROMATID-TYPE		CHROMOSOME-TYPE		OTHERS	TOTAL		JUDGE-MENT
						g	ctb	cte	csb	cse	x	-g	+g	
SOLVENT (DMSO)	24	0	100	0		0	0	0	0	0	0	0	0	
			100	0		1	1	0	0	0	0	1	2	
			200	0 (0.0)		1 (0.5)	1 (0.5)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.5)	2 (1.0)	
	48	0	100	0		2	0	0	0	1	0	1	3	
			100	4		0	0	0	0	0	0	0	0	
			200	4 (2.0)		2 (1.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.5)	0 (0.0)	1 (0.5)	3 (1.5)	
TEST SUBSTANCE TI-435	24	312.5	100	1		1	0	0	0	0	0	0	1	
			100	3	-	0	0	0	0	0	0	0	0	-
			200	4 (2.0)		1 (0.5)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.5)	
		625	100	0		0	2	1	0	0	0	3	3	
			100	0	-	2	3	2	0	0	0	5	7	±
			200	0 (0.0)		2 (1.0)	5 (2.5)	3 (1.5)	0 (0.0)	0 (0.0)	0 (0.0)	8* (4.0)	10* (5.0)	
		937.5	100	1		4	2	0	0	0	0	2	5	
			100	0	-	1	1	0	0	1	0	2	3	-
			200	1 (0.5)		5 (2.5)	3 (1.5)	0 (0.0)	0 (0.0)	1 (0.5)	0 (0.0)	4 (2.0)	8 (4.0)	
	48	156.25	100	0		2	0	0	0	0	0	0	2	
			100	3	-	0	0	0	0	1	0	1	1	-
			200	3 (1.5)		2 (1.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.5)	0 (0.0)	1 (0.5)	3 (1.5)	
		312.5	100	0		0	1	1	0	0	0	2	2	
			100	0	-	1	0	1	0	0	0	1	2	-
			200	0 (0.0)		1 (0.5)	1 (0.5)	2 (2.0)	0 (0.0)	0 (0.0)	0 (0.0)	3 (1.5)	4 (2.0)	
		625	100	3		13	9	2	0	0	0	11	24	
			100	4	-	17	7	3	0	0	0	8	23	+
			200	7 (3.4)		30 (15.0)	16 (8.0)	5 (2.5)	0 (0.0)	0 (0.0)	0 (0.0)	19*** (9.5)	47***(23.5)	
POSITIVE CONTROL (MMC)	24	0.05	50	1		8	10	12	0	1	0	19	23	
			100	2	-	9	19	12	0	0	0	25	32	+
			150	3 (2.0)		17 (11.3)	29 (19.3)	24 (16.0)	0 (0.0)	1 (0.7)	0 (0.0)	44***(29.3)	55***(36.7)	
	48	0.025	100	1		2	5	16	0	0	0	19	21	
			50	0	-	5	6	12	0	0	0	17	20	+
			150	1 (0.7)		7 (4.7)	11 (7.3)	28 (18.7)	0 (0.0)	0 (0.0)	0 (0.0)	36***(24.0)	41***(27.3)	

* represents $p \leq 0.05$ *** represents $p \leq 0.001$

TI-435 : CHROMOSOME ABERRATION TEST IN CHL CELLS *IN VITRO*
A P P E N D I X I (continued)
EXPERIMENT 2: RESULTS OF CHROMOSOME ABERRATION TEST WITH AND WITHOUT S9

6. RESULTS

TREATMENT	WITH (+) OR WITHOUT (-) S9-MIX	CONCEN-TRATION (μg/ml)	NUMBER OF CELLS OBSERVED	NUMBER OF POLYPLOIDS	JUDGE-MENT	NUMBER AND PERCENTAGES (%) OF CELLS SHOWING STRUCTURAL CHROMOSOMAL ABERRATIONS								
						GAP	CHROMATID-TYPE		CHROMOSOME-TYPE		OTHERS	TOTAL		JUDGE-MENT
						g	ctb	cte	csb	cse	x	-g	+g	
SOLVENT (DMSO)	-	0	100	5		0	0	0	0	0	0	0	0	
			100	0		0	0	0	0	0	0	0	0	
			200	5 (2.4)		0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	
	+	0	100	0		1	1	0	0	0	0	1	2	
			100	0		2	2	0	0	0	0	2	4	
			200	0 (0.0)		3 (1.5)	3 (1.5)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	3 (1.5)	6 (3.0)	
6 hr	-	312.5	100	2		0	1	0	0	0	0	1	1	
			100	3	-	0	0	0	0	0	0	0	0	-
			200	5 (2.4)		0 (0.0)	1 (0.5)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.5)	1 (0.5)	
		625	100	2		2	0	0	0	0	0	0	2	
			100	6	-	4	2	0	0	0	0	2	6	-
			200	8 (3.8)		6 (3.0)	2 (1.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	2 (1.0)	8 (4.0)	
TEST SUBSTANCE TI-435		1250	100	1		1	2	1	0	1	0	4	5	
			100	2	-	2	1	2	0	1	1	4	6	±
			200	3 (1.5)		3 (1.5)	3 (1.5)	3 (1.5)	0 (0.0)	2 (1.0)	1 (0.5)	8** (4.0)	11*** (5.5)	
	+	625	100	1		0	0	0	0	0	0	0	0	
			100	5	-	0	0	1	0	1	0	1	1	-
			200	6 (2.9)		0 (0.0)	0 (0.0)	1 (0.5)	0 (0.0)	1 (0.5)	0 (0.0)	1 (0.5)	1 (0.5)	
6 hr		1250	100	2		0	0	2	0	0	0	2	2	
			100	2	-	0	0	0	0	0	0	0	0	-
			200	4 (2.0)		0 (0.0)	0 (0.0)	2 (1.0)	0 (0.0)	0 (0.0)	0 (0.0)	2 (1.0)	2 (1.0)	
		1875	100	2		11	13	16	0	0	3	23	28	
			66	4	-	7	4	10	0	0	3	15	15	+
			166	6 (3.5)		18 (10.8)	18 (10.2)	26 (15.7)	0 (0.0)	0 (0.0)	6 (3.6)	38***(22.9)	43***(25.9)	
POSITIVE CONTROL (CP)	-	10	100	0		0	0	0	0	0	0	0	0	
			100	1	-	2	0	1	0	0	0	1	3	-
			200	1 (0.5)		2 (1.0)	0 (0.0)	1 (0.5)	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.5)	3 (1.5)	
	+	10	50	0		7	21	29	2	0	7	43	43	
			50	0	-	9	22	21	3	0	11	41	44	+
			100	0 (0.0)		16 (16.0)	43 (43.0)	50 (50.0)	5 (5.0)	0 (0.0)	18 (18.0)	84***(84.0)	87***(87.0)	

** represents $p \leq 0.01$ *** represents $p \leq 0.001$

TI-435 : CHROMOSOME ABERRATION TEST IN CHL CELLS *IN VITRO*
APPENDIX I (continued)
EXPERIMENT 2: RESULTS OF CHROMOSOME ABERRATION TEST WITH AND WITHOUT S9
6. RESULTS

TREATMENT	WITH (+) OR WITHOUT (-) S9-MIX	CONCENTRATION (μg/ml)	NUMBER OF CELLS OBSERVED	NUMBER OF POLYPLOIDS	JUDGEMENT	NUMBER AND PERCENTAGES (%) OF CELLS SHOWING STRUCTURAL CHROMOSOMAL ABERRATIONS								JUDGEMENT
						GAP	CHROMATID-TYPE		CHROMOSOME-TYPE		OTHERS	TOTAL		
						g	ctb	cte	csb	cse	x	-g	+g	
SOLVENT (DMSO)	-	0	100	0		0	0	0	0	0	0	0	0	
			100	1		0	0	0	0	0	0	0	0	
			200	1 (0.5)		0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	
	+	0	100	0		1	0	2	0	0	0	2	2	
			100	0		0	0	0	0	0	0	0	0	
			200	0 (0.0)		1 (0.5)	0 (0.0)	2 (1.0)	0 (0.0)	0 (0.0)	0 (0.0)	2 (1.0)	2 (1.0)	
12 hr	-	312.5	100	1		0	0	0	0	0	0	0	0	
			100	1		0	0	0	0	0	0	0	0	
			200	2 (1.0)		0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	
		625	100	2		0	0	1	0	0	0	1	1	
			100	0		1	0	0	0	0	0	0	1	
			200	2 (1.0)		1 (0.5)	0 (0.0)	1 (0.5)	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.5)	2 (1.0)	
TEST SUBSTANCE TI-435		937.5	100	0		2	1	2	0	0	0	3	4	
			100	1		0	0	1	0	0	0	1	1	
			200	1 (0.5)		2 (1.0)	1 (0.5)	3 (1.5)	0 (0.0)	0 (0.0)	0 (0.0)	4 (2.0)	5* (2.5)	
		937.5	100	0		1	0	0	0	0	0	0	1	
			100	0		0	0	0	0	0	0	0	0	
			200	0 (0.0)		1 (0.5)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.5)	
12 hr	+	1250	100	0		0	0	0	0	0	0	0	0	
			100	0		0	0	0	0	0	0	0	0	
			200	0 (0.0)		0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	
		1562.5	100	2		0	1	7	0	0	0	7	7	
			100	1		1	1	2	0	0	0	3	4	±
			200	3 (1.5)		1 (0.5)	2 (1.0)	9 (4.5)	0 (0.0)	0 (0.0)	0 (0.0)	10* (5.0)	11* (5.5)	
POSITIVE CONTROL (MMC)	-	0.075	100	0		1	0	1	0	0	0	1	2	
			100	0		2	2	2	0	0	0	4	6	
			200	0 (0.0)		3 (1.5)	2 (1.0)	3 (1.5)	0 (0.0)	0 (0.0)	0 (0.0)	5* (2.5)	8** (4.0)	
POSITIVE CONTROL (CP)	+	10	100	1		4	22	15	0	0	0	34	34	
			100	1		3	12	7	0	0	0	16	17	+
			200	2 (1.0)		7 (3.5)	34 (17.0)	22 (11.0)	0 (0.0)	0 (0.0)	0 (0.0)	50***(25.0)	51***(25.5)	

* represents $p \leq 0.05$ ** represents $p \leq 0.01$ *** represents $p \leq 0.001$

SPL PROJECT NUMBER: 178/111

APPENDIX VI

HISTORICAL CONTROL DATA FOR NEGATIVE AND POSITIVE CONTROLS IN CHL CELLS AT SAFEPHARM LABORATORIES (1996)

VEHICLE CONTROLS - CELLS WITH ABERRATIONS INCLUDING GAPS (%)

	24 HRS	48 HRS	6(18) -S9	6(18) +S9	12 -S9 Ex 1	4(8) +S9 Ex 1	12 -S9 Ex 2	4(8) +S9 Ex 2
MIN	0	0	0	0	0	0.5	0	0.5
MAX	1.5	3	2	6	2	4	2	3.5
MEAN	0.7	0.8	0.7	1.5	0.9	1.8	0.7	1.8
SD	0.4	0.7	0.7	1.4	0.6	1.1	0.8	1.1
No. OF VALUES	29	29	29	29	8	8	8	8

POSITIVE CONTROLS - CELLS WITH ABERRATIONS INCLUDING GAPS (%)

	24 HRS MMC 0.05	48 HRS MMC 0.025	6(18) -S9 CP 10	6(18) +S9 CP 10	12 -S9 Ex 1 MMC 0.075	4(8) +S9 Ex 1 CP 10	12 -S9 Ex 2 MMC 0.075	4(8) +S9 Ex 2 CP 10
MIN	13.5	19	0	0.2	1	1.5	1	3.5
MAX	53	57	3	87	12	10.5	13.5	25.5
MEAN	32.5	36.5	0.9	45.2	4.9	5.7	5.4	10.1
SD	12.2	11.3	1.0	27.2	3.4	3.3	4.3	6.9
No. OF VALUES	21	26	29	29	8	8	8	8

343

CLOTHIANIDIN *IN VIVO/IN VITRO* UNSCHEDULED DNA SYNTHESIS

EPA Reviewer: Irving Mauer, Ph.D. Date: 11/20/02
Registration Action Branch 3, HED (7509C)
Secondary Reviewer: Nancy McCarroll Date: 11/20/02
Toxicology Branch, HED (7509C)

TXR No.: 0050321

DATA EVALUATION RECORD

STUDY TYPE: Other Genotoxicity: Unscheduled DNA Synthesis in Primary Rat Hepatocytes from treated rats. OPPTS 870.5550 [84-2].

DP BARCODE: D279778 SUBMISSION CODE: S607124

P.C. CODE: 0044309 TOX. CHEM. NO.: None.

TEST MATERIAL (PURITY): TI-435 [(Batch No. 30034708 (T0053840/T9053902, 95.2%/96.2% a.i.)]

SYNONYMS: Clothianidin (iso). Chemically: N-[(2-chloro-5-thiazolyl)methyl]-N′-methyl-N″ nitroguanidine (from MRID 45422734).

CITATION: Brendler-Schwaab, S. (1999). TI-435: Test on Unscheduled DNA Synthesis with Rat Liver Cells *in vivo*, performed at Bayer AG's Department of Toxicology, Wuppertal (GERMANY). Bayer AG Report No. 28850/Bayer AG Study No. T0053840/T9053902 (Bayer Corporation Agricultural Division Report No. 109991), dated June 8, 1999. MRID 45422739. Unpublished.

SPONSOR: Takeda Chemical Industries, Agro Company, Tokyo (JAPAN).

EXECUTIVE SUMMARY: In an *in vivo/in vitro* unscheduled DNA synthesis (UDS) assay (MRID 45422739), four male Wistar rats/group were administered TI-435 (Batch No. 30034708, T0053840/T9053902, 95.2%/96.2% a.i.) in 0.5% aqueous Cremophor in a single oral dose of 2500 or 5000 mg/kg, and liver cells were withdrawn 4 or 16 hours post-dosing. In addition to animals treated with vehicle (4 mice for the 4-hr harvest and 6 mice for the 16-hr harvest), additional animals (4/group) were administered N,N^1-dimethylhydrazine (DMH, 40 mg/kg) and sacrificed for hepatocytes 4 hours later, or 2- acetylaminofluorene (2-AAF, 100 mg/kg), and sacrificed after 16 hours.

Rats treated at 5000 mg/kg TI-435 showed signs of clinical toxicity (*i.e.*, roughened fur, shivering, rapid breathing, twitching), but no cytotoxicity was observed.

There was also no evidence (or a dose related positive response) that UDS at either harvest time, as determined by radioactive tracer procedures (nuclear silver grain counts), was induced. This study is classified as acceptable even though only males were used. (See Section III. REVIEWER'S DISCUSSION/CONCLUSIONS.

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

I. MATERIALS AND METHODS

A. MATERIALS:

1. Test Material: TI-435.
   Description: Yellow powder.
   Lot/Batch No.: 30034708 (T 0053840/T 9053902).
   Purity: (95.2%/96.2% - analyzed on December 4, 1996 and May 28, 1997).
   Stability of compound: Stable in the vehicle at room temperature for at least two hours.
   CAS. No.: 205510-53-8
   Solvent used: 0.5% Aqueous Cremophor
   Storage: Room temperature (protected from light).

2. Control Materials:

   Negative: None.

   Solvent/Final concentration: Cremophor/0.5%.
   Positive (concentrations, solvent): 2-Acetylaminofluorene (2-AAF, 100 mg/kg, dissolved in corn oil -- 16-hr liver treatment); dimethylhydrazene [DMH, 40 mg/kg, dissolved in dimethylsulfoxide (DMSO) -- 4-hr treatment].

3. Test Compound Concentrations Used (for preliminary cytotoxicity test, if performed, and main assay):

   Dose-selection: 4000, 5000 mg/kg x 1 - oral gavage.
   Main Assay: 2500, 5000 mg/kg x 1 - oral gavage.

4. Media: Altromin 1324 Standard Diet, plus tap water, *ad libitum*.

5. Animals: Young (10-14 weeks) male Wistar rats, weighing 120-140 g.

6. Test Cells: Hepatocytes from treated male Wistar rats.

7. Cell Preparation:

Hepatocytes from perfused livers of treated animals were established as primary cultures in Williams Medium E, supplemented with L-glutamine, gentamycin, dexamethasone and heat-inactivated fetal calf serum (= WEC). All cultures were incubated for 90 minutes at 37°C in a humidified atmosphere containing 5% CO_2.

Prepared cell suspensions for each animal in all treatment groups (7.5 x 10^5 viable cells) were seeded into two 60 mm. Petri dishes, allowed to attach for 1.5 hours, and cell visibility was assessed by trypan blue exclusion.

8. Audioradiography and Staining:

For the assessment of genotoxicity, ≈ 3.78 x 10^5 viable cells were seeded into 6-well culture dishes containing coverslips pre-coated with collagen. Cultures were incubated for 90 minutes at 37° C. Air-dried coverslips from culture-vessels were mounted cell-side up on standard microscope slides. In the darkroom, these were dipped in Kodak NTB-2 photographic emulsion, diluted 1:1 with distilled water (DW), and left to dry in air overnight. Next day, the coated slides were stored in light-tight boxes containing Drierite for 11-14 days at -20°C. The photographic emulsions were developed in Kodak D-19, rinsed in DW, fixed in Kodak fixer and air-dried. Slides were then stained with H & E, coded and counted.

9. Grain Counting:

Grain counting was done by eye using a Zeiss microscope (100 x objective under oil immersion) interfaced to a TV color screen with a high resolution TV color camera. Unscheduled DNA synthesis (UDS) was determined as follows:

Each slide was examined by counting 50 cells per slide. Three slides per animal were evaluated (total of 150 nuclei for each rat). Only cells viable at the time of fixation were scored; isolated nuclei and cells with abnormal morphology were excluded. A starting point was randomly selected on each slide, and cells were scored in a regular fashion by bringing new cells into the field of view, moving along the X-axis. If the total number of 50 cells had not been reached before coming to the edge of the slide, the stage was moved on the Y-axis, and counting resumed in the opposite X-

direction, parallel to the first line.
UDS was measured by counting nuclear grains and subtracting the average number of grains in 3 cytoplasmic areas of the same size as the corresponding nucleus. This value was referred to as the nuclear net grain (NNG) count of the cell. The mean NNG count per animal was routinely determined from triplicate coverslips. The mean NNG value per dose group was calculated from the mean NNG value of each animal of the respective dose group. The number of cells in repair (nuclei with 5 or more net grains) was also determined.

10. Data Presentation:

The data are presented in tabular form. Decriptive statistical methods were used to calculate means and standard deviations. The means and standard deviations in the tables were calculated from the means calculated individually for each of the three coverslips per animal.

Net Grains per Nucleus = Average number of the mean nuclear net grain counts of each evaluable coverslip, 50 cells per coverslip.

Mean Grains per Nucleus = Average number of the mean nuclear grain counts of each evaluable coverslip, 50 cells per coverslip.

Mean Cytoplasmic Grain Count = Average number of the mean cytoplasmic grain counts (3 areas per cell) of each evaluable coverslip, 50 cells per coverslip.

Percentage Nuclei with 5 or More Grains = (Number of cells with 5 or more net nuclear grain counts per dose/number of evaluated cells per dose) x 100.

Absolute Survival (%) = Percentage of viable cells after isolation.

10. Assay Acceptance Criteria:

Standardized assay acceptance criteria were presented.

II REPORTED RESULTS:

Single oral administration of TI-435 at levels of 2500 and 5000 mg/kg produced clinical effects at 5000 mg/kg (rough-fur, palmospasms, shivering, rapid breathing, slit eyes, twitching). No cytotoxicity, however, was evident in any test group exposed for 16 or 4 hours (nor in positive controls). The number of heavily labelled nuclei (*i.e.*, blackened with numerous grains TNTC) representing cells undergoing DNA replication was in the normal range for hepatocytes.

The results for UDS are shown in Tables 3 and 4 for net nuclear grains (NNG) (MRID 4542739 - ATTACHMENT, single grain values per animal) and ATTACHMENT Tables 5 and 6 (mean grain values per dose group), which indicate TI-435 caused no biologically relevant increase in nuclear labelling.

The mean NNG values and the average number of cells in repair (greater or equal to 5 net grains) of hepatocytes from vehicle controls were in the range typical of negative controls.

Positive controls induced a significant ($p \leq 0.05$) increase in NNG and percentage of cells in repair.

The investigator concluded that TI-435 induced no biologically relevant increase in nuclear labeling. Therefore, TI-435 is considered to be negative for UDS in rat liver cells.

III. REVIEWER'S DISCUSSION/CONCLUSIONS:

A. The EPA reviewer agrees with the investigator's conclusions that TI-435 administered at clinically toxic level to Wistar rats was negative for UDS, even though only males were used, since the chemical did not induce a sex-specific toxic response in the micronucleus assay.

B. STUDY DEFICIENCIES:

None.

ATTACHMENT

THE FOLLOWING ATTACHMENT IS NOT AVAILABLE ELECTRONICALLY
SEE THE FILE COPY

TI 435
UDS Assay In Vivo
Study No. T 0053840/T 9053902

Table 3

SUMMARY OF DATA OF THE RAT HEPATOCYTE UDS ASSAY IN VIVO

Test Substance: TI 435
Test Date : February 10, 1997
Exposure : 16 h

TEST CONDITION	Net Grains[a] per Nucleus ± S.D.	Mean Grains[a] per Nucleus ± S.D.	Mean Cyto-[a] plasmic Grain Counts ± S.D.	Average[b] % Cells in repair	Absolute[c] Survival (%)
Vehicle Control (0.5% Cremophor)					
Animal 1	- 0.71 ± 0.42	2.43 ± 0.21	3.14 ± 0.62	0	77.4
Animal 2	- 0.68 ± 0.32	1.95 ± 0.10	2.63 ± 0.37	0	76.4
Animal 3	- 1.35 ± 0.33	1.51 ± 0.06	2.86 ± 0.40	0	74.0
Animal 4	- 1.78 ± 0.51	3.27 ± 0.64	5.05 ± 0.14	0	77.9
Animal 5	- 0.96 ± 0.49	0.80 ± 0.19	1.76 ± 0.41	0	81.2
Animal 6	- 0.88 ± 0.19	1.66 ± 0.31	2.54 ± 0.48	0	82.0
TEST SUBSTANCE					
2500 mg/kg bw					
Animal 1	- 0.82 ± 0.30	2.27 ± 0.56	3.09 ± 0.38	0	65.2
Animal 2	- 0.90 ± 0.45	3.87 ± 1.76	4.77 ± 1.32	0	65.9
Animal 3	- 0.52 ± 0.40	4.79 ± 0.81	5.32 ± 0.57	0	78.1
Animal 4	- 0.24 ± 0.18	4.75 ± 1.24	5.00 ± 1.14	0	74.8
5000 mg/kg bw					
Animal 1	- 0.51 ± 0.16	3.53 ± 0.30	4.04 ± 0.23	0	67.7
Animal 2	- 0.87 ± 0.45	3.52 ± 1.15	4.39 ± 0.72	0	75.8
Animal 3	- 0.92 ± 0.07	3.32 ± 3.17	4.24 ± 3.11	0	67.3
Animal 4	- 1.46 ± 0.41	3.77 ± 1.04	5.23 ± 0.77	0	64.9
Positive Control (100 mg/kg 2-AAF)					
Animal 1	6.77 ± 0.22	10.58 ± 0.69	3.81 ± 0.54	75.3*	75.8
Animal 2	4.55 ± 0.50	6.70 ± 1.10	2.15 ± 0.73	43.3*	75.3
Animal 3	4.87 ± 0.81	8.44 ± 1.54	3.57 ± 0.75	51.3*	79.6
Animal 4	4.88 ± 0.78	6.77 ± 1.18	1.90 ± 0.44	42.7*	75.7

a Average values of triplicate coverslips
b Average values of percentage of cells with 5 or more net nuclear grains on triplicate coverslips
c Percentage of viable cells after isolation
* Significant increase (significance level $\leq$ 0.05)

HED Records Center Series 361 Science Reviews - File R107689 - Page 367 of 721

Table 4

SUMMARY OF DATA OF THE RAT HEPATOCYTE UDS ASSAY IN VIVO

Test Substance: TI 435
Test Date : June 04, 1997
Exposure : 4 h

TEST CONDITION	Net Grains[a] per Nucleus ± S.D.	Mean Grains[a] per Nucleus ± S.D.	Mean Cyto-[a] plasmic Grain Counts ± S.D.	Average[b] % Cells in repair	Absolute[c] Survival (%)
Vehicle Control (0.5% Cremophor)					
Animal 1	- 1.26 ± 0.34	1.47 ± 0.26	2.72 ± 0.51	0	82.4
Animal 2	- 1.03 ± 0.37	2.43 ± 2.18	3.46 ± 2.54	0	86.5
Animal 3	- 0.79 ± 0.20	1.57 ± 0.59	2.36 ± 0.56	0	76.7
Animal 4	- 0.75 ± 0.05	0.86 ± 0.11	1.61 ± 0.06	0	78.3
TEST SUBSTANCE					
2500 mg/kg bw					
Animal 1	- 0.73 ± 0.34	2.30 ± 0.76	3.03 ± 1.10	0	78.0
Animal 2	- 0.78 ± 0.12	2.58 ± 0.82	3.36 ± 0.81	0	72.1
Animal 3	- 0.97 ± 0.50	4.32 ± 1.57	5.29 ± 1.71	0	67.5
Animal 4	- 0.64 ± 0.77	2.47 ± 0.36	3.11 ± 0.84	0	71.7
5000 mg/kg bw					
Animal 1	- 1.29 ± 0.23	3.17 ± 0.82	4.46 ± 1.02	0	75.0
Animal 2	- 0.77 ± 0.18	3.34 ± 1.25	4.11 ± 1.40	0	74.1
Animal 3	- 1.13 ± 0.51	3.13 ± 1.84	4.26 ± 2.22	0	71.2
Animal 4	- 0.75 ± 0.04	0.88 ± 0.09	1.63 ± 0.11	0	70.9
Positive Control (40 mg/kg DMH)					
Animal 1	2.34 ± 0.19	5.51 ± 0.45	3.18 ± 0.57	8.7*	69.3
Animal 2	2.82 ± 0.10	6.82 ± 1.58	4.00 ± 1.68	12.7*	74.5
Animal 3	2.93 ± 0.34	5.89 ± 0.41	2.97 ± 0.64	15.3*	71.5
Animal 4	2.93 ± 0.06	6.83 ± 1.08	3.90 ± 1.05	17.3*	75.0

a Average values of triplicate coverslips
b Average values of percentage of cells with 5 or more net nuclear grains on triplicate coverslips
c Percentage of viable cells after isolation
* Significant increase (significance level ≤ 0.05)

HED Records Center Series 361 Science Reviews - File R107689 - Page 368 of 721

AC Report No. 109991
Page 36 of 39

TI 435
UDS Assay In Vivo
Study No. T 0053840/T 9053902
BAYER AG

Table 5

Mean Grain Values per Dose Group

16 h Exposure

Dose Group	Mean NNG ± SD	Mean NG ± SD	Mean CG ± SD
Vehicle Control	- 1.06 ± 0.4	1.94 ± 0.8	3.00 ± 1.1
Test Substance			
2500 mg/kg	- 0.62 ± 0.3	3.92 ± 1.2	4.55 ± 1.0
5000 mg/kg	- 0.94 ± 0.4	3.54 ± 0.2	4.48 ± 0.5
Positive Control 2-AAF 100 mg/kg	5.27 ± 1.0	8.12 ± 1.8	2.86 ± 1.0

TI 435
UDS Assay In Vivo
Study No. T 0053840/T 9053902
BAYER AG

Table 6

Mean Grain Values per Dose Group

4 h Exposure

Dose Group	Mean NNG ± SD	Mean NG ± SD	Mean CG ± SD
Vehicle Control	- 0.96 ± 0.2	1.58 ± 0.6	2.85 ± 0.6
Test Substance			
2500 mg/kg	- 0.78 ± 0.1	2.92 ± 0.9	2.95 ± 0.5
5000 mg/kg	- 0.99 ± 0.3	2.63 ± 1.2	3.62 ± 1.3
Positive Control DMH 40 mg/kg	2.78 ± 0.3	6.26 ± 0.7	3.51 ± 0.5

354

DATA EVALUATION RECORD

TI-435 (CLOTHIANIDIN)

Study Type: §83-5a; Combined Chronic Toxicity/Carcinogenicity Study in Rats

Work Assignment No. 4-02-167 C (formerly 4-01-167 C) (MRIDs 45422719 and 45422720)

Prepared for
Health Effects Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
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Arlington, VA 22202

Prepared by
Pesticides Health Effects Group
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Primary Reviewer Ronnie J. Bever Jr., Ph.D.	Signature: Ronnie J. Bever Jr. Date: 8/1/02
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Program Manager Mary L. Menetrez, Ph.D.	Signature: Debbie S. Wally for Mary Menetrez Date: 8/1/02
Quality Assurance Steve Brecher, Ph.D.	Signature: Steven Brecher Date: 8/1/02

Disclaimer

This Data Evaluation Record may have been altered by the Health Effects Division subsequent to signing by Dynamac Corporation personnel.

TI-435 (CLOTHIANIDIN)/044309 OPPTS 870.4300/ OECD 453

PMRA/EPA Reviewers: Scott Hancock and Pamela Hurley
EPA Work Assignment Manager:Ghazi Dannan, Ph.D.
Registration Action Branch 3, Health Effects Division (7509C)

Signature: Pamela M Hurley
Signature: Ghazi Dannan
Date 1/6/05

Template version 11/01

TXR#: 0050321

DATA EVALUATION RECORD

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

PC CODE: 044309 **DP BARCODE:** D279596, D276450, and D277188
SUBMISSION NO.: S607124 and S600587

TEST MATERIAL (PURITY): TI-435 (95.2-95.5% a.i.)

SYNONYMS: Clothianidin; (E)-1-(2-chloro-5-thiazolylmethly)-3-methyl-2-nitroguanidine

CITATION: Biegel, L.B. (2000). 104-Week dietary combined chronic toxicity and carcinogenicity study with TI 435 in rats. Covance Laboratories Ltd., Madison, WI. Laboratory Project Id: Covance 6155-108, April 11, 2000. MRID 45422719. Unpublished.

Biegel, L.B. (2001). 104-Week dietary combined chronic toxicity and carcinogenicity study with TI 435 in rats. Covance Laboratories Ltd., Madison, WI. Laboratory Project Id: 6155-108, supplement no. 1, March 29, 2001. MRID 45422720. Unpublished.

SPONSOR: Takeda Chemical Industries Ltd., Agro Company, 13-10, Nihonbashi 2-chome, Chuo-ku, Tokyo, Japan

EXECUTIVE SUMMARY: In a combined chronic/carcinogenicity study (MRIDs 45422719 and 45422720), TI-435 (clothianidin, 95.2-95.5% a.i., Lot No. 30037120) was administered daily in the diet to 80 Crl:CD®(SD)BR VAF/Plus® rats/sex/dose at dose levels of 0, 150, 500, 1500, or 3000 ppm (equivalent to 0/0, 8.1/9.7, 27.4/32.5, 82.0/97.8, and 156.5/193.4 mg/kg/day in males and females respectively) for 24 months. At 12 months, 20 rats/sex/dose were sacrificed.

There were no compound-related effects on mortality, clinical signs, functional observational battery, food efficiency, water consumption, ophthalmology, hematology, clinical chemistry, or urinalysis.

Treatment-related decreases ($p<=0.05$) in body weights and food consumption were observed in females dosed at 1500 and 3000 ppm and in males dosed at 3000 ppm; decreases ($p<=0.01$) of

25-31% in cumulative body weight gains were observed at Weeks 1-13, 13-50, and 1-102 in high dose animals.

In the kidney in the 3000 ppm males, granular material or calculus was observed grossly in the males when all animals were combined. The incidences of pelvic mineralization and transitional cell hyperplasia were increased at the terminal ($p<=0.01$) and interim sacrifices in males. Pelvic angiectasis and tubular ectasia were increased ($p<=0.05$) in the females when all animals were combined. Relative (to body) kidney weight was increased ($p<=0.05$) in the females at the interim and terminal sacrifices. All these abnormalities occurred at an increase of 8-23% beyond the concurrent controls, and exceeded the historical control values. The observed microscopic lesions were of minimal to slight severity on average. In conclusion, slight signs of nephrotoxicity were observed.

In the liver in the 3000 ppm group, mottled livers were observed grossly in males when all animals were combined. Altered hepatocellular eosinophilic foci were observed at the terminal sacrifice in males and females ($p<= 0.05$), as well as the interim sacrifice in females. Altered hepatocellular eosinophilic focus in females (21% vs 11%; NS) were also observed at 1500 ppm when all animals were combined. Increased ($p<=0.01$) relative (to body) liver weights were observed in the 3000 ppm females at the interim and terminal sacrifices. The incidence of liver lymphohistiocytic infiltrate exceeded the concurrent controls at 1500 (69% treated vs 53% concurrent controls) and 3000 (69%) ppm in females. Minor increases in liver congestion in males at terminal sacrifice were observed at >=1500 ppm. All abnormalities observed at termination occurred at an increase incidence of 12-25% beyond the concurrent controls, and exceeded the historical control values. In conclusion, slight signs of hepatotoxicity were observed.

In the lung, increased ($p<=0.05$) relative (to body) lung weights were observed in the 3000 ppm females at the interim and terminal sacrifices (incr 23-27%), and the incidence of chronic lung inflammation was increased 11% ($p<=0.01$). The incidence of these lesions exceeded the historical control values. Other corroborating signs of lung toxicity were not observed.

Ovary interstitial gland hyperplasia was observed ($p<=0.01$) at 1500 (incr.19%) and 3000 (incr.30%) ppm when all animals were combined. The incidence of this lesion exceeded the historical control values.

Other minor effects were observed at 3000 ppm when all animals were combined ($p<=0.05$ by pair-wise comparison and dose-effect trend) as follows: (i) stomach edema and hemorrhage in males; (ii) thymus epithelial hyperplasia in females; (iv) pituitary angiectasis females; (v) stomach glandular erosion and edema in females; and (vi) uterus congestion. Thyroid follicular cyst(s) were observed at 3000 ppm in females (38% treated vs 21% controls).

The LOAEL is 1500 ppm in females and 3000 ppm in males (equivalent to 156.5/97.8 mg/kg/day in males/females), based on decreased body weight, and food consumption, ovary interstitial gland hyperplasia, increased lymphohistiocytic infiltrate and altered hepatocellular eosinophilic focus of the liver in females; and decreased body weight, and

food consumption and slightly increased incidences of pelvic mineralization and transitional cell hyperplasia in the kidney, mottled livers, and altered hepatocellular eosinophilic foci in the livers of males. The NOAEL for this study is 500 ppm in females and 1500 ppm in males (equivalent to 82.0/32.5 mg/kg/day in males/females).

At the doses tested, there was a treatment-related increase in thyroid C-cell adenoma incidence in females when compared to controls. A statistical analysis conducted by the Health Effects Division (HED) staff showed that the increase in thyroid C-cell tumors in females was not significant, especially when carcinomas and adenomas were combined. In males, an increase in the incidence of hepatocellular carcinomas was observed at 500 and 3000 ppm when all animals were combined. The increased incidences in the low and high dose groups were just outside the historical control incidences for the same testing laboratory but were within the historical control range for the animal supplier. In addition, there was no dose-response and there was no continuum (i.e., no preneoplastic lesions and no adenomas).

Based on all the factors summarized above, on September 2, 2003, the Hazard Identification Assessment Review Committee (HIARC) determined that there was no evidence of carcinogenicity under the conditions of this study. Dosing was considered adequate based on body weight, body weight gain, and food consumption decreases, and slight abnormalities in the kidney and liver.

This study is **acceptable/guideline** and does satisfy the guideline requirement for a chronic/ carcinogenicity study (OPPTS 870.4300; OECD 453) in rats.

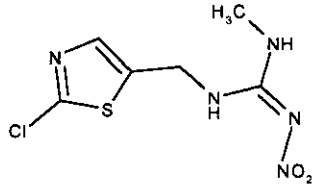
COMPLIANCE: Signed and dated GLP, Quality Assurance, Data Confidentiality and Flagging statements were provided.

I. MATERIALS AND METHODS

A. MATERIALS

1. **Test Material:** TI-435
★ **Description:** Pale yellow powder
Lot/Batch #: 30037120
Purity: 95.2-95.5% a.i.
Stability of compound: Stable in the diet for at least 14 days at room temperature
CAS #: 210880-92-5

Structure:



2. **Vehicle**: Diet

3. **Test animals:**

Species:	Rat	
Strain:	Crl:CD®(SD)BR VAF/Plus®	
Age/weight at dosing:	34-40 days old; 105-194 g males; 113-162 g females	
Source:	Charles River Laboratories, Raleigh, NC	
Housing:	Individually in stainless steel or polycarbonate cages	
Diet:	Certified rodent diet (#8728 CM meal, Harlan Teklad), *ad libitum*	
Water:	Mains water *ad libitum*	
Environmental conditions:	**Temperature:**	18 - 26 °C
	Humidity:	30 - 70 %
	Air changes:	Not reported
	Photoperiod:	12 hrs dark/ 12 hrs light
Acclimation period:	9 days	

B. STUDY DESIGN:

1. **In life dates** - Start: October 9, 1997 End: October 13, 1999

2. **Animal assignment** - Animals were randomly assigned, stratified by weight, to the test groups presented in Table 1.

Table 1. Study design[a]

Test Group	Dose to Animal (ppm)	Actual Dose (mg/kg/day; M/F)	Interim Sacrifice (#/sex) (12 Months)	Terminal Sacrifice (#/sex) (24 Months)
Control	0	0/0	20	60
Low	150	8.1/9.7	20	60
Mid	500	27.4/32.5	20	60
Mid-High	1500	82.0/97.8	20	60
High	3000	156.5/193.4	20	60

a Data obtained from MRID 45422719, page 24 and Table 11 on page 214.

3. **Dose-selection rationale** - The Sponsor submitted 4- and 13-Week rat subchronic studies concurrently with this chronic toxicity/carcinogenicity study. In the 13-Week subchronic oral toxicity study (MRIDs 45422809 and 45422708), TI-435 was administered to 15 Sprague-Dawley rats/sex/group in the diet at dose levels of 0, 150, 500, or 3000 ppm. The LOAEL for this 13-Week study was 3000 ppm based on decreased body weight, body weight gains, and food consumption in both sexes. In the 4-Week oral toxicity study (MRID 45422808), TI-435 was administered in the diet to 5 Crl:CD BR rats/sex/group at doses of 0, 1250, 2500, 5000, or 7500 ppm. The LOAEL for this 4-Week study was 2500 ppm based on decreased body weight gains and food consumption in both sexes.

359

Based on the results of these two studies, the doses summarized in Table 1 were selected for the chronic toxicity/carcinogenicity study.

4. **Treatment preparation, analysis, and administration** - Dietary formulations were prepared by diluting a concentrated test material-feed mixture (premix) with appropriate amounts of diet to achieve the desired test concentrations. Dietary formulations were prepared approximately every 2 weeks, and were stored at room temperature until used. Homogeneity analyses (top, middle, and bottom) were performed on the 100 and 3000 ppm dietary formulations prior to study initiation. Stability of the compound for up to 14 days at room temperature was tested in 25 and 5000 ppm dietary formulations (MRID 45422723). Concentration analyses were performed at Months 1, 3, 6, 9, 13, 16, 19, 22, and 25.

Results - Homogeneity Analysis - Range of % coefficient of variation over both doses (calculated by the reviewers): 0.548-2.05

Stability Analysis - Range of % of Day 0 over both doses: 90.8-106%

Concentration Analysis - Range as % of nominal: 88.7-109%

150 ppm	91.3-109
500 ppm	89.4-106
1500 ppm	88.7-104
3000 ppm	88.7-102

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

5. **Statistics** - Data were subjected to the statistical procedures listed below.

Parameter	Statistical procedure
FOB evaluations (continuous data), body weights, body weight gains, food consumption, food efficiency, and organ weights (absolute, relative to body, and relative to brain)	Levene's test was performed to test for variance homogeneity. The data was transformed when necessary to achieve variance homogeneity. One-way analysis of variance (ANOVA) followed by Dunnett's multiple comparison t-test (two-tailed) was applied.
Incidence of macroscopic and microscopic observations	Fisher-Irwin exact test
Mortality	Two-tailed log-rank test was used to compare the groups. One-tailed logistic regression was performed to test the dose-response relationship of mortality.

Tumor incidence	Peto's mortality-prevalence test was used to analyze the tumor data when the number of tumors was >8. Otherwise, the exact permutation test was used. Rare tumors were tested at the 5%, upper-tailed probability level, and common tumors were tested at the 1%, upper tailed probability level. Analysis of hyperplastic lesions from the same cell lines as the tumors, or analysis of a combination of hyperplastic lesions, benign tumors, and malignant tumors may have also been performed, at the discretion of the pathologist.

C. METHODS

1. Observations

1a. Cageside observations - Animals were observed twice daily for mortality and signs of toxicity.

1b. Clinical examinations - Detailed examinations were conducted weekly.

1c. Neurological evaluations - A functional observational battery (FOB) was conducted blindly on 10 random animals/sex/group during Weeks 66 and 67. Only animals without physical abnormalities that could hinder the evaluation were used. FOB scoring criteria (MRID45422719, Attachment 3, pages 802-809) are included in the Appendix of this DER. The FOB assessment included the following CHECKED (X) parameters.

	HOME CAGE OBSERVATIONS		HANDLING OBSERVATIONS		OPEN FIELD TEST OBSERVATIONS
X	Posture*		Reactivity*		Mobility
X	Activity	X	Lacrimation* / chromodacryorrhea	X	Rearing+
X	Convulsions*	X	Salivation*	X	Arousal/ general activity level*
X	Tremors*	X	Piloerection*	X	Convulsions*
	Abnormal Movements*	X	Fur appearance	X	Tremors*
	Palpebral closure*	X	Palpebral closure*	X	Abnormal movements*
X	Body tone	X	Respiratory rate+	X	Urination / defecation*
X	Vocalization	X	Vocalization	X	Grooming
X	Arousal	X	Writhing	X	Gait abnormalities*
		X	Red/crusty deposits*	X	Gait score*
	SENSORY OBSERVATIONS		Mucous membranes /eye /skin color	X	Bizarre / stereotypic behavior*
X	Approach response+	X	Eye prominence*		Backing
X	Touch response+		Muscle tone*	X	Time to first step
X	Startle response*	X	Ease of removal	X	Posture
X	Pain response*	X	Ease of handling	X	Body tone
X	Pupil status and response*				
X	Eyeblink response (corneal)		**PHYSIOLOGICAL OBSERVATIONS**		**NEUROMUSCULAR OBSERVATIONS**
	Forelimb extension	X	Body weight*		Hindlimb extensor strength
	Hindlimb extension	X	Body temperature+	X	Forelimb grip strength*
X	Air righting reflex+			X	Hindlimb grip strength*
	Olfactory orientation			X	Landing foot splay*
X	Catalepsy		**OTHER OBSERVATIONS**		Rotarod performance

*Required parameters/+Recommended parameters taken from Acute Neurotoxicity (Rats) OPPTS 870.6200a.

2. Body weight - All animals were weighed (g) prior to start of the study, weekly for the first 14 weeks, approximately monthly thereafter, and at termination. Cumulative body weight (g) gains were also presented.

3. Food and water consumption and food efficiency - Mean food consumption (g/animal/week) was determined weekly for the first 13 weeks, every four weeks thereafter, and at terminal sacrifice. Food efficiency (mean weekly body weight gain x 100 / mean weekly food consumption) was reported for the first 13 weeks. Compound intake (mg/kg/day) values were calculated from the nominal dose of the test substance, and food consumption and body weight gain data. Water consumption (g/animal/week) was determined daily during Weeks 12, 25, and 51 for the interim sacrifice animals.

4. Ophthalmoscopic examination - Ophthalmoscopic examinations were performed on all animals prior to the start of the study and on surviving animals scheduled for sacrifice (at 12 and 24 months).

5. Hematology & clinical chemistry - Animals were fasted overnight, and blood samples were obtained from 10 rats/sex/dose during Weeks 13, 27, 53, 79, 103/104 (females), and 105 (males). Additionally, blood samples were collected from animals sacrificed at unscheduled intervals when possible. Blood and reticulocyte smears were prepared at the scheduled sacrifices. The CHECKED (X) parameters were examined.

a. Hematology

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

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

b. Clinical chemistry

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

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

6. Urinalysis - Animals were fasted overnight, and urine samples were obtained from 10 rats/sex/dose during Weeks 13, 27, 53, 79, 103/104 (females), and 105 (males). The CHECKED (X) parameters were examined.

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

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

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

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

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

Microscopic examinations were performed on all tissues collected, except for the Harderian gland, head, and larynx.

II. RESULTS

A. OBSERVATIONS

1. Clinical signs of toxicity - No treatment-related effect was observed on clinical signs of toxicity.

2. Mortality - No treatment-related effect was observed on mortality. Survival generally exceeded guideline requirements of 50% at Week 78 and 25% at Week 104 in both sexes. At Week 78, survival was 60-93% in all dose groups. At Week 104, survival was 28-55% in the males and 25-50% in the females (except 20% in 300 ppm females). More 3000 ppm males and females survived than males and females in the other treatment groups. The primary cause of unscheduled deaths was adenoma/carcinoma in the pituitary (16-39 deaths/dose in each sex). Mammary gland carcinoma resulted in 2-7 deaths/dose in females. All other causes of death and undetermined causes of death resulted in ≤5 deaths/dose in each sex.

3. Neurological Evaluations - No treatment-related effect was observed during the FOB. A significant increase ($p \leq 0.05$) in forelimb grip strength of the 150 and 3000 ppm females was observed (↑25-27%). The increased strength was not dose-dependent and there were no other signs that indicated an adverse effect in the central nervous system.

B. BODY WEIGHT: Treatment-related decreases in body weight were observed in the 1500 ppm (↓4-11%, $p \leq 0.01$; during weeks 1-70) and 3000 ppm females (↓9-21%; $p \leq 0.01$ throughout the study) and in the 3000 ppm males through week 90 (↓9-14%; $p \leq 0.05$; Table 2). Treatment-related decreases ($p \leq 0.01$) in cumulative body weight gain were observed in the 3000 ppm females at Weeks 1-13, 13-50, and 1-102 (↓25-31%). Decreased ($p \leq 0.01$) body weight gains were observed during Weeks 1-50 in 3000 ppm males and 1500 ppm females (↓13%, each); overall body weight gain (Weeks 1-102) in these animals were decreased by (↓5-8%; not statistically significant [NS]). All other statistically significant differences in body weights and cumulative body weight gains were minor and/or sporadic.

Table 2. Selected mean (±SD) body weights (g) and cumulative body weight gains (g) in rats dosed with TI-435 for up to 24 months. [a]

Study week (s)	Dose (ppm)				
	0	150	500	1500	3000
Males					
1	152±20.0	150±19.7	151±20.3	150±19.7	149±19.9
2	214±22.6	211±21.5	213±22.5	205±21.3* (↓4)	184±21.9** (↓14)
50	778±103.4	766±95.9	743±91.8	741±84.7	691±93.6** (↓11)
70	818±129.9	784±122.1	790±137.1	779±113.1	741±103.6* (↓9)
102	759±141.2	784±134.7	799±155.4	754±116.5	725±122.5
Gain (1-13)	391±49.4	384±52.7	381±39.1	372±41.9	343±42.0** (↓12)
Gain (1-50)	626±99.6	616±93.2	592±85.5	591±76.5	543±87.8** (↓13)
Gain (1-102)	607±144.8	637±135.7	655±150.1	603±111.6	574±116.9 (↓5)
Females					
1	137±10.7	136±9.9	134±9.9	135±10.0	137±10.5
2	171±12.4	168±10.7	167±13.2	164±12.7** (↓4)	154±11.1** (↓10)
3	193±16.1	190±13.7	186±15.6* (↓4)	184±14.1** (↓5)	175±13.7** (↓9)
66	433±87.9	453±92.9	425±60.6	384±57.5** (↓11)	342±49.4** (↓21)
102	447±81.2	500±114.8	456±80.3	424±76.2	356±57.3** (↓20)
Gain (1-13)	150±25.8	146±23.5	142±21.0	132±19.7** (↓12)	112±18.0** (↓25)
Gain (13-50)	105±45.8	126±56.1	111±39.1	89±41.4	72±27.5** (↓31)
Gain (1-102)	313±78.6	370±111.6	321±80.2	289±74.5 (↓8)	220±55.7** (↓30)

a Data were obtained from MRID 45422719, Tables 7-8 on pages 191-201. Numbers listed parenthetically represent the percent difference from controls (calculated by reviewers).

* Statistically different ($p \leq 0.05$) from the controls

** Statistically different ($p \leq 0.01$) from the controls

C. FOOD CONSUMPTION AND FOOD EFFICIENCY

1. Food and water consumption - A treatment-related decrease (↓7-31%; $\bar{x}$=16%; $p \leq 0.05$) in food consumption was observed in the 3000 ppm females through Week 77 (Table 3). Decreased ($p \leq 0.05$) food consumption was generally observed in the 3000 ppm males (↓4-30%; $\bar{x}$=10%) and in the 1500 ppm females (↓6-16%; $\bar{x}$=10%) during Weeks 1-73. All other statistically significant differences in food consumption were minor and/or sporadic. No treatment-related effects were observed on water consumption.

Table 3. Food consumption (mean food consumed/rat/week [g]) at selected intervals in rats fed TI-435 for up to 24 months.[a]

Week	Dose (ppm)				
	0	150	500	1500	3000
Males					
1	167±13.0	164±12.2	165±12.8	152±11.5** (↓9)	117±13.7** (↓30)
29	199±19.6	201±20.1	201±18.7	197±18.1	191±18.8* (↓4)
101	172±62.8	173±51.5	178±41.4	183±31.4	168±50.7
Females					
1	132±10.6	125±10.2** (↓5)	121±10.5** (↓8)	111±11.8** (↓16)	91±11.2** (↓31)
37	145±20.2	144±20.9	141±18.8	137±19.2* (↓6)	126±16.1** (↓13)
73	150±35.1	156±32.6	151±28.0	147±25.2	139±15.8* (↓7)
101	133±37.7	137±35.4	120±45.1	130±26.7	124±34.4

a These data were obtained from MRID 45422719, Table 9 on pages 202-206. Numbers listed parenthetically represent the percent difference from controls (calculated by reviewers).
* Statistically different ($p \leq 0.05$) from the controls
** Statistically different ($p \leq 0.01$) from the controls

2. Food efficiency - No treatment-related effect was observed on food efficiency. Sporadic increases and decreases in food efficiency in the 3000 ppm males resulted in a minor overall effect. There were also sporadic increases and decreases in mean food efficiency in all treated females.

3. Compound consumption - The mean achieved dosages based on actual body weights and food consumption are shown in Table 1.

D. OPHTHALMOSCOPIC EXAMINATION - No treatment-related findings were observed during ophthalmoscopic examination.

E. BLOOD ANALYSES

1. Hematology - No treatment-related effects were observed in hematology parameters. Several differences from the controls were observed; however, these differences did not persist throughout the study, were minor, and/or were not dose-dependent.

2. Clinical chemistry - No treatment-related effects were observed in clinical chemistry parameters. Several differences from the controls were observed; however, these differences did not persist throughout the study, were minor, not dose-dependent, and/or within the historical control values.

F. URINALYSIS - A dose-dependent decrease in urine volume was observed in the females, and was significant ($p \leq 0.05$) at 3000 ppm (↓60%) at Week 13. However, this effect did not persist throughout the study; therefore, the decreased urine volume was not considered treatment-related. No treatment-related effects were observed in the males.

G. SACRIFICE AND PATHOLOGY:

1. Organ weights - Body weights were decreased ($p \leq 0.01$) in the 3000 ppm females (↓19-20%; Table 4) at the interim and terminal sacrifices. Increased ($p \leq 0.05$) relative (to body) liver, kidney, and lung weights were observed in the 3000 ppm females at the interim and terminal sacrifices (↑20-27%). There was not a statistically significant difference in these organ weights in the males, nor in the absolute or relative (to brain) weights in the females. Other observed differences ($p \leq 0.05$) were not dose-dependent, did not persist throughout the study, and/or were not corroborated by gross or microscopic pathological data. Since the liver, lung and kidney were all organs found to express treatment related macro and microscopic pathologies, the organ weight changes noted above were deemed to be treatment related and adverse.

Table 4. Terminal body weights (g) and selected relative (to body) organ weights (%) in female rats fed TI-435 for up to 12 and 24 months.[a]

Organ	Dose (ppm)				
	0	150	300	1500	3000
Interim Sacrifice (n=19-20)					
Terminal body weight	380±71.6	376±68.3	371±53.3	337±73.5	304±42.0** (↓20)
Liver	2.61±0.253	2.97±0.342** (↑14)	2.72±0.301	2.98±0.395** (↑14)	3.23±0.241** (↑24)
Kidney	0.651±0.0685	0.700±0.109	0.658±0.0698	0.700±0.0997	0.786±0.0880** (↑21)
Lung	0.403±0.0631	0.403±0.0729	0.418±0.161	0.440±0.0835	0.494±0.0590* (↑23)
Terminal Sacrifice (n=12-30)					
Terminal body weight	407±71.8	454±107	419±79.2	391±80.2	328±58.5** (↓19)
Liver	2.71±0.463	2.57±0.524	2.75±0.417	2.99±0.607	3.33±0.533** (↑23)
Kidney	0.692±0.182	0.657±0.221	0.736±0.156	0.676±0.139	0.833±0.165* (↑20)
Lung	0.387±0.0695	0.350±0.0934	0.374±0.0700	0.400±0.0849	0.490±0.0795** (↑27)

a Data were obtained from MRID 45422719, Tables 31-32 on pages 268-331. Numbers listed parenthetically represent the percent difference from controls (calculated by reviewers).

* *Statistically different ($p \leq 0.05$) from the controls*

* Statistically different ($p \leq 0.01$) from the controls

2. Gross pathology - When all animals are combined, including those that died and/or were killed *in extremis* (n=80), slightly increased incidences (treated vs controls) of mottled or red focal areas of the livers (18% vs 5%) and granular material or calculus(i) in kidneys (8% vs 0%) were observed in the 3000 ppm males (statistical analyses not performed; Table 5). In the combined unscheduled killed and terminal sacrifice females (n=60-61), there was an increase in the incidence of multiple mammary gland masses observed grossly in the 150 (28%), 300 (37%), 1500 (43%), and 3000 (43%) ppm animals vs (17%) in the controls. However, microscopic

analysis did not corroborate a treatment-related effect in this incidence of masses. In the 3000 ppm females red foci/areas in the pituitary were observed, but other data did not support an adverse effect on this organ. At 3000 ppm in both sexes, effects were also observed in the stomach (dark foci/areas), but were minor.

Table 5. Incidence (%) of selected macroscopic lesions found in all male rats fed TI-435 in the diet for up to 24 months. [a]

Macroscopic Lesion	Dose (ppm)				
	0	150	500	1500	3000
Liver, mottled	5	9	6	10	18
Liver, red focus area	5	7	8	16	13
Kidney, granular material or calculus(i)	0	0	1	4	8

a Data were obtained from MRID 45422719, Table 36 on pages 394-420; n=80.

3. Microscopic pathology

a. Non-neoplastic - When all animals are combined, including those that died and/or were killed *in extremis,* slightly increased incidences (treated vs controls) of the following microscopic lesions were observed at 3000 ppm (n=79-80; $p \leq 0.05$ by pair-wise comparison and dose-effect trend; Table 6): (i) kidney pelvic mineralization and transitional cell hyperplasia in males (64-65% vs 41-43%); (ii) altered hepatocellular eosinophilic focus in males (20% vs 8%) and in females (36% vs 11%); (iii) kidney pelvic angiectasis (26% vs 11%) and tubular ectasia (16% vs 5%) both in females; (iv) chronic lung inflammation in females (14% vs 3%); and (v) ovary interstitial gland hyperplasia (42% vs 6%). Additionally, increased incidences of ovary interstitial gland hyperplasia (25% vs 6%; $p \leq 0.01$) was observed at 1500 ppm when all animals were combined. The average severity for each abnormality (when reported) for the combined unscheduled killed and terminal sacrifice animals (n=60-62) was minimal to slight; severity increased only slightly with dose.

The following microscopic lesions that were increased (treated vs controls) when all animals were combined were also increased at the interim sacrifice (n=18-20; statistical analysis was not performed): (i) kidney pelvic mineralization and transitional cell hyperplasia at ≥1500 ppm in males (47-70% vs 22-39%); (ii) liver lymphohistiocytic infiltrate in all treated females (84-100% vs 60%); and (iii) altered hepatocellular eosinophilic focus in 1500 and 3000 ppm females (21-25% vs 5%). An increase in kidney tubular mineralization and transitional cell hyperplasia at ≥1500 ppm in females at terminal sacrifice was also noted.

Other minor effects were observed at 3000 ppm when all animals were combined ($p \leq 0.05$ by pair-wise comparison and dose-effect trend) as follows: (i) stomach edema and hemorrhage in males; (ii) thymus epithelial hyperplasia in females; (iv) pituitary angiectasis females; (v) stomach glandular erosion and edema in females; and (vi) uterus congestion. Thyroid follicular cyst(s) were observed at 3000 ppm in females (38% treated vs 21% controls). The incidence of liver lymphohistiocytic infiltrate exceeded the concurrent controls at 1500 (69% treated vs 53% concurrent controls) and 3000 (69%) ppm in females. Minor increases in kidney pelvic

mineralization in males (54% vs 43%; not statistically significant [NS]) and altered hepatocellular eosinophilic focus in females (21% vs 11%; NS) were observed at 1500 ppm when all animals were combined. Minor increases in liver congestion in males at terminal sacrifice were observed at ≥1500 ppm.

Table 6. Incidence (% [severity index]) of selected non-neoplastic microscopic lesions found in rats fed TI-435 in the diet for up to 24 months. [a]

Microscopic Lesion	Dose (ppm)					Historical Controls [b]
	0	150	500	1500	3000	
Males						
Kidney						
Mineralization, pelvic	43 (1.1)	38 (1.0)	43 (1.0)	54 (1.0)	65** (1.8)	5-43
Hyperplasia, transitional cell	41 (0.8)	38 (0.7)	36 (0.6)	36 (0.5)	64** (1.2)	0-41
Liver						
Congestion[d]	4	3	5	15	10	
Focus, altered hepatocellular, eosinophilic	8 (0.2)	15 (0.3)	8 (0.3)	10 (0.3)	20* (0.6)	0-8
Females						
Kidney						
Angiectasis, pelvic	11 (0.3)	15 (0.3)	6 (0.1)	9 (0.2)	26* (0.6)	0-11
Hyperplasia, transitional cell[d]	12	14	10	21	29	-
Mineralization, tubular[d]	3	5	6	14	16	-
Ectasia, tubular	5 (0.1)	5 (0.1)	10 (0.3)	5 (0.1)	16* (0.4)	0-5
Liver						
Infiltrate, lymphohistiocytic	53	56	66	69*	69*	53-72
Focus, altered hepatocellular, eosinophilic	11 (0.4)	18 (0.5)	8 (0.3)	21 (0.6)	36** (1.2)	0-11
Lung						
Inflammation, chronic	3	3	6	5	14**	2-3
Ovary						
Hyperplasia, interstitial gland	6	13	16	25**	42** [c]	0-6

a Data (n=80) were obtained from MRID 45422719, Text-Table 5 on page 67 and Text-Tables 3-4 on pages 78-95. The severity index (when reported) was for the unscheduled killed and terminal sacrifice animals (n=60-62), rather than all combined animals (n=80). The severity index is the average severity based upon a five point scale: minimal (1), slight (2), moderate (3), moderately severe (4), and severe (5).

b Historical control value data obtained from MRID 45422720, pages 19, 36, and 91 (n=80-100; three data sets).

c n=79

d Terminal sacrifice animals only.

* Statistically different ($p \leq 0.05$) from the controls by pair-wise comparison and dose-effect trend

** Statistically different ($p \leq 0.01$) from the controls by pair-wise comparison and dose-effect trend

b. <u>Neoplastic</u> - An increased incidence of thyroid adenomas was observed in females. The study author's analysis indicated that when all animals were considered, the incidence of C-cell adenoma in the thyroid was increased ($p \leq 0.05$) in the 1500 and 3000 ppm females (↑11-12%) over controls, a positive ($p \leq 0.05$) dose-effect trend was established, and the incidence exceeded the historical controls (20-21% treated vs 8.8-12% historical controls). The combined incidence

of C-cell adenoma and carcinoma was increased in the females at 1500 (↑12%; p≤0.05) and 3000 (↑10%; NS) ppm females. A statistically significant effect on combined C-cell adenoma and carcinoma was not demonstrated in the 3000 ppm females by pair-wise comparison, and a dose-effect trend was not established. Furthermore, a dose-dependent effect on combined C-cell adenoma and carcinoma was not present in the 3000 ppm females that survived until terminal sacrifice (n=12-30): 150 (51%), 500 (33%), 1500 (35%), and 3000 (20%) ppm vs 27% in the concurrent controls. A separate analysis conducted by the HED staff, using the Peto Prevalence Test did not show any statistically significant increases in the incidence of thyroid adenomas and/or carcinomas combined (either pairwise comparisons or a trend and with or without interim sacrifice animals) when compared to the control group. Therefore, the increased incidences of thyroid tumors were not considered to be treatment-related.

In the males, hepatocellular carcinoma incidence was increased at 500 (4% treated vs 0% controls; NS) and 3000 (5% treated; p≤0.05) ppm when all animals were combined (Table 8). A significant (p≤0.05) dose-effect trend was also reported. The increased incidence of hepatocellular carcinoma only slightly exceeded the maximum historical control values in the conducting laboratory (4% treated vs 3.3% historical controls), and the Sponsor stated that observed incidence did not exceed the historical control values reported by other laboratories (6%). A separate analysis conducted by HED indicated a statistically significant increase in the incidence of hepatocellular carcinoma in the 500 and 3000 ppm males, both pairwise (p = 0.02) and as an increasing trend (p=0.03) when compared to the control group. No adenomas were observed. However, as stated previously, the increased incidences in the low and high dose groups were just outside the historical control incidences for the same testing laboratory and were within the historical control range for the animal supplier. In addition, there was no dose-response and there was no continuum (i.e., no preneoplastic lesions and no adenomas). Therefore, the increased incidences were again not considered to be treatment-related.

Table 8. Incidence (%) of selected neoplastic microscopic lesions found in rats fed TI-435 in the diet for up to 24 months. [a]

Neoplastic Lesion	Dose (ppm) 0	150	500	1500	3000	Historical Controls (%)
Males						
Liver						
Adenoma	0	0	0	0	0	0-5.0
Carcinoma	0 [b]	0	3(4)	0	4(5)*	0-3.3 [c]
Adenoma and carcinoma	0	0	(4)	0	(5)	
Females						
Thyroid						
C-cell adenoma	7(9) [b]	13(16)	9(11)	17(21)*	16(20)*	8.8-12.0
C-cell carcinoma	2(3)	2(3)	1	1	1	0-2.5
C-cell adenoma and carcinoma	(11)	(18)	(13)	(23)*	(21)	

a Data were obtained from MRID 45422719, Text-Table 5 on page 96 and Text-Table 6 on page 102 (n=79-80). The sum of adenoma and carcinoma incidence as reported in the table differs from the incidence of combined adenoma and carcinoma due to rounding.

b Statistically significant (p≤0.05) dose-related trend; * statistically different (p≤0.05) from the controls

c Other labs have reported a maximum historical control rate of 6%.

III. DISCUSSION and CONCLUSIONS

A. INVESTIGATORS' CONCLUSIONS: Body weight and food consumption were decreased in 1500 and 3000 ppm males and ≥500 ppm females. Treatment-related gross and microscopic lesions were observed in the stomach, kidney, and ovary. Stomach edema and hemorrhage were observed in the 3000 ppm males, and stomach edema and mucosa erosion were observed in the 3000 ppm females. Grossly, granular material or calculi were increased in the kidneys of the 3000 ppm males, and pelvic mineralization and transitional cell hyperplasia were observed microscopically. Increased ovary interstitial gland hyperplasia was observed at ≥500 ppm in females. The LOAEL was 1500 ppm in males and 500 ppm in females. The NOAEL was 500 ppm in males and 150 ppm in females. It was concluded that there was no evidence of carcinogenicity in male and female rats.

B. REVIEWER COMMENTS: There were no compound related effects on mortality, clinical signs, functional observational battery, food efficiency, water consumption, ophthalmology, hematology, clinical chemistry, or urinalysis.

Systemically, treatment-related decreases in body weight and food consumption were observed in the ≥1500 ppm females and in the 3000 ppm males. Although the body weight and food consumption was not decreased in the 1500 ppm females at the end of the study, the decreases were consistant over much of the study, and other adverse effects were seen in animals of the same dose. Although the study author suggested an adverse effect in body weights and food consumption in females at 500 ppm, these deficits were of small magnitude and for only the first 13 weeks of the study. The effect in these animals (females dosed at 500 ppm) was considered to be treatment related but not adverse, as the body weights recovered and no other evidence of toxicity were seen in these animals.

In the kidney in the 3000 ppm males, granular material or calculus was observed grossly in the males when all animals were combined. The incidences of pelvic mineralization and transitional cell hyperplasia were increased at the terminal ($p \leq 0.01$) and interim sacrifices in males. Pelvic angiectasis and tubular ectasia were increased ($p \leq 0.05$) in the females when all animals were combined. Relative (to body) kidney weight was increased ($p \leq 0.05$) in the females at the interim and terminal sacrifices. All these abnormalities occurred at an increase of 8-23% beyond the concurrent controls, and exceeded the historical control values. The observed microscopic lesions were of minimal to slight severity on average. In conclusion, slight signs of nephrotoxicity were observed.

In the liver in the 3000 ppm group, mottled livers were observed grossly in males when all animals were combined. Altered hepatocellular eosinophilic foci were observed at the terminal sacrifice in males and females ($p \leq 0.05$), as well as the interim sacrifice in females. Altered hepatocellular eosinophilic focus in females (21% vs 11%; NS) were also observed at 1500 ppm when all animals were combined. Increased ($p \leq 0.01$) relative (to body) liver weights were observed in the 3000 ppm females at the interim and terminal sacrifices. The incidence of liver lymphohistiocytic infiltrate exceeded the concurrent controls at 1500 (69% treated vs 53% concurrent controls) and 3000 (69%) ppm in females. Minor increases in liver congestion in

males at terminal sacrifice were observed at ≥1500 ppm. All abnormalities observed at termination occurred at an increase incidence of 12-25% beyond the concurrent controls, and exceeded the historical control values. In conclusion, slight signs of hepatotoxicity were observed.

In the lung, increased ($p \leq 0.05$) relative (to body) lung weights were observed in the 3000 ppm females at the interim and terminal sacrifices (incr 23-27%), and the incidence of chronic lung inflammation was increased 11% ($p \leq 0.01$). The incidence of these lesions exceeded the historical control values. Other corroborating signs of lung toxicity were not observed.

Ovary interstitial gland hyperplasia was observed ($p \leq 0.01$) at 1500 (↑19%) and 3000 (↑30%) ppm when all animals were combined. The incidence of this lesion exceeded the historical control values.

Other minor effects were observed at 3000 ppm when all animals were combined ($p \leq 0.05$ by pair-wise comparison and dose-effect trend) as follows: (i) stomach edema and hemorrhage in males; (ii) thymus epithelial hyperplasia in females; (iv) pituitary angiectasis females; (v) stomach glandular erosion and edema in females; and (vi) uterus congestion. Thyroid follicular cyst(s) were observed at 3000 ppm in females (38% treated vs 21% controls).

The LOAEL is 1500 ppm in females and 3000 ppm in males (equivalent to 156.5/97.8 mg/kg/day in males/females), based on decreased body weight, and food consumption, ovary interstitial gland hyperplasia, increased lymphohistiocytic infiltrate and altered hepatocellular eosinophilic focus of the liver in females; and decreased body weight, and food consumption and slightly increased incidences of pelvic mineralization and transitional cell hyperplasia in the kidney, mottled livers, and altered hepatocellular eosinophilic foci in the livers of males. The NOAEL for this study is 500 ppm in females and 1500 ppm in males (equivalent to 82.0/32.5 mg/kg/day in males/females).

At the doses tested, there was a treatment-related increase in thyroid C-cell adenoma incidence in females when compared to controls. However, a statistical analysis showed that the increase in thyroid C-cell tumors in females was not significant, especially when carcinomas and adenomas were combined. In males, an increase in the incidence of hepatocellular carcinomas was observed at 500 and 3000 ppm when all animals were combined. The increased incidences in the low and high dose groups were just outside the historical control incidences for the same testing laboratory but were within the historical control range for the animal supplier. In addition, there was no dose-response and there was no continuum (i.e., no preneoplastic lesions and no adenomas).

Based on all the factors summarized above, it was determined that there was no evidence of carcinogenicity under the conditions of this study. Dosing was considered adequate based on body weight, body weight gain, and food consumption decreases, and slight abnormalities in the kidney and liver.

HED Records Center Series 361 Science Reviews - File R107689 - Page 390 of 721

C. STUDY DEFICIENCIES: Several minor deficiencies were observed, such as: (i) survival at Week 102 in the 500 ppm females (23.3%) was less than the guideline requirement of 25%; (ii) glutamate dehydrogenase and magnesium levels were not measured; (iii) bile ducts were not collected and examined microscopically; and (iv) the larynx was not examined microscopically. These deficiencies are minor and would not change the conclusions of this review.

DATA FOR ENTRY INTO ISIS

Chronic/Carcinogenicity Study - rodents (870.4300)

PC code	MRID #	Study type	Species	Duration	Route	Dosing method	Dose range mg/kg/day	Doses tested mg/kg/day	NOAEL mg/kg/day	LOAEL mg/kg/day	Target organ(s)	Comments
044309	45422719 45422720	chronic/onc	rat	24 months	oral	diet	8.1-193.4	M/F: 0/0, 8.1/9.7, 27.4/32.5, 82.0/97.8, 156.5/193.4	82.0	156.5	bw, bwg, fc, kidney, liver	

325

HED Records Center Series 361 Science Reviews - File R107689 - Page 391 of 721

APPENDIX

Attachment No. 3

FUNCTIONAL OBSERVATIONAL BATTERY

Observational measurements will be evaluated before and upon removal from the homecage. The animal will be placed into a circular arena for 2 minutes. The latency (time in seconds) to the first step will be recorded. During this time, behavior will be evaluated as indicated in the Open Field section. The number of rears, grooms, fecal boli, and urine pools during this 2-minute period will be recorded. After the open field test, a battery of manipulative tests will be initiated.

Observations
Before being removed from the home cage, the animal will be observed and evaluated for the following:

1. Position

    1. Sitting, standing, or walking
    2. Low carriage
    3. Hunched
    4. Prostrate

2. Activity

    1. Alert
    2. Hypoactive
    3. Hyperactive

3. Body tone

    1. Some muscle tone
    2. Flaccid
    3. Rigid

4. Tremor

    1. Absent
    2. Present - Mild
    3. Present - Moderate
    4. Present - Severe

Attachment No. 3 (Continued)

5. Convulsions

    1. No convulsions present
    2. Wet dog shake
    3. Clonic contractions
    4. Tonic contractions
    5. Clonic-tonic contractions

6. Vocalization

    1. No vocalization
    2. Intermittent vocalization
    3. Continuous vocalization

7. Arousal upon opening cage

    1. Alert
    2. Nonresponsive
    3. Hypersensitive

Each animal will be removed from its cage and observed for the following (any unusual observations will be described as they are observed):

8. Ease of removal

    1. Slight resistance
    2. No resistance
    3. Moderately difficult (evasive)
    4. Animal freezes (appears rigid) -
    5. Aggressive behavior

9. Ease of handling

    1. Easy
    2. Very easy (flaccid)
    3. Moderately difficult (animal resists restraint)
    4. Rigid in hand
    5. Difficult

Attachment No. 3 (Continued)

10. Vocalization

    1. No vocalization
    2. Vocalization upon removal only
    3. Vocalization during handling only
    4. Continuous vocalization

11. Palpebral closure

    1. Eyelids wide open
    2. Ptosis
    3. Eyelids completely closed

12. Exophthalmos

    1. Absent (-)
    2. Present (+)

13. Excessive Lacrimation

    1. None
    2. Slight
    3. Moderate
    4. Severe

14. Excessive Salivation

    1. None
    2. Slight
    3. Moderate
    4. Severe

15. Respiration

    1. Relaxed and continuous
    2. Rapid and shallow
    3. Labored
    4. Gasping

Attachment No. 3 (Continued)

16. Appearance of fur

1. Groomed
2. Stained
3. Unkempt
4. Very unkempt

17. Piloerection

1. Absent (-)
2. Present (+)

18. Writhing
1. Absent (-)
2. Present (+)

Open Field
Each animal will be placed into an open field arena for 2 minutes. Animals will be observed for the following (any unusual observations will be described as they are observed):

19. Latency (time in seconds) to the first step

20. Number of grooms

21. Number of rears

22 Number of urine pools

23. Number of fecal boli

24. Position

1. Sitting, standing, or walking
2. Low carriage
3. Hunched
4. Prostrate

Attachment No. 3 (Continued)

25. Gait abnormalities - type

1. None
2. Ataxia
3. Paralytic
4. Spastic
5. Scissor

26. Gait abnormalities - severity

1. None
2. Slight
3. Moderate
4. Severe

27. Activity

1. Moderate activity
2. Low activity
3. No movement
4. High activity

28. Stereotypy

1. Absent (-)
2. Present (+)

29. Body tone

1. Some muscle tone
2. Flaccid
3. Rigid

30. Tremor

1. Absent
2. Present - Mild
3. Present - Moderate
4. Present - Severe

Attachment No. 3 (Continued)

31. Convulsions

1. No convulsions present
2. Wet dog shake
3. Clonic contractions
4. Tonic contractions
5. Clonic-tonic contractions

32. Other unusual behavior (will be described)

1. Absent (-)
2. Present (+)

Reflexes and Physiological Parameters

33. Approach response. Animal will be approached head-on with a pen light.

1. Approaches
2. No reaction
3. Avoidance
4. Jumps, freezes, or both
5. Aggressive

34. Touch response. Animal will be touched on both sides of the rump.

1. Turns to stimulus
2. No response
3. Avoids stimulus
4. Jumps, freezes, or both
5. Aggressive

35. Catalepsy. Animal will be placed in a catalepsy testing box containing a support bar; its forelimbs are placed on the support bar. If animal displays waxy rigidity of the muscles and tends to remain in any position in which it is placed, the response will be recorded as present.

1. Absent (-)
2. Present (+)

Attachment No. 3 (Continued)

36. Pupillary status (relative to the room lighting conditions)

    1. Pupils constricted and considered normal for room lighting conditions
    2. Miosis (pupils constricted more that what would be observed at room lighting conditions)
    3. Pupils dilated

37. Pupillary response

    1. Pupillary response positive (both pupils contracted)
    2. Pupillary response negative (one or both pupils did not contract)

38. Corneal response to touch. The cornea will be touched with a rigid nylon thread.

    1. Positive (animal responds to touch of both eyes)
    2. Negative (no response in one or neither eye)

39. Startle response. A galton whistle will be held behind the animals head. A positive or negative reaction to the stimulus will be recorded.

    1. Positive
    2. Negative

40. Air drop righting reflex. Animal will be dropped from a height of approximately 30 cm from a horizontal position (abdomen up).

    1. Positive (animal rights mid-air)
    2. Negative (animal does not right mid-air)

41. Analgesic reflex. The animal will be placed into an automated system where a heat stimulus will be applied to the animal's tail. The time in seconds for the animal to remove its tail away from the nociceptive stimulus will be recorded. One trial will be done.

42. Grip strength. Animal will be held in a test trough, allowed to grip the forelimb grasping bar, and then pulled away from the grasping bar until grip is broken. The animal will then be held so its hindlimbs grip the hindlimb grasping bar and then pulled away from the grasping bar until grip is broken. Grip strength will be recorded for both forelimb and hindlimb grip.

Attachment No. 3 (Continued)

43. Foot splay. The hind feet of the animal will be moistened with water. The animal will be dropped from a height of approximately 30 cm from a horizontal position onto a chalkboard, and the hindlimb foot splay distance will be measured.

44. Body temperature

45. Body weight

DATA EVALUATION RECORD

TI-435 (CLOTHIANIDIN)

Study Type: §83-2b, Carcinogenicity Study in Mice

Work Assignment No. 4-02-167 B (Formerly 4-01-167 B)
MRIDs 45422721, 45422722, and 45422709

Prepared for
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Disclaimer

This Data Evaluation Record may have been altered by the Health Effects Division subsequent to signing by Dynamac Corporation personnel.

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DATA EVALUATION RECORD

STUDY TYPE: Carcinogenicity - mice/feeding. OPPTS 870.4200b [§83-2b]; OECD 451.

PC CODE: 044309 **DP BARCODE:** D279596, D276450, and D277188
SUBMISSION NO.: S607124 and S600587

TEST MATERIAL (PURITY): TI-435 (95.2-95.5% a.i.)

SYNONYMS: Clothianidin; (E)-1-(2-chloro-5-thiazolylmethly)-3-methyl-2-nitroguanidine

CITATION: Biegel, L.B. (2000). 78-week dietary carcinogenicity study with TI 435 in mice. Covance Laboratories Ltd, Madison, WI. Laboratory Project Id: Covance 6155-109, March 27, 2000. MRID 45422721. Unpublished.

Chambers, P.R. (1997). TI-435: Toxicity to mice by dietary administration for 4 weeks. Huntingdon Life Sciences Ltd, Huntingdon, Cambridgeshire, England. Laboratory Project Id: TDA 180/960497, February 19, 1997. MRID 45422709. Unpublished.

Biegel, L.B. (2001). 78-week dietary carcinogenicity study with TI 435 in mice. Covance Laboratories Ltd, Madison, WI. Laboratory Project Id: Covance 6155-109, Supplement No. 1, March 29, 2001. MRID 45422722. Unpublished.

SPONSOR: Takeda Chemical Industries Ltd, Agro Company, 13-10 Nihonbashi 2-chome, Chuo-ku, Tokyo, Japan.

EXECUTIVE SUMMARY: In this mouse oncogenicity study (MRIDs 45422721, 45422722, and 45422709), TI-435 (Clothianidin; 95.2-95.5% a.i.; Lot #: 30037120) was administered in the diet to 50 Crl:CD-1®(ICR)BR VAF/Plus® mice/sex/group for up to 18 months at doses of 0, 100, 350, or 1250 ppm (equivalent to 0, 13.5/17.0, 47.2/65.1, and 171.4/215.9 mg/kg/day in males/females, respectively). In addition, 50 mice/sex were treated with 700 ppm for weeks 1-4, 2000 ppm for weeks 5-10, 2500 ppm for weeks 11-34, and 2000/1800 ppm (males/females, respectively) (equivalent to 254.1/322.3 mg/kg/day in males/females, respectively) from week 35 until study termination at week 79 and will be referred to as the 2000/1800 ppm group.

No treatment-related effects on hematology, organ weights, or gross pathology were observed. There was no adverse effect at 100 or 350 ppm.

At week 78 in the 1800 ppm females, survival was significantly (p<=0.01) decreased (decr. 43%) compared to the concurrent controls, and a dose-related trend (p<=0.01) was demonstrated. In addition, at week 65 survival was 54% in the 1800 ppm females vs 88-92% in the other groups at week 65 (statistical analyses not reported). Decreases in survival began at week 20 and persisted throughout the study.

Clinical signs were restricted to an increased incidence of vocalization, observed at >=1250 ppm in both sexes (22-46 treated animals/dose group/sex vs 0 controls, n=50).

Body weights were decreased (p<=0.05) in the males at 2000/1800 ppm (decr. 4-18%), and in the females at 1250 (decr. 5-13%) and 2000/1800 ppm (decr. 9-17%). Overall (weeks 1-78) body weight gains decreased (p<=0.01) in the 2000/1800 ppm group (decr. 24% and 35% in males and females respectively) and in the 1250 ppm females (decr. 26%). The greatest reduction in body weight gain occurred at the high dose during weeks 11-33 (decr. 85% and 73% in males and females respectively), when the animals were being treated at 2500 ppm.

Food consumption was frequently decreased (p<=0.05) in the 2000/1800 ppm group (decr. 8-21% in males and decr. 11-24% in females), beginning at week 5 (when the dose was increased to 2000 ppm). Decreases (p<=0.05) in food efficiency in the 2000/1800 ppm group lead to cumulative decreases of 33-82% at weeks 5-10 (2000 ppm) and 117-151% at weeks 11-33 (2500 ppm).

In the 1800 ppm females, increased (p<=0.01) incidences of pulmonary congestion (26% treated vs 0% controls; 0%, historical controls), adrenal cortex congestion (22% treated vs 2% controls; 0-2%, historical controls), and cervix fibromuscular hyperplasia (58% treated vs 28% controls; 0-28.0%, historical controls) were observed; however, these effects were considered equivocal without further evidence of an adverse effect in these organs.

The LOAEL is 1250 ppm (215.9 mg/kg/day) based on decreased body weights and body weight gains in females. The NOAEL is 350 ppm (65.1 mg/kg/day).

There was no treatment-related increase in tumor incidences in the treated animals when compared to controls. Dosing was considered adequate based on decreased body weights, body weight gains, food consumption, and food efficiency in both sexes and decreased survival in females.

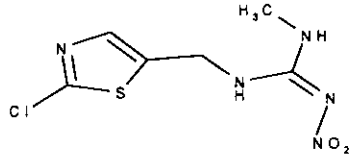
Under the conditions of this study, the carcinogenic potential of TI-435 is considered negative.
This study is **acceptable/guideline** and satisfies the requirements for a carcinogenicity study [OPPTS 870.4200; OECD 451] in mice.

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

I. MATERIALS AND METHODS

A. MATERIALS:

1. **Test material:** TI-435

Description:	Pale yellow powder
Lot/Batch #:	30037120
Purity:	95.2-95.5% a.i.
Stability of compound:	Stable in the diet for at least 22 days at 21°C
CAS #:	210880-92-5
Structure:	

2. **Vehicle:** Diet

3. **Test animals**

Species:	Mice
Strain:	Crl:CD-1®(ICR)BR VAF/Plus®
Approximate age and weight at the start of dosing:	35-41 days; 19.8-33.8 g males and 15.8-25.9 g females
Source:	Charles River Laboratory, Inc., Portage, MI
Housing:	Individually in stainless steel or polycarbonate cages
Diet:	Certified rodent diet (#8728 CM meal, Harlan Teklad), *ad libitum*
Water:	Tap water (assumed by reviewers), *ad libitum*
Environmental conditions	
Temperature:	18-26°C
Humidity:	30-70%
Air changes:	Not reported
Photoperiod:	12 hours light/ 12 hours dark
Acclimation period:	11 days

B. STUDY DESIGN

1. **In life dates** - Start: October 10, 1997 End: April 19, 1999

2. **Animal assignment** - Animals were randomly assigned (stratified by weight) to the test groups shown in Table 1.

Table 1. Study design [a]

Dose Group	Nominal Dose (ppm)	Actual Dose (mg/kg/day; M/F)	Assigned animals (#/sex)
Control	0	0/0	50
Low	100	13.5/17.0	50
Mid	350	47.2/65.1	50
Mid-High	1250	171.4/215.9	50
High	2000/1800 (M/F) [b]	254.1/322.3	50

a Data obtained from MRID 45422721, text-table on page 22 and Table 9 on page 140.

b Animals received 700 ppm for Weeks 1-4, 2000 ppm for Weeks 5-10, 2500 ppm for Weeks 11-34, and 2000/1800 ppm (males/females) for Weeks 35 through termination at Week 79.

3. Dose-selection rationale - A concurrently reviewed 4-week oral toxicity study in mice (MRID 45422709; see the appendix), provided information for the dose-selection rationale. In addition, the Sponsor reported that the doses were selected based on a previous 13-week toxicity study; however, the data was not provided.

In the present carcinogenicity study, minimal toxicity was observed at 1250 ppm during the first 4 weeks; therefore, the 700 ppm dose was increased to 2000 ppm and became the high dose group. However, 2000 ppm also did not appear to be the maximum tolerated dose; consequently, the dose was increased to 2500 ppm. Following substantial decrease in body weight and body weight gain, increased clinical abnormalities and increased mortality, the dosage was reduced to 2000/1800 ppm in males/females at Week 35. These changes in the high dose concentration were made following consultation with personnel from the EPA.

4. Treatment preparation, analysis, and administration - Dietary formulations were prepared by diluting a concentrated test material-feed mixture (premix) with appropriate amounts of diet to achieve the desired test concentrations. Dietary formulations were prepared approximately every 2 weeks, and were stored in covered containers at room temperature until used. Concentration analyses were performed at Months 1, 3, 6, 9, 12, 15, and 18. Homogeneity analyses (top, middle, and bottom) were performed on the 50 and 1500 ppm dietary formulations prior to study initiation, and on the 2500 ppm dietary formulation at Month 16. Stability of the compound for up to 22 days at 21°C was tested in the 100 and 30,000 ppm dietary formulations in a concurrently reviewed 4-week oral toxicity study in mice (MRID 45422709).

Results - Homogeneity Analysis: Range of % coefficient of variation over all doses: 1.22-2.84

Stability Analysis: Range of % of Day 0 over both doses: 88.9-109.9%

Concentration Analysis: Concentration Analysis: Range as % of nominal: 86.0-107%

100 ppm	86.8-107
350 ppm	86.9-97.4
700 ppm	88.6
1250 ppm	89.6-91.2
1800 ppm	88.3-97.8
2000 ppm	86.0-98.5
2500 ppm	90.4-106

At Month 15, the concentrations at 100 and 350 ppm were 82.1 and 76.3% nominal, respectively. Upon retesting, the concentrations at 100 and 350 ppm were found to be 96.8 and 97.4%, respectively. Only the retested values were considered in the concentration ranges reported above. The analytical data indicated that the mixing procedure was adequate and that the variation between nominal and actual dosages to the animals was acceptable.

5. Statistics - Data were subjected to the statistical procedures listed below at 5 and 1% levels of probability when determining differences between control and treatment groups.

Parameter	Statistical procedure
Body weights, body weight gains, food consumption, food efficiency, and organ weights (absolute, relative to body, and relative to brain)	Levene's test was performed to test for variance homogeneity. The data were transformed when necessary to achieve variance homogeneity. One-way analysis of variance (ANOVA) followed by Dunnett's multiple comparison t-test was applied.
Incidence of macroscopic and microscopic observations	Fisher-Irwin exact test
Mortality	Two-tailed log-rank test was used to compare the groups. One-tailed logistic regression was performed to test the dose-response relationship of mortality.
Tumor incidence	Peto's mortality-prevalence test was used to analyze the tumor data when the number of tumors was >8. Otherwise, the exact permutation test (SAS PROC MULTTEST, 1996) was used. Rare tumors were tested at the 5%, upper-tailed probability level, and common tumors were tested at the 1%, upper tailed probability level. Analysis of hyperplastic lesions from the same cell lines as the tumors, or analysis of a combination of hyperplastic lesions, benign tumors, and malignant tumors may have also been performed, at the discretion of the pathologist.

C. METHODS

1. Observations - All animals were observed twice daily for signs of toxicity and mortality. Detailed examination, including palpation for masses, was performed at least once a week.

2. Body weight - All animals were weighed prior to treatment, the first day of treatment, weekly from weeks 1-14, every 4 weeks thereafter until study termination and week 55. Body weights were also recorded at death.

3. Food consumption/efficiency and compound intake - Food consumption (mean food consumed/mouse/week [g]) was determined for each animal weekly for weeks 1-14 and every 4 weeks thereafter. Food efficiency was calculated weekly as mean body weight gain/mean food consumption x 100. Compound intake (mg/kg bw/day) values were calculated from the nominal dose of the test substance, food consumption and body weight gain data.

4. Ophthalmoscopic examination - Ophthalmoscopic examinations were not performed and are not required for carcinogenicity studies based on Guideline 870.4200 & OECD 451.

5. Hematology and clinical chemistry - Blood was collected from the orbital venous sinus at week 53 from all surviving animals and from the tail of all animals sacrificed on schedule or sacrificed *in extremis*. Animals were fasted for at least 4 hours prior to scheduled sacrifice. Differential leukocyte counts and cellular morphology quantization were performed for all unscheduled sacrificed animals, as well as for the controls and the 2000/1800 ppm group after 52 and 78 weeks of treatment. Clinical chemistry was not performed and is not required for carcinogenicity studies based on Guideline 870.4200 & OECD 451. The CHECKED (X) hematological parameters were examined.

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

* Minimum required for carcinogenicity studies (Control and HDT unless effects are observed) based on Guideline 870.4200 & OECD 451

6. Urinalysis - Urinalysis was not performed and is not required for carcinogenicity studies based on Guideline 870.4200 and OECD 451.

7. Sacrifice and Pathology - All animals from each group that died and those sacrificed *in extremis* or on schedule were subjected to macroscopic examinations. The CHECKED (X)

tissues were collected and examined microscopically (except for the Harderian gland, head, and larynx). The (XX) organs from all animals that survived to the scheduled sacrifice were weighed.

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

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

II. RESULTS

A. OBSERVATIONS

1. **Clinical signs of toxicity** - An increased incidence of vocalization was observed at ≥1250 ppm in both sexes (22-46 treated animals/dose group/sex vs 0 controls, n=50). This behaviour was considered to be treatment related, and a reaction to an adverse stimulus (test material).

2. **Mortality** - Survival exceeded guideline requirements of 50% at week 65 and 25% at week 78 in both sexes. At week 78, survival was 62-84% in the males, and there was no statistically significant effect at any dose nor a dose-dependent effect. Among females, at week 78, survival was significantly ($p \leq 0.05$) decreased at 350 (↓23%) and 1800 (↓43%) ppm compared to the concurrent controls, and a dose-related trend ($p \leq 0.01$) was demonstrated (Table 2). In addition, survival was 54% in the 1800 ppm females vs 88-92% in the other groups at week 65 (statistical analyses not reported). Decreases in survival in the 1800 ppm females began at week 20 and persisted throughout the study. Consequently, the significant severe effect on mortality at the high dose in females is considered treatment-related. The decreased survival in the 350 ppm females was regarded as incidental.

Table 2. Survival (%) in male and female mice fed TI-345 for up to 78 weeks.[a]

Dose (ppm)	0	100	350	1250	2000/1800 (M/F)[d]
Male					
Week 20	100	100	94	100	96[c]
Week 65	86	94	76	92	76
Week 78	70	84	62	82	70
Female					
Week 20	96	100	100	100	92[c] (↓4)
Week 65	88	92	90	88	54(↓39)
Week 78	80[b]	86	62* (↓23)	78	46**(↓43)

a Data were obtained from MRID 45422721, Table 3 on pages 99-103. The results of statistical analysis of survival data were only reported for Week 78 (MRID 45422721, Text Tables 1 and 2, page 55). Numbers listed parenthetically represent the percent difference from controls (calculated by the reviewers).

b Dose-related trend ($p \leq 0.01$)

c Dose was 2500 ppm at Week 20

d Animals received 700 ppm for Weeks 1-4, 2000 ppm for Weeks 5-10, 2500 ppm for Weeks 11-34, and 2000/1800 ppm (males/females) for Weeks 35 through termination.

* Statistically different ($p \leq 0.05$) from the control group

** Statistically different ($p \leq 0.01$) from the control group

B. <u>BODY WEIGHT AND WEIGHT GAIN</u>: Treatment resulted in decreased body weights and body weight gains in the 2000 ppm males and the ≥1250 ppm in females (Table 3). Body weights decreased ($p \leq 0.05$) in the males at 2000 ppm (↓4-18%) and in the females at 1250 (↓5-13%) and 1800 ppm (↓9-17%). Decreased ($p \leq 0.05$) body weights were also observed in the 100 and 1250 ppm males; however, these decreases were minor, sporadic and were considered unrelated to treatment. Overall (Weeks 1-78) body weight gains decreased ($p \leq 0.01$) in the 2000 ppm males (↓24%), in the 1800 ppm females (↓35%) and in the 1250 ppm females (↓26%). The greatest reduction in body weight gain occurred at the high dose during Weeks 11-33 (↓73-85%), when the animals were being treated at 2500 ppm.

Table 3. Selected body weights and cumulative body weight gains (g, mean ± SD) in mice fed TI-435 for up to 78 weeks.[a]

Study week(s)	Dose (ppm)				
	0	100	350	1250	2000/1800 (M/F)[b]
Males					
1	28.0±2.29	27.5±2.14	27.9±2.35	27.9±2.34	27.3±2.29
6	35.4±2.88	34.0±2.55* (↓4)	34.7±2.78	34.2±2.63	33.9±2.64* (↓4)
7	36.7±3.01	34.9±2.65** (↓5)	35.5±2.88	35.1±2.73* (↓4)	34.9±2.69** (↓5)
22	41.6±4.62	39.7±3.72	40.4±4.03	39.0±3.35** (↓6)	34.2±3.80** (↓18)
78	44.3±4.40	42.4±5.46	42.5±4.15	42.5±5.16	39.2±4.47** (↓12)
1-4	6.5±1.42	5.8±1.50	5.7±1.57	5.4±1.55** (↓17)	7.2±1.89
5-10	3.6±1.47	2.9±1.51	3.8±1.48	3.3±1.11	0.8±1.40** (↓78)
11-33	4.8±2.92	5.1±2.56	4.6±2.75	4.6±2.33	0.7±2.14** (↓85)
37-77	0.9±2.10	1.6±2.82	0.9±3.56	0.9±2.92	3.4±2.47** (↑278)
1-78	16.1±3.45	15.0±4.67	14.5±4.26	14.6±4.41	12.3±3.39** (↓24)
Females					
1	22.1±1.50	21.8±1.45	22.0±1.56	21.9±1.82	21.8±1.91
12	31.1±3.25	31.1±3.89	30.3±2.77	30.0±2.71	28.4±2.73** (↓9)
13	31.9±3.30	31.7±3.64	31.2±2.79	30.2±2.98* (↓5)	28.6±2.87** (↓10)
34	37.2±5.50	36.8±6.29	35.9±4.82	34.5±4.40* (↓7)	31.0±2.71** (↓17)
78	41.5±6.91	40.8±7.09	38.5±5.04	36.2±4.45** (↓13)	34.9±3.54** (↓16)
1-4	5.5±1.53	5.9±1.64	5.1±1.90	5.5±1.86	5.8±1.94
5-10	3.0±2.14	2.9±1.88	3.0±1.54	2.2±1.37* (↓27)	1.6±1.63** (↓47)
11-33	6.6±3.46	6.2±3.63	5.8±3.08	4.9±2.45* (↓26)	1.8±1.71** (↓73)
37-77	4.0±4.39	3.7±3.46	2.8±4.18	2.0±2.94	4.2±3.12
1-78	19.3±6.40	19.0±6.36	16.7±4.62	14.2±4.37** (↓26)	12.5±4.01** (↓35)

a Data were obtained from MRID 45422721, Tables 5-6 on pages 117-126. Numbers listed parenthetically represent the percent difference from controls (calculated by the reviewers).

b Animals received 700 ppm for Weeks 1-4, 2000 ppm for Weeks 5-10, 2500 ppm for Weeks 11-34, and 2000/1800 ppm (males/females) for Weeks 35 through termination.

* Statistically different ($p \leq 0.05$) from the control group

** Statistically different ($p \leq 0.01$) from the control group

C. FOOD CONSUMPTION, COMPOUND INTAKE AND FOOD EFFICIENCY

1. Food consumption - Food consumption was frequently decreased ($p \leq 0.05$) in the 2000 ppm males (↓8-21%) and 1800 ppm females (↓11-24%), beginning at Week 5 (when the dose was increased to 2000 ppm; Table 4). Incidental changes in food consumption were observed in other dose groups including the first week of the high dose group (700 ppm during Week 1), and these changes were considered unrelated to treatment.

Table 4. Mean food consumption (g) at selected intervals in mice fed TI-435 for up to 78 weeks.[a]

Study week	Dose (ppm)				
	0	100	350	1250	2000/1800 (M/F)[b]
Males					
1	36.0±3.61	36.3±3.30	36.5±3.76	35.4±3.99	38.0±3.62
5	37.4±5.43	36.2±4.46	36.7±4.24	36.6±5.23	32.8±5.60** (↓12)
13	36.6±3.76	34.6±3.92	35.3±3.62	35.7±3.98	28.9±5.13** (↓21)
45	36.0±4.31	33.3±4.22* (↓8)	34.1±3.93	34.5±3.05	33.3±5.28* (↓8)
77	35.9±4.16	34.8±5.01	34.4±6.11	34.7±2.95	33.3±5.29
Females					
1	33.7±3.93	34.6±3.39	36.0±4.08* (↑7)	32.0±3.97	36.2±4.07* (↑7)
5	42.0±7.27	38.5±4.20	40.2±5.15	38.8±4.97	35.1±5.51** (↓16)
13	38.6±5.39	38.3±5.41	38.9±4.08	37.0±3.78	29.3±4.89** (↓24)
77	38.6±6.31	39.5±4.76	41.1±4.94	37.4±5.55	34.3±5.12* (↓11)

a These data were obtained from MRID 45422721, Table 7 on pages 127-130. Numbers listed parenthetically represent the percent difference from controls (calculated by the reviewers).

b Animals received 700 ppm for Weeks 1-4, 2000 ppm for Weeks 5-10, 2500 ppm for Weeks 11-34, and 2000/1800 ppm (males/females) for Weeks 35 through termination.

* Statistically different ($p \leq 0.05$) from the control group

** Statistically different ($p \leq 0.01$) from the control group

2. **Compound consumption** - Compound intake values (mg/kg/day) are presented in Table 1 of this DER.

3. **Food efficiency** - Decreases ($p \leq 0.05$) in food efficiency in the 2000/1800 ppm group lead to cumulative decreases of 33-82% at weeks 5-10 (2000 ppm) and 117-151% at weeks 11-33 (2500 ppm; Table 5). No differences ($p > 0.05$) were detected between treated animals and controls at weeks 37-77. Changes in food efficiency were also observed in other groups, but these changes were sporadic and considered unrelated to treatment.

Table 5. Cumulative food efficiency (%) in mice fed TI-435 for up to 78 weeks.[a]

Study weeks	Dose (ppm)				
	0	100	350	1250	2000/1800 (M/F)[b]
Males					
1-4	4.48	4.12	4.08	3.68 (↓18)	4.68
5-10	1.64	1.40	1.80	1.58	0.29 (↓82)
11-33	0.77	0.74	0.72	0.73	-0.39 (↓151)
37-77	-0.10	0.02	-0.01	0.03	0.20
Females					
1-4	3.97	4.37	3.76	4.40	4.85
5-10	1.37	0.96	1.56	0.90 (↓34)	0.92 (↓33)
11-33	0.66	0.97	0.80	0.64	-0.11 (↓117)
37-77	0.16	0.28	0.14	0.17	0.44

a These data were obtained from MRID 45422721, Table 8 on page 135. Numbers listed parenthetically represent the percent difference from controls (calculated by the reviewers). Statistical analyses of cumulative food efficiency data was not performed.

b Animals received 700 ppm for Weeks 1-4, 2000 ppm for Weeks 5-10, 2500 ppm for Weeks 11-34, and 2000/1800 ppm (males/females) for Weeks 35 through termination.

D. BLOOD ANALYSES: No treatment related effect on hematology was observed. Increased ($p \leq 0.05$) segmented neutrophils (↑29%) and decreased ($p \leq 0.05$) lymphocytes (↓21%) were observed in the 1800 ppm females (Table 6). No other dose groups were analyzed, and these slight effects were considered to be not biologically adverse.

Table 6. Clinical Hematology data in mice fed TI-435 for up to 78 weeks.[a]

Dose ppm	NRBC /100 WBC	N-SEG %	N-BAND %	LYMPH %	MONO %	EOSIN %	BASO %
Males							
0	0 ±0.2	41 ±16.8	0 ±0.0	56 ±17.0	2 ±1.6	1 ±1.4	0 ±0.2
2000 [b]	0 ±0.0	40 ±15.4	0 ±0.0	56 ±14.9	3 ±1.9	1 ±1.3	0 ±0.0
Females							
0	0 ±0.2	41 ±18.5	0 ±0.0	56 ±18.6	2 ±1.8	1 ±1.3	0 ±0.0
1800 [b]	0 ±0.0	53* ±16.6 (↑29)	0 ±0.0	44* ±16.6 (↓21)	2 ±1.3	1 ±1.5	0 ±0.0

a Blood sampled week 79. These data were obtained from MRID 45422721, Table 10 & 11 on page 143-144. Numbers listed parenthetically represent the percent difference from controls (calculated by the reviewers).

b Animals recieved 700ppm for weeks 1-4, 2000 ppm for weeks 5-10, 2500 ppm fr weeks 11-34, and 2000/1800 ppm (males/females) for weeks 35 through termination.

E. SACRIFICE AND PATHOLOGY

1. Organ weight - No treatment-related effects on organ weights were observed. Changes in organ weights (absolute, relative to body, and relative to brain) were observed, such as: increased ($p \leq 0.05$) relative (to body) brain weight in the 1800 ppm females (↑10%), decreased ($p \leq 0.01$) and ($p \leq 0.05$) absolute and relative (to brain) kidney weight in males at ≥1250 ppm (↓11-17%) and decreased ($p \leq 0.05$) absolute and relative (to brain) heart weight in the males at ≥350 ppm (↓8-13%). However, an adverse effect was not corroborated by microscopic pathology. Additionally, these changes were generally minor and were likely due to decreased body weight.

2. Gross pathology - No treatment-related macroscopic abnormalities were observed. Increased incidences of irregular shaped kidneys in the 1800 ppm females (5/23 treated vs 0/40 control) and large preputial glands in the 2000 ppm males (4/34 vs 0/34) were observed in terminally sacrificed animals; however, an adverse effect was not corroborated by microscopic pathology.

3. Microscopic pathology

a) Non-neoplastic - In the 1800 ppm females, increased ($p \leq 0.01$) incidences of pulmonary congestion (26% treated vs 0% controls; 0%, historical controls), adrenal cortex congestion (22% treated vs 2% controls; 0-2%, historical controls), and cervix fibromuscular hyperplasia (58% treated vs 28% controls; 0-28.0%, historical controls) were observed. Increased ($p \leq 0.05$) hepatocellular hypertrophy was observed in the 2000 ppm males (80% vs 34%) and females (30% treated vs 10% controls); however, this effect was considered an adaptive response in the absence of evidence demonstrating an adverse effect. Other changes ($p \leq 0.05$) were observed, but these changes were not dose-dependent and/or not adverse.

b) Neoplastic - No treatment-related effect on the incidence of neoplasms was observed.

Table 7. Total neoplastic lesions benign and malignant in mice fed TI-435 for up to 78 weeks. [a]

Dose ppm	0	100	350	1250	2000/1800 (M/F)[b]
Male					
Total benign neoplasms	11	13	10	7	8
Animals with 1≤	8	12	10	6	7
% with 1≤	16	24	20	12	14
Total malignant neoplasms	4	9	9	3	3
Animals with 1≤	4	7	9	3	3
% with 1≤	8	14	18	6	6
Females					
Total benign neoplasms	15	15	13	11	6
Animals with 1≤	14	13	12	9	6
% with 1≤	28	26	24	18	12
Total malignant neoplasms	13	14	20	8	4
Animals with 1≤	12	12	19	8	4
% with 1≤	24	24	38	16	8

a Data was obtained from MRID 45422721, Tables 30 & 33 on pages 429 &432. n = 50
b Animals recieved 700 ppm for weeks 1-4, 2000 ppm for weeks 5-10, 2500 ppm for weeks 11-34, and 2000/1800 ppm (males/females) for week 35 through termination.

III. DISCUSSION AND CONCLUSIONS

A. <u>INVESTIGATORS' CONCLUSIONS</u>: At ≥1250 ppm, adverse reductions in mean food consumption (high dose only), mean body weights, and body weight gain were observed. No other treatment-related effects were observed. There was no tumorigenic effect in this study. The NOAEL was 350 ppm.

B. <u>REVIEWER'S COMMENTS</u>: No treatment related effect on hematology, organ weights, or macroscopic abnormalities were observed. There was no adverse effect at 100 or 350 ppm.

At week 78, survival was significantly ($p \leq 0.01$) decreased at 1800 ppm (↓43%) compared to the concurrent controls, and a dose-related trend ($p \leq 0.01$) was demonstrated. In addition, survival was 54% in the 1800 ppm females vs 88-92% in the other dose groups at week 65 (statistical analyses not reported). Decreases in survival in the 1800 ppm females began at week 20 and persisted throughout the study. Consequently, the significant severe effect on mortality at the high dose in females is considered treatment-related.

An increased incidence of vocalization was observed at ≥1250 ppm in both sexes (44-92% treated animals/dose group/sex vs 0% controls). This behavior was only evident in animals

treated with an adverse dose of test material. As such, the behavior is considered to be a display of discomfort or distress and is considered to be an adverse, treatment related finding.

Treatment resulted in decreased body weights and body weight gains in the 2000 ppm males and at ≥1250 ppm in females. Body weights decreased ($p \leq 0.05$) in the males at 2000 ppm (↓4-18%) and in the females at 1250 (↓5-13%) and 1800 ppm (↓9-17%). Overall (Weeks 1-78) body weight gains decreased ($p \leq 0.01$) in the 2000 ppm males (↓24%), 1800 ppm females (↓35%), and in the 1250 ppm females (↓26%). The greatest reduction in body weight gain occurred at the high dose during Weeks 11-33 (↓73-85%), when the animals were being treated at 2500 ppm.

Food consumption was frequently decreased ($p \leq 0.05$) in the 2000 ppm males (↓8-21%) and 1800 ppm females (↓11-24%), beginning at week 5 (when the dose was increased to 2000 ppm). Decreases ($p \leq 0.05$) in food efficiency in the 2000/1800 ppm group lead to cumulative decreases of 33-82% at weeks 5-10 (2000 ppm) and 117-151% at weeks 11-33 (2500 ppm). No differences ($p > 0.05$) were detected between treated animals and controls at Weeks 37-77.

In the 1800 ppm females, increased ($p \leq 0.01$) incidences of pulmonary congestion (26% treated vs 0% controls; 0%, historical controls), adrenal cortex congestion (22% treated vs 2% controls; 0-2%, historical controls), and cervix fibromuscular hyperplasia (58% treated vs 28% controls; 0-28.0%, historical controls) were observed; however, these effects were considered equivocal without further evidence of an adverse effect in these organs.
The LOAEL is 1250 ppm (equivalent to 171.4/215.9 mg/kg/day in males/females) based on decreased body weights and body weight gains in females. The NOAEL is 350 ppm (equivalent to 47.2/65.1 mg/kg/day in males/females).

There was no treatment-related increase in tumor incidences in the treated animals when compared to controls. Dosing was considered adequate based on decreased: body weights, body weight gains, food consumption, and food efficiency in both sexes and decreased survival in females.

Under the conditions of this study, the carcinogenic potential of TI-435 is considered negative.

C. <u>STUDY DEFICIENCIES</u>: The larynx, pharynx, and nose were not examined microscopically. The uterus was not weighed. These deficiencies were minor and would not change the conclusions of this review.

DATA FOR ENTRY INTO ISIS

Carcinogenicity study - mice (870.4200b)

PC code	MRID #	Study type	Species	Duration	Route	Dosing method	Dose range mg/kg/day	Doses tested mg/kg/day	NOAEL mg/kg/day	LOAEL mg/kg/day	Target organ(s)	Comments
44309	45422721; 45422722; 45422709	Carcinogenicity	Mice	78 Weeks	Oral	Diet	13.5-322.3	0/0, 13.5/17.0, 47.2/65.1, 171.4/215.9, and 254.1/322.3 in males/females	65.1	215.9	BW; BWG in females	

HED Records Center Series 361 Science Reviews - File R107689 - Page 416 of 721

APPENDIX

4-Week Dietary Range-Finding Study in Mice

This range-finding study was performed to determine adequate dose levels for subsequent studies. A summary is provided in order to confirm that this purpose was achieved.

In this oral toxicity study (MRID 45422709), TI-435 (Clothianidin; 97.5% a.i.; Lot# 30033623) was administered in the diet to 6 Crl:CD-1(ICR)BR mice/sex/group for up to 4 weeks at doses of 0, 500, 1000, 2000, or 4000 ppm (equivalent to 0, 90/122, 190/248, 383/491, and 683/619 mg/kg/day in males/females, respectively).

Decreased ($p<=0.01$) overall (Weeks 1-4) food consumption (decr. 16-53%) was observed in all treated animals throughout the study. Relative (to body) lung weights were increased ($p<=0.05$) in all surviving treated females (incr. 38-42%).

At 1000 ppm, food conversion ratios (food consumption [g] / body weight gain [g]) increased (statistical analyses not performed) by 21% in males and 177% in females. There was insufficient body weight gain at higher doses to provide a meaningful food conversion ratio. Neutrophils were increased ($p<=0.05$) in 1000 ppm males by 81%. In females, potassium increased ($p<=0.05$) by 20%.

At 2000 ppm, other evidence of toxicity was observed in addition to the abnormalities observed at 1000 ppm. Mortality was increased in males (2/6 died following the stress of the blood sampling on Week 4). A thin appearance and hunched posture was observed in some mice of each sex from Week 3. Body weights were reduced in both sexes throughout the study. Overall (Weeks 0-4) body weight gains were decreased ($p<=0.01$) in males (-4.1 g treated vs 5.8 g controls) and females (-4.0 g treated vs 2.9 g controls). Aspartate aminotransferase levels were increased ($p<=0.05$) by 61% in males. In females, eosinophils and alanine aminotransferase levels increased (incr.100-180%; $p<=0.05$) and glucose and triglycerides decreased (decr.31-52%; $p<=0.05$).

At 2000 ppm, absolute, relative (to body) and/or relative (to brain) organ weights were decreased ($p<=0.05$) compared to controls as follows: brain, liver, spleen, and kidney in both sexes; lung and heart in males; and pituitary, thymus, and ovaries in females. Absolute, relative (to body) and/or relative (to brain) organ weights were increased ($p<=0.05$) relative to controls as follows: brain and adrenal in both sexes; pituitary in males; and thyroid and lungs in females. Gross examinations revealed, minimal adipose tissue in 2 males and 5 females in treated animals and 0 in controls (n=6). Atrophy of the red pulp of the spleen was observed in 2/6 treated females vs 0 controls.

At 4000 ppm, numerous abnormalities were observed in addition to the abnormalities observed at 2000 ppm. Increased mortality (4/6 males and 6/6 females at Week 2) was observed as well as many clinical signs indicative of poor health (such as lethargy, body tremors, and unsteady gait). Packed cell volume, hemoglobin, erythrocytes, mean corpuscular volume, and lymphocytes decreased (statistical analyses not appropriate) in males by 11-73% . Decreased ($p<=0.05$) absolute, relative (to body), and/or relative (to brain) organ weights were observed in males, including the: thymus, spleen, testes, thyroids, and lungs. Gross examination revealed, small

thyroids, thymuses, spleens, seminal vesicles, prostate, testes, and epididymides, dark stomach contents, and depression(s) in stomach corpus mucosa in males. Gross examination of females revealed, congested lungs and adrenals, distended gall bladders, and small, pale spleens. Microscopically, various abnormalities were observed in the spleen of both sexes; reduced colloid in the prostate and seminal vesicles, testicular atrophy; and lung and adrenal congestion in females.

Decreases in food consumption was observed at 500 ppm, which did not result in decreased body weight gain. The increased relative (to body) lung weights were only observed in the females. Histological corroboration of lung abnormality (congestion; only in the female) was not observed until 4000 ppm. In the concurrently reviewed oncogenicity study (MRID 45422721), a treatment-related effect was seen in food consumption and efficiency at >=1800 ppm. An equivocally treatment-related pulmonary congestion was observed in the 1800 ppm females.

The LOAEL for this study is 1000 ppm (equivalent to 190/248 mg/kg/day) based on decreased food consumption in both sexes, increased relative (to body) lung weights in females, and decreased food efficiency. The NOAEL for this study is 500 ppm (equivalent to 90/122 mg/kg/day).

The submitted study is classified as **acceptable/guideline** and satisfies the purpose for which is was intended. This study was adequate to suggest a dose range for subsequent studies.

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

DATA EVALUATION RECORD

TI 435 (CLOTHIANIDIN)

Study Type (§83-6a): Developmental Neurotoxicity Study in the Rat

Work Assignment No. 4-02-191A (MRID 45422804)

Prepared for

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Office of Pesticide Programs
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Disclaimer

This Data Evaluation Record may have been altered by the Health Effects Division subsequent to signing by Dynamac Corporation personnel.

405

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Toxicology Branch, Health Effects Division (7509C) **Date** 11-21-04
EPA Work Assignment Manager: Ghazi Dannan, Ph.D. **Signature:** [signature]
Registration Action Branch 3, Health Effects Division (7509C) **Date** 1/6/05

TXR#: 0050321

DATA EVALUATION RECORD

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

PC CODE: 044309 **DP BARCODE:** D282308
SUBMISSION NO.: S613887

TEST MATERIAL (PURITY): TI 435 (Clothianidin, 95.5-95.9% a.i.)

SYNONYMS: (E)-1-(2-chloro-5-thiazolylmethyl)-3-methyl-2-nitroguanidine

CITATION: Hoberman, A.M. (2000) Developmental neurotoxicity study of TI 435 administered orally via the diet to CRL:CD® presumed pregnant rats. Argus Research Laboratories, Inc., Horsham, PA. Laboratory Project ID: 1120-003, October 20, 2000. MRID 45422804. Unpublished.

SPONSOR: Takeda Chemical Industries Ltd. Agro Company, 13-10, Nihonbashi 2-chome, Chuo-Ku, Toyko 103, Japan

EXECUTIVE SUMMARY: In a developmental neurotoxicity study (MRID 45422804) TI 435 (clothianidin, 95.5-95.9% a.i.; Batch # LOT30037120-97) was administered in the diet to pregnant Crl:CD®(SD)IGS BR VAF/Plus® rats (25/dose) from gestation day (GD) 0 to lactation day (LD) 22 at doses of 0, 150, 500, or 1750 ppm (0, 12.9, 42.9, and 142 mg/kg/day during gestation; 0, 27.3, 90.0, and 299.0 mg/kg/day during lactation). The day that litter delivery was completed was designated postnatal day (PND) 1 (or LD 1). Body weight, and food consumption data were recorded for dams. Although the dams were not subjected to a formal functional observational battery (FOB), detailed clinical observations included most of the FOB parameters. Dams were killed and necropsied on LD 22. On PND 5, litters were standardized to yield 5 males and 5 females (as closely as possible), and 10 randomly selected pups/sex/group were subjected to detailed clinical examination outside the home cage. On PND 12, pups were randomly assigned to each of the following four subsets: 1) fixed brain weights and/or neuropathological evaluation on PND 12 (10/sex/group); 2) passive avoidance testing (on PND 24-25 and 31-32) and water maze testing (on PND 59-61 and 66-68) (20/sex/group); 3) motor activity testing (on PND 14, 18, 22, and 60) and auditory startle habituation (on PND 23 and 61) (20/sex/group); 4) detailed clinical exam outside the home cage on PND 12 and weekly during PND 22-79 (20/sex/group), fixed brain weights and neuropathological evaluation on PND 80-83

(10/sex/group). In addition, the pups from subsets 2-4 were observed for the age of attainment of balanopreputial separation or vaginal patency (60/sex/group).

At 1750 ppm, maternal body weights were consistently decreased ($p \leq 0.05$ or NS) throughout gestation and lactation (↓2-8%). Body weight gains were decreased ($p \leq 0.05$) during GDs 0-3 (↓63%) and LDs 4-7 (↓67%). These decreases corresponded with the significant reductions noted in absolute (↓7-23%) and relative (↓6-22%) food consumption during the gestation and lactation periods. These findings were considered to be minimal in nature. There were no treatment-related effects on maternal survival, clinical or functional observations, or reproductive function at 1750 ppm. No treatment-related findings were observed in the 500 or 150 ppm groups.

The maternal LOAEL is 1750 ppm (142 mg/kg/day) based on decreased body weights, body weight gains, and food consumption. The maternal NOAEL is 500 ppm (42.9 mg/kg/day).

No treatment-related differences in live litter size, postnatal survival (through PND 22), sex ratios, clinical signs, food consumption, sexual maturation, or physical landmarks were observed in any treated group. Learning, memory, brain weights, gross pathology, neuropathology, and morphometric measurements were unaffected by the test substance. It was stated that no treatment-related FOB findings were observed; however, no FOB data were provided.

Significantly decreased body weight and body weight gains were observed from approximately PND 14-22 in female pups at 500 ppm and in both sexes at 1750 ppm. Overall pre-weaning (PND 5-22) body weight gains were decreased ($p \leq 0.05$) by 7% in the 500 ppm females and by 18% at 1750 ppm in both sexes, as compared to controls. Immediately after weaning, decreased ($p \leq 0.05$) body weights were noted at 1750 ppm in both sexes (↓4-15%), and two male and three female weanlings died (PNDs 25-27). However, within 3 weeks post-weaning (i.e., following the cessation of treatment) the treatment-related body weight deficits in 1750 ppm pups had essentially been reversed.

There were no effects of treatment on other developmental landmarks examined, including pinna unfolding, eye opening, pupil constriction, acoustic startle response, surface righting reflex, balanopreputial separation or vaginal patency. No treatment-related differences in learning or memory were noted in any treated group relative to concurrent controls in the passive avoidance or water maze tests.

On PND 22, a treatment-related decrease (↓24%; not statistically significant) in mean total motor activity (number of movements) and mean time spent in movement was observed in the 1750 ppm males. There did not appear to be any treatment-related differences in habituation between control and treated rats. There were no apparent effects of treatment in motor activity data measured on PND 14, 18, or 62.

In the 1750 ppm females on PND 23, the average magnitude of the acoustic startle response over 5 blocks and the mean response for each individual block were substantially decreased ($p \leq 0.01$)

by 45-50% (average of 48%) as compared to controls. This finding was considered to be treatment-related.

There were no treatment-related differences in brain weights or brain-to-body weight ratios between control and treated groups for offspring at PND 12 or at termination (PND 83-87). No treatment-related macroscopic findings were noted at necropsy and no treatment-related microscopic findings were noted at subjective histopathological evaluation of nervous system tissues. Morphometric evaluations of PND 12 pup brains revealed an increased thickness of the hippocampal gyrus (4% in males and 9% in females), an increased thickness of the cerebellum height (4% in males and 10% in females), and a decreased thickness of the external germinal layer of the cerebellum (11%) at PND 12. In adult offspring at termination (PND 83-87), the thickness of the hippocampal gyrus was decreased for both males and females (5%) and the measurement of the caudate putamen was decreased 6% in females.

The offspring systemic LOAEL is 500 ppm (42.9 mg/kg/day), based on decreased body weights and body weight gains of female pups during PND 14-21. The offspring systemic NOAEL is 150 ppm (12.9 mg/kg/day).

The offspring neurotoxicity LOAEL is 1750 ppm (142 mg/kg/day), based on decreased motor activity (number and duration of movements) in PND 22 male pups, decreased magnitude of the auditory startle response in PND 23 females, increases in the thickness of the hippocampal gyrus and cerebellum height and decreases in the external germinal layer in the brains of PND 12 pups; and decreases in the thickness of the caudate putamen and hippocampal gyrus in adult offspring at termination (PND 83-87). The offspring neurotoxicity NOAEL is 500 ppm (42.9 mg/kg/day).

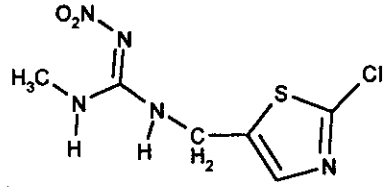
This study is classified as **acceptable/non-guideline** and does not satisfy the guideline requirement (OPPTS 870.6300; OECD 426) for a developmental neurotoxicity study in rats. Classification may be upgradable to guideline upon submission of procedural information for functional observation assessments.

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

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Material: TI 435 technical (Clothianidin)

Description:	Pale yellow powder
Batch #:	LOT30037120-97
Purity:	95.5-95.9 % a.i.
Compound Stability:	It was stated that the test substance was stable in the diet for at least 7 days at room temperature or refrigerated for 28 days.
CAS # of TGAI:	210880-92-5
Structure:	

2. Vehicle: Diet

3. Test animals (P)

Species:	Rat	
Strain:	Crl:CD®(SD)IGS BR VAF/Plus®	
Age at study initiation:	Approximately 10 weeks	
Wt. at study initiation:	207-254 g (females)	
Source:	Charles River Laboratories, Inc., Portage, MI	
Housing:	Following successful mating, females were housed individually in nesting boxes with Bed-o'cobs® bedding. The females and offspring were housed together in these cages through lactation day (LD) 21. After weaning, offspring selected for evaluation of developmental landmarks, neurobehavioral testing, and neuropathological examination were housed individually in wire bottomed cages until their scheduled termination.	
Diet:	Purina Mills Rodent LabChow® 5001-4 in "etts" form (PMI Nutrition International Inc., St. Louis, MO), *ad libitum*	
Water:	Reverse osmosis, chlorine-treated tap water, *ad libitum*	
Environmental conditions:	**Temperature:**	18-26 °C (nominal)
	Humidity:	30-70% (nominal)
	Air changes:	Approximately 10/hour
	Photoperiod:	Not reported
Acclimation period:	6 days	

B. PROCEDURES AND STUDY DESIGN

1. In life dates - Start: 4/20/99 End: 8/06/99

2. Study schedule: P females were administered the test substance continuously in the diet from gestation day (GD) 0 until LD 22. Females were allowed to deliver naturally; the day of completion of delivery was designated lactation day (postnatal day 1). All P females (including those that did not deliver) were killed on LD 22. On postnatal day (PND) 5, ten pups/litter were randomly selected in order to reduce variability among the litters; the remaining offspring were weighed and euthanized. On PND 12, twenty litters/dose were randomly selected for continued

examination, and the litters were standardized to 8 pups/litter. Subsequently, ten pups/sex/group were selected for neurobehavioral testing (Subsets 2 and 3) and neuropathological examination (Subsets 1 and 4). Pups not selected for behavioral evaluations (Subset 5) were sacrificed on PND 22. All pups in Subsets 2, 3, and 4 were evaluated for either vaginal patency or balanopreputial separation.

3. **Mating procedure:** At approximately 10 weeks of age, females were paired with males (1:1) for mating for up to 4 days. Successful mating was determined by the presence of a copulatory plug or sperm in a vaginal smear. The day on which successful mating was determined was designated as GD 0. Following successful mating, females were housed individually in nesting boxes. The females and offspring were housed together in these cages through LD 22.

4. **Animal Assignment:** Mated females were randomly assigned (stratified by body weight) to test groups as shown in Table 1. Offspring were assigned to testing subgroups at the time of litter standardization on PND 5.

Table 1. Study design [a]

Experimental Parameter	Dose (ppm)				Subsets
	0	150	500	1750	
Maternal Animals					
No. of maternal animals assigned	25	25	25	25	NA
Detailed clinical observations (daily from GD 0 through LD 22)	25	25	25	25	NA
Offspring					
Developmental landmarks	All pups/litter	All pups/litter	All pups/litter	All pups/litter	1, 2, 3, 4, 5
Detailed clinical observations (PND 5) (PND 12)	1 pup/sex/litter b 1 pup/sex/litter	1 pup/sex/litter 1 pup/sex/litter	1 pup/sex/litter 1 pup/sex/litter	1 pup/sex/litter 1 pup/sex/litter	1, 2, 3, 4, 5 4
Motor activity (PND 14, 18, 22, 62)	1 pup/sex/litter	1 pup/sex/litter	1 pup/sex/litter	1 pup/sex/litter	3
Auditory startle habituation (PND 23, 63)	1 pup/sex/litter	1 pup/sex/litter	1 pup/sex/litter	1 pup/sex/litter	3
Passive avoidance (PND 23, 24, 30, 31)	1 pup/sex/litter	1 pup/sex/litter	1 pup/sex/litter	1 pup/sex/litter	2
Watermaze (PND 62, 63, 69, 70)	1 pup/sex/litter	1 pup/sex/litter	1 pup/sex/litter	1 pup/sex/litter	2
Brain weight PND 12 PND 83-87	 10/sex 10/sex	 10/sex 10/sex	 10/sex 10/sex	 10/sex 10/sex	 1 4
Neuropathology PND 12 PND 83-87	 10/sex 10/sex	 10/sex 10/sex	 10/sex 10/sex	 10/sex 10/sex	 1 4

a Data obtained from MRID 45422804, pages 42-48.
b 20 pups/sex/group.
NA Not applicable

5. Dose selection rationale: In a previous developmental toxicity study (Argus Research #1120-001), reduced maternal body weight gain and food consumption were observed at 125 mg/kg/day (approximately 1900-2000 ppm). No developmental toxicity was observed at doses up to 125 mg/kg/day. In a two-generation reproduction study (study # not reported), decreased maternal body weight gains and pup body weights and body weight gains were observed at doses as high as 2500 ppm. No reproductive toxicity was observed at doses as high as 500 ppm. Additionally, data from a subchronic neurotoxicity screening battery (doses of 150, 1000, and 3000 ppm) showed reduced body weight and food consumption at 3000 ppm. The preliminary evaluations indicated that the 1000 ppm group was not significantly affected. Based on these studies, the doses shown in Table 1 were selected for the current study.

6. Dosage preparation, administration, and analysis: Test diets were prepared by the Diet Preparation Lab at Bayer Corporation, Toxicology (Stilwell, KS), and weekly aliquots were shipped refrigerated as needed to the testing facility. Weekly aliquots were stored at room temperature during use. P generation females were supplied appropriate dietary admixtures beginning on GD 0 and continuing through GD 24 (rats that did not deliver a litter) or LD 22 (rats that did deliver a litter). F1 animals were not directly supplied with the test diets. Each batch of diet admixture was sampled at Bayer Corporation prior to shipping. It was stated that homogeneity and stability of the test material in the diet were determined at 50 and 5000 ppm in previous studies. Concentration was determined for each dose formulation prepared during the study.

Results

Homogeneity: It was stated that the homogeneity of the test material in the diet was confirmed at 50 and 5000 ppm.

Stability: It was stated that the test material was stable in the diet for at least 7 days at room temperature or refrigerated for 28 days.

Concentration (mean % of nominal): (MRID 45422804, page 738)

100 ppm = 99.3% (n=1; on GDs 0-1, 12 rats in the 150 ppm group were inadvertently offered diets of 100 ppm)
150 ppm = 92.5% (Range: 82.7-93.3%, n=4)
500 ppm = 90.7% (Range: 87.8-94.0%, n=4)
1750 ppm = 96.3% (Range: 82.6-106%, n=4)

On one occasion each for the 150 and 1750 ppm dose groups, the dietary formulations were found to be approximately 17% below nominal concentrations, which is considered somewhat lower than optimal. It appeared that there was no effort made to correct this deficiency (i.e., reformulate the dietary mixes) during the two-week periods that the formulated feeds were fed to the test animals. The impact of this formulation problem on the study results is not expected to be significant, since in each case it occurred during the final two weeks of treatment, when relative feed consumption for pups would tend to be somewhat high. Overall for the duration of test substance administration, the mean concentration of test substance in feed was within 10% of nominal, indicating that the variation between nominal and actual dosage to the study animals was generally acceptable.

C. OBSERVATIONS

1. In-life observations

a. Maternal animals: The P animals were checked for mortality twice daily; clinical signs were recorded daily from GD 0 though LD 22. Maternal behavior of the dams was evaluated on PNDs 1, 5, 8, 14, and 22.

Dams were not subjected to a full functional observational battery (FOB). However, during treatment (GD 0 through LD 22), the dams were observed at approximately the same time each day by an individual who was unaware of each animal's dosage group. The functional

observations described below were recorded; however, the study report did not describe the procedures used for these observations, e.g., whether the same technicians were used throughout testing, where the testing was done (no mention was made as to whether animals were observed outside the home cage), when the testing was done with respect to time of dosing, the environmental conditions, whether a scoring or ranking system was used, or the duration of the observation period.

	FUNCTIONAL OBSERVATIONS
X	Signs of autonomic function, including: 1) Assessment of lacrimation and salivation, and respiration 2) Presence or absence of piloerection, 3) Observations of urination and/or defecation, 4) Degree of palpebral closure and "prominence of the eye"
X	Incidence of abnormal movements.
X	Incidence of abnormal postures.
X	Incidence of abnormal behavior patterns and/or unusual appearance.

Data taken from text, p. 42, MRID 45422804.

Body weights were measured weekly prior to treatment, daily beginning on GD 0, and at necropsy. Food consumption was recorded daily during the treatment period. Body weight gains were calculated for each corresponding gestation or lactation interval, as well as for GDs 0-20 and LDs 1-22.

P females that did not deliver a litter were sacrificed on presumed GD 25, necropsied, and examined for gross lesions and evidence of pregnancy. All other P dams were sacrificed on LD 22 and subjected to a gross pathological examination. Gross lesions were preserved in 10% neutral-buffered formalin for possible future histopathological examination.

b. Offspring

1) Litter observations: Litter size, live litter size, and pup sex and viability at birth were evaluated. For pups that died prior to the initial viability examination, the lungs were removed and immersed in water to determine whether the pup was live- or stillborn. Litters were checked for dead pups at least twice daily, and numbers of pups in each litter and clinical observations were recorded once daily preweaning. Live pups were weighed individually on PND 1, 5, 8, 12, 14, 18, and 22.

On PND 5, litters were standardized using a table of random units to a maximum of 10 pups/litter (5/sex/litter, as nearly as possible), and the excess pups were killed and necropsied. Litters with fewer than 9 pups were retained until PND 12, at which time the offspring were assigned to Subsets 1-5, as previously described

2) Developmental landmarks: Pups were evaluated for the following physical and sexual landmarks. Testing continued until the day that all pups in the group achieved the criterion.

Landmark	Subsets	First Day of Observation
Surface righting reflex (ability to right in 5 seconds)	1-5	PND 1
Pinna unfolding	1-5	PND 2
Eye opening	2-5	PND 12
Acoustic startle response	2-5	PND 13
Pupil constriction	2-5	Once on PND 21
Vaginal patency	2-4 females	PND 28
Preputial separation	2-4 males	PND 39

3) Postweaning observations: After weaning on PND 22, offspring were examined twice daily for mortality and morbidity. Clinical observations and body weights were recorded weekly until sacrifice. Food consumption was recorded weekly beginning on PND 30

4) Neurobehavioral evaluations: The offspring subsets were assigned to the following tests. The same animals were used for passive avoidance and water maze testing, and the same animals were used for motor activity and auditory startle habituation.

i) Functional observational battery (FOB): On PND 5, 10 randomly selected pups/sex/group (1/sex/litter from 10 litters) were examined outside the home cage (no information regarding performance of this examination was provided). On PND 12 and weekly during the postweaning period, the offspring in Subset 4 were examined; examination outside the home cage was specified for PND 12 only. Offspring were not subjected to a full FOB; however, observations were made as described above for the dams. Rats assigned to Subsets 2 and 3 were "examined for gross signs of toxicity" when they were removed from their cages for behavioral testing and/or weighed.

ii) Motor activity testing: Motor activity was evaluated in the pups from Subset 3 on PND 14, 18, 22, and 60; the same pups were evaluated each time. A passive infrared sensor mounted outside a stainless-steel 40.6 x 25.4 x 17.8 cm cage (with Plexiglas® flooring during preweaning) was used to record the number of movements and time spent in movement over the course of a 1-hour session, with tabulation at each 5-minute interval. A rack of up to 32 cages and sensors was monitored during each session. Each rat was tested in the same location on the rack across test sessions, and groups were counterbalanced according to sex and treatment level across testing sessions and cages, where possible. No information was provided as to whether testing was performed at the same time of day across sessions.

iii) Auditory startle reflex habituation: Auditory startle reflex habituation testing was evaluated in the pups from Subset 3 on PND 23 and 63, using a microcomputer to control the test

session. Testing was conducted in a sound-attenuated chamber, using sets of 4 rats per session. Each rat was placed in a small cage above a platform that contained a force transducer in its base. There was an initial adaptation period of 5 minutes, and during the last minute of this period 10 "blank" trials were given to sample the baseline force in the absence of a stimulus. The rats were then given 50 trials of 30 msec, 120 dB bursts of noise at 10-second intervals, followed by an additional 10 "blank" trials. The microcomputer sampled the output of the force transducer and recorded the peak amplitude of each response. The response magnitude was calculated by subtracting the average response on baseline trials, and the average response magnitude and the pattern of responses over 10-trial blocks were compared among treatment groups.

iv) **Learning and memory testing:** Learning and memory testing was performed on animals in Subset 2 (1 pup/sex/litter/dose group).

Passive avoidance test: A passive avoidance test was conducted on PNDs 23 or 24 and again seven days later to assess learning, short-term retention, long-term retention, and hyperactivity; each animal was tested twice, with a one-week interval between test sessions. For each trial, the animal was placed in the "bright" compartment of a two-compartment chamber, the sliding door between compartments was opened, and the light was turned on. When the animal entered the "dark" compartment, the sliding door was closed, the light was turned off, and a 1 second pulse of 1 mA electric current was delivered to the grid floor of the compartment. The animal was then removed from the apparatus and placed in a holding cage for 30 seconds before the start of the next trial. The criterion for learning was that the rat remained in the "bright" compartment for 60 seconds on two consecutive trials, and trials were repeated until the criterion had been met or until 15 trials had been completed. For each trial the latency to enter the dark compartment was recorded.

The following measures were compared among treatment groups: the number of trials to criterion in the first session (for overall learning performance); the latency to enter the "dark" compartment on trial 1 of the first test session (activity levels and exploratory tendencies in a new environment); the latency to enter the "dark" compartment on trial 2 of the first session (short-term retention); the number of trials to criterion in the second test session (long-term retention); and the latency to enter the "dark" compartment on trial 1 of the second session (long-term retention).

Water maze: Water maze testing was conducted on PND 62 and 63 and again seven days later to assess overt coordination, swimming ability, learning, and memory. Testing was conducted using a watertight, 16-gauge stainless-steel modified M-maze filled with 21±1°C water at a depth of approximately nine inches, and each animal was tested twice, with a one-week interval between test sessions. For each trial, the rat was placed in the starting position at the base of the M-maze, farthest from the two arms and required to swim to one of the two goals to be removed from the water. On the initial trial, the rat had to enter both arms of the maze before being removed from the water, and the first arm chosen was designated as the incorrect goal during the remaining trials of both test sessions. For each trial, the animals were given 60 seconds to make a correct goal choice, and animals failing to make a correct choice within that time were guided to the correct goal and then removed from the water. The inter-trial interval was 15 seconds.

The criterion for learning was five consecutive errorless trials, and trials were repeated with a 15-second inter-trial interval until the criterion had been met or until 15 trials had been completed. For each trial, the latency to choose the correct goal and the number of errors, i.e., incorrect turns in the maze, were recorded. No information was provided regarding criteria for scoring errors.

The following measures were compared among treatment groups: the number of trials to criterion in the first session (for overall learning performance); the average number of errors for each trial on the first day of testing (for overall learning performance); the latency to reach the correct goal on trial 2 of the first session (short-term retention); the number of trials to criterion in the second test session (long-term retention); the average number of errors for each trial in the second session (long-term retention); and the latency to reach the correct goal on trial 1 of the second session (long-term retention).

2. Postmortem observations

a. Maternal animals: Maternal animals were sacrificed by carbon dioxide asphyxiation on either GD 25 (rats that did not deliver) or LD 22 (following weaning of their litter) and subjected to gross necropsy of the thoracic, abdominal, and pelvic cavities. The number and distribution of implantation sites was recorded, and tissues were preserved in 10% neutral buffered formalin for possible future histopathological examination. Additionally, dams with no surviving pups were sacrificed after the last pup was found dead or missing and presumed cannibalized. Postpartum data for these dams were excluded from summary tables.

b. Offspring: Pups that died prior to litter examinations for pup viability were evaluated for vital status at birth, as previously described. Gross necropsies were conducted on all pups found dead or sacrificed moribund, as well as the pups that were culled on PND 5, the pups from Subset 5 not used as replacement animals (sacrificed on PND 22), and the animals from Subset 4 that were not selected for neuropathological examination (sacrificed on PND 83-87). All pups were sacrificed by carbon dioxide asphyxiation, except for those in Subset 4, which were sacrificed by overdose of sodium pentobarbital. For necropsies conducted on pups dying or sacrificed on or before PND 5, pups with gross lesions were preserved in Bouin's solution. For necropsies conducted after PND 5, gross lesions were preserved in 10% neutral buffered formalin for possible future evaluation. All gross lesions were subjected to histological examination.

The offspring selected for brain weight or neuropathological evaluation were sacrificed on PNDs 12 (subset 1) or 83-87 (subset 4). These animals were subjected to postmortem examinations as described below.

On postnatal day 12, the approximately twenty pups/sex/group of Subset 1 were sacrificed by carbon dioxide asphyxiation and subjected to gross necropsy. The head of each pup was severed just behind the back of the skull and the calvarium was removed from the top of each skull prior to immersion fixation of the entire head in 10% neutral buffered formalin. The heads were then sent to Consultants in Veterinary Pathology, Inc. (Murrysville, PA) for additional processing and evaluation. Upon arrival, the brains were removed and weighed, and 10 undamaged brains/sex/group were randomly selected for microscopic evaluation. Prior to sectioning, the

following gross measurements were taken (in a blinded manner) using a Vernier caliper: the anterior to posterior (AP) length of the cerebrum, extending from the anterior pole to the posterior pole, exclusive of the olfactory bulbs; and the AP length of the cerebellum, extending from the anterior edge of the cortex to the posterior. The brains were then cut into six coronal slices, by means of the following cuts: 1) half-way between the ventral base of the olfactory bulbs and the optic chiasm; 2) through the optic chiasm; 3) through the infundibulum; 4) through the midbrain just posterior to the mammillary body; 5) through the cerebellum just anterior to its midpoint; and 6) through the anterior portion of the medulla. The tissues were embedded in paraffin, sectioned at 7μ, and stained with hematoxylin and eosin, and histopathological examination was performed on tissues from control and high-dose pups. In addition, the following microscopic measurements were taken (in a blinded manner), using a calibrated, ocular micrometer: 1) thickness of the dorsal portion of the frontal cortex within the coronal section passing through the region of the optic chiasm; 2) thickness of the parietal cortex (the dorsolateral portion of the cerebral cortex within the coronal section taken through the optic chiasm), 3) diagonal width of the caudate putamen and underlying globus pallidus within the coronal section taken at the level of the optic chiasm, 4) thickness of the corpus callosum within the section taken at the level of the optic chiasm, 5) thickness of the dorsal portion of the dentate gyrus of the hippocampus within the section taken at the level of the infundibulum; 6) the maximum height of the cerebellum at the level of the deep cerebellar nuclei, extending from the roof of the fourth ventricle to the dorsal surface, and 7) the thickness of the external germinal layer of the cerebellum (measurements were taken from six areas over the dorsum of the cerebellum, and the mean value was reported). For those areas measured bilaterally, only the mean was provided in the data report.

On postnatal days 83-87, the 10 animals/sex/group from Subset 4 selected for neurohistological evaluation were sacrificed by administration o
f heparin and sodium pentobarbital, perfused *in situ* with 10% neutral buffered formalin, and subjected to gross necropsy. The head of each animal was severed between the back of the skull and the first cervical vertebra, and the calvarium was removed from the top of each skull prior to immersion of the entire head in 10% neutral buffered formalin for additional fixation. The dorsal arches of the vertebrae were removed to expose the spinal cord, and the hind limbs were dissected to expose the peripheral nerves. The spinal column and legs were placed in neutral buffered formalin for additional fixation. All tissues were sent to Consultants in Veterinary Pathology, Inc. (CVP, Murrysville, PA); neural tissues other than the brain were forwarded to Experimental Pathology Laboratories, Inc. (Herndon, VA) for processing and examination.

The brains were processed and evaluated as follows. Upon arrival at CVP, the brains were removed and weighed, and 10 brains/sex/group were selected for microscopic evaluation. Prior to sectioning, the following gross measurements were taken (in a blinded manner) using a Vernier caliper: the anterior to posterior (AP) length of the cerebrum, extending from the anterior pole to the posterior pole, exclusive of the olfactory bulbs; and the AP length of the cerebellum, extending from the anterior edge of the cortex to the posterior pole (uvula) (this measurement was taken on the diagonal). The brains were then cut into eleven coronal slices approximately 2-3 mm in thickness, by means of the following cuts: 1) just posterior to the olfactory bulbs; 2) midway between the optic chiasm and the plane of the first section; 3) just anterior to the optic

chiasm; 4) through the median eminence just anterior to the infundibulum; 5) just anterior to the posterior edge of the mammillary body; 6) immediately in front of the anterior edge of the pons; 7) just anterior to the middle of the cerebellar cortex; 8) through the posterior portion of the cerebellar cortex; and 9) through the anterior portion of the medulla. The brain tissues and gasserian ganglia and associated trigeminal nerve were embedded in paraffin, sectioned, and stained with hematoxylin and eosin, luxol fast blue/cresyl violet, and the Bielschowsky's technique. Histopathological examination was performed on tissues from control and high-dose animals. In addition, the following microscopic measurements were taken (in a blinded manner), using a calibrated, ocular micrometer: 1) thickness of the dorsal portion of the frontal cortex within the coronal section passing through the region of the optic chiasm; 2) thickness of the parietal cortex (the dorsolateral portion of the cerebral cortex within the coronal section taken through the optic chiasm), 3) diagonal width of the caudate putamen and underlying globus pallidus within the coronal section taken at the level of the optic chiasm, 4) thickness of the corpus callosum within the section taken at the level of the optic chiasm, 5) thickness of the dorsal portion of the dentate gyrus of the hippocampus within the section taken at the level of the mamillary body; and 6) the maximum height of the cerebellum at the level of the deep cerebellar nuclei, extending from the roof of the fourth ventricle to the dorsal surface. For those areas measured bilaterally, only the mean was provided in the data report.

Peripheral nervous system tissues were processed as follows at Experimental Pathology Laboratories, Inc., Herndon, VA. The Gasserian ganglia and associated trigeminal nerve tissue were removed from the floor of the cranial vault. After remaining portions of the dorsal arches were trimmed away, the cord was lifted out of the spinal canal with the spinal nerve roots and ganglia attached. The dorsal and ventral nerve roots (including the dorsal root ganglia) were then removed, and sections were taken from the cervical and lumbar swellings, the mid-thoracic cord, and the cervical cord. The sciatic nerve and its branches were removed from one hind leg; sections of the sciatic, tibial, common peroneal (fibular) and sural nerves were taken.

The following central and peripheral nervous tissues (X) were dissected, preserved in paraffin (CNS tissues) or glycol methacrylate (PNS tissues), blocked, sectioned, and stained with hematoxylin and eosin, Bielschowsky's technique, and luxol fast blue/cresyl violet (paraffin tissue blocks, 5 micrometer sections) or hematoxylin and eosin, Bielschowsky's technique, and toluidine blue (glycol methacrylate blocks, 2 micrometer sections). Neurohistological evaluation was performed on tissues from males and females in the control and high dose groups.

The following CHECKED (X) tissues from the control and 1750 ppm groups, as well as the 500 ppm females were examined microscopically:

X	CENTRAL NERVOUS SYSTEM	X	PERIPHERAL NERVOUS SYSTEM
	BRAIN		**PERIPHERAL NERVES**
X	Olfactory bulbs	X	Sciatic (cross- and longitudinal sections)
X	Cerebral cortex	X	Tibial (cross- and longitudinal sections)
X	Hippocampus	X	Common peroneal (longitudinal section)
X	Basal ganglia	X	Sural (longitudinal section)
X	Thalamus		
X	Hypothalamus		
X	Midbrain		
X	Cerebellum		
X	Pons		
X	Medulla oblongata		
	SPINAL CORD		**OTHER**
	(Cross and longitudinal sections)		
X	Cervical	X	Dorsal root ganglia (longitudinal sections)
X	Thoracic	X	Spinal nerve roots (longitudinal sections)
X	Lumbar		
	OTHER		
X	Gasserian ganglion		
X	Trigeminal nerves		

Data taken from Appendix M, pp. 754-756 and 808-811, MRID 45422804.

D. DATA ANALYSIS

1. Statistical analyses: In general, continuous data (body weight, food consumption, latency and errors per trial scores in behavioral tests, and percent mortality per litter) were initially assessed for equality of variance using Bartlett's test. Group means with equal variances were analyzed further using ANOVA, followed by Dunnett's test as necessary. Group means with unequal variances were analyzed using non-parametric procedures; either a Kruskal-Wallis test followed by Dunn's test (if ≤75% of the scores were tied) or Fisher's Exact test (if >75% of the scores were tied).

Motor activity and auditory startle habituation interval data were analyzed using a repeated measures ANOVA for a Dosage effect or a Dosage x Block interaction. If the Dosage effect was significant the data were analyzed using Dunnett's test. If the Dosage x Block interaction was

significant, the data were analyzed further using ANOVA, followed by Dunnett's test as necessary.

Litter size, number of trials to criterion, and developmental landmark data were analyzed using non-parametric procedures; either a Kruskal-Wallis test followed by Dunn's test (if ≤75% of the scores were tied) or Fisher's Exact test (if >75% of the scores were tied).

Clinical observations and other proportion data were analyzed as contingency tables using Variance Test for Homogeneity of the Binomial Distribution. The level of significance was set at $p \leq 0.05$ for all tests.

2. Indices: The following indices were calculated:

a. Reproductive indices: The following reproductive indices were calculated from breeding and parturition records of animals in the study:

Gestation index = (Number of animals with live offspring/number of pregnant animals) x 100

b. Offspring viability indices: The following viability (survival) indices were calculated from lactation records of litters in the study:

Viability index (%) = (# live pups on PND 5 precull/# live pups on PND 1) x 100

LD 5-12 Lactation index (%) = (# live pups on PND 12/# live pups on PND 5 postcull) x 100

LD 12-22 Lactation index (%) = (# live pups on PND 22/# live pups on PND 12) x 100, where the number of live pups on PND 22 excludes those not selected for further evaluation, and the number of live pups on PND 12 excludes those sacrificed for fixed brain weights and/or neurohistological evaluations.

3. Positive and historical control data:

A number of positive control study reports were included in an appendix (pp. 959-1412) to the clothianidin study report (se Appendix A to this DER for a detailed summary), and included functional observational battery, motor activity, auditory startle, passive avoidance, water maze, and subjective and morphometric neuropathology assessments following exposures to known neurotoxic agents. Some of these studies were unacceptable for use to support the current study since they predated the current study by nearly a decade and used procedures and technical staff that are no longer current to the performing laboratory or relevant to the current study. On the other hand, there were some studies in the package that were conducted within the past 5 years, that focused on the assessment of young animals, and were able to demonstrate the ability of the performing laboratories (Argus Laboratory and Consultants in Veterinary Pathology, Inc.) to detect adverse neurobehavioral and/or neuropathological effects of known neurotoxic agents. While the positive control data submission is not optimal, it does provide a certain degree of confidence in the performance of the DNT study with clothianidin.

Historical control data were provided for standard functional observational battery parameters, developmental landmarks, motor activity, passive avoidance, auditory startle habituation, water maze, brain weights, and brain morphometrics (pp. 860-958). Some of these data were derived from different procedures than those used in the clothianidin study (e.g., FOB and motor activity).

II. RESULTS

A. PARENTAL ANIMALS

1. **Mortality, clinical signs, and functional observations:** All dams survived until scheduled termination. No treatment-related findings were observed during the conduct of detailed clinical observations. One high-dose dam exhibited limited use of the right forelimb during GD 1-7; no other abnormal autonomic functions were reported in any group. Incidental findings of localized alopecia, abrasions, or scabs on the extremities were observed in all groups. Soft or liquid feces occurred in one low-dose dam on LD 11-13 and in one high-dose dam on LD 12; however, normal autonomic function was consistently reported for these dams.

2. **Body weight and food consumption:** Body weights and body weight gains for the P females are presented in Tables 3a and 3b; food consumption values are summarized in Table 4. At 1750 ppm, body weights were consistently decreased ($p \leq 0.05$ or not statistically significant [NS]) throughout gestation and lactation (↓2-8%). Body weight gains were decreased ($p \leq 0.05$) during GDs 0-3 (↓63%) and LDs 4-7 (↓67%). These decreases corresponded with the reductions ($p \leq 0.05$) noted in absolute (↓7-23%) and relative (↓6-22%) food consumption during the gestation and lactation periods (Table 4). Body weight gains increased ($p \leq 0.05$) during LDs 14-22 at 500 (↑162%) and 1750 (↑215%) ppm; and overall body weight gains were not significantly different from controls for the gestation (GD 0-20) and lactation (LD 1-22) periods.

Table 3a. Selected mean (± SD) body weights (g) for P females administered TI 435 from GD 0 to LD 22. [a]

Treatment interval (days)	Dose (ppm)			
	0	150	500	1750
Gestation				
0	231.0±11.3	232.0±11.1	231.3±11.6	232.4±10.5
7	259.6±12.7	260.0±11.7	259.5±14.9	250.3±11.7* (↓4)
15	305.0±14.5	306.4±13.9	307.8±20.0	295.4±17.3
17	327.8±16.3	329.5±17.0	332.0±21.7	316.4±19.2* (↓3)
20	367.2±20.8	374.0±21.0	376.3±26.5	356.7±22.8
Lactation				
1	280.3±12.7	280.4±15.6	278.7±20.1	267.1±15.2** (↓5)
4	277.3±21.3	283.8±20.2	280.4±22.4	272.6±13.5
7	294.1±16.0	289.7±16.5	290.7±21.7	279.2±17.9** (↓5)[b]
14	319.2±21.6	318.5±18.1	309.4±25.4	294.4±20.3** (↓8)
19	334.0±16.4	339.6±21.6	334.2±21.7	319.2±24.6* (↓4)
22	330.0±28.9	337.8±16.2	337.7±16.2	328.4±21.3

a Data were obtained from MRID 45422804, Tables B3 and B5, pages 95, 96, 98, and 99. Percent difference from controls is presented parenthetically; n= 23-25.
b Excludes values from dams that had fewer than 9 pups on LD 5; n=22.
* Significantly different from controls at p≤0.05
** Significantly different from controls at p≤0.01

Table 3b. Selected mean (± SD) body weight gains (g) for P females administered TI 435 from GD 0 to LD 22 [a]

Treatment interval (days)	Dose (ppm)			
	0	150	500	1750
Gestation				
0-3	13.9±6.5	11.6±8.7	12.8±7.6	5.1±6.8** (↓63)
3-6	12.3±7.7	14.9±6.9	14.1±5.6	13.0±6.8
18-20	25.4±14.1	28.4±6.8	28.8±5.9	26.1±5.0
Overall (0-20)	136.2±21.7	142.0±20.7	145.1±18.0	124.3±18.0
Lactation				
1-4	3.0±22.0	3.5±12.9	1.7±17.2	5.5±11.6
4-7	16.4±17.5	5.8±8.6* (↓65)	10.3±15.5	5.4±12.2* (↓67)[b]
14-22	10.8±25.5	19.4±13.7	28.3±15.1* (↑162)[c, d]	34.0±17.3** (↑215)[c]
Overall (1-22)	51.6±30.8	57.4±9.0	62.1±10.1	62.4±14.8

a Data were extracted from MRID 45422804, Tables B4 and B6, pages 97 and 100. Percent difference from controls is presented parenthetically; n=23-25.
b Excludes values from dams that had fewer than 9 pups on LD 5; n=22.
c Excludes values from dams that were not selected for continued observation; n=20.
d Excludes values from dam 5550, which had no surviving pups on LD 10; n=20.
* Significantly different from controls at p≤0.05
** Significantly different from controls at p≤0.01

When compared to concurrent controls, absolute (g/animal/day) food consumption (Table 4) was reduced ($p \leq 0.05$) in the 1750 ppm dams during GDs 0-9 (↓11-23%), 15-20 (↓7-8%), and 0-20 (↓10%) and LDs 7-22 (↓10%). Relative (g/kg/day) food consumption was reduced ($p \leq 0.01$) in the 1750 ppm dams during GDs 0-9 (↓8-22%) and 0-20 (↓7%), and LDs 14-22 (↓7%, $p \leq 0.05$) and 1-22 (↓6%, $p \leq 0.05$). In addition, relative food consumption was decreased ($p \leq 0.05$) at 500 ppm during GDs 0-3 (↓7%).

Table 4. Selected mean (± SD) absolute (g/animal/day) and relative (g/kg/day) food consumption for P females administered TI 435 from GD 0 to LD 22[a]

Treatment interval (days)		Dose (ppm)			
		0	150	500	1750
Gestation					
Absolute	0-3	19.6±2.2	19.2±3.0	18.2±2.3	15.1±1.9** (↓23)
	3-6	21.6±2.6	21.8±2.2	21.7±2.2	18.9±2.6** (↓13)
	6-9	24.4±2.2	23.9±2.8	23.7±2.6	21.7±2.6** (↓11)
	15-18	29.4±2.4	29.1±2.4	30.0±2.6	27.4±2.8* (↓7)
	18-20	27.0±3.1	27.4±2.7	27.6±2.8	24.8±2.4* (↓8)
	Overall (0-20)	24.9±1.6	24.6±1.8	24.5±2.0	22.4±1.6** (↓10)
Relative	0-3	82.2±7.6	80.5±11.0	76.6±8.0* (↓7)	64.5±6.8** (↓22)
	3-6	85.9±9.7	87.0±8.4	86.4±6.6	77.3±8.8** (↓10)
	6-9	92.5±5.3	90.8±9.2	89.8±7.6	85.4±8.4** (↓8)
	Overall (0-20)	87.6±3.6	86.1±4.9	85.8±4.3	81.2±3.4** (↓7)
Lactation					
Absolute	1-4	29.3±7.3	29.0±6.1	29.4±5.0	26.9±4.7
	7-14	55.1±6.4	55.2±5.4	52.4±5.3	49.4±6.1** (↓10)[b]
	14-22	70.3±5.2	71.7±6.9	69.5±5.9	63.1±6.4** (↓10)[b]
	Overall (1-22)	55.7±4.7	56.4±4.5	54.8±4.2	50.3±5.0** (↓10)[b]
Relative	1-4	104.7±25.2	102.5±19.3	104.7±16.5	100.3±16.2
	7-14	179.8±15.8	180.2±12.9	175.7±17.7	169.8±16.4
	14-22	215.0±11.5	215.8±20.0	213.3±17.1	200.6±16.5* (↓7)[b]
	Overall (1-22)	180.9±11.1	181.8±12.0	180.0±13.0	170.8±12.9* (↓6)[b]

a Data were extracted from MRID 45422804 Tables B7-B10, pages 101-104. Percent difference from controls is listed parenthetically; n=23-25.

b Excludes values from dams that were not selected for continued observation; n=20.

* Significantly different from controls at $p \leq 0.05$

** Significantly different from controls at $p \leq 0.01$

3. Test substance intake: Mean compound intake (mg/kg bw/day) during the gestation and lactation periods was determined based on maternal food consumption and body weight (Table 5). The investigators stated that it was presumed that pups began to consume maternal feed after

approximately LD 14. The overall average lactation test substance intake values are similar to average intake values for LD 7-14 (i.e., based upon maternal intake alone).

Table 5. Mean (±SD) test substance intake (mg/kg/day) for P females administered TI 435 from GD 0 to LD 22 [a]

Observation	Dose (ppm)			
	0	150	500	1750
GD 0-20	0.0±0.0	12.9±0.7	42.9±2.1	142±6.0
LD 1-22[b]	0.0±0.0[c]	27.3±1.8[c]	90.0±6.5[c, d]	299.0±22.5[c]

a Data were extracted from MRID 45422804 Table B1, pages 92-93; n=23-25.
b It was presumed that the pups began to consume maternal feed after approximately LD 14.
c Excludes values from dams that were not selected for continued observation; n=19-20.
d Excludes values from dam 5550, which had no surviving pups on LD 10; n=20.

4. **Reproductive performance:** Pregnancy rate, number of implantations/dam, gestation length, and sex ratio were comparable between treated and control animals (Table 6). The gestation index was 100% for all groups.

Table 6. Delivery observations in P females administered TI 435 from GD 0 to LD 22 [a]

Observation	Dose (ppm)			
	0	150	500	1750
# Animals Mated	25	25	25	25
# Animals Pregnant Pregnancy Rate (%)	25 (100)	25 (100)	23 (92)	23 (92)
# Nonpregnant	0	0	2	2
Mean (±SD) gestation length (days)	22.6±0.5	22.6±0.5	22.6±0.5	22.9±0.3
Total # Implantations Mean (±SD) Implantations/Delivered Litter	366 14.6±2.0	380 15.2±2.0	355 15.4±1.6	347 15.1±1.8
Total # of Litters Examined	25	25	23	23
Sex Ratio (% Male, ±SD)[b]	44.4±15.9[c]	45.9±13.8	50.1±16.7	50.9±11.1

a Data extracted from MRID 45422804 Tables B11 and B12, pages 105 and 109.
b Includes pups born alive, found dead on PND 1.
c Excludes values for litters 5504 and 5524; the dams delivered one additional pup each on LD 2; n=23.

5. **Maternal postmortem results**

a. **Macroscopic examination:** No treatment-related pathological abnormalities were observed in any treated group.

b. Microscopic examination: Microscopic examinations were not conducted on adult animals.

B. OFFSPRING

1. Viability and clinical signs: No treatment-related differences in live litter size, postnatal survival, or sex ratios were observed in any treated group through PND 22 (Table 7). At 1750 ppm, two male and three female rats were found dead on PNDs 25-27 post-weaning. The study report stated that this finding was considered related to a failure to thrive post-weaning, based on the patterns of body weight gains and losses, and was related to treatment. The lactation index was decreased ($p \leq 0.05$) in the 150 and 500 ppm animals (92.7-94.8% treated vs 99.6% controls); however, this finding was considered unrelated to treatment because it was not dose-dependent and the lactation index at 1750 ppm was comparable to controls. The majority of pup deaths that contributed to the decreased lactation index at 150 and 500 ppm occurred between PND 9-12; at 150 ppm, 5 of the 18 pup deaths were observed in two litters and at 500 ppm, 9 of the 10 pup deaths were observed in one litter. No treatment-related clinical signs were observed in pups.

An apparent increase in the number of pups and litters with stillborn pups was noted at 500 and 1750 ppm (Table 7). Although a dose-response relationship was not observed (the number of stillborn pups and litters with stillborn pups was greater at 500 ppm than at 1750 ppm), this finding is considered noteworthy since an increase of stillborn pups was found in the reproduction study with clothianidin.

Table 7. F_1 live litter size and survival [a]

Observation	Dose (ppm)			
	0	150	500	1750
Number of litters	25	25	23	23
Mean live litter size (pups/litter)				
PND 1	13.5±2.2 [d]	14.1±2.1	14.2±1.7	13.8±2.3
PND 5 [b]	13.2±2.0	13.8±2.2	13.6±1.8	13.3±2.3
PND 5 [c]	10.0±0.2 [g]	9.9±0.3	10.0±0.0	10.0±0.0 [g]
PND 8	10.0±0.3 [g]	9.9±0.3	9.9±0.3	10.0±0.0 [g]
PND 12	8.3±0.8 [g]	7.6±1.7	7.8±1.8	8.2±0.6 [g]
PND 14	8.0±0.2 [h]	7.8±0.5 [h]	8.1±0.3 [h]	8.0±0.2 [h]
PND 18	8.0±0.2 [h]	7.7±0.7 [h]	8.0±0.4 [h]	7.9±0.3 [h]
PND 22	8.0±0.2 [h]	7.7±0.7 [h]	8.0±0.4 [h]	7.8±0.5 [h]
No. pup deaths (litters)				
Stillborn	1	1	6 (5)	3 (3)
PND 1	2 (2)	1	2 (2)	2 (2)
PND 2-5	11 (9)	7 (6)	12 (10)	11 (8)
PND 6-8	1	0	2 (2)	0
PND 9-12	0	18**(4)	10*(2)	0
PND 13-14	0	2 (2)	0	1
PND 15-22	0	2 (2)	1	3 (2)
Total preweaning deaths	15	31	33	20
No. postweaning deaths (PND 25-27)	0	0	0	5
Sex ratio (% male)	44.4±15.9 [d]	45.9±13.8	50.1±16.7	50.9±11.1
Viability index (%)	96.1	97.7	95.7	95.9
Lactation index (%) [e]	99.6	92.7**	94.8*	100.0
Lactation index (%) [f]	100.0	97.4	99.4	97.5

a Data extracted from MRID 45422804, Table B12, pages 106-111, and Table B22, pages 159-166.
b Before standardization (culling).
c After standardization (culling).
d Excludes values for litters 5504 and 5524; the dams delivered one additional pup each on LD 2.
e Number of live pups in Subsets 1-5 on PND 12/ number of live pups on PND 5 post-culling. Excludes values for litters with fewer than 9 pups on LD 5.
f Number of live pups on PND 22 in Subsets 2-5 on PND 12/ number of live pups in Subsets 2-5 on PND 12. Excludes values for litters that were not selected for continued observation and pups in Subset 1.
g Excludes values for litters with fewer than 9 pups on LD 5.
h Excludes values for litters that were not selected for continued observation.
* Significantly different from controls at $p \leq 0.05$
** Significantly different from controls at $p \leq 0.01$

2. Offspring body weight and food consumption: Selected pre- and post-weaning body weights and body weight gains for F_1 pups are presented in Tables 8a and 8b. At 1750 ppm, pre-weaning body weights were decreased ($p \leq 0.05$) in the males on PNDs 12-22 (↓6-16%) and in the females on PNDs 14-22 (↓13-16%). Additionally, pre-weaning body weights were decreased ($p \leq 0.05$) in the 500 ppm females on PNDs 14-22 (↓6-7%). At 1750 ppm, post-weaning body

weights were sporadically decreased ($p \leq 0.05$) in the males on PNDs 23-27 (↓4-15%) and in the females on PNDs 23-30 (↓6-13%). Body weight gains were decreased ($p \leq 0.05$) during pre-weaning between PNDs 12-22 in the males (↓11-44%) and females (↓11-48%). Overall pre-weaning (PND 5-22) body weight gains were decreased by 18% in both sexes compared to controls. In the 500 ppm females, body weight gains were decreased ($p \leq 0.05$) during PNDs 12-18 (↓8-24%) and overall pre-weaning weight gain was decreased ($p \leq 0.05$) by 7% compared to controls. Post-weaning body weight gains in the 1750 ppm females were decreased (↓21%, $p \leq 0.05$) on PNDs 65-72. Post weaning body weights in the 1750 ppm males were similar to controls. Overall (PNDs 5-79) weight gains were similar between treated animals and controls.

Table 8a. Mean (± SD) F_1 pup body weights (g) [a]

Postnatal Day	Dose (ppm)			
	0	150	500	1750
Males				
Pre-weaning				
5	9.1±1.1	9.1±1.5	9.0±1.0	8.9±1.0
12	18.6±3.6	19.0±3.3	18.4±3.1	17.4±3.0* (↓6)
18[b]	31.6±4.4	32.0±5.0	30.3±4.5	26.5±4.4** (↓16)
22[b]	41.6±6.1	42.8±6.5	40.5±5.6	35.3±5.4** (↓15)
Post-weaning				
23[b]	44.0±6.8	45.2±8.0	42.8±6.6	37.4±6.5** (↓15)
30[b]	79.7±10.3	82.3±13.7	75.4±12.7	72.9±12.0** (↓8)
37[b]	137.6±16.9	142.9±19.3	135.6±16.4	130.3±17.2* (↓5)
44[b]	196.4±20.4	203.8±22.7	195.3±19.5	188.9±21.6
72[b]	381.2±30.8	392.7±32.6	379.6±30.6	367.6±28.0* (↓4)
79[b]	409.4±33.8	412.4±29.7	414.3±36.8	401.6±31.2
Females				
Pre-weaning				
5	8.5±1.1	8.8±1.4	8.3±0.9	8.4±1.0
14[b]	22.0±3.8	22.1±3.6	20.5±3.6* (↓7)	19.2±4.2** (↓13)
18[b]	30.8±3.8	30.8±4.2	28.6±4.3** (↓7)	25.9±5.3** (↓16)
22[b]	40.3±5.3	41.4±5.2	38.0±5.5* (↓6)	34.4±6.3** (↓15)
Post-weaning				
23[b]	42.1±5.6	43.9±6.9	40.3±6.4	36.5±7.6** (↓13)
30[b]	73.9±8.1	77.2±10.0	69.5±11.5	69.6±9.0* (↓6)
37[b]	118.6±11.1	123.4±12.3	114.5±14.9	115.1±12.1 (↓3)
44[b]	150.6±16.0	155.0±18.8	147.1±16.6	148.0±13.5
79[b]	246.7±24.0	249.9±23.8	246.9±32.2	248.0±19.0

a Data obtained from MRID 45422804, Tables C3 and C5, pages 268-269 and 272-273. Percent difference from controls is presented parenthetically; n = 56-80 through PND 12

b Excludes values for rats from Subset 1, these rats were sacrificed on PND 12; n=56-60

* Significantly different from controls at $p \leq 0.05$

** Significantly different from controls at $p \leq 0.01$

Table 8b. Selected F_1 pup mean (±SD) body weight gains (g) [a]

Postnatal Day	Dose (ppm) 0	150	500	1750
Males				
Pre-weaning				
5-8	4.0±2.7	4.1±1.3	3.9±1.2	3.6±1.0
12-14 [b]	4.3±1.2	3.9±1.9	3.6±2.2	2.4±1.9** (↓44)
14-18 [b]	8.9±1.3	9.1±1.5	8.3±1.8* (↓7)	6.8±1.5** (↓24)
18-22 [b]	9.9±2.5	10.8±2.3	10.2±1.7	8.8±1.7** (↓11)
5-22 [b]	32.5±5.4	33.6±6.0	31.4±5.1	26.5±5.2** (↓18)
Post-weaning				
23-79 [b]	365.4±31.8	366.5±27.2	371.4±34.0	363.8±28.0
(Overall) 5-79 [b]	400.2±33.1	403.3±29.3	405.2±36.2	392.8±30.4
Females				
Pre-weaning				
5-8	3.6±1.4	4.0±1.4	3.7±1.1	3.4±0.9
12-14 [b]	4.2±1.3	3.8±1.6	3.2±1.9** (↓24)	2.2±1.9** (↓48)
14-18 [b]	8.8±1.3	8.7±1.2	8.1±1.5* (↓8)	6.8±1.8** (↓23)
18-22 [b]	9.5±2.5	10.6±2.0	9.4±1.8	8.5±1.7* (↓11)
5-22 [b]	31.8±4.8	32.6±4.4	29.7±5.0* (↓7)	26.0±5.9** (↓18)
Post-weaning				
65-72 [b]	16.1±6.7	16.1±7.9	15.2±7.1	12.7±6.9* (↓21)
23-79 [b]	204.0±22.9	205.1±22.0	205.6±29.8	211.0±16.6
(Overall) 5-79 [b]	238.0±24.1	241.0±23.0	238.6±32.1	239.6±18.8

a Data obtained from MRID 45422804, Tables C4 and C6, pages 270-271 and 274-275. Percent difference from controls is presented parenthetically; n = 56-80 through PND 12.

b Excludes values for rats from Subset 1, these rats were sacrificed on PND 12; n=56-60

* Significantly different from controls at $p \leq 0.05$

** Significantly different from controls at $p \leq 0.01$

Absolute (g/animal/day) and relative (g/kg/day) food consumption were comparable between treated and control animals. Differences from control ($p \leq 0.05$) noted in the 1750 ppm males on PND 51-58 and 65-72 and in the 150 ppm females on PND 30-37 were considered unrelated to treatment as they were minor, transient, and not dose-dependent (MRID 45422804, Tables C7 and C9, pages 276 and 278; not presented in DER).

3. Developmental landmarks

a. Sexual maturation: No treatment-related differences in balanopreputial separation or vaginal patency were observed between treated and control F_1 animals (Table 9a). A slight delay in vaginal patency (35 days treated vs. 34 days controls, $p \leq 0.01$) was observed in the 500 ppm females; however, it was not dose-dependent and considered unrelated to treatment.

Table 9a. Sexual maturation (mean days ± SD) in F_1 generation rats [a]

Parameter	Dose (ppm)			
	0	150	500	1750
N (M/F)	60/60	56/56	60/60	58/57
Preputial separation (Males)	47.1±3.0	46.8±3.0	47.7±2.7	48.3±3.5
Vaginal patency (Females)	34.0±1.4	33.7±1.2	35.0±1.7**	34.1±1.3

a Data extracted from MRID 45422804, Table C11, page 280.
** Significantly different from controls at $p \leq 0.01$

b. Physical landmarks: No treatment-related effects on pinna unfolding (examined on PND 2-6), acoustic startle response (examined on PND 13-19), eye opening (examined on PND 12-20), or pupil constriction (examined on PND 21) were observed (MRID 45422804, Table B14, pages 114-116; not presented in DER).

A significant delay in development of the surface righting reflex (evaluated on PND 1-11) was observed on PND 3 at 150 (↓34%), 500 (↓34%), and 1750 (↓47%) ppm compared to controls (Table 9b). Initiation of the reflex was similar for control and high dose pups at PND 1. By PND 2, the response was delayed (NS) in an apparent dose-dependant manner at 500 and 1750 ppm, with significance achieved at PND 3 for all treated groups. Delays remained on PND 4 (NS), and at PND 5, the mean percent of pups reaching the criterion was similar between treated and control groups. Historical control data from 7 studies conducted in Sprague Dawley rats at the performing laboratory (MRID 45422804, page 876) demonstrate that on PND 3 an average of 40.2±11.7 percent of the pups met the criterion for surface righting, with a minimum of 23.6% and a maximum of 52.0% on that day postpartum. This historical control value is similar to the PND 3 control value from the current study.

The average day postpartum that surface righting was observed in at least 50% of the pups was similar between control and treated litters, and the control value of 3.8 ± 2.0 (Table 9b) was similar to the historical control value of 3.3 ± 1.1. For the 1750 ppm group, this value was slightly decreased (NS) as compared to control, suggesting achievement at a slightly earlier age, a result which is not consistent with the significant delay in the mean percent of pups reaching the criterion, as described above for PND 3.

The inconsistent findings reported for this parameter can be attributed to the manner in which the endpoint is evaluated, specifically that all pups in a litter are examined on each day of assessment. In other words, pups that have shown the ability to right themselves are not excluded from testing on subsequent days. Some pups do not consistently demonstrate the reflex, which can result in erratic results within a litter. (An example is control litter 5575, which demonstrated 3, 1, 4, 11, 6, and 10 pups (of 10) with the righting reflex on PND 1 through 6, respectively.) The reason for this inconsistent response is not apparent. However, it results in lower mean numbers of treated pups with the righting reflex on PND 3 than were observed to have the same reflex on PND 2. Positive control data, demonstrating the ability of this testing

paradigm to identify treatment-related alterations in the development of the righting reflex, were not provided.

In summary, apparent significant delays in development of the surface righting reflex noted on PND 3 for pups at 175, 500, and 1750 ppm were not considered toxicologically meaningful.

Table 9b. Surface righting reflex (mean % of pups meeting the criterion ±SD) in F_1 generation rats [a]

Postnatal Day	Dose (ppm)				Historical Control
	0	150	500	1750	
2	32.8±19.6	32.9±22.4	29.2±22.1	27.2±21.6	35.1±14.5
3	38.2±14.9	25.4±24.5* (↓34)	25.3±19.6* (↓34)	20.1±19.2** (↓47)	40.2±11.7
4	47.3 ±18.0	36.3±24.7	34.1±15.4	38.4±20.1	51.3±12.3
5	48.0 ±16.2	48.8±23.1	41.1±21.7	52.6±26.0	70.1±11.7
Overall [b]	3.8 ±2.0	3.8±2.3	4.4±2.1	3.5±2.1	3.3±1.1

a Data obtained from MRID 45422804, Table B14, page 113. Historical control data from 7 studies conducted from 1994-1999, page 878. Percent difference from controls is presented parenthetically.
b Average day postpartum that at least 50% of pups/litter had the developmental measure present.
* Significantly different from controls at $p \leq 0.05$
** Significantly different from controls at $p \leq 0.01$

4. Behavioral assessments

a. Functional observational battery: A formal FOB was not conducted on the offspring. Detailed clinical observations revealed one control male with tip-toe walk on week 10 postpartum, with subsequent hyper-reactivity in weeks 11-13 and one control male with tremors on week 7 postpartum. There were no clinical findings consistent with "abnormal autonomic functions" in offspring treated with TI 435.

b. Motor activity: Mean total activity count and mean time spent in movement data are summarized in Tables 10a and 10b, respectively. Mean sub-session (block) data for total activity count on all days of testing, and for time spent in movement on PND 22, are summarized in Tables 11a and 11b.

There were no apparent effects of treatment in motor activity data measured on PND 14, 18, or 62. On PND 22, mean total motor activity (number of movements) was decreased (NS) in the 1750 ppm males (↓24%) and in the females at 500 ppm (↓21%) and 1750 ppm (↓10%) (Table 10b). The mean time spent in movement on PND 22 was also decreased (NS) in the 1750 ppm males (↓27%) and in the females at 500 ppm (↓24%) and 1750 ppm (↓19%) (Table 10a). Subsession data (Tables 11a and 11b) also demonstrate significant decreases ($p \leq 0.05$) in mean motor activity counts during one to four testing blocks for 1750 ppm offspring on PND 22 (↓17-32% in males and ↓27% in females) and nonsignificant decreases (↓19-37% in males) after the

fourth testing block. Subsession data for mean time spent in movement on PND 22 (not presented) show significant decreases ($p \leq 0.05$) in 1750 ppm males (↓30% to 43% during the first 15 minutes of testing) and females (↓44% in the fourth testing block) and in 500 ppm females (↓36% in the fourth testing block). Overall, interpretation of the motor activity data is complicated by the variability in the data, as demonstrated by the large coefficients of variation, particularly for PND 14, 18, and 21 offspring. However, it is noted that the decreases in motor activity counts and the time spent in movement at PND 22 for 1750 ppm male offspring are of substantial magnitude and supported by sub-session data; therefore, they are likely related to treatment. Effects noted for 500 ppm female offspring at PND 22, while nearly of the same magnitude as those observed in PND 22 males at 1750 ppm, demonstrate a lack of dose-response and an absence of significant alterations in the subsession data. Therefore, the apparent decreases in motor activity in PND 22 females at 500 ppm are not considered to be treatment-related. (It is noted that historical control data included in the study report do not contribute to the interpretation of these data since they report total mean activity values which are substantially lower than those measured in the current study.)

In general, habituation was not observed on PND 14 or 18. Some habituation was evident in both male and female offspring on PND 22 and PND 62. There did not appear to be any treatment-related differences in habituation between control and treated rats. On all of the testing days, individual motor activity data demonstrate some animals with little or no habituation.

Table 10a. Mean (±SD) number of movements (counts) during motor activity assessment in F_1 pups in Subset 3 [a]

Postnatal Day	Dose (ppm)			
	0	150	500	1750
Males				
14	147.2±159.5 [108]	162.7±121.0	218.8±164.5	169.0±117.4
18	472.0±240.8 [51]	367.9±245.6	398.6±300.8	447.8±242.8
22 [b]	558.5±171.9 [31]	490.4±212.4	542.5±224.3 (↓3) [41]	422.2±237.4 (↓24) [56]
62	723.0±175.0 [24]	709.6±128.1	758.8±97.1	707.2±140.4 (↓2)
Females				
14	219.6±128.6 [59]	260.5±200.4	199.8±181.6	211.7±154.8
18	475.1±307.8 [65]	470.5±241.8	476.3±317.5	474.6±310.4
22 [b]	579.5±153.6 [27]	563.9±195.1	457.0±229.4 (↓21) [50]	518.7±203.2 (↓10) [39]
62	749.4±136.5 [18]	663.4±122.8	708.2±113.3	651.3±150.0 (↓13)

a Data obtained from MRID 45422804 Table F1, pages 450 through 465; n=18-20. Percent difference from control presented parenthetically. Coefficients of variance are presented in brackets.

b Historical control data for PND 21 rats (pages 892-895 of the study report), indicate total mean values of approximately 370 (241 min-513 max) for males and 349 (181 min-529 max) for females. [The values for twelve 5-minute blocks were calculated by the reviewer from mean subsession data for eighteen 5-minute blocks and may be less accurate than if they had been generated from individual animal data.]

Table 10b. Mean (±SD) time spent in movement (seconds) during motor activity assessment in F_1 pups in Subset 3 [a]

Postnatal Day	Dose (ppm)			
	0	150	500	1750
Males				
14	114.1±171.0	136.6±175.4	190.2±170.9	128.6±132.0
18	578.6±384.1	513.3±502.2	548.2±486.8	548.1±330.2
22	801.8±328.0	710.2±411.9	773.2±364.6 (↓4)	588.7±409.4 (↓27)
62	1523.5±350.4	1586.3±306.3	1759.5±317.4	1637.0±418.7
Females				
14	162.9±162.9	229.9±264.7	151.0±180.2	141.5±144.0
18	595.6±466.6	602.7±420.8	627.4±560.1	570.8±460.2
22	790.2±289.9	800.9±357.7	597.9±354.8 (↓24)	639.6±319.6 (↓19)
62	1545.2±405.1	1326.0±359.6	1497.5±397.7 (↓3)	1454.6±440.7 (↓6)

a Data obtained from MRID 45422804 Table F1, pages 450 through 465; n=18-20. Percent difference from control presented parenthetically.

Table 11a. Mean (±SD) sub-session motor activity (counts) in F_1 male pups in Subset 3 [a]

Block [b]		Dose (ppm)			
		0	150	500	1750
PND 14	1	15.3±18.6	15.7±15.9	13.4±15.0	15.6±18.2
	2	14.0±18.2	13.6±12.9	14.4±15.8	13.8±11.1
	3	17.8±21.8	16.1±17.2	17.0±19.7	13.8±13.2
	4	14.6±18.7	18.9±18.7	25.0±24.2	15.6±16.8
	5	12.4±16.8	18.8±17.5	20.1±22.8	13.1±10.5
	6	9.9±15.1	18.5±22.1	18.8±19.3	16.4±15.0
	7	11.4±14.2	12.6±15.2	14.2±20.7	12.0±14.5
	8	17.8±24.2	13.8±17.5	19.4±20.2	14.6±12.8
	9	9.4±17.2	8.2±12.5	17.8±20.1	13.0±15.1
	10	7.5±15.9	7.3±10.5	20.6±25.0	10.0±15.5
	11	9.0±14.5	6.8±8.5	19.6±24.6	15.4±19.0
	12	8.2±11.2	12.4±18.2	18.4±20.7	15.4±19.0

(Table continues next page)

Block [b]		Dose (ppm)			
		0	150	500	1750
PND 18	1	35.4±26.3	31.0±24.2	23.6±28.1	22.1±19.8
	2	41.8±27.2	36.4±22.6	27.8±28.4	33.2±25.5
	3	50.6±20.6	38.0±22.0	33.8±24.8	39.6±26.9
	4	48.4±29.2	39.7±24.4	34.5±29.1	42.2±28.3
	5	47.5±28.2	36.6±27.0	40.3±30.5	37.2±23.8
	6	47.3±25.5	32.2±29.4	31.8±26.2	42.2±24.2
	7	44.0±30.2	28.3±25.4	37.2±30.7	36.6±26.2
	8	37.2±25.2	25.7±25.6	39.2±33.0	38.0±25.0
	9	37.8±30.0	24.6±27.5	31.4±29.2	42.2±28.4
	10	27.6±27.3	23.6±22.1	33.8±32.5	38.6±27.8
	11	25.0±28.7	27.9±28.6	32.5±31.2	38.0±28.6
	12	29.6±29.2	23.9±25.0	32.6±31.6	37.8±30.0
PND 22	1	64.0±9.9	59.2±10.5	62.7±14.3	53.0±20.1* (↓17)
	2	56.4±14.0	56.3±17.1	55.2±15.7	40.2±21.1* (↓29)
	3	55.4±14.8	51.6±13.1	49.0±25.1	38.2±23.1* (↓31)
	4	54.0±15.9	44.2±26.0	50.5±20.8	36.9±25.9* (↓32)
	5	45.1±22.5	46.7±24.0	43.0±23.3	33.9±24.4 (↓25)
	6	45.2±19.8	45.2±22.3	46.6±24.6	31.8±24.1 (↓30)
	7	43.6±23.0	39.0±29.2	44.4±28.2	35.4±22.9 (↓19)
	8	43.9±24.4	36.3±28.1	41.8±27.3	27.8±27.5 (↓37)
	9	44.2±23.2	32.9±26.4	42.0±26.7	34.0±28.5 (↓23)
	10	32.3±26.8	28.8±29.5	35.2±26.9	30.4±25.6
	11	34.9±28.4	25.7±30.7	36.4±28.3	32.2±26.8
	12	39.4±24.2	24.4±31.0	35.6±28.1	28.4±28.0

(Table continues next page)

Block [b]		Dose (ppm)			
		0	150	500	1750
PND 62	1	70.0±7.9	62.9±9.0	64.7±10.2	61.4±10.1* (↓12)
	2	67.6±9.1	66.4±9.5	65.2±7.2	64.0±11.3
	3	67.8±6.8	66.0±8.9	66.7±7.6	64.7±11.5
	4	69.2±8.0	65.6±11.7	63.8±9.6	60.1±9.1* (↓13)
	5	63.0±17.9	67.0±10.1	66.0±6.7	62.4±14.2
	6	61.8±19.0	63.2±19.7	64.4±12.3	57.9±18.6
	7	61.1±21.2	61.2±19.1	63.9±10.9	59.1±14.2
	8	56.6±20.1	55.5±20.9	65.8±10.8	60.3±16.5
	9	55.2±25.1	57.2±23.6	61.0±17.3	57.6±21.2
	10	52.0±27.9	54.3±21.3	62.2±18.5	57.2±19.1
	11	53.0±31.2	47.2±24.7	55.3±20.2	53.3±23.6
	12	45.8±28.4	43.0±27.8	59.9±19.6	49.0±22.6

a Data obtained from MRID 45422804 Table F1, pages 450 through 465; n=18-20. Percent difference from control presented parenthetically.
b Each block is a 5-minute period.
* Significantly different from controls at $p \leq 0.05$

Table 11b. Mean (±SD) sub-session motor activity (counts) in F_1 female pups in Subset 3 [a]

Block [b]		Dose (ppm)			
		0	150	500	1750
PND 14	1	19.2±16.6	23.7±22.3	19.2±20.3	13.3±11.8
	2	23.4±18.8	22.0±19.9	13.8±18.5	10.4±13.9
	3	26.0±16.6	23.7±26.5	21.3±23.4	15.0±14.2
	4	21.0±17.7	24.8±23.2	25.3±24.4	19.4±17.4
	5	21.8±18.3	24.7±25.7	17.0±22.3	23.0±22.4
	6	21.1±20.8	24.1±24.1	16.2±21.4	20.5±19.6
	7	21.8±21.6	19.5±19.1	13.8±18.4	18.6±16.2
	8	13.0±17.5	17.3±16.4	9.0±22.7	16.5±22.1
	9	16.8±19.0	23.3±23.8	11.8±19.4	14.0±17.2
	10	14.2±19.2	21.6±23.2	13.6±22.1	18.7±20.7
	11	12.0±15.6	19.6±25.4	20.9±26.2	20.8±19.1
	12	9.4±16.4	16.2±22.3	17.9±19.8	21.6±20.2

(Table continues next page)

Block [b]		Dose (ppm)			
		0	150	500	1750
PND 18	1	30.8±23.3	36.5±24.5	23.9±26.3	41.2±29.1
	2	39.8±26.5	45.1±22.1	34.5±29.2	43.6±28.2
	3	49.4±30.5	44.3±20.4	45.8±29.0	45.6±28.5
	4	51.2±32.5	49.2±22.7	47.4±33.4	45.4±30.3
	5	45.9±30.3	42.3±24.7	44.8±30.5	45.0±28.0
	6	49.6±29.1	44.9±30.4	46.4±32.0	44.9±30.4
	7	41.8±29.5	39.3±26.4	38.9±31.4	39.6±31.7
	8	38.8±29.0	36.9±29.6	31.9±28.7	35.8±29.1
	9	31.8±33.4	40.2±26.5	43.3±35.3	36.4±30.9
	10	31.4±31.6	31.9±31.6	39.5±29.9	30.4±31.1
	11	32.4±32.6	28.8±33.6	43.8±32.9	33.1±31.9
	12	32.0±31.4	31.2±30.2	36.0±35.0	33.6±30.0
PND 22	1	66.7±11.1	68.8±9.2	58.6±19.5	55.3±19.4 (↓17)
	2	59.8±18.2	62.7±12.8	50.0±18.6	53.8±17.1
	3	55.4±22.6	56.4±20.3	47.8±19.7	49.8±23.3
	4	56.6±14.5	52.4±17.2	43.2±24.3	41.2±19.9* (↓27)
	5	50.0±21.9	48.2±23.2	38.4±27.2	44.3±23.3
	6	51.1±18.0	46.2±26.4	41.4±24.3	45.2±19.2
	7	48.5±21.0	41.2±32.1	32.0±27.9	37.2±22.8
	8	46.6±21.0	40.4±28.4	35.4±25.3	43.4±26.9
	9	38.2±24.1	44.2±29.0	39.0±30.0	42.6±25.1
	10	35.0±25.7	38.5±27.8	27.5±27.9	39.1±28.6
	11	35.9±25.5	35.7±28.5	27.5±25.5	33.2±26.6
	12	35.8±33.0	29.2±29.0	16.1±21.4	33.6±27.5

(Table continues next page)

Block [b]		Dose (ppm)			
		0	150	500	1750
PND 62	1	66.4±11.0	68.5±10.0	64.2±8.9	60.9±8.9
	2	68.4±8.9	70.4±11.0	70.2±8.0	64.8±9.8
	3	72.6±7.5	70.8±12.5	69.0±8.7	67.0±8.4
	4	71.0±9.0	69.0±10.0	68.9±8.9	63.2±10.4* (↓11)
	5	71.3±14.1	68.7±9.2	67.8±12.6	64.2±19.0
	6	69.0±12.3	62.3±20.8	63.6±12.3	58.6±17.2
	7	63.0±12.6	60.1±21.7	62.0±15.0	58.2±20.8
	8	64.4±23.6	53.0±22.7	51.8±24.5	54.1±22.8
	9	55.4±27.0	40.5±30.2	54.4±23.2	48.7±25.5
	10	58.6±31.6	38.2±28.3	52.0±27.1	41.3±31.7
	11	46.7±30.6	34.3±30.4	44.2±28.3	32.6±26.2
	12	42.6±27.5	27.5±26.5	40.2±26.7	37.7±29.8

a Data obtained from MRID 45422804 Table F1, pages 450 through 465; n=18-20. Percent difference from control presented parenthetically.
b Each block is a 5-minute period.
* Significantly different from controls at $p \leq 0.05$

c. Auditory startle reflex habituation: Auditory startle reflex magnitude data are summarized in Table 12. In the 1750 ppm females on PND 23, the average magnitude of response over 5 blocks and the mean response for each individual block were substantially decreased ($p \leq 0.01$) by 45-50% (average of 48%) as compared to controls. This finding was considered to be treatment-related. Decreases from control values (not significant) in the mean sub-session (block) values and the overall average magnitude of the auditory startle response were also observed on PND 23 in the 1750 ppm males (decreased by 17-34%; average of 29%) and in the 500 ppm females (decreased by 13-36%; average of 27%). These non-significant decreases observed in PND 23 pups were not judged to be related to treatment due to the following factors: 1) The sub-session (block) data for the 1750 ppm males and the 500 ppm females were not significantly different from control values, with the isolated exception of the second block of testing for the 500 ppm females ($p \leq 0.05$). 2) Examination of the overall average startle magnitude values across study groups did not reveal a dose-response relationship in the PND 23 male data; average response magnitude was increased at 500 ppm. 3) The magnitude of the difference between the average response magnitude in treated pups and controls was similar for the 1750 ppm males (↓29%) and the 500 ppm females (↓27%). There were no apparent effects of treatment on auditory startle habituation on PND 63.

Historical control auditory startle habituation data from two developmental neurotoxicity studies previously conducted by the performing laboratory (study report pages 918 and 920) did not assist in the interpretation of the data for the DNT study with clothianidin. In this very limited historical database, reported average response magnitudes were 11.6 and 12.2 for PND 23 males and 12.5 and 11.6 for PND 23 females. Positive control data for auditory startle habituation were provided (see summary in Appendix A to this DER) but were not reported in sufficient detail to

aid in the interpretation of the study findings. Comparison of the PND 23 control data from the current study with the historical control data indicate that the female control values were higher than those seen historically.

Table 12. Mean (±SD) auditory startle reflex magnitude (g) data from F_1 rats in Subset 3. [a]

Observation [b]		Dose (ppm)			
		0	150	500	1750
Males					
PND 23	Block 1	18.3±10.9	15.0±7.0	23.0±14.2	13.5±3.5 (↓26)
	Block 2	13.2±9.8	12.8±6.3	17.9±14.0	10.9±4.2 (↓17)
	Block 3	15.8±11.1	10.5±4.9	16.6±13.4	9.7±5.3 (↓39)
	Block 4	15.5±10.4	10.9±7.5	16.8±14.2	10.8±5.5 (↓30)
	Block 5	15.1±9.3	14.1±13.5	17.4±13.1	9.9±3.9 (↓34)
	Average	15.6±8.5	12.6±6.3	18.3±12.8	11.0±3.6 (↓29)
PND 63	Block 1	62.8±54.8	59.9±53.0	85.8±56.4	75.8±38.0
	Block 2	39.2±49.8	34.8±40.4	55.9±53.9	46.2±25.5
	Block 3	33.7±32.4	34.5±41.5	46.4±44.6	45.3±31.9
	Block 4	30.5±24.9	28.7±26.5	39.3±26.4	34.2±25.0
	Block 5	23.3±17.9	22.4±17.5	31.2±25.9	32.3±20.6
	Average	37.9±31.0	36.1±33.3	51.7±37.9	46.7±23.3
Females					
PND 23	Block 1	23.0±11.2	17.7±7.6	20.1±13.0 (↓13)	12.7±5.8** (↓45)
	Block 2	20.7±12.9	16.1±8.1	13.3±7.5* (↓36)	10.1±7.6** (↓50)
	Block 3	19.5±14.3	18.5±11.2	13.9±9.2 (↓29)	10.0±6.8* (↓49)
	Block 4	21.0±14.2	17.4±12.4	14.1±9.8 (↓33)	11.5±7.8* (↓45)
	Block 5	22.6±16.7	17.8±11.4	16.1±10.8 (↓29)	11.2±6.2** (↓50)
	Average	21.3±12.7	17.5±8.1	15.5±8.2 (↓27)	11.1±5.7** (↓48)
PND 63	Block 1	44.9±28.2	46.8±36.9	34.2±24.2	48.8±57.9
	Block 2	30.7±22.1	28.3±24.2	22.4±14.3	33.6±29.3
	Block 3	26.8±22.9	23.0±20.2	19.6±14.1	20.7±20.9
	Block 4	22.6±15.5	16.6±18.8	14.2±11.0	23.0±25.5
	Block 5	25.0±22.1	15.0±14.3	15.2±10.2	24.9±33.3
	Average	30.0±18.0	26.0±19.6	21.1±12.5	30.2±29.7

a Data obtained from MRID 45422804 Table F2, pages 466-467; n=18-20. Numbers presented parenthetically represent percent difference from control (calculated by reviewers).

b Block=10 consecutive trials

* Statistically different from control, $p \leq 0.05$

** Statistically different from control, $p \leq 0.01$

d. Learning and memory testing: Passive avoidance and water maze data are summarized in Tables 13 and 14, respectively). No treatment-related differences in learning or memory were noted in any treated group relative to concurrent controls in the passive avoidance or water maze tests. A nonsignificant 19% decrease in the latency time for trial 2 in the passive avoidance test for 1750 ppm males at PND 24 was not judged to be indicative of a treatment-related response.

Table 13. Mean (±SD) passive avoidance performance data in F_1 rats [a]

Session/Parameter		Dose (ppm)			
		0	150	500	1750
Males					
Session 1 PND 23	Trials to criterion	3.6±0.8	3.8±0.8	4.0±0.9	3.7±0.9
	Latency trial 1 (sec)	10.0±8.8	13.5±13.5	9.5±9.8	13.8±16.6
	Latency trial 2 (sec)	50.4±16.3	40.9±22.2	37.0±21.0	40.6±20.5 (↓19)
	Failed to learn (n)	0	0	0	0
Session 2 PND 30	Trials to criterion	2.9±0.8	2.7±0.6	3.1±1.0	2.7±0.6 [b]
	Latency trial 1 (sec)	33.2±24.2	29.0±23.2	30.6±25.4	35.4±22.2 [b]
Females					
Session 1 PND 24	Trials to criterion	3.6±0.5	4.0±1.2	3.6±0.7	3.6±1.0
	Latency trial 1 (sec)	12.8±9.5	12.3±14.8	15.5±14.3	14.8±13.3
	Latency trial 2 (sec)	40.8±20.2	35.2±23.6	39.9±19.9	44.2±19.6
	Failed to learn (n)	0	0	0	0
Session 2 PND 31	Trials to criterion	2.8±0.8	3.4±2.1	3.2±1.2	2.8±0.7 [c]
	Latency trial 1 (sec)	35.6±22.7	35.0±25.1	28.8±19.8	39.2±22.8 [c]

a Data obtained from MRID 45422804 Table E1, page 421; n=18-20.
b Excludes values for rats found dead; n=19.
c Excludes values for rats found dead; n=18.

Table 14. Mean (±SD) water maze performance data in F_1 rats [a]

Session/Parameter		Dose (ppm)			
		0	150	500	1750
Males					
Session 1	Trials to criterion	9.1±3.1	9.1±2.5	7.9±1.9	7.3±1.4 [b]
	Errors per trial	0.43±0.18	0.43±0.25	0.35±0.19	0.31±0.15 [b]
	Latency trial 2 (sec)	13.4±5.7	15.0±9.4	14.2±8.5	12.3±7.8 [b]
	Failed to learn (n)	2	0	0	0
Session 2	Trials to criterion	5.7±1.3	5.4±0.6	6.6±2.6	5.9±1.4
	Errors per trial	0.04±0.07	0.10±0.18	0.11±0.12	0.09±0.12
	Latency trial 1 (sec)	7.5±3.0	10.5±8.4	11.0±9.4	8.9±4.4
Females					
Session 1	Trials to criterion	7.7±2.2	8.6±2.7	8.2±2.2	7.7±2.1 [c]
	Errors per trial	0.46±0.32	0.41±0.38	0.44±0.22	0.44±0.24 [c]
	Latency trial 2 (sec)	19.5±15.4	13.9±7.4	17.7±12.3	13.8±11.0 [c]
	Failed to learn (n)	0	1	0	0
Session 2	Trials to criterion	6.4±2.3	7.9±3.4	7.2±3.1	6.6±1.9
	Errors per trial	0.17±0.25	0.22±0.26	0.18±0.16	0.18±0.20
	Latency trial 1 (sec)	13.0±13.5	9.9±5.6	11.8±5.6	15.6±12.2

a Data obtained from MRID 45422804 Table E2, page 422; n=18-20.
b Excludes values for rats found dead; n=19.
c Excludes values for rats found dead; n=18.

5. Postmortem results

a. Brain weights: No treatment-related differences in brain weights or brain-to-body weight ratios were noted between treated and control groups for PND 12 pups or for offspring at termination (Table 15).

Table 15. Mean (±SD) brain weights in F_1 rats [a]

Parameter	Dose (mg/kg/day)			
	0	150	500	1750
Males				
PND 12 (subset 1)				
Terminal Body Weight (g)	19.0±3.4	18.9±3.6	18.1±3.8	17.8±3.3
Brain Weight (g)	1.14±0.12	1.14±0.15	1.09±0.15	1.15±0.15
Brain-to-body weight ratio (%)	6.108±0.746	6.110±0.589	6.164±0.706	6.573±0.827
Termination (subset 4)				
Terminal Body Weight (g)	429.1±45.0	427.1±31.7	425.7±38.0	405.1±30.5
Brain Weight (g)	2.229±0.131	2.291±0.085	2.200±0.118	2.170±0.119
Brain-to-body weight ratio (%)	0.522±0.047	0.539±0.037	0.519±0.035	0.537±0.028
Females				
PND 12 (subset 1)				
Terminal Body Weight (g)	17.2±3.1	17.8±4.1	17.0±2.1	16.8±2.6
Brain Weight (g)	1.08±0.13	1.08±0.17	1.06±0.08	1.11±0.12
Brain-to-body weight ratio (%)	6.407±0.728	6.233±0.714	6.251±0.454	6.710±0.460
Termination (subset 4)				
Terminal Body Weight (g)	261.4±26.1	253.3±28.0	258.6±33.1	248.2±16.8
Brain Weight (g)	2.053±0.086	2.084±0.101	2.034±0.080	2.033±0.145
Brain-to-body weight ratio (%)	0.791±0.076	0.830±0.097	0.799±0.109	0.818±0.046

a Data obtained from MRID 45422804 Tables D3-D4 and G3-G4, pages 402-403 and 625-626; n=10 for subset 4 and 20 for subset 1.

b. Macroscopic examination: Gross necropsy findings at study termination revealed slight to marked dilation of the pelvis of the kidney in male rats at all treatment levels (3 males at 175 ppm, one male at 500 ppm, and one male at 1750 ppm). Additionally, a small, purple, and flaccid left testis and small, flaccid left epididymis was observed in one 1750 ppm male. No treatment-related gross pathological findings of the nervous system were noted in any treated group.

c. Microscopic examination:

1) Urogenital system of male offspring: The pelvic dilation of the kidneys observed at necropsy was confirmed histopathologically. Histopathological evaluation revealed diffuse, moderate

testicular degeneration and minimal focal mononuclear cell infiltration with mild, necrotic germ cell in the epididymis.

2) Neurological system: No treatment-related microscopic findings were noted at subjective histopathological evaluation of nervous system tissues. Morphometric evaluations (Table 16) revealed a number of significant differences from control at 1750 ppm. In the 1750 ppm females, differences ($p \leq 0.05$) were noted in the thickness of the hippocampal gyrus (↑9%), cerebellum height (↑10%), and the external germinal layer of the cerebellum (↓11%) on PND 12. Additionally, on PND 83-87, decreases ($p \leq 0.05$) in the thickness of the caudate putamen (↓6%) and hippocampal gyrus (↓5%) were noted in the 1750 ppm females. Although the measurements of the hippocampus demonstrate an increase in weanlings, and a decrease in adults, their relationship to treatment was nevertheless considered to be biologically plausible. This could be explained if the effects noted at termination are not representative of a persistence of effects, but are rather the latent consequences of early exposure.

Due to the presence of apparently treatment-related findings at 1750 ppm, morphometric evaluations were conducted in PND 12 and adult 500 ppm females; no significant differences from control were observed. A dose-response relationship was noted in the responses observed in the female cerebellum height at PND 12 and in the hippocampal gyrus at PND 12 and termination; for these findings, a similar trend was noted in males, although no significance was identified. The PND 12 male hippocampal gyrus thickness and cerebellum height were each increased 4% from control, and the termination hippocampal gyrus thickness was decreased 5% from control. In summary, the following findings were judged to be related to treatment at 1750 ppm: the increased thickness of the hippocampal gyrus (both sexes), the increased thickness of the cerebellum height (both sexes), and the decreased thickness of the external germinal layer at PND 12 (females), and the decreased thickness of hippocampal gyrus (both sexes) and caudate putamen (females) at termination.

Table 16. Mean (±SD) morphometric data for F_1 rats [a]

Parameter	Dose (ppm)							
	0	150	500	1750	0	150	500	1750
	Males				Females			
PND 12 (subset 1)								
Cerebrum (mm)	12.14±0.59	NA	NA	12.35±0.50	11.97±0.56	NA	11.94±0.46	12.14±0.57
Cerebellum (mm)	5.40±0.47	NA	NA	5.55±0.50	5.28±0.60	NA	5.32±0.43	5.49±0.57
Frontal Cortex (µm)	1356.0±97.5	NA	NA	1413.6±114.0	1356.0±132.1	NA	1377.0±83.0	1416.0±141.3
Parietal Cortex (µm)	1408.8±68.9	NA	NA	1461.6±102.7	1423.2±70.7	NA	1431.0±56.7	1488.0±86.9
Caudate Putamen (µm)	2548±185.8	NA	NA	2568.0±118.1	2529.6±128.1	NA	2442.0±102.2	2572.8±158.7
Corpus Callosum (µm)	282.1±32.6	NA	NA	293.9±31.0	261.1±37.3	NA	288.0±29.4	273.5±56.5
Hippocampal Gyrus (µm)	948.0±42.7	NA	NA	981.6±88.0 (↑4)	919.2±67.9	NA	942.0±69.6	1003.2±81.4* (↑9)
Cerebellum Height (µm)	3004.8±340.3	NA	NA	3129.6±309.3 (↑4)	2856.0±277.4	NA	2946.0±194.8	3148.8±259.2* (↑10)
Ext. Germinal Layer (µm)	34.3±4.2	NA	NA	33.9±3.3	37.2±3.5	NA	38.1±4.0	33.2±4.9* (↓11)
Termination (subset 4)								
Cerebrum (mm)	15.83±0.27	NA	NA	15.66±0.30	15.59±0.28	NA	15.48±0.28	15.53±0.56
Cerebellum (mm)	7.09±0.36	NA	NA	7.18±0.39	7.25±0.24	NA	7.17±0.37	7.15±0.28
Frontal Cortex (µm)	1848.4±123.9	NA	NA	1893.6±165.7	1711.2±118.1	NA	1710.0±78.7	1730.4±107.6
Parietal Cortex (µm)	1956.0±105.7	NA	NA	1992.0±160.8	1800.0±35.8	NA	1758.0±63.6	1795.2±71.4
Caudate Putamen (µm)	3542.4±218.0	NA	NA	3700.8±221.1	3379.2±206.4	NA	3480±174.4	3192.0±155.5* (↓6)
Corpus Callosum (µm)	281.4±37.8	NA	NA	272.6±30.1	266.9±43.7	NA	266.4±38.7	253.5±26.5
Hippocampal Gyrus (µm)	1819.2±68.6	NA	NA	1735.2±112.0 (↓5)	1562.4±69.2	NA	1506±73.2	1483.2±94.5* (↓5)
Cerebellum Height (µm)	5419.2±444.8	NA	NA	5424.0±569.3	4915.2±267.0	NA	4926±114.7	4771.2±255.2 (↓3)

a Data obtained from MRID 45422804, pages 765-770 and 816-821; n=10. Numbers presented parenthetically represent percent difference from control (calculated by reviewers).

* Statistically different from control, $p \leq 0.05$

NA Not applicable

III. DISCUSSION and CONCLUSIONS

A. INVESTIGATORS' CONCLUSIONS: The investigators concluded that maternal toxicity at 1750 ppm was characterized by decreased body weight and food consumption. Developmental and/or neonatal toxicity at ≥500 ppm was characterized by decreases in body weight gains during pre-weaning, motor activity on PND 22, and acoustic startle response in females on PND 23. The maternal NOAEL was 500 ppm. The offspring NOAEL was 150 ppm.

B. REVIEWER'S COMMENTS: In this study, maternal toxicity was observed as decreased body weight, body weight gain, and food consumption during gestation and lactation at 1750 ppm. Maternal body weights were consistently decreased ($p \leq 0.05$ or NS) throughout gestation and lactation (↓2-8%). Body weight gains were decreased ($p \leq 0.05$) during GDs 0-3 (↓63%) and

LDs 4-7 (↓67%). These decreases corresponded with the significant reductions noted in absolute (↓7-23%) and relative (↓6-22%) food consumption during the gestation and lactation periods.

Overall effects on maternal body weight gain and food consumption at 1750 ppm were minimal in nature. Decreased body weight and body weight gain were correlated with decreased food consumption, suggesting that there was little difference in food efficiency between control and treated rats (not calculated). At 1750 ppm, decreased body weight gain and food consumption from GD 0-3 is suggestive of a palatability problem. Body weight was essentially recovered by the end of gestation. Decreased body weight gain during lactations days 4-7 was similar for the low (150 ppm) and high (1750 ppm) dose groups (i.e., there was no dose-response), and body weight gain was substantially increased at 500 and 1750 ppm during lactation days 14-22 (i.e., when pups would have also been beginning to consume treated feed). Overall gestation (GD 0-20) and lactation (LD 1-22) body weight gains were not statistically different from control for any treated group.

The maternal LOAEL is 1750 ppm (142 mg/kg/day) based on decreased body weights, body weight gains, and food consumption. The maternal NOAEL is 500 ppm (42.9 mg/kg/day).

During the pre-weaning period, significantly decreased body weight was noted on PND 14-22 in the females pups at 500 ppm (↓6-7%) and in both sexes at 1750 ppm (↓6-16%). Body weight gains were decreased ($p \leq 0.05$) in the female pups at 500 ppm (↓8-24%) and in both sexes at 1750 ppm (↓11-48%). Overall pre-weaning (PND 5-22) body weight gains were decreased ($p \leq 0.05$) by 7% in the 500 ppm females and by 18% at 1750 ppm in both sexes. Immediately after weaning, decreased ($p \leq 0.05$) body weights were noted at 1750 ppm in both sexes (↓4-15%). Also during this time period, two male and three female rats at 1750 ppm were found dead on PNDs 25-27; this finding was considered related to a failure to thrive post-weaning, based on the patterns of body weight gains and losses. However, within 3 weeks post-weaning (i.e., following the cessation of treatment) the treatment-related body weight deficits in 1750 ppm pups had essentially been reversed.

There were no effects of treatment on other developmental landmarks examined, including pinna unfolding, acoustic startle response, eye opening, pupil constriction, balanopreputial separation or vaginal patency. A slight retardation in surface righting reflex was observed on PND 3 in all treated groups compared to controls; however, following a careful examination of the data and methodologies used, it was concluded that this finding could not be unequivocally attributed to treatment.

In a modified functional observational battery, no clinical findings consistent with "abnormal autonomic functions" in offspring were observed.

There were no apparent effects of treatment in motor activity data measured on PND 14, 18, or 62. On PND 22, mean total motor activity (number of movements) was decreased (NS) in the 1750 ppm males (↓24%) and in the females at 500 ppm (↓21%) and 1750 ppm (↓10%). The mean time spent in movement on PND 22 was also decreased (NS) in the 1750 ppm males (↓27%) and in the females at 500 ppm (↓24%) and 1750 ppm (↓19%). The study author

considered all of these effects to be evidence of treatment-related toxicity. Overall, interpretation of the motor activity data is complicated by the variability in the data. Nevertheless, the decreases in motor activity counts and the time spent in movement at PND 22 for 1750 ppm male offspring are of substantial magnitude and supported by sub-session data; therefore, they were judged to be related to treatment. Agency reviewers considered the decreases in motor activity in 1750 ppm female weanlings to be minimal in nature and not likely to be treatment-related. Additionally, it was noted that effects noted for 500 ppm female offspring at PND 22, while nearly of the same magnitude as those observed in PND 22 males at 1750 ppm, demonstrated a lack of dose-response and an absence of significant corollary alterations in the sub-session data. Therefore, the apparent decreases in motor activity in PND 22 females at 500 ppm were also not considered to be treatment-related by Agency reviewers.

In general, habituation was not observed in the motor activity data on PND 14 or 18, but some habituation was evident in both male and female offspring on PND 22 and PND 62. There did not appear to be any treatment-related differences in habituation between control and treated rats. On all of the testing days, individual motor activity data demonstrated some animals with little or no habituation.

In the 1750 ppm females on PND 23, the average magnitude of the acoustic startle response over 5 blocks and the mean response for each individual block were substantially decreased ($p \leq 0.01$) by 45-50% (average of 48%) as compared to controls. This finding was considered to be treatment-related. Decreases from control values (not significant) in the mean sub-session (block) values and the overall average magnitude of the auditory startle response were observed on PND 23 in the 1750 ppm males (decreased by 17-34%; average of 29%) and in the 500 ppm females (decreased by 13-36%; average of 27%). These non-significant decreases observed in PND 23 pups were not judged to be related to treatment since: 1) the sub-session (block) data for the 1750 ppm males and the 500 ppm females were not significantly different from control values, with the isolated exception of the second block of testing for the 500 ppm females ($p \leq 0.05$). 2) examination of the overall average startle magnitude values across study groups did not reveal a dose-response relationship in the PND 23 male data; average response magnitude was increased at 500 ppm, and 3) the magnitude of the difference between the average response magnitude in treated pups and controls was similar for the 1750 ppm males (↓29%) and the 500 ppm females (↓27%). There were no apparent effects of treatment on auditory startle habituation on PND 63.

No treatment-related differences in learning or memory were noted in any treated group relative to concurrent controls in the passive avoidance or water maze tests.

There were no treatment-related differences in brain weights or brain-to-body weight ratios between control and treated groups for offspring at PND 12 or at termination (PND 83-87). No treatment-related macroscopic findings were noted at necropsy and no treatment-related microscopic findings were noted at subjective histopathological evaluation of nervous system tissues. Morphometric evaluations revealed a number of significant differences from control at 1750 ppm. In the 1750 ppm females, differences ($p \leq 0.05$) were noted in the thickness of the hippocampal gyrus (↑9%), cerebellum height (↑10%), and the external germinal layer of the cerebellum (↓11%) on PND 12. Additionally, on PND 83-87, decreases ($p \leq 0.05$) in the

thickness of the caudate putamen (↓6%) and hippocampal gyrus (↓5%) were noted in the 1750 ppm females. Morphometric evaluations of PND 12 and adult 500 ppm females revealed no significant differences from control. A dose-response relationship was noted in the responses observed in the female cerebellum height at PND 12 and in the hippocampal gyrus at PND 12 and termination; for these findings, a similar trend was noted in males, although no significance was identified. The PND 12 male hippocampal gyrus thickness and cerebellum height were each increased 4% from control, and the termination hippocampal gyrus thickness was decreased 5% from control. In summary, based upon the magnitude and/or statistical significance of the response and/or on apparent dose-response relationships, the following findings were judged to be related to treatment at 1750 ppm: the increased thickness of the hippocampal gyrus (both sexes), the increased thickness of the cerebellum height (both sexes), and the decreased thickness of the external germinal layer at PND 12, and the decreased thickness of hippocampal gyrus (both sexes) and caudate putamen (females) at termination.

The offspring systemic LOAEL is 500 ppm (42.9 mg/kg/day), based on decreased body weights and body weight gains of female pups during PND 14-21. The offspring systemic NOAEL is 150 ppm (12.9 mg/kg/day).

The offspring neurotoxicity LOAEL is 1750 ppm (142 mg/kg/day), based on decreased motor activity (number and duration of movements) in PND 22 male pups, decreased magnitude of the auditory startle response in PND 23 females, increases in the thickness of the hippocampal gyrus and cerebellum height and decreases in the external germinal layer in the brains of PND 12 pups; and decreases in the thickness of the caudate putamen and hippocampal gyrus in adult offspring at termination (PND 83-87). The offspring neurotoxicity NOAEL is 500 ppm (42.9 mg/kg/day).

This study is classified as **acceptable/non-guideline** and does not satisfy the guideline requirement (OPPTS 870.6300; OECD 426) for a developmental neurotoxicity study in rats. Classification may be upgradable to guideline upon submission of procedural information for functional observation assessments.

C. **STUDY DEFICIENCIES:** The following deficiencies were noted:
1) The method used for detection of functional changes was not adequately described in the text of the report. The procedures used were not described, including whether the same technicians were used throughout testing, where the testing was done (including whether the animals were removed from the cage), when testing was done with respect to time of test substance administration, what the environmental conditions were (e.g., noise level, etc.), whether scoring criteria were used for the measured parameters, the duration of the observation period for open field observations. There was no mention of evaluation of pupillary function such as constriction of the pupil in response to light, or a measure of pupil size.
2) According to the histopathology report, gross morphometric measurements (A/P cerebrum and cerebellum) were performed on all brains evaluated histopathologically, however data were reported only for high dose and control animals, and any mid-dose females that were processed for further analysis. In addition, morphometric measurements were made bilaterally for a number of areas of the brain in both PND 12 and adult offspring (see report pages 756 and 811),

but only the mean values were reported. Values for morphometric measurements evaluated, but not included in the current report, should be submitted.
3) Latency was not reported for auditory startle data.

Appendix A – Positive Control Data

The following positive control data were provided:

Parker, R. (1999) Neurotoxicity evaluation of positive control substances in Crl:CD®(SD)IGS BR VAF/Plus® rats. Argus Research Laboratories, Inc., 905 Sheehy Drive, Building A, Horsham, Pennsylvania 19044-1297. Laboratory project number 012-075, August 6, 1999. Unpublished. This study used a functional observational battery (FOB) to evaluate the positive control substances acrylamide, trimethyltin, MK-801, carbaryl, and DDT and was not acceptable for use with the current study because the current study did not use a formal FOB. (MRID 45422804, pp. 959-1137)

Foss, J. (1992) Neurotoxicity evaluation of DDT in Crl:CD®(SD)IGS BR VAF/Plus® rats. Argus Research Laboratories, Inc., 905 Sheehy Drive, Building A, Horsham, Pennsylvania 19044-1297. Laboratory project number 012-015. Unpublished. This study used an FOB to evaluate the positive control substance DDT and was not acceptable for use with the current study because the current study did not use a formal FOB. (MRID 45422804, pp. 1138-1147)

Lochry, E., J. Foss, and M. Christian. (1990) Validation of a functional observational battery and motor activity measure using positive control substances. Argus Research Laboratories, Inc., 905 Sheehy Drive, Building A, Horsham, Pennsylvania 19044-1297. Poster presented at the 11th annual meeting of the American College of Toxicology; Orlando, Florida; October, 1990. This study used an FOB to evaluate the positive control substances DDT, physostigmine monosalicylate, or acrylamide and used a motor activity assessment to evaluate the positive control substances chlorpromazine or amphetamine. It was not acceptable for use with the current study because the current study did not use a formal FOB, and because the motor activity sessions in the positive control study were 2 hours in duration and comprised of 24 5-minute blocks, while the current study used 1-hour sessions comprised of twelve 5-minute blocks. There was also insufficient information provided to determine whether the same equipment was used as was used in the current study. (MRID 45422804, pp. 1148-1181)

Foss, J. (1991) Neurotoxicity evaluation of positive control substances in Crl:CD® VAF/Plus® rats. Argus Research Laboratories, Inc., 905 Sheehy Drive, Building A, Horsham, Pennsylvania 19044-1297. Laboratory project number 012-014. Unpublished. This study used an FOB and motor activity assessment to evaluate the positive control substances acrylamide, IDPN, carbaryl, DDT, and triadimefon. It was not acceptable for use with the current study because the current study did not use a formal FOB, and because the motor activity sessions in the positive control study were 1.5 hours in duration/comprised of 18 5-minute blocks, while the current study used 1-hour sessions comprised of twelve 5-minute blocks. There was also insufficient information provided to determine whether the same equipment was used as was used in the current study. (MRID 45422804, pp. 1182-1249)

Foss, J. and E. Lochry (1991) The assessment of motor activity in neonatal and adult rodents using passive infrared sensors. Argus Research Laboratories, Inc., 905 Sheehy Drive, Building A, Horsham, Pennsylvania 19044-1297. Poster presented at the 12th annual meeting of the

American College of Toxicology; Savannah, Georgia; October, 1991. This study used passive infrared sensors to monitor motor activity of untreated adult rats, untreated adult mice, and neonatal rats on postnatal days 13, 17, 21, and 58-59. The positive control substances d-Amphetamine and chlorpromazine were evaluated in rats at approximately postnatal day 60, and the positive control substances acrylamide, IDPN, carbaryl, DDT, and triadimefon were evaluated in adult rats. Test sessions with positive control substances were 90-115 minutes in duration and comprised of 5-minute blocks, while the current stuy used 1-hour sessions comprised of twelve 5-minute blocks. (MRID 45422804, pp. 1250-1257)

Neurotoxicity evaluation of positive control substances in Crl:CD® BR VAF/Plus® rats. Argus Research Laboratories, Inc., 905 Sheehy Drive, Building A, Horsham, Pennsylvania 19044-1297. Laboratory project number 012-058. Unpublished. This study used motor activity assessment, auditory startle habituation, and neurohistological examination to evaluate the positive control substances acrylamide, trimethyltin chloride, or MK-801. Motor activity assessment was conducted using similar equipment to that used in the current study; however, sessions were 1.5 hours in duration and comprised of 5 minute blocks, while the current study used 1-hour sessions comprised of 10-minute blocks. Auditory startle habituation testing was conducted using similar equipment and methods as those used in the current study. Similar processing and staining methods were used, and the positive control study evaluated the same brain sections for neuropathology as those evaluated in the F_1 adults in the current study. (MRID 45422804, pp. 1258-1296)

Lochry, E. and E. Riley (1980) Retention of passive avoidance and T-maze escape in rats exposed to alcohol prenatally. Neurobehavioral Toxicology, Vol. 2, pp. 107-115. This study used different equipment than that used in the current study to assess passive avoidance and learning acquisition and retention and is not acceptable for use as positive control data. (MRID 45422804, pp. 1297-1306)

Lochry, E., J. Foss, and M. Christian (1990) Learning and retention paradigms in developmental neurotoxicity test batteries: passive avoidance and water maze. Argus Research Laboratories, Inc., 905 Sheehy Drive, Building A, Horsham, Pennsylvania 19044-1297. Poster presented at the 18th European Teratology Society Conference; Edinburgh, Scotland; September 1990. This was a collection of historical control data from passive avoidance and water maze testing conducted in 1988-1989. No further details were provided. (MRID 45422804, pp. 1307-1312)

Foss, J., E. Lochry, and A. Hoberman (1990) Automated monitoring systems for motor activity and auditory startle applicable for both developmental and adult neurotoxicity studies. Poster presented at the 8th International Neurotoxicity Conference; Little Rock, Arkansas; October, 1990. Motor activity was assessed on postnatal days 13, 17, 21, and 60, using similar equipment to that used in the current study; however, the test session was 1.5 hours long and comprised of 18 5-minute blocks, while the current study used 1 hour test sessions comprised of twelve 5-minute blocks. Auditory startle habituation was assessed on postnatal days 22 and 60, using similar equipment and methods to those used in the current study. (MRID 45422804, pp. 1313-1327)

Foss, J. and E. Riley (1989) Elicitation and modification of the acoustic startle reflex in animals prenatally exposed to cocaine, Neurotoxicology and Teratology 13:541-546. This study was conducted using different equipment than that used in the current study and is not acceptable for use as positive control data for the current study. (MRID 45422804, pp. 1328-1334)

E. Lochry, A. Hoberman, and M. Christian (1985) Detection of prenatal effects on learning as a function of differential criteria, Neurotoxicology and Teratology 7:697-701. This study was conducted using different equipment than that used in the current study and is not acceptable for use as positive control data for the current study. (MRID 45422804, pp. 1335-1340)

Garman, R.H. (1998) Neuropathology Validation Report, Consultants in Veterinary Pathology, Inc., P.O. Box 68, Murrysville, PA 15668, dated 8-21-96. Unpublished. This consisted of a brief description of the consulting neuropathologist's credentials, experience, and publications, and also included the neuropathology methods and results from Argus Research Laboratories, laboratory project number 012-058 [mentioned above]. (MRID 45422804, pp. 1341-1390)

Garman, R.H. (1998) Morphometric measurement validation study comparing day 10 and day 12 pups Report, Consultants in Veterinary Pathology, Inc., P.O. Box 68, Murrysville, PA 15668. Unpublished. This study compared 9 different morphometric measurements between 10 and 12 day old pups. The brains were measured grossly and sectioned similarly to those of the PND 12 pups used in the current study. It was concluded that increases in the thickness of the frontal cortex, height of the cerebellar cortex, and cross-sectional width of the caudate-putamen correlated best with brain maturation between PND 10 and 12. Only the previous two of these three measurements were used in the current study, which also included measurement of the dentate gyrus of the hippocampus. (MRID 45422804, pp. 1391-1398)

Foss, J., A. Hoberman, and M. Christian (1992) Developmental neurotoxicity evaluation of lead nitrate in in Crl:CD® BR VAF/Plus® rats. Argus Research Laboratories, Inc., 905 Sheehy Drive, Building A, Horsham, Pennsylvania 19044-1297. Poster presented at the Annual Meeting of the Society of Toxicology; Seattle, Washington; February 1992. Motor activity assessment was conducted using similar equipment to that used in the current study; however, the test session was comprised of 5-minute blocks, while the current study used 10-minute blocks. The equipment and methods used for auditory startle habituation, passive avoidance, and water maze testing were similar to those used in the current study, however no effects of treatment were detected. (MRID 45422804, pp. 1399-1412)

DATA FOR ENTRY INTO ISIS

Developmental Neurotoxicity Study - rats (870.6300)

PC code	MRID #	Study type	Species	Duration	Route	Dosing method	Dose range ppm	Doses tested mg/kg/day	NOAEL mg/kg/day	LOAEL mg/kg/day	Endpoint(s)	Comments
044309	45422804	dev neurotox	rats	GD 0-LD 22	oral	dietary	0, 150, 500, 1750	0, 12.9, 42.9, 142	42.9	142	decr BW, BWG, FC	Maternal
044309	45422804	dev neurotox	rats	GD 0-LD 22	oral	dietary	0, 150, 500, 1750	0, 12.9, 42.9, 142	12.9	42.9	decr BW, BWG in female pups (PND 14-21)	Offspring systemic
044309	45422804	dev neurotox	rats	GD 0-LD 22	oral	dietary	0, 150, 500, 1750	0, 12.9, 42.9, 142	42.9	142	decr motor activity in PND 22 males; decr auditory startle amplitude in PND 23 females; alterations in brain morphometry in PND 12 and 83-87 offspring	Offspring neurotox

450

HED Records Center Series 361 Science Reviews - File R107689 - Page 465 of 721

DATA EVALUATION RECORD

CLOTHIANIDIN (TI-435)

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

MRID 45422707

Prepared for

Health Effects Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
1921 Jefferson Davis Highway
Arlington, VA 22202

Prepared by

Toxicology and Hazard Assessment Group
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Task No. 02-44

Primary Reviewer:
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Disclaimer

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

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PMRA/EPA Reviewers: Scott Hancock/Pamela Hurley Pamela M Hurley
EPA WAM Ghazi A. Dannan 12/10/04
Date June 28 /02

TXR No. 0050321
STUDY TYPE: 28-Day Dermal Toxicity - rat; OPPTS 870.3200 [§82-2] (rodent); OECD 410.

TEST MATERIAL (PURITY): Clothianidin (TI-435) (95.2%)

SYNONYMS: (E)-1-(2-Chloro-5-thiazolylmethyl)-3-methyl-2-nitroguanidine

CITATION: Weiler, M.S. (2000). 28-Day dermal toxicity study with TI-435 in rats. Covance Laboratories, Inc., 3301 Kinsman Boulevard, Madison, Wisconsin 53704-2595. Laboratory Study Identification: Covance 6155-120. October 13, 2000. MRID 45422707. Unpublished.

SPONSOR: Takeda Chemical Industries, Ltd., Tokyo, Japan.

EXECUTIVE SUMMARY:

In a 28-day dermal toxicity study (MRID 45422707), TI-435 (95.2% a.i., Lot #30037120) was applied under a semi-occlusive dressing, to the shaved skin of 10 Crl:CD(SD)IGS BR rats/sex/dose at dose levels of 0 (reverse osmosis water), 100, 300, or 1000 mg/kg bw/day, 6 to 6.5 hours/day, 7 days/week for 29 days.

There were no compound-related dermal observations, mortalities, clinical signs, or adverse effects on motor activity, opthalmoscopic observations, body weights, food consumption, organ weights, or gross or histopathology. A 60% decrease ($p<0.05$) in mean body weight gain, accompanied by a 9% ($p<0.05$) decrease in food consumption, was noted in males treated with 1000 mg/kg/day compared to controls during week 1. This effect is considered of questionable toxicological significance since no body weight gain effects were noted in high-dose males during weeks 2-4. Overall body weight gain for high-dose males was 20% lower than controls and was attributed to the decrease observed during week 1. No effects on body weight gain were noted in females or in low- or mid-dose males. **Based on the results of this study, the systemic and dermal LOAELs for TI-435 in male and female rats are not identified, and the systemic and dermal NOAELs are the limit dose of 1000 mg/kg/day.**

This 28-day dermal toxicity study in the rat is Acceptable and satisfies the guideline requirement for a 28-day dermal toxicity study (OPPTS 870.3200; OECD 410) in rats.

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

I. MATERIALS AND METHODS

A. MATERIALS:

1. **Test Material:** TI-435
   - **Description:** Pale yellow powder
   - **Lot/Batch #:** 30037120
   - **Purity:** 95.2% a.i.
   - **Compound Stability:** At least 53 weeks when stored at 2 to 8 °C
   - **CAS #:** Not available
   - **Structure:** Not available

2. **Vehicle and/or positive control:** Reverse osmosis water

3. **Test animals:**
   - **Species:** Rat
   - **Strain:** Crl:CD(SD)IGS BR
   - **Age/weight at study initiation:** Males: 8 weeks/ 240-317 g; Females: 8 weeks/ 171-213 g
   - **Source:** Charles River Laboratories, Raleigh, NC
   - **Housing:** Singly in suspended stainless steel cages
   - **Diet:** Certified rodent diet #8728C, Harlan Teklad, *ad libitum*
   - **Water:** Tap water, *ad libitum*
   - **Environmental conditions:** **Temperature:** 18 - 26 °C; **Humidity:** 30 - 70 %; **Air changes:** Not stated; **Photoperiod:** 12 hrs dark/ 12 hrs light
   - **Acclimation period:** 16 days.

B. STUDY DESIGN:

1. **In life dates** - Start: April 29, 1999; End: May 28, 1999

2. **Animal assignment**: Animals were assigned randomly by computer to the test groups noted in Table 1 so that mean group body weights would be normalized.

TABLE 1: Study design. Dose Levels

TABLE 1: Study design.			
Test group	**Dose level (mg/kg bw/d)**	**# Male**	**# Female**
Control	0	10	10
Low	100	10	10
Mid	300	10	10
High	1000	10	10

Data taken from p. 16, MRID 45422707.

3. **Dose selection rationale**

The dose levels were selected with the goal of providing a gradient of toxic effects. The high-dose was the limit dose of 1000 mg/kg/day. Dose concentrations were based on the test material as supplied, and test material was weighed for each day of dosing. Each dose level was prepared independently by

weighing a specified amount of TI-435 required for the calculated day's dose (based on most recent body weight). Dose preparations were stored in a refrigerator between 2-8°C. Homogeneity, stability, and concentration analyses were not performed since the test substance was applied directly (without vehicle) to the skin.

4. **Preparation and treatment of animal skin**
Before the first application and as needed thereafter, the fur of each test animal was clipped from the dorsal area of the trunk over an area of approximately 10% of the body surface. The skin application sites were moistened prior to dose application. Individual doses were based on the most recent body weight. The test material or reverse osmosis water was applied directly to the skin of each animal and evenly distributed over the application site. During exposure, the test material or vehicle was covered with a porous gauze dressing and non irritating tape. The application site and entire trunk of the rat were then wrapped with Vetwrap® and secured with surgical tape. After each 6-6.5 hour exposure period, the dressings were removed and the exposure area was wiped with a water-moistened towel to remove any remaining test material. Animals were collared with flexible plastic collars during the exposure period to minimize preening of the application site.

4. **Statistics** - Levene's test was done to test for variance homogeneity. In the case of heterogeneity of variance at $p \leq 0.05$, transformations were used to stabilize the variance. One-way ANOVA was used to analyze body weight, body weight change, food consumption, motor activity, grip strength, continuous clinical pathology data, and organ weight data. If the ANOVA was significant, Dunnett's t-test was used for pairwise comparison between treated and control groups. Treatment group comparisons for each sex were compared with the control group and were evaluated at the 5% two-tailed probability level. The reviewer considers the analyses used to be appropriate.

C. **METHODS:**

1. **Observations:**

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

1b. **Clinical examinations:** Clinical examinations, including detailed examination of the skin, were performed weekly.

1c. **Neurological evaluations:** Open field observations were performed pretest on the first day of treatment and weekly thereafter. The following parameters were evaluated: changes in skin, fur, eyes, and mucous membranes; occurrences of secretions and excretions; lacrimation, piloerection, pupil size, unusual respiratory pattern, posture, reactivity to handling, clonic or tonic movements, stereotypic behavior, bizarre behavior, and changes in gait.

Expanded clinical observations were conducted during week 4, and included hand-held observations, open field observations, and sensory reactivity to stimuli. Motor activity testing was also done during week 4 on the same day as the expanded clinical testing. The rats were placed into an automated photocell activity recording device and activity counts were recorded at 2-minute intervals for a total of 40 minutes.

2. **Body weight** Animals were weighed prior to initiation of the study (day -1), on the first day of treatment, and on days 7, 14, 21, 28, and 30 (day of necropsy).

3. **Food consumption** Individual food consumption was determined weekly.

4. **Ophthalmoscopic examination:** Eyes were examined before initiation of treatment and during week 4 by indirect opthalmoscopy after dilation with 0.5% Mydriacyl.

5. **Haematology & Clinical Chemistry:**
After an overnight fast, blood was collected from a jugular vein of all surviving animals prior to terminal sacrifice. The CHECKED (X) parameters were examined.

a. Haematology

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

* Recommended for dermal toxicity studies based on Guideline 870.3200

b. Clinical Chemistry

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

* Recommended for dermal toxicity studies based on Guideline 870.3200

6. Urinalysis*

Urinalysis was not required and was not performed.

7. Sacrifice and Pathology

All surviving animals were sacrificed on day 30 under sodium pentobarbital anesthesia and the exsanguinated animals were subjected to gross pathological examination. (A necropsy was also done on the two rats that died on day 15). Selected tissues were fixed in 10% neutral buffered formalin, embedded in paraffin, and stained with Hematoxylin and Eosin. Microscopic examination was performed on control and high-dose tissues and on all gross lesions and treated skin from low- and mid-dose animals as well. The CHECKED (X) tissues were collected for histological examination. The (XX) organs, in addition, were weighed.

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

* Recommended for dermal toxicity studies based on Guideline 870.3200
+ Organ weights required.

II. RESULTS

A. Observations :

1. Mortality - There were no treatment-related deaths. One female treated with 100 mg/kg/day and one female treated with 1000 mg/kg/day died on day 15. There were no clinical signs reported before death. The low-dose female exhibited a large thickened liver with moderately severe congestion, mineralization of the kidney, a cyst on the thyroid, and minimal dilatation of the uterus at necropsy. The high-dose female was found with its collar caught between the cage and hanging feeder. At necropsy it had mineralization in the kidney, slight congestion of the lungs, minimal edema of the lung alveoli, eosinophilic or macrophage infiltrates in the lung tissue, and a cyst in the thyroid.

2. Clinical signs of toxicity - No treatment-related effects were noted.

3. Dermal Irritation - No treatment-related dermal effects were noted.

B. Body weight and weight gain: A 60% decrease ($p<0.05$) in mean body weight gain, accompanied by a 9% ($p<0.05$) decrease in food consumption, was noted in males treated with 1000 mg/kg/day compared to controls during week 1. This effect is considered of questionable toxicological significance since no body weight gain effects were noted in high-dose males during weeks 2-4. Overall body weight gain for high-dose males was 20% lower than controls and was attributed to the decrease observed during week 1. No effects on body weight gain were noted in females or in low- or mid-dose males. Body weight and body weight gain data are summarized in Table 2.

TABLE 2. Average body weights and body weight gains during 28 days of treatment

Dose rate (ppm)	Body Weights (g)					Total Weight Gain	
	Day -1	Day 7	Day 14	Day 21	Day 28	g	% of control
Male							
0	279 ± 16.0	297 ± 22.0	330 ±25.7	363 ±28.6	380 ± 29.5	101 ± 19.5	100
100	279 ± 16.7	296 ± 16.5	331 ± 19.4	358 ± 23.2	371 ± 27.0	92 ± 15.3	91
300	278 ± 14.1	296 ± 10.8	322 ± 18.0	350 ±20.6	366 ± 23.4	87 ± 14.9	86
1000	279 ± 16.6	286 ± 18.6	315 ± 20.4	343 ± 24.8	358 ± 26.7	80* ± 13.9	79
Female							
0	188 ± 8.3	196 ± 8.3	213 ± 9.6	225 ± 12.8	234 ± 14.2	45 ± 11.6	100
100	188 ± 9.1	196 ± 8.3	214 ± 10.7	226 ± 8.3	231 ± 9.0	44 ± 4.9	98
300	188 ± 9.2	197 ± 11.3	219 ± 10.0	227 ± 10.1	233 ± 9.6	45 ± 7.3	100
1000	188 ± 8.0	195 ± 7.9	214 ± 11.7	219 ± 14.1	229 ± 12.1	42 ± 11.4	93

[a] Data obtained from pages (56-57) in the study report.
* Significantly different ($p<0.05$) from the control.

C. Food consumption:

1. Food consumption - There were sporadic differences in food consumption for mid- and high-dose males and females; however, the effects are considered toxicologically insignificant due to the small magnitude of change, lack of a clear dose-response relationship, and the fact that food consumption was decreased in males and increased in females. Statistical significance ($p<0.05$) was achieved for mid-dose males during days 14-21 (9.7% decrease) and for high-dose males during days 1-7 (8.7% decrease) and days 14-21 (9.7% decrease). Statistical significance ($p<0.05$) was achieved for mid- and high-dose females during days 7-14 (10% increase).

2. Food efficiency Food efficiency was decreased (55% decrease, N.S.) for high-dose males during days 1-7, correlating with the decreased body weight gain. Food efficiency was also decreased (24% decrease, $p<0.05$) for mid-dose males from days 7-14.

D. Ophthalmoscopic examination: No treatment-related effects were noted.

E. Blood analyses

1. Haematology - No treatment-related effects were noted.

2. Clinical Chemistry - No treatment-related effects were noted.

F. Urinalysis Not performed.

G. Sacrifice and Pathology:

1. Organ weight - No treatment-related effects were noted.

2. Gross pathology - No treatment-related effects were noted.

3. Microscopic pathology - No treatment-related effects were noted.

III. DISCUSSION

A. Investigators' conclusions: "Based on the results of this study, daily dermal administration of TI-435 to male and female Crl:CD®(SD)IGS BR rats for at least 28 consecutive days is well-tolerated at dose levels up to and including 1,000 mg/kg/day. The no-observable-effect level is considered to be 1,000 mg/kg/day."

B. Reviewer comments:
There were no toxicologically significant treatment-related deaths. One female treated with 100 mg/kg/day and one female treated with 1000 mg/kg/day died on day 15; however, in the absence of clinical observations and macroscopic or microscopic compound-related pathology, these deaths are of questionable toxicological significance. There were no compound- related dermal observations, clinical signs, or effects on motor activity, opthalmoscopic observations, body weights, food consumption, organ weights, or gross or histopathology. The decrease in mean body weight gain noted in males treated with 1000 mg/kg/day during week 1 is considered treatment-related but not adverse since no body weight gain effects were noted in high-dose males during weeks 2-4. Overall body weight gain for high-dose males was 20% lower than controls and was attributed to the decrease observed during week 1.

Based on the results of this study, the systemic and dermal LOAELs for TI-435 in male and female rats are not identified, and the systemic and dermal NOAELs are the limit dose of 1000 mg/kg/day.

C. Study deficiencies: None noted.

DATA EVALUATION RECORD

CLOTHIANIDIN (TI-435)

STUDY TYPE: PRENATAL DEVELOPMENTAL TOXICITY STUDY - RAT [ORAL] [OPPTS 870.3700a (§83-3a)] OECD 414.
MRID 45422711 (main study) 45422710

Prepared for

Health Effects Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
1921 Jefferson Davis Highway
Arlington, VA 22202

Prepared by

Toxicology and Hazard Assessment Group
Life Sciences Division
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Disclaimer

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

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Date 12/10/04
Template version 11/01

DATA EVALUATION RECORD
TXR#: 0050321

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

PC CODE: 044309

DP BARCODE: D282578
SUBMISSION NO.: S614462

TEST MATERIAL (PURITY): TI-435 (Clothianidin (TI-435) (95.2% a.i.)

SYNONYMS: none stated

CITATION: York, R.G. (1998). Oral (gavage) developmental toxicity study of TI-435 in Rats. Argus Research Laboratories, Inc., 905 Sheehy Drive, Horsham, PA 19044. Report No. 109971, April 16, 1998. MRID 45422711. Unpublished

York, R.G. (1998). Oral (gavage) Dosage-Range Developmental Toxicity Study of TI-435 in Rats. Argus Research Laboratories, Inc., 905 Sheehy Dr., Horsham, PA 19044. Report 109996, January 15, 1998, MRID 45422710. Unpublished.

SPONSOR: Takeda Chemical Industries Ltd., Agro Company, 13-10, Nihonbashi 2-chome, Chuo-ku, Tokyo 103, Japan.

EXECUTIVE SUMMARY: In a developmental toxicity study (MRID 45422711) TI-435 (95.2% a.i., Lot # 30037120) was administered to 25 Crl:CD®BR VAF/Plus® (Sprague-Dawley) rats/dose by gavage (in a vehicle of 0.5% methyl cellulose) at dose levels of 0(vehicle), 10, 40, or 125 mg/kg bw/day from days 6 through 19 of gestation (dosage volume of 10ml/kg adjusted daily on the basis of individual weights before intubating). On gestation day (GD) 20, dams were sacrificed, subjected to gross necropsy, and all fetuses examined externally. The total numbers of fetuses examined (number of litters) for the 0, 10, 40, and 125 mg/kg bw/day groups were 299 (23), 294 (22), 330 (24), and 340 (25), respectively. Approximately one-half of the fetuses were examined viscerally, and the other one-half of the fetuses were examined for skeletal malformations/variations.

No statistically significant decreases in food consumption, body weight, or body weight gain were observed in the 10 mg/kg/day group. Dams in the 40 mg/kg/day group had decreased mean body weight gain ($p<0.01$) and decreased food consumption ($p<0.05$) over GDs 6-9. Mean body weight gain, body weight and food consumption values of the 40 mg/kg/day group were comparable to controls for the other treatment intervals including the treatment interval of GDs

6-20. The 125 mg/kg/day group had statistically significant decreased mean absolute body weights during GD 7-20 ($p<0.05$; 0.01), as well as decreased ($p<0.01$) corrected final body weights (93% of control), decreased ($p<0.01$) body weight gains over the intervals GD 6-9 and GD 6-20, and decreased corrected body weight gains for GDs 6-20 (55% of controls). With the exception of GDs 18-20, the highest dose group had statistically significant decreased food consumption at all treatment intervals ($p<0.01$). Overall food consumption of the 125 mg/kg/day group during the interval GDs 6-20 was 83% of controls.

Treatment with TI-435 did not adversely affect clinical signs or gross necropsy findings.

The maternal LOAEL is 40 mg/kg bw/day based on decreased body weight gain and food consumption. The maternal NOAEL is 10 mg/kg bw/day.

No treatment-related, statistically significant effects on pregnancy rates, number of corpora lutea, pre- or post-implantation losses, resorptions/dam, fetuses/litter, fetal body weights, or fetal sex ratios were observed in the treated groups as compared with the controls.

No treatment-related external, visceral, or skeletal malformations/variations were observed in any groups.

The developmental NOAEL is ≥ 125 mg/kg bw/day, and a developmental LOAEL could not be established.

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 the rat.

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

I. MATERIALS AND METHODS

A. MATERIALS:

1. Test material: TI-435

Description:	Pale, yellow powder
Lot/Batch #:	30037120
Purity:	95.2 % a.i.
Compound Stability:	Stored refrigerated protected from light
CAS #of TGAI:	210880-92-5
Structure:	Not available

2. Vehicle and/or positive control: aqueous 0.5% methylcellulose, Lot # 56H0439

3. Test animals:

Species:	Rat	
Strain:	Crl:CD®BR VAF/Plus (Sprague-Dawley)	
Age/weight at study initiation:	63 days; 201-231 g	
Source:	Charles River Laboratories, Inc., Portage, MI	
Housing:	Individually housed	
Diet:	Certified Rodent Diet #5002 (PMI Feeds, Inc., St. Lois, MO) *ad libitum*	
Water:	Reverse osmosis water *ad libitum*	
Environmental conditions:	**Temperature:**	18-26°C
	Humidity:	30-70%
	Air changes:	10 changes/hr
	Photoperiod:	12 hrs dark/12 hrs light
Acclimation period:	6-10 days	

B. PROCEDURES AND STUDY DESIGN:

1. **In life dates:** Start: May 27, 1997; End: June 19, 1997

2. **Mating:** Sexually mature, virgin females were mated with sexually mature males (one male rat per female rat in the male rat's cage). Confirmation of mating was determined by detection of sperm in a vaginal smear or a copulatory plug and was designated as day 0 of gestation.

3. **Animal assignment:** Animals were assigned to dose groups as indicated in Table 1 based on a computer-generated (weight-ordered) randomization procedure based on body weights.

TABLE 1. Animal assignment

Dose (mg/kg bw/day)	0	10 (LDT)	40 (MDT)	125 (HDT)
# Females	25	25	25	25

4. **Dose selection rationale:** The dose levels were selected based on the results from a developmental range-finding study (MRID 45422710) where groups of 8 presumed pregnant Sprague-Dawley rats/sex/dose were administered TI-435 orally by gavage at dose levels of 0,

125, 250, 500, or 1000 mg/kg bw/day from days 6 through 19 of gestation (see Appendix for details). Maternal toxicity was evident at all dosages as indicated by decreased body weights and food consumption. Dosages of 250 mg/kg/day and greater resulted in treatment-related clinical signs, and all 8 dams in the 1000 mg/kg/day group died before study termination. Cesarean section findings included a dose-related decrease in gravid uterine weights in all groups, and an increased average number of early resorptions, decreased average litter size, increased percentage of resorbed conceptuses, and decreased average fetal body weights as compared with concurrent controls and/or historical controls. The 500 mg/kg/day group had 6 dams with totally resorbed litters, and a total of only 3 fetuses. The 125 and 250 mg/kg/day group had a comparable number of corpora lutea, implantations, and sex ratio as controls. External examination of the fetuses revealed one 250 mg/kg/day group fetus with multiple malformations (short snout, flexed fore and hindlimbs, curled tail, exencephaly, gastroschisis, depressed eye bulges and short trunk) and anasarca in all three fetuses in the 500 mg/kg/day group (one additionally had a short trunk while another also had a short trunk, short tail and umbilical hernia). Based on these data, dosages of 0, 10, 40, and 125 mg/kg/day were recommended for the developmental toxicity study in rats.

5. **Dosage preparation and analysis:** Test material-vehicle mixture was prepared weekly at concentrations of 0, 1, 4, and 12.5 mg/mL by mixing appropriate amounts of test substance with 0.5% methylcellulose. As part of the rat range-finding developmental study (MRID 45422710), stability of the test mixture (in 0.5% methylcellulose) at a concentration of 1 and 200 mg/mL was evaluated for a period of 7 and 14 days (temperature not provided). Homogeneity (top, middle, and bottom) of 1, 12.5, and 100 mg/mL test mixtures was evaluated in previous studies: the 1 mg/mL test mixture in a rabbit developmental study (MRID 45422713) and the 12.5 and 100 mg/mL test mixtures in the rat range-finding developmental study (MRID 45422710). Concentrations of 0, 1, 4, and 12.5 mg/mL test mixtures were evaluated at the beginning and the end of the dosing period of this study (MRID 45422711).

Results:

Homogeneity analysis: The mean concentrations of the samples taken from the top, middle, and bottom of the 1, 12.5 and 100 mg/mL test mixtures ranged from 97.5-106% of nominal, 101-108% of nominal, and 97.4-102% of nominal, respectively, indicating homogenous distribution of the test compound in the solutions.

Stability analysis: The mean concentrations of 1 mg/mL and 200 mg/mL test mixtures at Day 7 as a percentage of nominal were 103% and 95.5%, respectively; and at day 14 were 101% and 94.5% of nominal, respectively.

Concentration analysis: The mean concentrations of the 1, 4, and 12.5 mg/mL test mixtures ranged from 98.4-115%, 99.0-104.5%, and 100.8-102.4% of nominal, respectively.

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 oral gavage, on gestation days 6 through 19, in a volume of 10 mL/kg of body weight/day. Dosing was based on the most recent body weight determination.

C. OBSERVATIONS:

1. **Maternal observations and evaluations**: The animals were checked for mortality twice per day and for clinical signs before and one hour after dosing (GDs 6-19) and on GD20. Body weights were recorded on GD 0 and GD 6-20, and food consumption data were recorded on GDs 0, 6, 9, 12, 15, 18, and 20. Dams were sacrificed on day 20 of gestation. Examinations at sacrifice consisted of: gross necropsy of the thoracic, abdominal, and pelvic viscera; staining of the uteri from apparent nonpregnant rats; excise and weighing of gravid uteri; recording the number of corpora lutea in each ovary; and determining the number and distribution of viable and nonviable fetuses, early and late resorptions, and implantations.

2. **Fetal evaluations:** The fetuses were examined in the following manner: each fetus was removed from the uterus, weighed, and examined for sex and gross alterations. Approximately one-half of the fetuses in each litter were examined for soft tissue alterations by using a variation of the technique of Staples. The heads of the fetuses were fixed in Bouin's solution and examined by free-hand sectioning. The remaining fetuses in each litter were eviscerated, cleared, and stained with alizarin red S and alcian blue for skeletal examinations.

D. DATA ANALYSIS:

1. **Statistical analyses:** The Variance Test for Homogeneity of the Binomial Distribution was used to analyze clinical observation and other proportion data. Maternal body weights and body weight changes, feed consumption, litter averages for percent male fetuses, percent resorbed conceptuses, fetal body weights, fetal anomaly data, and fetal ossification site data were analyzed for homogeneity of variance using Bartlett's Test of Homogeneity of Variance. If variances were homogenous, the data were analyzed using the Analysis of Variance, followed by Dunnett's Test if significant. The Kruskal-Wallis Test was used when Bartlett's was significant (i.e., variances were not homogeneous). Dunn's Method of Multiple Comparisons was used if the Kruskal-Wallis Test was significant. If there were greater than 75% ties, Fisher's Exact Test was used to analyze the data. The count data obtained at Cesarean-sectioning of the dams was analyzed using the procedures already described for the Kruskal-Wallis Test.

2. **Indices:** No cesarean section indices (conception rate, pre-implantation loss, post-implantation loss) were calculated.

3. **Historical control data:** Historical control data were provided to allow comparison with concurrent controls. Data (reproductive indices, maternal necropsy observations, fetal external alterations, fetal soft tissue alterations, fetal skeletal alterations, and a summary of averages of fetal ossification sites) were obtained from studies in CD rats collected over a period of 2 years.

II. RESULTS:

A. MATERNAL TOXICITY:

1. **Mortality and clinical observations:** All animals survived to terminal sacrifice. No treatment-related clinical signs were observed. Alopecia was observed in all groups and was not related to dose.

2. **Body weight** - Mean body weight data is given in Table 2 and body weight gain data is summarized in Table 3. Statistically significant decreases in body weights were observed in the high-dose dams beginning at GD 7 and continuing throughout the study (93-96% of controls; $p<0.05$; 0.01). High-dose dams also had decreased corrected maternal body weights (GD 20 b.w.-gravid uterine weight), decreased body weight gains over GD 6-9 and 6-20, and decreased corrected body weight gains for GDs 6-20. Mid-dose females had statistically decreased body weight gains for GDs 6-9 ($p<0.01$). No other statistically significant differences were observed.

TABLE 2. Mean (± SD) maternal body weight (g) [a]

Gestation Day	Dose in mg/kg bw/day			
	0	10	40	125
0	214.3 ± 8.2	213.4 ±7.8	213.9 ±7.6	214.7 ±7.8
6	239.7 ± 13.6	240.9 ±10.4	241.6 ±8.8	240.8 ±11.6
7	241.6 ±13.8	240.9 ±11.4	240.5 ±10.3	232.4 * ±10.9
8	247.0 ±11.2	245.8 ±11.6	242.1 ±10.5	229.7 ** ±9.8
9	251.6 ±10.9	250.3 ±12.0	248.4 ±8.4	234.7 ** ±10.6
10	257.0 ±12.4	253.9 ±12.2	253.4 ±10.0	240.9 ** ±11.0
11	263.9 ±12.1	262.4 ±12.7	259.4 ±10.3	247.6 ** ±11.3
12	268.6 ±13.8	268.4 ±14.8	265.0 ±11.9	252.3 ** ±12.4
13	275.4 ±14.9	273.0 ±14.3	270.7 ±12.0	257.8 ** ±11.9
14	279.8 ±14.0	278.3 ±15.6	275.4 ±11.5	262.7 ** ±13.0
15	286.3 ±15.2	286.4 ±16.0	281.8 ±12.0	269.1 ** ±14.4
16	296.3 ±14.5	297.0 ±17.5	293.5 ±11.5	278.3 ** ±15.8
17	308.8 ±14.9	310.4 ±19.5	306.5 ±13.0	288.7 ** ±16.5
18	324.1 ±16.2	324.7 ±18.9	320.6 ±13.8	303.0 ** ±17.4
19	338.2 ±17.8	337.9 ±19.9	334.7 ±15.8	320.1 ** ±19.7
20	360.6 ±22.0	362.7 ±22.8	358.6 ±18.1	341.6 ** ±21.6

[a] Data obtained from Table 3, p. 42; MRID 45422711
* Statistically different ($p<0.05$) from the control.
** Statistically different ($p<0.01$) from the control.

TABLE 3. Mean (±SD) maternal body weight gain (g) [a]				
Interval	Dose in mg/kg bw/day (# of Dams)			
	0 (23)	10 (22)	40 (24)	125 (25)
Pretreatment: GDs 0-6	25.5 ± 10.1	26.7 ± 6.7	27.7 ± 6.7	26.1 ± 7.6
Treatment: GDs 6-9	11.8 ± 6.2	10.2 ± 4.3	6.8** ± 5.1	-6.1** ± 7.1
Treatment: GDs 6-20	120.9 ± 18.3	122.6 ± 15.0	117.0 ± 14.1	100.8** ± 13.6 (83) [b]
Corrected BW Gain GDs 6-20	46.7 ± 11.4	46.4 ± 16.1	37.7 ± 11.2	25.8** ± 9.9 (55)

[a] Data obtained from Table 4, p. 44; MRID 45422711.
[b] Percentage of controls; calculated by reviewer.
** Statistically different ($p < 0.01$) from the control.

3. **Food consumption:** Selected mean maternal food consumption data are summarized in Table 4. High-dose females had decreased food consumption values at all treatment intervals (53-90% of controls; $p<0.01$) except for GD 18-20, with the largest decrease occurring over GDs 6-9. Over the entire treatment interval of GDs 6-20, food consumption values for high-dose dams was 83% of controls ($p<0.01$). Mid-dose dams had a statistically significant decrease in food consumption over GDs 6-9 (91% of controls; $p<0.05$). No other statistically significant differences in food consumption values were observed.

TABLE 4 Selected mean (±SD) maternal food consumption (g/animal/day) [a]				
Interval	Dose in mg/kg bw/day (# of Dams)			
	0 (23)	10 (22)	40 (24)	125 (25)
Pretreatment: GD 0 - 6	19.6 ± 2.3	20.0 ± 2.5	19.7 ± 1.4	20.2 ± 2.2
GD 6-9	20.9 ± 1.7	20.5 ± 2.4	19.0* ± 2.4 (91) [b]	11.1** ± 2.9 (53)
GD 12-15	24.0 ± 2.0	24.0 ± 3.0	23.0 ± 2.4 [c]	21.3** ± 2.4 (89)
GD 18-20	24.9 ± 2.1	25.4 ± 3.2	24.4 ± 2.9 [c]	24.0 ± 3.2
Treatment: GD 6-20	23.6 ± 1.5	23.6 ± 2.1	22.7 ± 1.5 [c]	19.5** ± 2.0 (83)

[a] Data obtained from Tables 5, pages 45; MRID 45422711.
[b] Percentage of controls; calculated by reviewer.
[c] N = 21
* Statistically different ($p < 0.05$) from the control.
** Statistically different ($p < 0.01$) from the control.

4. **Gross pathology:** No gross pathological changes related to the substance were observed.

5. **Cesarean section data:** Data are summarized in Table 5. No statistically significant, substance-related changes were observed. The study author did not calculate pre-

implantation loss or post-implantation loss; therefore, the reviewer calculated these indices (see Table 5). No treatment-related changes were observed in these indices.

TABLE 5 Cesarean section observations [a]

Observation	Dose (mg/kg bw/day)			
	0	10	40	125
# Animals Assigned (Mated)	25	25	25	25
# Animals Pregnant	23	22	24	25
Pregnancy Rate (%)	92	88	96	100
# Nonpregnant	2	3	1	0
Maternal Wastage				
# Died	0	0	0	0
# Died Pregnant	0	0	0	0
# Died Nonpregnant	0	0	0	0
# Aborted	0	0	0	0
# Premature Delivery	0	0	0	0
Total # Corpora Lutea	365	347	381	400
Corpora Lutea/Dam	15.9 ± 2.3	15.8 ± 2.5	15.9 ± 2.0	16.0 ± 2.2
Total # Implantations	314	314	340	357
(Implantations/Dam)	13.6 ± 2.8	14.3 ± 1.6	14.2 ± 2.2	14.3 ± 1.7
Total # Litters	23	22	24	25
Total # Live Fetuses	299	294	330	340
(Live Fetuses/Dam)	13.0 ± 3.0	13.4 ± 1.7	13.8 ± 2.0	13.6 ± 1.7
Total # Dead Fetuses	0	0	0	0
Total # Resorptions	15	20	10	17
Early	14	20	10	15
Late	1	0	0	2
Resorptions/Dam	0.6 ± 1.0	0.9 ± 1.0	0.4 ± 0.6	0.7 ± 0.7
Early	0.6 ± 0.9	0.9 ± 1.0	0.4 ± 0.6	0.6 ± 0.8
Late	0.0 ± 0.2	0.0 ± 0.0	0.0 ± 0.0	0.1 ± 0.3
Litters with Total Resorptions	0	0	0	0
Mean Fetal Weight (g)	3.52 ± 0.31	3.50 ± 0.18	3.55 ± 0.24	3.34 ± 0.33
Males	3.60 ± 0.32	3.57 ± 0.19	3.63 ± 0.21	3.40 ± 0.33
Females	3.43 ± 0.33	3.44 ± 0.19	3.46 ± 0.26	3.28 ± 0.34
Sex Ratio (% Male)	49.3 ± 14.4	49.1 ± 9.3	52.0 ± 11.2	48.0 ± 13.4
Preimplantation Loss (%) [c]	14.0	9.5	10.8	10.8
Postimplantation Loss (%) [d]	4.8	6.4	2.9	4.8
Mean Gravid Uterine Weight (g)	74.2 ± 15.0	76.2 ± 14.2	79.3 ± 11.1	75.1 ± 7.9

[a] Data obtained from Tables 3, 7-8, and 18; pp. 43, 47-48 76-79; MRID 45422711.
[b] Totaled by reviewer using Table 18, pp. 76-79.
[c] Calculated by reviewer using the following equation: $\frac{\text{\# corpora lutea - \# implantations}}{\text{\# corpora lutea}} \times 100$
[d] Calculated by reviewer using the following equation: $\frac{\text{\# implantations - \# live fetuses}}{\text{\# implantations}} \times 100$

B. DEVELOPMENTAL TOXICITY:

1. **External examination:** External examination data are presented in Table 6. No treatment-related external malformations or variations were observed. In the control group, one fetus had an umbilical hernia, and another fetus from another litter had whole body edema (anasarca). A depressed eye bulge was observed in one fetus from the low-dose and one fetus from the mid-dose group. No other findings were reported.

2. **Visceral examination:** Visceral examination data are presented in Table 6. No treatment-related visceral alterations were noted. Two control fetuses from two litters and one low-dose fetus had the umbilical artery descending to the left of the bladder One mid-dose fetus had microphthalmia (this is the same fetus that had a depressed eye bulge noted during external examination), while another mid-dose fetus had an absent innominate artery. One high-dose fetus had multiple soft-tissue variations including an absent innominate artery, aortic arch passing dorsal to the trachea and esophagus, and the left carotid arising to the right of the right subclavian.

3. **Skeletal examination:** Skeletal examination data are presented in Table 6. No treatment-related skeletal malformations or variations were noted. Malformations were present as follows: one control fetus had a short fibula and tibia (the same one that had anasarca noted during external examination), one low-dose fetus had a small eye socket (had depressed eye bulge noted during visceral examination), and one high-dose fetus had fused ribs (also had bifid centrum in the 12th thoracic vertebra). The only statistically significant difference observed was a decreased incidence of wavy ribs in all dose groups as compared to the controls. The most common skeletal variations were incomplete ossification or unossification. The study author analyzed the average numbers of fetal ossification sites per litter and did not find any biologically or statistically significant differences among the groups.

TABLE 6. External, visceral, and skeletal examinations [a]				
Observations [b]	Dose (mg/kg bw/day)			
	0	10	40	125
External examinations				
#Fetuses(litters) examined	299(23)	294(22)	330(24)	340(25)
#Fetuses(litters) affected with malformations [c]	2(2)	1(1)	1(1)	0(0)
Visceral examinations				
#Fetuses(litters) examined	143(23)	142(22)	160(24)	164(25)
#Fetuses(litters) affected with malformations [c]	0(0)	0(0)	1(1)	0(0)
#Fetuses(litters) affected with variations [c]	2(2)	1(1)	1(1)	1(1)
Skeletal examinations				
#Fetuses(litters) examined	156(23)	152(22)	170(24)	176(25)
#Fetuses(litters) affected with malformations [c]	1(1)	1(1)	0(0)	1(1)
#Fetuses(litters) affected with variations [c]	11(7)	8(8)	11(9)	16(10)

[a] Data obtained from Tables 10-13 and 21, pp. 50-54 and 92-104; MRID 45422711.
[b] Some observations may be grouped together.
[c] Reviewer totaled the number of fetuses(litter) affected.

III. DISCUSSION AND CONCLUSIONS:

A. INVESTIGATORS' CONCLUSIONS: The investigators concluded that the NOAEL for TI-435 is 10 mg/kg/day. The LOAEL is 40 mg/kg/day based on the decreased body weight gain and food consumption observed over GDs 6-9. The developmental NOAEL is greater than 125 mg/kg/day. There were no adverse effects on embryo-fetal development as evaluated in the study.

B. REVIEWER COMMENTS:

1. **Maternal toxicity:** Maternal toxicity was evident at a dose of 40 or 125 mg/kg/day as indicated by decreased body weight gain and food consumption. Dams in the mid-dose group had decreased mean body weight gain accompanied by decreased food consumption over GDs 6-9. High-dose dams generally had decreases in mean absolute body weights and food consumption throughout the study, with significant decreases in body weight gain noted during treatment intervals 6-9 and 6-20. High-dose dams additionally had decreased values for the final corrected body weight and corrected body weight gain for GDs 6-20. Treatment with TI-435 did not result in abnormal clinical signs or gross necropsy observations.

   Therefore, the maternal LOAEL is 40 mg/kg bw/day based on decreased body weight gain and food consumption, and the maternal NOAEL is 10 mg/kg bw/day.

2. **Developmental toxicity:**

   a. **Deaths/resorptions:** Treatment of pregnant rats with TI-435 did not result in increased embryonic or fetal death.

   b. **Altered growth:** Treatment with TI-435 did not result in any alterations of fetal growth.

   c. **Developmental variations:** No adverse, treatment-related increases in developmental variations were observed. Variations noted occurred equally in all groups or at low incidences.

   d. **Malformations:** No major fetal malformations could be attributed to maternal treatment with TI-435.

   Therefore, the developmental NOAEL is ≥ 125 mg/kg bw/day, and a developmental LOAEL could not be established.

C. **STUDY DEFICIENCIES:** No major deficiencies were observed.

APPENDIX: Oral (gavage) Dosage-Range Developmental Toxicity Study of TI-435 in Rats

TEST MATERIAL (PURITY): TI-435 (96.0% a.i.)

CITATION: York, R.G. (1998) Oral (gavage) dosage-range developmental toxicity study of TI-435 in rats. Argus Research Laboratories, Inc., 905 Sheehy Dr., Horsham, PA 19044. Report 109996, January 15, 1998, MRID 45422710. Unpublished.

In a 28-day oral toxicity study (MRID 45422710) TI-435 [(96.0% a.i., Lot nos.30032305 and 30034708)] was administered to 8 presumed pregnant Crl:CD®BR VAF/Plus® (Sprague-Dawley) rats/dose by gavage at dose levels of 0, 125, 250, 500, or 1000 mg/kg bw/day from days 6 through 19 of gestation. On gestation day 20, dams were sacrificed and the following observations recorded: number and distribution of corpora lutea, implantation sites and uterine contents; results of gross necropsy of the thoracic, abdominal, and pelvic viscera; gravid uterine weights; and spleen weights. Fetuses were examined for gross external alterations, gender, and were weighed.

Maternal toxicity was evident at all dosages. All 8 animals in the 1000 mg/kg/day group died before study termination: 5 were found dead and 3 were sacrificed in moribund condition. Clinical signs observed included red perivaginal substance and scant feces in dams dosed with 250 mg/kg/day or greater; decreased motor activity, tremors, chromorhinorrhea, ptosis, coldness to touch, emaciation, dehydration, piloerection, urine-stained abdominal fur, and no stool in dams dosed with 500 mg/kg/day or higher; and discolored urine and chromodacryorrhea in dams dosed with 1000 mg/kg/day. No treatment-related clinical signs were observed in dams dosed with 125 mg/kg/day.

Necropsy revealed small spleens in 0, 0, 1, 6, and 7 animals dosed with 0, 125, 250, 500, or 1000 mg/kg/day, respectively. Absolute spleen weights were reduced in the 500 mg/kg/day group (55% of controls), but spleen weight relative to body weight was not significantly reduced (94% of controls).

Treatment with all dosages resulted in decreased body weights and food consumption. Corrected maternal body weights were 88, 81, and 69% of controls for the 125, 250, and 500 mg/kg/day groups, respectively. Body weight losses were observed in all treatment groups over GDs 6-9, and body weight gains over the treatment interval of GDs 6-20 were 70 and 42% of controls for the 125 and 250 mg/kg/day groups, respectively. The 500 mg/kg/day group lost weight over this interval. The corrected body weight gain for the 125 mg/kg bw/day group during GDs 6-20 was 25% of the controls, while the 250 and 500 mg/kg bw/day groups had decreases in weight during the treatment interval. Decreases in absolute and relative food consumption accompanied the body weight decreases. Total food consumption over GDs 6-20 was 76, 56, and 31% of controls for the 125, 250, and 500 mg/kg/day groups, respectively.

Therefore, the maternal toxicity LOAEL was ≤ 125 mg/kg/day based on decreased body weights and food consumption, and a maternal toxicity NOAEL was not established.

A dose-related decrease in gravid uterine weights was observed (92.7, 74.2, and 4.9% of controls for the 125, 250, and 500 mg/kg/day groups, respectively). Dams from the 250 and 500

mg/kg/day groups had an increased average number of early resorptions (1.8 and 13.8 times the control, respectively), a decreased average litter size (96% and 3% of the control litter size, respectively), and an increased percentage of resorbed conceptuses (10.3% and 89.2%, respectively compared to 6% for the control). The 500 mg/kg/day group had 6 dams with totally resorbed litters, and a total of only 3 fetuses. Average fetal body weights were decreased in the 250 and 500 mg/kg/day groups (2.57 g and 1.04 g, respectively) compared to concurrent controls (3.50 g) and historical controls (3.46 g). Although the average fetal body weights were decreased in the 125 mg/kg/day group (3.22 g), they were within the historical control range (3.10-3.72 g). The 125 and 250 mg/kg/day groups had a comparable number of corpora lutea, implantations, and sex ratio as compared to controls.

The total number of fetuses examined externally for alterations in the 0, 125, 250, 500, and 1000 mg/kg/day groups were 116, 103, 70, 3, and 0 fetuses, respectively. One 250 mg/kg/day group fetus had multiple malformations including a short snout, flexed fore and hindlimbs, curled tail, exencephaly, gastroschisis, depressed eye bulges and short trunk. All three fetuses in the 500 mg/kg/day group had anasarca: one additionally had a short trunk while another also had a short trunk, short tail and umbilical hernia.

Dosages of 0, 10, 40, and 125 mg/kg/day were recommended for the developmental toxicity study in rats. The 125 mg/kg/day dosage was expected to produce maternal toxicity and minimal developmental toxicity.

DATA FOR ENTRY INTO ISIS

Developmental Study - rats (870.3700a)

PC code	MRID	Study	Species	Duration	Route	Admin	Dose range mg/kg/day	Doses mg/kg/day	NOAEL mg/kg/day	LOAEL mg/kg/day	Target organ	Comments
044309	45422711, 45422710	developmental	rats	GD 6-19	oral	gavage	10-125	0, 10, 40, 125	10	40	decreased body weight gain and food consumption	Maternal
044309	45422711, 45422710	developmental	rats	GD 6-19	oral	gavage	10-125	0, 10, 40, 125	≥ 125	not established	none identified	Developmental

473

HED Records Center Series 361 Science Reviews - File R107689 - Page 488 of 721

DATA EVALUATION RECORD

CLOTHIANIDIN (TI-435)

STUDY TYPE: PRENATAL DEVELOPMENTAL TOXICITY STUDY - RABBIT [ORAL] [OPPTS 870.3700b (§83-3b]; OECD 414. MRID 45422713 (main study), 45422712

Prepared for

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Task No. 02-44B

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Template version 11/01

DATA EVALUATION RECORD
TXR#: 0050321

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

PC CODE: 044309

DP BARCODE: D282578
SUBMISSION NO.: S614462

TEST MATERIAL (PURITY): TI-435 (Clothianidin (TI-435)) (95.2% a.i.)

SYNONYMS: (E)-1-(2-Chloro-5-thiazolylmethyl)-3-methyl-2-nitroguanidine

CITATION: York, R.G. (1998). Oral (stomach tube) developmental toxicity study of TI-435 in rabbits. Argus Research Laboratories, Inc., 905 Sheehy Drive, Horsham, PA 19044. Report No. 1120-002, April 16, 1998. MRID 45422713. Unpublished

York, R.G. (1998). Oral (stomach tube) dosage-range developmental toxicity study of TI-435 in rabbits. Argus Research Laboratories, Inc., 905 Sheehy Dr., Horsham, PA 19044. Report 1120-002P, January 15, 1998, MRID 45422712. Unpublished.

SPONSOR: Takeda Chemical Industries Ltd., Agro Company, 13-10, Nihonbashi 2-chome, Chuo-ku, Tokyo 103, Japan.

EXECUTIVE SUMMARY: In a developmental toxicity study (MRID 45422713), TI-435 (95.2% a.i., Lot # 30037120) dissolved in aqueous 0.5% methylcellulose was administered to 23 female New Zealand White [Hra:(NZW)SPF] rabbits/dose by gavage at dose levels of 0, 10, 25, 75, or 100 mg/kg bw/day from days 6 through 28 of gestation. On gestation day (GD) 29, does were sacrificed, subjected to gross necropsy, and all fetuses examined externally. The total numbers of fetuses examined (number of litters) was 150 (18), 201 (23), 179 (22), 133 (17), and 85 (11) for the 0, 10, 25, 75, and 100 mg/kg bw/day groups, respectively. All of the fetuses were examined viscerally and for skeletal malformations/variations.

Maternal toxicity was evident at doses of 75 mg/kg/day and higher. Increased incidences of scant feces in cage and orange urine were observed in does treated with 75 mg/kg/day and higher. At the 75 mg/kg/day dose, does additionally exhibited mortality (2/23), early delivery (2/23), abortion (1/23), and decreased body weight gain (53% of controls; n.s.) and food consumption (83% of controls; $p<0.05$) for the treatment interval of GDs 6-29. At 100 mg/kg/day, maternal toxicity was additionally indicated by: mortality (3/23); abortions (6/23**); early deliveries (2/23); increased incidence of red substance in the cage pan (8/4 vs. 0/0 for controls; $p<0.05$);

decreased mean absolute body weights starting at GD 14 and continuing throughout the study (90-94% of controls; $p<0.05$; 0.01), loss in body weight over treatment intervals GDs 9-12, 12-15, and 6-29; and decreased food consumption at all treatment intervals (34-90% of controls). No dose-related gross necropsy findings were noted.

The maternal LOAEL is 75 mg/kg/day based on increased incidences of clinical signs (scant feces and orange urine) and mortalities and decreased food consumption. The maternal NOAEL is 25 mg/kg/day.

Developmental toxicity was evident at doses of 75 and 100 mg/kg/day. Two does in each of these dose groups delivered early, six 100 mg/kg/day does aborted, and dose-related decreases in gravid uterine weights were observed (89 and 81% of controls, respectively; n.s.). Examination of cesarean section data revealed a number of differences in the 100 mg/kg/day group, including: an increase in the total number of resorptions (15 vs. 5 for controls; n.s.) and resorptions/doe (1.4 vs. 0.3 for controls; n.s.); total number of early resorptions (10 vs. 2 for controls; n.s.) and early resorptions/doe (0.9 vs. 0.1 for controls; n.s.), and post implantation loss (18.3% vs. 3.3% for controls), and decreased mean fetal body weights (84% of controls; $p<0.01$). One doe from the 100 mg/kg/day group had complete litter resorption, along with two 100 mg/kg/day group does that died early.

External examination of fetuses did not reveal any treatment-related alterations. Visceral examination revealed a statistically increased ($p<0.01$) litter incidence of an absent lung lobe in fetuses from the 75 and 100 mg/kg/day groups [fetus(litter) incidence: 3(3) and 8(5), respectively, vs. 0(0) for controls; 18 and 45% of litters affected, respectively]. Skeletal examination found a statistically decreased ($p<0.01$) litter average for ossified sternal centra per fetus in the 75 and 100 mg/kg/day groups [3.83 and 3.76, respectively, vs. 3.99 for controls]. In the 100 mg/kg/day group, other findings included a statistically increased ($p<0.01$) litter incidence of incomplete ossification of the sternal centra and absent hindpaw phalanges [fetus(litter) incidence for both: 2(2) vs. 0(0) for controls; 18% of litters affected].

The developmental LOAEL is 75 mg/kg bw/day, based on premature deliveries, decreased gravid uterine weights, an increased litter incidence of a missing lobe of the lung and decreased litter average for ossified sternal centra per fetus. The developmental NOAEL is 25 mg/kg bw/day.

The developmental toxicity study in the rabbit is classified **Acceptable/Guideline**, and satisfies the guideline requirement for a developmental toxicity study (OPPTS 870.3700; OECD 414) in rabbits.

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

I. MATERIALS AND METHODS:

A. MATERIALS:

1. Test material: TI-435

Description:	pale, yellow powder
Lot/Batch #:	30037120
Purity:	95.2 % a.i.
Compound Stability:	Stored refrigerated protected from light
CAS #of TGAI:	Not available
Structure:	Not available

2. Vehicle and/or positive control: aqueous 0.5% methylcellulose, Lot # 56H0439

3. Test animals:

Species:	Rabbits	
Strain:	New Zealand White [Hra:(NZW)SPF]	
Age/weight at study initiation:	6-8 months; 3.3-4.6 kg	
Source:	Covance Research Products, Inc., Denver, Pennsylvania	
Housing:	Individually housed in units of 6-8 stainless steel cages	
Diet:	Certified Rabbit Chow® #5322 (PMI Feeds, Inc., St. Louis, MI); 150 g daily from day of arrival to GD 6; 180 g daily from GD 6 to end of study	
Water:	Reverse osmosis water *ad libitum*	
Environmental conditions:	**Temperature:**	16-22°C
	Humidity:	30-70%
	Air changes:	minimum of 10 changes/hr
	Photoperiod:	12 hrs dark/12 hrs light
Acclimation period:	1-5 days	

B. PROCEDURES AND STUDY DESIGN:

1. In life dates: Start: May 24, 1997 ; End: June 20, 1997

2. Mating: The female rabbits were naturally bred by breeder male rabbits of the same source and strain before shipment to the testing laboratory. Rabbits were mated on five consecutive days and shipped the day after the last day of mating. The day of mating was designated as gestation day 0.

3. Animal assignment: Animals were randomly assigned to dose groups as indicated in Table 1 using a computer-generated (weight-ordered) randomization procedure based on GD 0 body weights.

TABLE 1: Animal assignment

Dose (mg/kg bw/day)	0	10	25	75	100
# Females	23	23	23	23	23

4. **Dose selection rationale:** The dose levels were selected based on the results from a developmental range-finding study (MRID 45422712) in which groups of 5 time-pregnant New Zealand White rabbits/dose were administered TI-435 orally by gavage at dose levels of 0, 62.5, 125, 250, or 500 mg/kg bw/day from days 6 through 28 of gestation (see Appendix for details). Maternal toxicity was evident at all doses. The 62.5 mg/kg/day group had an increased incidence of dark urine. All does in the 125, 250, and 500 mg/kg/day groups died or were sacrificed early. Treatment-related clinical signs, body weight loss, and decreased food consumption were observed throughout treatment in does treated with 125 mg/kg/day or higher. No abnormal gross necropsy findings were observed. All animals were pregnant, but only 5, 4, 0, 0, and 0 does in the 0, 62.5, 125, 250, or 500 mg/kg/day groups were subjected to Cesarean section because of mortality or early sacrifice. The 62.5 mg/kg/day group had a comparable number of corpora lutea, implantations, litter sizes, early and late resorptions, fetal weights, and sex ratio compared to controls. No treatment-related, external fetal alterations were observed. Based on these data, doses of 0, 10, 25, 75, and 100 mg/kg/day were recommended for the developmental toxicity study in rabbits.

5. **Dose preparation and analysis**: Test material-vehicle mixture was prepared weekly at concentrations of 0, 1, 2.5, 7.5, or 10 mg/mL by mixing appropriate amounts of test substance with 0.5% methylcellulose. As part of another study conducted by the same laboratory (rat range-finding developmental study; MRID 45422710), stability of the test mixture (in 0.5% methylcellulose) at concentrations of 1 and 200 mg/mL was evaluated for a period of 7 and 14 days (temperature not provided). Homogeneity (top, middle, and bottom) of 1 mg/mL test mixtures was evaluated in this rabbit developmental study (45422713) and the 12.5 and 100 mg/mL test mixtures in the rabbit range-finding developmental study (MRID 45422712). Concentrations of 0, 1, 2.5, 7.5 and 10 mg/mL test mixtures were evaluated at the beginning and the end of the dosing period of this study (MRID 45422713).

Results:

Homogeneity analysis: The mean concentrations of the samples taken from the top, middle, and bottom of the 1, 12.5 and 100 mg/mL test mixtures ranged from 97.5-106% of nominal, 101-107% of nominal, and 96.5-105% of nominal, respectively, indicating homogenous distribution of the test compound in the solutions.

Stability analysis: The mean concentrations of 1 mg/mL and 200 mg/mL test mixtures at Day 7 as a percentage of nominal were 103% and 95.5%, respectively; and at day 14 were 101% and 94.5% of nominal, respectively.

Concentration analysis: The mean concentrations of the 1, 2.5, 7.5, and 10 mg/mL test mixtures ranged from 101%, 95.6-98%, 103.6-104%, and 101-103% of nominal, respectively.

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

6. **Dose administration:** All doses were administered once daily by oral gavage, on gestation days 6 through 28, in a volume of 10 mL/kg body weight/day. Dosing was based on the most recent body weight determination.

C. OBSERVATIONS:

1. **Maternal observations and evaluations**: The animals were checked for mortality or clinical signs twice daily. Body weight and food consumption data were recorded daily during GDs 6-29. Body weights were additionally recorded on GD 0 and upon arrival. Does were sacrificed on day 29 of gestation. Examinations at sacrifice consisted of: gross necropsy of the thoracic, abdominal, and pelvic viscera; staining of the uteri from apparent nonpregnant rabbits; excise and weighing of gravid uteri; recording the number of corpora lutea in each ovary; and determining the number and distribution of viable and nonviable fetuses, early and late resorptions, and implantations.

2. **Fetal evaluations:** The fetuses were examined in the following manner: each fetus was removed from the uterus, weighed, and examined for gross alterations. All fetuses were examined internally to determine sex. All fetuses were examined for soft tissue alterations using dissection. A single cross-section was made between the parietal and frontal bones, and the brain was examined *in situ* for approximately one-half the fetuses in each litter. The remaining half of the fetuses in each litter were decapitated and the heads were fixed in Bouin's solution and examined by Wilson's sectioning technique. All fetuses were eviscerated, cleared, and stained with alizarin red S for skeletal examinations.

D. DATA ANALYSIS:

1. **Statistical analyses:** The Variance Test for Homogeneity of the Binomial Distribution was used to analyze clinical observation and other proportion data. Maternal body weights and body weight changes, feed consumption, litter averages for percent male fetuses, percent resorbed conceptuses, fetal body weights, fetal anomaly data, and fetal ossification site data were analyzed for homogeneity of variance using Bartlett's Test of Homogeneity of Variance. If variances were homogenous, the data were analyzed using the Analysis of Variance, followed by Dunnett's Test if significant. The Kruskal-Wallis Test was used when Bartlett's was significant (i.e., variances were not homogeneous). Dunn's Method of Multiple Comparisons was used if the Kruskal-Wallis Test was significant. If there were greater than 75% ties, Fisher's Exact Test was used to analyze the data. The count data obtained at Caesarean-sectioning of the does was analyzed using the procedures already described for the Kruskal-Wallis Test.

2. **Indices:** No cesarean section indices (conception rate, pre-implantation loss, post-implantation loss) were calculated.

3. **Historical control data:** Historical control data were provided to allow comparison with concurrent controls. Data (reproductive indices, maternal necropsy observations, fetal external alterations, fetal soft tissue alterations, fetal skeletal alterations, and a summary of averages of fetal ossification sites) were obtained from 28-56 studies in New Zealand White rabbits collected over a period of 2 years.

II. RESULTS:

A. MATERNAL TOXICITY:

1. **Mortality and clinical observations:** Mortality and clinical observations are presented in Table 2. Three control does were sacrificed due to aborting (one on GD 19 and two on GD 25). In the 75 mg/kg/day group, two does were found dead on GDs 25 and 27; 1 doe was sacrificed due to aborting on GD 27, and two does were killed on GD 29 because of premature delivery. In the 100 mg/kg/day group, 3 does were found dead or sacrificed in moribund condition (GDs 17, 19, 20), 6 does were sacrificed due to aborting (GDs 20, 22, 23, 24, 26, 28), and two were sacrificed on GD 29 because of premature delivery.

   An increased incidence of clinical signs was noted in the 75 and 100 mg/kg/day groups (see Table 2). The incidence of scant feces in the cage pan and orange urine was statistically increased in both groups. The 100 mg/kg/day dose group additionally had a statistically increased incidence of no feces in the cage pan and red substance in the cage pan. At 25 mg/kg bw/day there was a treatment related increase in the incidences of scant feces and orange urine. However, in the absence of other indications of toxicity at this dose level, these clinical signs were not considered to be adverse to the health of the animals.

   Clinical signs in the premature decedents are as follows: no clinical signs were observed in the control does that aborted; the 75 mg/kg/day doe that aborted had orange urine and scant feces; one 75 mg/kg/day doe that delivered early had orange urine, scant feces, and localized alopecia; the six 100 mg/kg/day does that aborted generally had scant or no feces, orange urine, and red substance in the cage pan; the two 100 mg/kg/day does that delivered early had absent and/or scant feces and/or localized alopecia. All other clinical signs were not related to dose.

Table 2. Mortality and Clinical Signs [a]

Observation [No. observations/ No. animals]	Dose in mg/kg bw/day				
	0	10	25	75	100
Maximum possible incidence	534/23	552/23	552/23	544/23	489/23
Mortality					
Found dead/sacrificed moribund	0	0	0	2	3
Aborted (sacrificed early)	3	0*	0*	1	6*
Early delivery (GD 29)	0	0	0	2	2
Scant feces in cage	1/1	4/3	10/4	40/10**	79/16**
No feces in cage	0/0	0/0	0/0	1/1	22/11**
Orange urine	0/0	0/0	4/2	38/9**	64/9**
Red substance in cage pan	0/0	0/0	1/1	0/0	8/4*

[a] Data obtained from Table 1, pp. 51-52; MRID 45422713.
* Statistically different ($p < 0.05$) from the control.
** Statistically different ($p < 0.01$) from the control.

2. **Body weight:** Body weight data are summarized in Table 3. Does in the high-dose group had decreased mean absolute body weights beginning at GD 14 and continuing throughout the study (90-94% of controls; $p<0.05$; 0.01), with GD 29 body weights 91% of controls ($p<0.01$). The corrected mean body weight, however, was comparable to controls (96% of controls). Maternal body weight gains of high-dose does were generally decreased compared to controls, with the decreases reaching statistical significance for treatment intervals GDs 9-12 (-0.08 kg vs. 0.03 kg for controls), 12-15 (-0.08 kg vs. 0.06 kg for controls), and 6-29 (-0.02 kg vs. 0.32 kg for controls). The corrected body weight change for the entire treatment interval of GD 6-29 was decreased compared to controls.

   No other statistically or biologically significant differences in body weights were noted.

   Body weights of the animals that died or were sacrificed before study termination were generally severely reduced.

Table3. Mean (±SD) Maternal body weight and body weight gain (kg)[a]

Interval	Dose in mg/kg bw/day (# of Does)				
	0(21)	10(23)	25(20)	75(22)	100(21)
Pretreatment: Days 0-6	0.05 ± 0.11	0.07 ± 0.09	0.08 ± 0.08	0.08 ± 0.07	0.06 ± 0.10
Treatment: Days 6-9	0.01 ± 0.12	0.01 ± 0.08	0.05 ± 0.05	0.02 ± 0.08	-0.04 ± 0.10
Treatment: Days 9-12	0.03 ± 0.07	0.05 ± 0.06	0.02 ± 0.06	0.02 ± 0.06	-0.08** ± 0.10
Treatment: Days 12-15	0.06 ± 0.08	0.07 ± 0.06	0.07 ± 0.07	0.01 ± 0.07	-0.08** ± 0.10
Treatment: Days 6-29	0.32 ± 0.16	0.32 ± 0.19	0.30 ± 0.30	0.17 ± 0.34	-0.02** ± 0.25
Corrected BW Gain: Days 6-29	-0.13 ± 0.21	-0.13 ± 0.25	-0.14 ± 0.21	-0.12 ± 0.23	-0.31 ± 0.17
BW Day 14	4.15±0.33	4.20±0.30	4.21±0.31	4.10±0.25	3.92±0.28*
BW Day 18	4.20±0.33	4.25±0.30	4.25±0.30	4.25±0.30	3.88±0.32**
BW Day 29	4.36±0.32	4.40±0.33	4.37±0.31	4.22±0.34	3.98±0.41**
Corrected BW	3.84±0.33	3.88±0.33	3.86±0.28	3.83±0.25	3.67±0.26

[a] Data obtained from Table 5, pages 60-61; MRID 45422713.
* 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 4. Mean absolute food consumption was significantly reduced ($p<0.05$; 0.01) in high-dose does at all treatment intervals (34-73% of controls) up to GD 24, after which food consumption was decreased but

did not achieve statistical significance (59-90% of controls). Overall mean absolute food consumption during treatment (GD 6-29) was significantly decreased in high-dose does (63% of controls; $p<0.01$). In the 75 mg/kg/day group, mean absolute food consumption was significantly decreased over GDs 21-24 (71% of controls) and GDs 6-29 (83% of controls).

Food consumption in the animals that died or were sacrificed early was generally severely reduced.

TABLE 4. Selected Food Consumption Values (g/animal/day) [a]

Treatment interval	Dose in mg/kg bw/day (# of Does)				
	0 (21)	10 (23)	25 (20)	75 (21)	100 (22)
GD 6-9	141.3 ± 55.4	154.2 ± 39.6	168.7 ± 20.5	142.9 ± 37.8	90.9* ± 58.0 (64)
GD 12-15	134.4 ± 53.8	157.0 ± 36.2	141.2 ± 52.4	100.6 ± 54.5	45.7** ± 66.4 (34)
GD 27-29	73.4 ± 54.0	84.3 ± 50.7	73.5 ± 60.7	92.8 ± 62.3	66.0 ± 61.3 (90)
GD 6-29	142.8 ± 24.7	145.2 ± 27.6	139.5 ± 31.7	118.8* ± 31.3 (83)	89.6** ± 33.5 (63)

[a] Data obtained from Table 6, p. 62; MRID 45422713.
* Statistically different ($p<0.05$) from the control.
** Statistically different ($p<0.01$) from the control.

4. **Gross pathology:** No gross pathological changes related to treatment were noted in animals surviving to study termination. Gross necropsy findings of the does that died or were sacrificed early are as follows: one control doe that aborted had a trichobezoar; one doe in the 75 mg/kg/day group found dead had a friable stomach; one 75 mg/kg/day doe that aborted had a trichobezoar; one 100 mg/kg/day group doe found dead had yellow perioral and perianal substance, spongy lungs with hemorrhagic area, white mucus on the mucosal surface of the stomach and parovarian cysts; and one 100 mg/kg/day doe that aborted had a trichobezoar while another had a trichobezoar and black substance in the cecum and colon. The other does that did not survive to terminal sacrifice did not have any abnormal gross findings.

5. **Cesarean section data:** Cesarean section data summarized in Table 5. In the 75 mg/kg/day group, 2 does were found dead, 1 aborted, and 2 delivered on GD 29. Although not statistically significant, the decreases observed in gravid uterine weights were biologically significant (89% of controls). No other significant effects were observed in this group. A number of differences were observed in the 100 mg/kg/day group. Two does were found dead (with complete litter resorption), one doe was sacrificed in moribund condition, two does delivered on GD 29, six does aborted ($p<0.05$), and one doe had complete litter resorption. This resulted in only 11 litters for evaluation. Again, although the decreases observed in gravid uterine weights were not statistically significant, they were biologically significant (81% of controls) and related to dose. Other effects at 100 mg/kg/day included: an increase in the total number of resorptions, number of total early resorptions and early resorptions/doe, and post implantation loss. Fetal body weights were significantly decreased for both sexes combined (84% of controls; $p<0.01$) and for female fetuses (84% of controls;

p<0.01). Although male fetal body weights were not statistically significantly decreased, the decrease was still biologically significant at 85% of controls.

TABLE 5. Cesarean section observations [a]

Observation	Dose (mg/kg bw/day)				
	0	10	25 [b]	75	100 [b]
# Animals Assigned (Mated)	23	23	23	23	23
# Animals Pregnant	21	23	22	22	23
Pregnancy Rate (%)	91.3	100	95.6	95.6	100
# Nonpregnant	2	0	1	1	0
Maternal Wastage					
# Died or Killed Moribund	0	0	0	2	3
# Aborted	3	0*	0*	1	6*
# Premature Delivery	0	0	0	2	2
Total # Corpora Lutea [c]	169	220	198	148	108
Corpora Lutea/Doe	9.4 ± 1.4	9.6 ± 2.3	9.9 ± 1.1	8.7 ± 1.8	9.8 ± 2.3
Total # Implantations [c]	155	204	183	137	97
(Implantations/Doe)	8.6 ± 2.0	8.9 ± 2.5	9.2 ± 1.3	8.0 ± 2.0	8.8 ± 2.4
Total # Litters	18	23	22	17	11
Total # Live Fetuses	150	201	176	133	82
(Live Fetuses/Doe)	8.3 ± 2.0	8.7 ± 2.5	8.8 ± 1.5	7.8 ± 2.1	7.4 ± 4.1
Total # Dead Fetuses	0	0	0	0	0
Total # Resorptions	5	3	7	4	15
Early	2	0	2	0	10
Late	3	3	5	4	5
Resorptions/Doe	0.3 ± 0.5	0.1 ± 0.3	0.4 ± 0.9	0.2 ± 0.4	1.4 ± 2.2
Early	0.1 ± 0.3	0.0 ± 0.0	0.1 ± 0.4	0.0 ± 0.0	0.9 ± 1.8
Late	0.2 ± 0.4	0.1 ± 0.3	0.2 ± 0.7	0.2 ± 0.4	0.4 ± 0.8
Litters with Total Resorptions	0	0	0	0	1
Mean Fetal Weight (g)	43.85 ± 5.08	42.91 ± 5.23	40.37 ± 5.92	40.59 ± 5.98	36.66** ± 5.81 (84)
Males	44.16 ± 5.45	43.24 ± 6.31	40.73 ± 6.24	40.67 ± 6.33	37.73 ± 6.66 (85)
Females	42.96 ± 5.92	42.23 ± 4.90	39.98 ± 5.74	40.24 ± 6.00	36.05** ± 5.41 (84)
Sex Ratio (% Male)	51.8 ± 15.4	47.2 ± 22.3	50.2 ± 11.8	48.8 ± 18.4	39.6 ± 16.1
Preimplantation Loss (%) [d]	8.3	7.3	7.6	7.4	10.2
Postimplantation Loss (%) [e]	3.2	1.5	3.8	2.9	15.5
Mean Gravid Uterine Weight (g)	517.8 ± 80	524.5 ± 117	516.5 ± 94	461.6 ± 95	420.0 ± 175

[a] Tables 4, 8,9, and 19 pp. 59, 64-66, 119-122; MRID 45422713.

[b] The 25 mg/kg/day group had two does with litters of 2 or 1 conceptuses, and the 100 mg/kg/day group had a doe with a litter with 3 conceptuses. These values have been excluded from group averages and statistical analyses

[c] Totaled by reviewer using Table 19, pp. 119-122.

[d] Calculated by reviewer using the following equation: $\frac{\text{\# corpora lutea - \# implantations}}{\text{\# corpora lutea}} \times 100$

[e] Calculated by reviewer using the following equation: $\frac{\text{\# implantations - \# live fetuses}}{\text{\# implantations}} \times 100$

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

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

B. DEVELOPMENTAL TOXICITY:

1. **External examination:** External examination data are presented in Table 6. No treatment-related external malformations or variations were observed. One fetus in the 10 mg/kg/day group had an umbilical hernia. In the 25 mg/kg/day group, one litter contained 3 fetuses with rotated hindlimb(s) ($p<0.01$), and 1 fetus with a flexed hindpaw, while another litter contained one fetus with a short tail and one fetus with a short tail and meningocele. The only alteration observed in the 75 mg/kg/day group was a fetus with a short snout. In the 100 mg/kg/day group, one litter contained a fetus with an absent nail in the hindpaw, and another litter contained a fetus with multiple alterations including: a depressed eye bulge, absent digits in the fore- and hindpaws, and a short, kinked tail.

2. **Visceral examination:** Visceral examination data are presented in Table 6. A statistically significant increase ($p<0.01$) in the litter incidence of an absent lung lobe was observed in the 75 and 100 mg/kg/day group [given as fetus(litter): 3(3) and 8(5), respectively; % litters affected: 17.6% and 45.5%, respectively] compared to 0(0) controls. Included in the incidence for the high-dose group is a fetus that was missing the right and left apical, right cardiac, and intermediate lung lobe and 7 fetuses missing the intermediate lobe of the lung. Three of the affected high-dose fetuses also had small kidneys, with one additionally having an underdeveloped right and left apical, right cardiac, and right diaphragmatic lung. One of the affected 75 mg/kg/day fetuses also had persistent truncus arteriosis. Other soft tissue alterations were of low incidence or comparable to controls and were clearly not related to treatment.

3. **Skeletal examination:** Skeletal examination data are presented in Table 6. The high-dose group had a statistically increased litter incidence of a fused caudal vertebrae, incompletely ossified sternal centra (1st), and absent hindpaw phalanges [fetal(litter) incidence: 2(2) for all three alterations; % litters affected: 18.2% for all three alterations] compared to the controls [0(0); 0%]. The study author analyzed the average numbers of fetal ossification sites per litter and found statistically reduced ossification of sternal centers of the sternum in the 75 and 100 mg/kg/day groups (3.83 and 3.76%, respectively, vs. 3.99% for controls) and of hindlimb phalanges in the high-dose group (11.78% vs. 12.0% for controls). Other skeletal alterations occurred in low incidence and in a non-dose related manner.

TABLE 6. External, Viseral and Skeletal Examinations[a]					
Observations[b]	Dose (mg/kg bw/day)				
	0	10	25	75	100
External Examinations					
#Fetuses(litters) examined	150(18)	201(23)	179(22)	133(17)	85(11)
#Fetuses(litters) affected	0(0)	1(1)	6(2)	1(1)	3(2)
Visceral Examinations					
#Fetuses(litters) examined	150(18)	201(23)	179(22)	133(17)	85(11)
#Fetuses(litters) affected[c]	0(0)	3(3)	1(1)	4(4)	8(5)
Total: Lung lobe absent [d,e]	0(0)	0(0)	0(0)	3(3)**	8(5)**
Kidneys: small	0(0)	0(0)	0(0)	0(0)	3(1)**
Skeletal Examinations					
#Fetuses(litters) examined	150(18)	201(23)	179(22)	133(17)	85(11)
#Fetuses(litters) affected	4(4)	9(7)	16(9)	6(4)	9(5)
Caudal vertebraee: fused[d]	0(0)	0(0)	2(1)	0(0)	2(2)**
Sternal centra (incomplete ossification)[d]	0(0)	0(0)	0(0)	0(0)	2(2)**
Hindpaw phalanges: absent[d]	0(0)	0(0)	0(0)	0(0)	2(2)**
Fetal Ossification sites (mean ± SD)					
Sternum: sternal centers	3.99± 0.03	3.91± 0.14	3.94± 0.13	3.83**± 0.20	3.76**±0.25
Hindlimb: phalanges	12.00± 0.0	12.00± 0.0	12.00± 0.0	12.00± 0.0	11.78*± 0.6

[a] Data taken from Tables 11-14 and 22 pp. 68-78 and 139-159; MRID 45422713.
[b] Some observations may be grouped together.
[c] Statistical analysis not conducted.
[d] Fetal (litter) incidence
[e] Intermediate + one incidence of absent right and left apical, right cardiac lobe, and intermediate absent
** Statistically different ($p < 0.01$) from the control

III. DISCUSSION AND CONCLUSIONS:

A. INVESTIGATORS' CONCLUSIONS: The maternal toxicity NOAEL is 10 mg/kg/day, and the LOAEL is 25 mg/kg/day based on adverse clinical signs (reduced fecal output and discolored urine). Adverse clinical observations of toxicological and/or statistical significance were produced by the 75 and 100 mg/kg/day doses that also resulted in reduced maternal weight gains and feed consumption, deaths, a moribund sacrifice, and premature deliveries. Additionally, abortions were noted in the 100 mg/kg/day group.

The developmental NOAEL is 75 mg/kg/day, and the LOAEL is 100 mg/kg/day based on increased post-implantation loss, reduced fetal body weights, and retarded sternal ossification.

B. REVIEWER COMMENTS:

1. **Maternal toxicity:** Maternal toxicity was evident at doses of 75 mg/kg/day and higher. At 75 mg/kg/day, clinical signs of scant feces and orange urine were observed and are considered adverse, since these effects were evident at doses which produced other symptoms of toxicity including mortality and reduced food consumption. Additionally, dark urine was observed in the range-finding developmental toxicity study in rabbits (MRID 45422712). No gross renal changes were evident during necropsy of affected animals.

   Other maternal toxicity at the dose of 75 mg/kg/day consisted of mortality, premature deliveries, and decreased body weight gain and food consumption for the treatment interval of GDs 6-29. The study author did not call the abortion an effect of treatment because the incidence for abortions in this group (1/23) was below that of the controls (3/23); however, the author did call the premature deliveries an effect of treatment. One of the does that delivered prematurely exhibited orange urine and scant feces (also localized alopecia). Therefore, the reviewer agrees that the premature deliveries could be an effect of treatment.

   At 100 mg/kg/day, maternal toxicity was indicated by mortality; an increased incidence of abortions; premature deliveries, statistically increased incidences of scant or no feces in the cage, orange urine, and red substance in the cage pan; and statistically decreased mean absolute body weights, body weight gains, and food consumption. The two does that delivered early exhibited scant feces and one additionally had localized alopecia.

   Therefore, the maternal toxicity LOAEL is 75 mg/kg/day based on increased incidences of clinical signs (scant feces and orange urine), mortalities and decreased food consumption and the maternal toxicity NOAEL is 25 mg/kg/day.

2. **Developmental toxicity:**

   a. **Deaths/resorptions:** Treatment with 100 mg/kg/day resulted in an increased number of abortions; premature deliveries; complete litter resorptions in 3 does (2 of does died early); decreased gravid uterine weights; an increased number of total resorptions and resorptions/doe due to increased total number of early resorptions and early resorptions/doe, respectively; and an increased percentage of post-implantation loss. Effects in the 75 mg/kg/day group included premature delivery and decreased gravid uterine weights.

Although the decreases in gravid uterine weights observed in the 75 and 100 mg/kg/day groups (89 and 81% of controls, respectively) were not statistically significant, they were biologically significant and related to dose. Therefore, the reviewer considers them to be an adverse effect of treatment. As already discussed in the maternal toxicity discussion, the early deliveries are considered an effect of treatment because of associated clinical signs observed in these does before delivery.

b. **Altered growth:** The litter average for ossified sternal centra per fetus was statistically decreased in the 75 and 100 mg/kg/day groups (3.83 and 3.76, respectively, vs. 3.99 for controls; historical control range: 3.81-3.97). Although the value for the 75 mg/kg/day group was within the historical control range, the reviewer still considers the decrease an effect of treatment because the decrease was dose-related. Other signs of altered growth in the 100 mg/kg/day group included decreased mean fetal body weights and statistically increased litter incidence of incomplete ossification of the sternal centra [fetus(litter) incidence: 2(2) vs. 0(0) for controls; 18% of litters affected; historical control rage: 0-10.5% of litters affected]). These effects are considered an effect of treatment and are most likely the result of the maternal toxicity (particularly the body weight decreases) evident at this dose.

c. **Developmental variations:** Missing intermediate lobe of the lung is a common occasional finding in rabbit fetuses. However in this study, the incidence of a missing lobe of the lung was statistically increased in the 75 and 100 mg/kg/day groups [fetus(litter) incidence: 3(3) and 8(5), respectively, vs. 0(0) for controls; 18 and 45% of litters affected, respectively; historical control range: 0-27.8% of litters affected]. Although the value for the 75 mg/kg/day group was within the historical control range, the reviewer still considers the increase an effect of treatment because the increase was dose-related.

As discussed in the preceding section addressing altered fetal growth, a treatment-related decrease in the litter average for ossified sternal centra per fetus was observed in the 75 and 100 mg/kg/day groups and an increased litter incidence of incomplete ossification of the sternal centra was noted in the 100 mg/kg/day group.

No other treatment-related developmental variations were observed.

d. **Malformations:** Increased incidences of fused caudal vertebrae and absent hindpaw phalanges were noted in the 100 mg/kg/day group [for both alterations: 2(2) affected vs. 0(0) for controls; 18% of litters affected]. These were both outside of the historical control range (0-5.9% litters affected for both). The incidence of fused caudal vertebrae was not considered an effect of treatment because it was not definitively related to dose. Absent hindpaw phalanges, however, was considered an effect of treatment. No other treatment-related malformations were observed.

The developmental LOAEL is 75 mg/kg bw/day, based on premature deliveries, decreased gravid uterine weights, an increased litter incidence of a missing lobe of the lung and decreased litter average for ossified sternal centra per fetus. The developmental NOAEL is 25 mg/kg bw/day.

C. **STUDY DEFICIENCIES**: No major deficiencies were observed. A minor deficiency was that the study author did not calculate the reproductive indices, but the data were available for the reviewer to do so.

APPENDIX: Oral (Gavage) Dosage-Range Developmental Toxicity Study of TI-435 in Rabbits

TEST MATERIAL (PURITY): TI-435 (96.0% a.i.)

CITATION: York, R.G. (1998) Oral (Gavage) dosage-range developmental toxicity study of TI-435 in rabbits. Argus Research Laboratories, Inc., 905 Sheehy Dr., Horsham, PA 19044. Report 1120-002P, January 15, 1998, MRID 45422712. Unpublished.

In a range-finding oral developmental toxicity study (MRID 45422712), TI-435 [(96.0% a.i., Lot No. 30034708)] was administered to 5 timed-pregnant New Zealand White [Hra:(NZW)SPF] rabbits/dose orally by gavage at dose levels of 0, 62.5, 125, 250, or 500 mg/kg bw/day from days 6 through 28 of gestation. On gestation day 29, does were sacrificed and the following observations recorded: number and distribution of corpora lutea, implantation sites and uterine contents; results of gross necropsy of the thoracic, abdominal, and pelvic viscera; and gravid uterine weights. Fetuses were examined for gross external alterations, gender, and were weighed.

Maternal toxicity was evident at all doses. All does in the 62.5 mg/kg/day group survived to study termination except one doe that started to deliver before Cesarean section on GD 29 and was sacrificed. The 62.5 mg/kg/day group had an increased incidence of dark urine (# of observations/# of rabbits affected: 85/5 vs. 0/0 for controls). Body weights, food consumption, and gross necropsy findings were comparable to controls.

All does in the 125, 250, and 500 mg/kg/day groups died or were sacrificed early. Clinical signs included: scant, absent, or mucoid feces and/or red substance in the cage pan in does treated with 125 mg/kg/day or higher; emaciation, labored breathing, perinasal substance, and/or decreased motor activity in does treated with 250 mg/kg/day or higher; and lacrimation, impaired or lost righting reflex, and/or head tilt in does treated with 500 mg/kg/day. Does in the 125 mg/kg/day group additionally had dark urine (2/2). Body weight loss and decreased food consumption were observed throughout treatment in does treated with 125 mg/kg/day or higher. No abnormal gross necropsy findings were observed. It was determined at necropsy that two of the deaths in the 125 mg/kg/day group were due to intubation error.

Therefore, the maternal toxicity LOAEL was ≤ 62.5 mg/kg/day based on clinical signs (dark urine), and a maternal toxicity NOAEL was not established.

All animals were pregnant, but only 5, 4, 0, 0, and 0 does in the 0, 62.5, 125, 250, or 500 mg/kg/day groups were subjected to Cesarean section because of mortality, moribund sacrifice, abortion, or premature delivery. The 62.5 mg/kg/day group had a comparable number of corpora lutea, implantations, litter sizes, early and late resorptions, fetal weights, and sex ratio compared to controls.

The total number of fetuses examined externally for alterations in the 0 and 62.5 mg/kg/day groups were 48 and 28 fetuses, respectively. No external alterations were observed.

Doses of 0, 10, 25, 75, and 100 mg/kg/day are recommended for the developmental toxicity study in rabbits. The 100 mg/kg/day dose is expected to produce maternal toxicity and minimal developmental toxicity.

DATA FOR ENTRY INTO ISIS

Developmental Study - rabbits (870.3700b)

PC code	MRID	Study	Species	Duration	Route	Admin	Dose range mg/kg/day	Doses mg/kg/day	NOAEL mg/kg/day	LOAEL mg/kg/day	Target organ	Comments
44309	45422713, 45422712	developmental	rabbits	GD 6-29	oral	gavage	10-100	0, 10, 25, 75, 100	10	25	clinical signs: scant feces, orange urine	Maternal
44309	45422713, 45422712	developmental	rabbits	GD 6-29	oral	gavage	10-100	0, 10, 25, 75, 100	25	75	premature deliveries, decreased gravid uterine weights, an increased litter incidence of a missing lobe of the lung and decreased litter average for ossified sternal centra per fetus	Developmental

HED Records Center Series 361 Science Reviews - File R107689 - Page 505 of 721

DATA EVALUATION RECORD

CLOTHIANIDIN (TI-435)/044309

REPRODUCTION AND FERTILITY EFFECTS STUDY - RAT; OPPTS 870.3800 [§83-4]; OECD 416
MRIDs 45422715 (Main Study), 45422714, 45422716

Prepared for

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DATA EVALUATION RECORD
TXR#: 0050321

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

PC CODE: 044309

DP BARCODE: D282578
SUBMISSION NO.: S614462

TEST MATERIAL (PURITY): TI-435 (Clothianidin; 95.3-96.0% a.i.)

SYNONYMS: (E)-1-(2-Chloro-5-thiazolylmethyl)-3-methyl-2-nitroguanidine

CITATION: Freshwater, K. and A. Astroff (2000). A two generation reproductive toxicity study with TI-435 in the Sprague-Dawley rat. Bayer Corporation, Agriculture Division, Toxicology Department, 17745 South Metcalf, Stilwell, Kansas 66085-9104. Laboratory report number 109282, March 27, 2000. MRID 45422715. Unpublished.

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Young, A. (2001). Supplemental Submission to Bayer Report No. 109282: two generation reproductive toxicity study with TI-435 in the Sprague-Dawley rat. Bayer Corporation, Agriculture Division, Toxicology Department, 17745 South Metcalf, Stilwell, Kansas 66085-9104. Laboratory report number 109282-1, April 6, 2001. MRID 45422716. Unpublished.

Moore, K. and L. Shelton (1999). The homogeneity, room temperature and freezer stability of TI 435 in rodent ration. Bayer Corporation, Agriculture Division, Toxicology, 17745 South Metcalf, Stilwell, KS 66085-9104. Laboratory report number 108687, January 4, 1999. MRID 45422825. Unpublished.

Moore, K. and L. Shelton (1999). The homogeneity, room temperature and freezer stability of TI 435 in rodent ration. Bayer Corporation, Agriculture Division, Toxicology, 17745 South Metcalf, Stilwell, KS 66085-9104. Laboratory report number 108690, February 12, 1999. MRID 45422826. Unpublished.

SPONSOR: Takeda Chemical Industries, Ltd., Agro Company, 2-13-10, Nihonbashi, Chuo-ku Tokyo, Japan, 103-8668.

EXECUTIVE SUMMARY: In a 2-generation reproduction study (MRID 45422715) TI-435 (Clothianidin; 95.3-96.0% a.i., lot/batch #300371200) was administered to 30 Sprague-Dawley rats/sex/dose in the diet at concentrations of 0, 150, 500, or 2500 ppm. One litter was produced by each generation. Pre-mating doses were 0, 9.8, 31.2, or 163.4 mg/kg bw/day, respectively, for F_0 males, 0, 11.5, 36.8, or 188.8 mg/kg bw/day, respectively, for F_0 females, 0, 10.7, 34.3, or 195.7 mg/kg bw/day, respectively, for F_1 males, and 0, 12.2, 39.0, or 237.0 mg/kg bw/day, respectively, for F_1 females; the values for the F_0 generation were considered to be representative of the test substance intake for the entire study. F_0 and F_1 male and female parental animals were administered test or control diet for at least 70 days prior to mating, throughout mating, gestation, and lactation, and until necropsy. Additional information pertaining to diet analyses was obtained from two separately provided reports (MRIDs 45422825 and 45422826), and historical control data were also provided (MRID 45422716).

There were no treatment-related effects on parental clinical signs or mortality. High-dose F_0 males had decreased absolute body weights during weeks 4-10 of pre-mating (87-92% of controls) and decreased cumulative body weight gain during pre-mating (80% of controls), and high-dose F_1 males had decreased absolute body weights throughout pre-mating (79-83% of controls). High-dose F_0 females had decreased absolute body weights during weeks 2-10 of pre-mating (86-91% of controls), throughout gestation (86-88% of controls), and throughout lactation (82-89% of controls), with decreased body weight gains during pre-mating and gestation (61 and 83% of controls, respectively). High-dose F_1 females had decreased absolute body weights throughout pre-mating (83-84% of controls), throughout gestation (84-87% of controls), and throughout lactation (85-88% of controls), with decreased body weight gains during pre-mating and gestation (82 and 92% of controls, respectively). These groups generally also had significantly increased food consumption. Absolute thymus weights were decreased in high-dose F_0 and F_1 males and females (84, 71, 65, and 59% of controls for F_0 males, F_1 males, F_0 females, and F_1 females, respectively) and relative (to body) thymic weights were also decreased in high-dose F_1 males and high-dose females of both generations. **The parental systemic toxicity LOAEL is 2500 ppm (163.4 mg/kg bw/day in males, 188.8 mg/kg bw/day in females), based on decreased absolute body weights and body weight gains with decreased absolute and relative thymus weights in both sexes. The parental systemic NOAEL is 500 ppm (31.2 mg/kg bw/day in males, 36.8 mg/kg bw/day in females).**

Mean absolute body weights of F_1 high-dose male and female pups were significantly decreased from LD 4 through the end of weaning (74-84% of controls; $p<0.01$), and mean absolute body weights of F_2 high-dose male and female pups were significantly decreased from LD 7 through the end of weaning (79-87% of controls; $p<0.01$). Pup body weight gains of high-dose males and females of both generations were significantly decreased throughout lactation (F_1 combined sexes: 67-76% of controls, $p<0.01$; F_2 combined sexes: 72-81% of controls, $p<0.05$ or $p<0.01$), and the body weight gains of F_1 mid-dose pups of both sexes were slightly decreased during LD 7-14 (90% of controls for both sexes and 89% of controls for combined sexes; $p<0.05$). Decreased mean absolute thymus weights were noted in high-dose pups of both generations (71-72% of controls for high-dose F_1 males and females; 75-76% of controls for high-dose F_2 males and females) as well as in mid-dose F_1 pups (87-90% of controls). The spleen weights (relative

and absolute) were reduced ($p<0.01$) in females of high dose in the F_1 generation and in males and females of high dose in the F_2 generation as well. Mid- and high-dose F_1 males had increased mean ages of acquisition of balanopreputial separation (42.5 [$p<0.05$] and 47.9 [$p<0.01$] vs. 41.2 days for controls) and high-dose F_1 females had increase mean age of vaginal perforation (34.7 vs. 32.4 days for controls; $p<0.01$). A dose-related increased number of stillbirths was noted in the mid- and high-dose groups of both generations in the absence of effects on related parameters such as live birth index, live litter size, and/or numbers of implantations. **Therefore, the offspring toxicity LOAEL is 500 ppm (31.2 mg/kg bw/day in males, 36.8 mg/kg bw/day in females), based on decreased body weight gains, delayed sexual maturation (males), and decreased absolute thymus weights in F_1 pups of both sexes and an increase in stillbirths in both generations. The offspring toxicity NOAEL is 150 ppm (9.8 mg/kg bw/day in males, 11.5 mg/kg bw/day in females).**

There were no treatment-related effects on mating performance, fertility, estrous cyclicity, gestation length, or precoital intervals. There were no treatment-related abnormal gross or microscopic findings related to the reproductive organs of the parental animals or offspring. The mean primordial follicle, antral follicle, and corpora lutea counts of high-dose F_1 adults were similar to controls. The percentage of progressively motile sperm was decreased in high-dose F_0 males (88% of controls; $p<0.01$), in the absence of an effect on the percent motile, while in high-dose F_1 males decreases were noted in both the percent motile (90% of controls; $p<0.01$) and the percent progressively motile (77% of controls; $p<0.01$). Males of both generations also had increased percentages of sperm with detached heads (F_0: 2.1 vs. 0.6% for controls; F_1: 4.0 vs. 0.7% for controls). **The reproductive toxicity LOAEL for males is 2500 ppm (163.4 mg/kg bw/day) in males, based on decreased sperm motility, and increased number of sperm with detached heads in both generations. The reproductive toxicity NOAEL for males is 500 ppm (31.2 mg/kg bw/day). The reproductive toxicity LOAEL for females is not identified, and the reproductive toxicity NOAEL for females is 2500 ppm (188.8 mg/kg bw/day).**

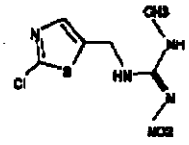
This study is classified **Acceptable/Guideline** and satisfies the guideline requirements for a 2-generation reproductive study in the rat [OPPTS 870.3800; OECD 416]. However, it must be noted that significant variation in the analytical dietary concentrations at all dose levels make the actual dosages to the animals uncertain.

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

I. MATERIALS AND METHODS:

A. MATERIALS:

1. Test material:

Description:	TI-435 pale yellow powder
Lot/Batch #:	300371200
Purity:	95.3-96.0 % a.i.
Compound Stability:	At least 12 months
CAS # of TGAI:	210880-92-5
Structure:	

2. **Vehicle and/or positive control:** The test material was fed to the animals in the diet (see below), and corn oil was used as a carrier for the test material and to help control the dust. There was no positive control used in the study.

3. Test animals:

Species:	Rat	
Strain:	Sprague-Dawley	
Age at study initiation:	(P) 7-8 wks; (F_1) 3 wks	
Wt. at study initiation:	(P) Males: 261.0-272.3 g; Females: 185.1-188.6 g (F_1) Males: 108.2-317.8 g; Females: 84.7-217.8 g	
Source:	Charles River Laboratories, Raleigh, NC	
Housing:	Individually (except during mating) in suspended, stainless steel cages. During gestation and lactation, dams and litters were housed in plastic cages with corn cob bedding.	
Diet:	Purina Mills Rodent Lab Chow 5001-4 *ad libitum*	
Water:	Municipal tap water *ad libitum*	
Environmental conditions:	**Temperature:**	18-26 °C
	Humidity:	30-70 %
	Air changes:	not reported
	Photoperiod:	12 hrs dark/12 hrs light
Acclimation period:	At least 6 days.	

B. PROCEDURES AND STUDY DESIGN:

1. **In life dates:** Start: June 29, 1998; End: April 6, 1999.

2. **Mating procedure:** Each female was caged with a male from the same treatment group for up to 14 consecutive days, and each morning vaginal smears were examined for the presence of sperm and/or an internal copulatory plug. Inseminated females were placed in a plastic nesting cage, and the day evidence of mating was observed was designated gestation day (GD) 0. At the end of the 14-day mating period, females without evidence of mating were placed in plastic nesting cages. The report stated that approximately four animals from each dose group were co-housed daily beginning on the first day of the mating phase and

continuing until all animals had been co-housed. Sibling matings were avoided. F_1 and F_2 generations were produced and one litter was produced in each generation.

3. **Study schedule:** F_0 and F_1 male and female parental animals were administered test or control diet for at least 70 days prior to mating, throughout mating, gestation, and lactation, and until sacrifice, beginning at either 7-8 weeks of age (F_0 generation) or at 3 weeks of age/weaning (F_1 generation). The approximate duration of treatment for each generation was 16 and 20 weeks for the males and females, respectively, of the F_0 generation and 20 and 24 weeks for the males and females, respectively of the F_1 generation.

4. **Animal assignment:** P animals were randomly assigned to the test groups given in Table 1 based on their body weight, using software provided by DATATOX.

TABLE 1. Animal assignment

Test group	Dose in diet [a] ppm	Parental animals/group			
		F_0 Males	F_0 Females	F_1 Males	F_1 Females
Control	0	30	30	30	30
Low (LDT)	150	30	30	30	30
Mid (MDT)	500	30	30	30	30
High (HDT)	2500	30	30	30	30

Data taken from text, pp. 20-21, MRID 45422715.
[a] Diets were administered from the beginning of the study until sacrifice, and dosages were adjusted to account for the purity of the test material.

5. **Dose selection rationale:** Dietary concentrations were selected based on preliminary results from a combined chronic toxicity/carcinogenicity study in the rat (MRID 45422719) and a range-finding reproduction study (MRID 45422714; summarized in the Appendix). In the chronic study, groups of rats received the test material in feed at dietary concentrations of 0, 150, 500, 1500, or 3000 ppm. Through week 17, decreased body weights were noted at 3000 ppm (11 and 14% less than controls for males and females, respectively) and at 1500 ppm (4 and 8% less than controls for males and females, respectively). In the range-finding reproduction study, there were no treatment-related parental, offspring, or reproductive effects when the test material was administered in feed to groups of 20 Sprague-Dawley rats/sex at dietary concentrations of 0, 50, 100, 500, or 1000 ppm for 8 weeks prior to mating, throughout mating, gestation, and lactation, and until termination. The dietary concentrations selected for the current study were 0, 150, 500, and 2500. The highest dietary concentration was expected to produce evidence of toxicity, and no parental or reproductive effects were expected at the lowest dietary concentration.

6. **Dosage preparation and analysis:** Test diets were prepared weekly and were stored under refrigeration until use. The method used to prepare the diets was not reported, although the study report did state that corn oil was used as a carrier for the test material and to help control the dust. Homogeneity was evaluated in conjunction with the pilot study (MRIDs 45422714 and 45422825), using 50 and 5000-ppm mixtures of the test material dissolved in corn oil/acetone and mixed in feed. A total of nine samples were taken, three from each third

of the mixture (i.e. top, middle, and bottom) by weight. Stability of the same 50 and 5000-ppm diets was also evaluated in conjunction with the pilot study (MRIDs 45422714 and 45422825), in which the mixtures were either stored in the freezer for up to 56 days or stored in the freezer for 7 days followed by up to 14 days at room temperature. A separate study (MRID 45422826) evaluated the stability of 50 and 5000-ppm mixtures of the test material dissolved in acetone/corn oil and mixed with feed, when either stored under refrigeration for up to 28 days or stored under refrigeration for 7 days followed by up to 7 days at room temperature. During the study, samples of all of the test diets were analyzed for concentration during weeks 1-3 and every 4 or 5 weeks thereafter.

Results:

Homogeneity analysis: The mean (± S.D.) concentration of the samples from the 50 ppm diet was 42.2±4.1 ppm (84.3% of nominal), and the individual samples varied from 75.6 to 92% of nominal. The mean (± S.D.) concentration of the samples from the 5000 ppm diet was 4400±380 ppm (88% of nominal), and the individual samples varied from 79.1 to 102% of nominal. Although the coefficients of variation for both diets were less than 10% (9.8 and 8.6% for the 50- and 5000-ppm diets, respectively), there was a significant amount of variation between the concentrations of the individual samples of both diets.

Stability analysis: After 7, 14, 21, 28, 40, and 56 days of storage in the freezer, the measured concentrations of the 50 ppm diet were 96.0, 90.5, 92.8, 80.1, 78.0, and 95.6% of initial, respectively, and the measured concentrations of the 5000 ppm diet were 100, 95.2, 107, 85.6, 94.6, and 99.5% of initial, respectively. After 1, 4, 7, 11, and 14 days of storage at room temperature, the measured concentrations of the 50 ppm diet were 104, 101, 108, 101, and 103% of initial, respectively, and the measured concentrations of the 5000 ppm diet were 108, 109, 117, 95.2, and 96.7% of initial, respectively. After 7, 14, and 28 days of storage under refrigeration, the measured concentrations of the 50 ppm diet were 100, 109, and 104% of initial, respectively. After 7, 16, and 28 days of storage under refrigeration, the measured concentrations of the 5000 ppm diet were 105, 90.6, and 114% of initial, respectively.

Concentration analysis: Concentrations of the 150, 500, and 2500-ppm diets were 74.7-117.7, 78.9-99.9, and 80.1-101.7% of nominal, respectively, with 13/33 of the analyzed samples differing from target by greater than ±15%.

The increases in the concentration of the test material in the diets over time indicate that there may have been a problem with either homogeneity or the analytical method; however, it is the opinion of the reviewer that the test material was stable in the diet under the storage and usage conditions used in the study. The analytical data from homogeneity and concentration analyses indicated that the mixing procedure may have been inadequate and that there may have been some variance between nominal and actual dosage to the study animals.

C. OBSERVATIONS:

1. **Parental animals:** The animals were observed for clinical signs or mortality/moribundity at least once daily, and if a possible clinical sign was noted, the animal was removed from the cage for a detailed examination. A physical examination with a detailed assessment of clinical signs was conducted once per week. For males, body weights and food consumption

were recorded weekly throughout the study. For females, body weights and food consumption were recorded weekly during pre-mating, on GD 0, 6, 13, and 20, and on lactation days (LD) 0, 4, 7, 14, and 21.

Estrous cycle staging was conducted over a 3-week period prior to mating for all F_0 and F_1 females by examining daily vaginal smears, and the estrous cycle stage was also determined for all females just prior to termination.

At termination, sperm was collected from one testes and one epididymis of all F_0 and F_1 males for enumeration of homogenization-resistant spermatids and cauda epididymal sperm reserves, respectively. Sperm was also collected from the distal portion of the vas deferens (closest to the urethra) to evaluate sperm morphology and motility.

2. **Litter observations:** The following litter observations (X) were made (see Table 2).

TABLE 2. F_1 / F_2 Litter observations

Observation	Time of observation (lactation day)					
	Day 0	Day 4 [a]	Day 4 [b]	Day 7	Day 14	Day 21
Number of live pups	Daily					
Survival/Clinical signs	Daily					
Pup weight	x	x	x	x	x	x
Sex of each pup (M/F)	x					
Detailed physical examination	x	x	x	x	x	x
Anogenital distance	x [c]					

Data taken from text, pp. 21-22, MRID 45422715.
[a] Before standardization (culling)
[b] After standardization (culling)
[c] Anogenital distance was measured for F_2 pups only.

On day 4 postpartum, litters were standardized to a maximum of 8 pups/litter (4/sex/litter, as nearly as possible), and excess pups were killed and discarded. The pups that were not culled remained with the dam until weaning (LD 21). F_1 pups selected as parental animals of the next generation were observed for vaginal patency or preputial separation, and each pup's body weight was recorded on the day the change was noted.

Pups born or found dead were examined grossly for external and internal abnormalities and/or a possible cause of death.

3. **Postmortem observations:**

1) **Parental animals:** At completion of the mating phase, all surviving parental males were sacrificed. Maternal animals were sacrificed following the weaning of their respective litters on LD 21. Females that were sperm positive and/or had an internal vaginal plug but did not deliver were sacrificed after gestation day 24, and females that were never observed as being inseminated and/or with an internal vaginal plug were sacrificed and

necropsied at least 24 days after the completion of the mating phase if they did not deliver. Animals that died or were sacrificed moribund as well as those killed at terminal sacrifice were subjected to gross necropsy consisting of external and internal examinations including body orifices and the cervical, thoracic, and abdominal viscera. In addition, in the females that did not deliver litters, the patency of the cervical/uterine os was evaluated via flushing of the uterine horns with an appropriate solution/fixative. As previously mentioned, just prior to termination the estrous cycle stage of all females was determined by examination of vaginal smears, and at the time of termination, sperm was collected from one testes, one epididymis, and the distal portion of the vas deferens of all males.

The CHECKED (X) tissues were collected, and the (XX) organs, in addition, were weighed. Histopathological examination was conducted on selected tissues [*] from the control and high-dose animals, with the following exception: the reproductive organs were examined in any animal with abnormal estrous cyclicity, affected sperm number, motility, or morphology, or potential fertility effects, i.e. those who failed to mate, conceive, sire, or deliver healthy offspring. A quantitative evaluation of the primordial (preantral), antral, and corpora lutea follicles was conducted on ten randomly selected F_1 females from the control and high-dose groups that had viable litters.

XX	Ovaries*	XX	Brain
XX	Uterus* with oviducts* and cervix*	XX	Pituitary*
X	Vagina*	XX	Thymus
XX	Testes*	XX	Liver*
XX [a]	Epididymides (caput, corpus, and cauda)*	XX	Kidneys
XX	Prostate*	XX	Adrenal glands*
XX	Seminal vesicles* with coagulating glands* and fluids	XX	Spleen
X	Gross lesions*		

[a] Total weight for both epididymides, and cauda weight for the side not being used for sperm analysis.

2) **Offspring:** The F_1 offspring not selected as parental animals and all F_2 offspring were sacrificed at 21 days of age and examined macroscopically for any structural abnormalities or pathological changes, with particular emphasis on the reproductive organs. In addition, the brain, spleen, and thymus were weighed from one randomly selected pup/sex/litter. Pups found dead were also examined for possible defects and/or cause of death.

D. DATA ANALYSIS:

1. **Statistical analyses:** With the exception of pathology data, the following statistical methods were used. Parametric data (including body weight gain and food consumption) were analyzed using a univariate Analysis of Variance (ANOVA), followed by Dunnett's Test if significant. Nonparametric data (e.g., number of estrous cycles, litter size, and number of implantation sites) were analyzed using the Kruskal-Wallis test, followed by Dunn's Test if significant. Nonparametric dichotomous data (e.g. fertility and gestation indices) were initially analyzed using the Chi-Square Test, followed by the Fisher's Exact Test with the

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Bonferroni adjustment if significant. Significance levels of $p<0.05$ and $p<0.01$ were used for all tests.

Sperm morphology and count were analyzed by testing the homogeneity of variances using the folded form of the F statistic at the 0.01 significance level. If variances were not significantly different, a two sample t-test was used to compare the high dose group with the control group. If variances were significantly different, the Cochran and Cox t-test was used to compare the high dose group with the control group. Animals with less than 100 available sperm were not included in the analysis of sperm morphology. Sperm motility was analyzed with a trend test conducted in SAS PROC MULTTEST using the ordinal dose scale (0,1,2,3) and the permutation adjusted p-value. If the trend test was significant with 4 groups at the $p<0.05$ level, the high dose group was deemed significantly different from control, and the trend test was repeated with the 3 remaining groups (control, low, and mid dose), and this analysis process continued until the trend test was not statistically significant.

Necropsy and histopathology data were analyzed using the following methods. Terminal body weight and organ weight data were analyzed using Bartlett's test to determine homogeneity of variance. Homogeneous data were then analyzed using an Analysis of Variance (ANOVA), followed by Dunnett's test if significant, and non-homogeneous data were analyzed using a Kruskal-Wallis Analysis of Variance, followed by a pairwise Mann-Whitney U Test if significant. Micropathology data for adult animals were analyzed using the Chi-Square test followed by a one-tailed Fisher's Exact Test if significant. Pup necropsy data were screened visually for potential effects, and lesions of questionable significance were further evaluated using the following gross lesion index (%) which was calculated for each litter: (number of pups with the lesion/number of pups necropsied) x 100. The mean gross lesion index for the lesion in question was then calculated for each dose group and analyzed using the Kruskal-Wallis Analysis of Variance followed by a Mann-Whitney U Test if significant. Ovarian follicles and corpora lutea count data (mean data/animal values) were analyzed using Bartlett's test; homogeneous data were then analyzed using Analysis of Variance (ANOVA) followed by Student's t-test if significant, and non-homogeneous data were analyzed using a Kruskal-Wallis Analysis of Variance followed by a pairwise Mann-Whitney U Test if significant. A significance level of $p<0.001$ was used for Bartlett's test, and a significance level of $p<0.05$ was used for all other statistical tests used to analyze pathology data.

The reviewer considers the particular statistical analyses used in the study to be appropriate.

2. **Indices:**

Reproductive indices: The following reproductive indices were calculated from breeding and parturition records of animals in the study:

Mating Index (%) = [# inseminated females/# of females co-housed] × 100, where "inseminated females" includes pregnant females not observed sperm positive or to have had an internal vaginal plug.

Fertility Index (%) = [# of pregnant females/# of inseminated females] × 100, where "pregnant females" includes females that did not deliver, but had implantation sites.

Gestation Index (%) = [# of females with live pups/# of pregnant females] × 100

Offspring viability indices: The following viability indices were calculated from lactation records of litters in the study:

Birth Index (%) = [total # of pups per litter/total # of implantation sites per litter] × 100

Live Birth Index (%) = [# of live pups per litter/total # of pups per litter] × 100

Viability Index (%) = [# of live pups per litter on LD 4 (pre-cull)/# of live pups per litter] × 100

Lactation Index (%) = [# of live pups per litter on LD 21/# of live pups per litter on LD 4 (post-cull)] × 100

3. **Historical control data:** Historical control data were provided (MRID 45422716) and included data from 6 reproduction studies conducted between 1995 and 1998, using the Sprague-Dawley rat. The data were presented in aggregate form only, rather than giving the data from the individual studies. No information was provided regarding the source(s) of the animals or the vehicles used.

II. RESULTS:

A. PARENTAL ANIMALS:

1. **Mortality and clinical signs:** One control F_0 female was found dead during week 8 without exhibiting any abnormal clinical signs, and a cause of death was not determined. One high-dose F_0 female was found dead post-partum during week 14 and had a prolapsed uterus with congestion and hemorrhage and marked diffuse centrilobular liver necrosis. One low dose F_1 female was sacrificed moribund during week 7 after exhibiting lacrimation, gasping, and urine staining and had a severe lung abscess with inhalation pneumonia. One mid-dose F_1 female was sacrificed due to dystocia; however, the report did not mention the day of gestation on which this occurred. All of the early deaths were considered to be incidental to treatment. No treatment-related clinical signs were observed during pre-mating, mating, gestation, or lactation.

2. **Body weight and food consumption:**

   a. **Pre-mating:** Selected body weight and food consumption data for the F_0 and F_1 adults are given in Tables 3a and 3b, respectively. Absolute body weights of F_0 high-dose males were significantly decreased by week 2 (6% less than controls; $p<0.01$) and remained slightly decreased (8-9% less than controls; $p<0.01$) for the remainder of pre-mating. Absolute body weights of F_0 high-dose females were significantly decreased by week 2 (9% less than controls; $p<0.01$) and remained decreased for the remainder of pre-mating

(13-14% less than controls; $p<0.01$). Cumulative body weight gain during pre-mating was also decreased in both high-dose males and high-dose females of the F_0 generation (20 and 39% less than controls, respectively). Absolute body weights of F_1 high-dose males and females were significantly decreased throughout pre-mating (17-21% and 16-17% less than controls for males and females, respectively; $p<0.01$), and the cumulative body weight gain during pre-mating was also decreased in both sexes (15 and 18% less than controls for males and females, respectively).

At the highest dietary concentration, a transient decrease in food consumption of both sexes of the F_0 generation was noted during the first week of treatment and was attributed to decreased palatability of the test article. F_0 high-dose males had increased food consumption during week 9 (11% greater than controls; $p<0.01$), and F_0 high-dose females had increased food consumption during weeks 8-10 (24-18% greater than controls; $p<0.01$). F_1 generation high-dose males and females had increased food consumption throughout pre-mating (10-27% and 10-30% greater than controls, respectively; $p<0.05$ or $p<0.01$).

b. **Gestation:** Selected body weight and food consumption data for pregnant dams of the F_0 and F_1 generations are given in Table 4. Pregnant high-dose females of both generations had decreased absolute body weights throughout gestation and decreased cumulative body weight gain during gestation compared to their respective controls. High-dose F_0 dams had increased food consumption during GD 6-13 and 13-20 (10-11% than controls; $p<0.05$ for GD 6-13 only), and high-dose F_1 dams had increased food consumption during GD 6-13 (11% greater than controls; $p<0.05$).

c. **Lactation:** Selected body weight and food consumption data for nursing dams of the F_0 and F_1 generations are given in Table 5. Nursing high-dose females of both generations had decreased absolute body weights throughout lactation, and high-dose F_1 females also had increased cumulative weight gain during lactation. High-dose F_0 dams had increased food consumption compared to controls during LD 0-4, 7-14, and 14-21, and high-dose F_1 dams had increased food consumption throughout lactation compared to controls.

TABLE 3a. Mean body weight and food consumption for F_0 adults during pre-mating [a]

Observations/study week	Treatment level (ppm)			
	Control	150	500	2500
F_0 Generation Males				
Mean body weight (g) **Week 0**	261.0±3.5	264.4±3.3	272.3±3.4	266.7±4.4
Week 2	345.4±5.3	348.9±5.3	351.2±4.3	323.2±4.5 (94) b **
Week 4	401.4±7.3	402.4±6.8	403.9±5.5	365.2±5.7 (91) **
Week 6	436.9±8.6	438.1±8.4	440.9±6.8	400.6±6.0 (92) **
Week 8	465.9±10.1	467.0±9.3	470.5±6.8	428.2±6.3 (92) **
Week 10	487.3±9.7	492.5±9.9	498.0±7.1	446.7±7.2 (92) **
Week 14	527.9±10.8	533.2±11.3	526.8±7.4	476.1±7.1 (90) **
Week 18	561.0±12.0	567.6±13.2	558.1±8.7	506.7±7.8 (90) **
Body weight gain (g) c **Weeks 0-10 (Pre-mating)**	226.3	228.1	225.7	180.0 (80)
Weeks 0-18 (Entire Study)	300.0	303.2	285.8	240.0 (80)
Mean food consumption (g/kg /day) d **Weeks 0-10**	71.7	71.3	70.4	73.3
F_0 Generation Females				
Mean body weight (g) **Week 0**	188.5±2.6	185.1±2.5	188.4±2.5	188.6±2.6
Week 2	222.0±2.9	220.5±3.0	217.1±3.0	201.1±2.3 (91) **
Week 4	246.2±4.6	242.5±3.9	240.1±4.2	212.0±2.9 (86) **
Week 6	267.5±4.3	261.2±4.42	259.3±4.2	230.1±2.7 (86) **
Week 8	276.4±4.9	268.8±4.8	266.7±4.7	236.4±3.11 (86) **
Week 10	283.3±4.5	279.2±5.0	276.6±4.6	246.0±3.0 (87) **
Body weight gain (g) c **Weeks 0-10 (Pre-mating)**	94.8	94.1	88.2	57.4 (61)
Mean food consumption (g/kg/day) [d] **Weeks 0-10 (Pre-mating)**	81.8	83.1	83.0	84.6

Data taken from Tables 2 and 3, pp. 43-46 and 52-53, respectively, MRID 45422715.

[a] Data are given as Mean ± Standard Error.

[b] Numbers in parentheses equal percent of control, calculated by reviewer.

[c] Calculated by reviewer, using group mean body weight data; not subjected to statistical analysis.

[d] Calculated by reviewer, using group mean food consumption data; not subjected to statistical analysis.

Significantly different from control: * $p<0.05$; ** $p<0.01$.

TABLE 3b. Mean body weight and food consumption for F_1 adults during pre-mating [a]

Observations/study week	Treatment level (ppm)			
	Control	150	500	2500
F_1 Generation Males				
Mean body weight (g)				
Week 0	258.8±5.0	252.3±5.7	251.4±4.8	203.7±5.5 (79) [b] **
Week 2	366.6±6.2	360.2±6.2	357.6±6.5	296.2±5.5 (81) **
Week 4	439.6±7.3	431.6±7.5	426.3±7.9	357.7±5.46 (81) **
Week 6	486.9±8.3	483.2±8.4	482.4±8.1	402.6±5.8 (83) **
Week 8	523.0±9.1	519.6±9.0	519.5±7.7	431.0±5.9 (82) **
Week 10	550.3±9.7	551.3±9.9	551.3±8.5	451.8±6.2 (82) **
Week 14	587.9±10.5	591.9±11.3	594.6±9.1	486.1±7.0 (83) **
Week 17	622.7±11.4	629.2±11.7	627.8±10.4	507.9±7.6 (82) **
Body weight gain (g) [c]				
Weeks 0-10 (Pre-mating)	291.5	299	299.9	248.1 (85)
Weeks 0-18 (Entire Study)	363.9	376.9	376.4	304.2 (84)
Mean food consumption (g/kg/day)				
Weeks 0-10 (Pre-mating) [d]	75.8	77.2	77.3	87.7 (116)
F_1 Generation Females				
Mean body weight (g)				
Week 0	184.3±3.3	181.5±3.2	179.3±2.7	154.3±3.8 (84) **
Week 2	225.5±4.1	226.1±3.8	220.4±3.2	190.3±4.0 (84) **
Week 4	255.4±4.6	257.2±4.2	246.8±3.6	212.5±4.4 (83) **
Week 6	280.9±5.4	278.8±5.3	270.8±3.9	235.1±4.0 (84) **
Week 8	294.9±5.5	294.6±5.3	284.4±4.6	244.4±4.0 (83) **
Week 10	306.1±5.5	307.5±5.9	295.4±4.8	253.7±4.0 (83) **
Body weight gain (g) [c]				
Weeks 0-10 (Pre-mating)	121.8	126	116.1	99.4 (82)
Mean food consumption (g/kg/day) [d]				
Weeks 0-10 (Pre-mating)	88.4	88.4	88.1	106.2 (120)

Data taken from Tables 2 and 3, pp. 47-50 and 54-55, respectively, MRID 45422715.

[a] Data are given as Mean ± Standard Error.

[b] Numbers in parentheses equal percent of control, calculated by reviewer.

[c] Calculated by reviewer, using group mean body weight data; not subjected to statistical analysis.

[d] Calculated by reviewer, using group mean food consumption data; not subjected to statistical analysis.

Significantly different from control: * $p<0.05$; ** $p<0.01$.

TABLE 4. Mean body weight and food consumption of F_0 and F_1 dams during gestation [a]				
Observations/gestation day	Treatment level (ppm)			
	Control	150	500	2500
F_0 Generation				
Mean body weight (g)				
GD 0	286.6±5.8	283.8±5.3	280.5±5.0	251.2±3.2 (88) b **
GD 6	307.1±5.4	301.9±5.1	298.0±4.4	266.4±3.8 (87) **
GD 13	330.6±5.6	326.9±5.2	318.9±4.5	287.8±3.8 (87) **
GD 20	407.5±7.0	401.5±6.8	397.9±4.7	351.7±5.4 (86)
Body weight gain (g)				
GD 0-20	120.9±4.4	117.7±4.5	117.4±3.2	100.5±3.6 (83)
Mean food consumption (g/kg/day)				
GD 0-6	64.4±1.9	66.8±2.9	64.3±1.5	63.3±3.8
GD 6-13	67.7±1.4	71.7±1.9	69.3±1.5	75.3±2.7 (111) *
GD 13-20	70.5±1.4	72.0±1.7	74.4±1.8	77.2±2.8 (110)
F_1 Generation				
Mean body weight (g)				
GD 0	304.6±6.2	309.0±6.5	300.6±4.9	257.2±4.2 (84) **
GD 6	325.9±6.7	329.6±6.4	322.5±4.6	274.6±4.3 (84) **
GD 13	354.0±7.2	358.6±6.9	351.5±4.8	303.8±4.9 (86) **
GD 20	427.8±12.3	436.5±9.2	428.5±6.4	370.5±6.3 (87) **
Body weight gain (g)				
GD 0-20	123.2±8.4	127.5±5.2	128.0±5.2	113.3±3.5 (92)
Mean food consumption (g/kg/day)				
GD 0-6	66.6±1.5	65.6±1.7	66.2±1.8	68.3±2.8
GD 6-13	73.6±2.1	75.9±2.1	74.0±1.3	81.9±2.2 (111) *
GD 13-20	74.2±3.0	74.1±2.3	71.3±1.7	75.5±1.6

Data taken from Tables 7 and 8, pp. 68-69 and 71-72, respectively, MRID 45422715.
[a] Data are given as Mean ± Standard Error.
[b] Numbers in parentheses equal percent of control, calculated by reviewer.
Significantly different from control: * $p<0.05$; ** $p<0.01$.

TABLE 5. Mean body weight and food consumption of F_0 and F_1 dams during lactation [a]

Observations/lactation day	Treatment level (ppm)			
	Control	150	500	2500
F_0 Generation				
Mean body weight (g)				
LD 0	308.6±7.5	304.2±5.2	295.3±5.0	270.3±4.4 (88) [b] **
LD 4	331.2±6.1	323.5±5.2	318.1±4.5	277.4±4.2 (84) **
LD 7	336.1±6.2	329.8±4.9	327.0±4.0	288.4±3.5 (86) **
LD 14	360.3±5.8	351.1±5.1	336.8±6.7 (93) *	296.9±4.2 (82) **
LD 21	338.4±4.2	333.0±4.2	330.4±3.4	302.6±3.2 (89) **
Body weight gain (g) [c]				
LD 0-21	29.8	28.8	35.1 (118)	32.3 (108)
Mean food consumption (g/kg/day)				
LD 0-4	102.4±3.9	107.8±3.5	110.7±3.7	115.3±6.3 (113)
LD 4-7	129.8±3.6	135.3±2.6	138.4±2.7	139.5±6.0 (107)
LD 7-14	165.8±4.8	171.5±2.9	165.9±3.8	181.3±7.0 (109)
LD 14-21	183.8±6.0	193.6±3.9	204.9±6.6 (111)	207.6±6.9 (113)
F_1 Generation				
Mean body weight (g)				
LD 0	337.4±7.3	344.0±6.8	340.2±6.5	288.4±4.6 (86) **
LD 4	350.6±6.9	356.6±7.4	346.7±6.7	299.4±4.5 (85) **
LD 7	361.1±6.7	359.6±7.0	354.8±5.6	307.1±4.4 (85) **
LD 14	376.8±6.9	377.7±6.9	377.7±5.0	323.3±5.0 (86) **
LD 21	350.1±5.8	354.6±5.5	350.8±4.7	305.2±6.4 (88) **
Body weight gain (g) c				
LD 0-21	12.7	10.6	10.6	16.8 (132)
Mean food consumption (g/kg/day)				
LD 0-4	100.2±4.1	95.3±3.5	99.9±4.4	123.5±10.3 (123)
LD 4-7	136.2±4.2	127.0±3.4	126.5±8.2	161.7±12.2 (119)
LD 7-14	161.9±3.4	160.6±4.6	163.1±4.5	183.7±6.6 (113) *
LD 14-21	176.7±4.3	182.2±5.6	179.6±4.7	200.6±8.8 (114) *

Data taken from Tables 11 and 12 pp. 80-81 and 83-84, respectively, MRID 45422715.
[a] Data are given as Mean ± Standard Error.
[b] Numbers in parentheses equal percent of control, calculated by reviewer.
[c] Calculated by reviewer, using group mean body weight data; not subjected to statistical analysis.
Significantly different from control: * $p<0.05$; ** $p<0.01$.

3. **Test substance intake:** The doses expressed as mean daily mg test substance/kg body weight during the 10-week pre-mating period are given in Table 6. Individual test substance intakes were calculated for each animal using body weight and food consumption data; there was no mention of adjustment for analytical results. The values for the F_0 generation are considered to be representative of the test substance intake for the entire study.

TABLE 6. Mean test substance intake during pre-mating (mg/kg body weight/day)[a]						
Generation	Males			Females		
	Treatment level (ppm)			Treatment level (ppm)		
	150	500	2500	150	500	2500
F_0	9.8±0.4	31.2±1.1	163.4±4.3	11.5±0.3	36.8±0.8	188.8±2.5
F_1	10.7±0.5	34.3±1.6	195.7±8.4	12.2±0.4	39.0±1.2	237.0±5.2

Data taken from Table 4, pp. 57-60, MRID 45422715.
[a] Data are given as Mean ± Standard Error.

4. **Reproductive function:**

   a. **Estrous cycle length and periodicity:** Results from the evaluation of vaginal smears indicated that the regularity and duration of estrous were not affected. The mean lengths of the estrous cycles for the control, low-, mid-, and high-dose groups were 4.2, 4.3, 4.2, and 4.1 days, respectively, for the F_0 females and 4.3, 4.4, 4.3, and 4.4 days, respectively, for the F_1 females.

   b. **Sperm measures:** Sperm motility, count and morphology results are summarized in Table 7. Sperm morphology and total sperm counts were evaluated only for the high-dose and control groups of both generations. In high-dose F_0 males there was a significant decrease in the percent progressively motile as compared to controls, in the absence of an effect on the percent motile. In high-dose F_1 males, both the percent motile (↓10%) and the percent progressively motile (↓23%) were statistically significantly decreased compared to control. These decreases may be related to increases in the percent of sperm with detached heads; (2.1% in high-dose F_0 males vs 0.6% in controls and 4.0% in high-dose F_1 males vs 0.7% in controls).

   c. **Ovarian follicle counts:** The ovarian follicle counts of the high-dose F_1 females were similar to those of controls. The mean primordial (preantral) follicle, antral follicle, and corpora lutea counts were 121.7, 39.4, and 37.1, respectively, for the control F_1 females and 122.2, 45.3, and 32.4, respectively for the high-dose F_1 females.

5. **Reproductive performance:** The reproductive performances of the F_0 and F_1 animals are summarized in Table 8. Mating performance and fertility were not affected by test article administration in either generation. Mating, gestation, and fertility indices, mean precoital intervals, and mean gestation lengths were similar between the treated and control groups of both generations. Assessment at necropsy found that the number of implantation sites was not affected by treatment. Numbers of grossly visible corpora lutea were not reported.

TABLE 7. Sperm measures of F_0 and F_1 males [a]

Parameter	Dose Group (ppm)			
	Control	150	500	2500
F_0 Generation Males				
Sperm morphology				
% Normal	83.5±1.1	n/a b	n/a	81.9±1.8
% Abnormal	15.9±1.2	n/a	n/a	16.0±1.2
% Detached	0.6±0.1	n/a	n/a	2.1±1.1
Total sperm count				
Epididymis	146.1±7.7	n/a	n/a	141.6±8.1
Testis	129.9±4.7	n/a	n/a	129.5±3.9
Sperm motility				
% Motile	82.9±1.9	82.6±1.9	82.6±1.6	79.2±1.5
% Progressive	64.1±2.5	66.2±1.9	61.5±2.2	56.2±1.7 (88) c **
F_1 Generation Males				
Sperm morphology				
% Normal	69.3±2.0	n/a	n/a	67.0±2.0
% Abnormal	30.0±2.0	n/a	n/a	29.0±2.1
% Detached	0.7±0.2	n/a	n/a	4.0±1.7
Total sperm count				
Epididymis	149.5±9.0	n/a	n/a	133.7±11.4 (89)
Testis	105.7±5.1	n/a	n/a	103.8±5.5
Sperm motility				
% Motile	81.7±1.5	82.6±1.6	79.0±1.9	73.4±2.5 (90) **
% Progressive	59.9±2.0	61.7±2.0	53.9±2.2 (90)	46.1±2.7 (77) **

Data taken from Table 19, p. 113, MRID 45422715.

[a] Data are given as Mean ± Standard Error.

[b] Not applicable, as sperm morphology and total sperm counts were only evaluated in control and high-dose animals.

[c] Numbers in parentheses equal percent of control, calculated by reviewer.

Significantly different from control: ** $p<0.01$.

TABLE 8. Reproductive Performance [a]

Observation	Treatment level (ppm)			
	Control	150	500	2500
F_0 Generation (F_1 litters)				
Number males/females paired	29/29	30/30	30/30	30/30
Number females with evidence of mating	26	30	30	30
Number of females delivered	24	29	28	29
Number of females with implantations	24	29	28	29
Mean precoital interval (days)	2.2±0.19	2.7±0.38	3.1±0.31	2.3±0.47
Mean gestation length (days)	22.0±0.04	22.3±0.08	22.1±0.09	22.0±0.10
Mating index (%)	89.7	100.0	100.0	100.0
Fertility index (%)	92.3	96.7	93.3	96.7
Gestation index (%)	100.0	100.0	100.0	100.0
Number of females that died [b]	0	0	0	1
F_1 Generation (F_2 litters)				
Number males/females paired	30/30	29/29	30/30	30/30
Number females with evidence of mating	28	28	28	28
Number of females delivered	23	25	20	28
Number of females with implantations	23	25	21	28
Mean precoital interval (days)	3.4±0.47	2.5±0.19	2.9±0.29	2.5±0.23
Mean gestation length (days)	22.0±0.04	22.2±0.09	22.1±0.10	22.3±0.11
Mating index (%)	93.3	96.6	93.3	93.3
Fertility index (%)	82.1	89.3	75.0	100.0
Gestation index (%)	100.0	100.0	95.2	100.0
Number of females that died [b]	0	0	1	0

Data taken from Table 5, pp. 62-63, Appendix I, pp. 123, 129, and 142, and Attachment 3, p. 452, MRID 45422715.
[a] Data are given as Mean ± Standard Error, where applicable.
[b] During gestation or parturition.

6. Parental postmortem results:

a) **Organ weights:** Representative organ weight data are given in Tables 9 and 10. There were numerous statistically significant differences between the absolute and relative (to body) organ weights of the high-dose groups of both generations and their respective controls; however, many of these differences were of insufficient magnitude to be considered biologically relevant. The decreases in absolute and/or relative thymus weights of the high-dose F_0 and F_1 males and females were considered to be treatment-related and were most likely secondary to thymic atrophy due to the body weight decrements noted in these groups and/or stress. As there were no histopathologic correlates noted in any of the organs routinely examined (reproductive organs, liver, pituitary, and adrenals), the remaining differences were most likely attributable to the decreased terminal body weights of the animals in these groups, rather than the result of test article toxicity on the organs themselves.

b) **Pathology:**

1) **Macroscopic examination:** There were no treatment-related abnormal gross necropsy findings noted in the parental animals of either generation.

2) **Microscopic examination:** There were no treatment-related abnormal histopathology observations noted in the parental animals of either generation.

TABLE 9. Selected absolute and relative organ weights for adult F_0 and F_1 males [a]

Observation		Treatment level (ppm)			
		Control	150	500	2500
F_0 Males					
Terminal body weight (g)		559.9±64.2	571.0±73.3	561.9±48.3	508.7±42.6 (91) *
Liver	Absolute wt (g)	22.29±3.40	22.62±3.53	22.65±2.72	20.21±3.04 (91) *
	Relative wt (%)	3.98±0.35	3.96±0.30	4.03±0.26	3.96±0.39
Brain	Absolute wt (g)	2.22±0.09	2.22±0.09	2.24±0.08	2.21±0.11
	Relative wt (%)	0.40±0.04	0.40±0.05	0.40±0.03	0.44±0.04 (110) *
Testes	Absolute wt (g)	3.47±0.24	3.38±0.27	3.47±0.48	3.28±0.65
	Relative wt (%)	0.63±0.08	0.60±0.09	0.62±0.09	0.65±0.14
Epididymis	Absolute wt (g)	1.47±0.17	1.42±0.13	1.41±0.22	1.41±0.30 (96) *
	Relative wt (%)	0.27±0.04	0.25±0.04	0.25±0.04	0.28±0.06
Prostate	Absolute wt (g)	1.74±0.38	1.67±0.37	1.69±0.40	1.50±0.26
	Relative wt (%)	0.31±0.07	0.30±0.07	0.30±0.07	0.30±0.05
Seminal vesicles	Absolute wt (g)	1.99±0.43	1.92±0.36	1.85±0.26	1.66±0.29 (84) *
	Relative wt (%)	0.36±0.10	0.34±0.08	0.33±0.05	0.33±0.05
Thymus	Absolute wt (g)	0.43±0.09	0.47±0.15	0.43±0.10	0.36±0.08 (84) *
	Relative wt (%)	0.077±0.014	0.082±0.021	0.077±0.015	0.071±0.015
F_1 Males					
Terminal body weight (g)		630.6±62.5	634.8±67.0	635.3±57.5	510.0±44.2 (81) *
Liver	Absolute wt (g)	25.03±3.61	24.99±2.98	24.77±5.17	20.04±2.72 (80) *
	Relative wt (%)	3.96±0.35	3.94±0.27	3.90±0.75	3.92±0.31
Brain	Absolute wt (g)	2.28±0.08	2.27±0.13	2.27±0.09	2.17±0.10
	Relative wt (%)	0.36±0.04	0.36±0.04	0.36±0.03	0.43±0.04 (120) *
Testes	Absolute wt (g)	3.75±0.27	3.54±0.73	3.70±0.31	3.48±0.36 (93) *
	Relative wt (%)	0.60±0.07	0.56±0.13	0.59±0.05	0.69±0.09 (115) *
Epididymis	Absolute wt (g)	1.50±0.14	1.48±0.18	1.50±0.12	1.31±0.11
	Relative wt (%)	0.24±0.03	0.24±0.03	0.24±0.02	0.26±0.03 (108) *
Prostate	Absolute wt (g)	1.65±0.35	1.60±0.35	1.47±0.34	1.32±0.23 (80) *
	Relative wt (%)	0.26±0.06	0.25±0.05	0.23±0.05	0.26±0.05
Seminal Vesicles	Absolute wt (g)	1.70±0.27	1.78±0.25	1.77±0.32	1.71±0.36
	Relative wt (%)	0.27±0.05	0.28±0.05	0.28±0.05	0.34±0.08
Thymus	Absolute wt (g)	0.520±0.099	0.542±0.126	1.37±4.63	0.370±0.087 (71) *
	Relative wt (%)	0.083±0.015	0.085±0.014	0.218±0.746	0.072±0.015 (87) *

Data taken from Tables OW1K-SUM and OW2K-SUM, pp. 517-520 and 526-529, respectively, MRID 45422715.
[a] Data are given as Mean ± Standard Error.
Significantly different from control: * $p<0.05$.

511

TABLE 10. Selected absolute and relative organ weights for adult F_0 and F_1 females [a]

Observation		Treatment level (ppm)			
		Control	150	500	2500
F_0 Females					
Terminal body weight (g)		325.1±21.8	323.6±26.4	324.6±18.2	305±23.8 (94) *
Liver	Absolute wt (g)	16.57±2.62	17.14±1.90	16.86±1.50	16.01±2.48
	Relative wt (%)	5.10±0.76	5.30±0.45	5.20±0.41	5.24±0.60
Brain	Absolute wt (g)	2.05±0.07	2.04±0.09	2.04±0.09	2.00±0.09
	Relative wt (%)	0.63±0.04	0.63±0.05	0.63±0.04	0.65±0.06
Ovaries	Absolute wt (g)	0.103±0.018	0.097±0.013	0.103±0.014	0.083±0.015 (81) *
	Relative wt (%)	0.032±0.005	0.030±0.004	0.032±0.005	0.027±0.005 (84) *
Uterus	Absolute wt (g)	0.675±0.201	0.611±0.148	0.642±0.204	0.504±0.124 (75) *
	Relative wt (%)	0.21±0.07	0.19±0.05	0.20±0.06	0.17±0.05
Thymus	Absolute wt (g)	0.252±0.066	0.228±0.060	0.217±0.060	0.163±0.060 (65) *
	Relative wt (%)	0.078±0.020	0.071±0.020	0.067±0.017	0.053±0.019 (68) *
F_1 Females					
Terminal body weight (g)		345.9±32.3	345.1±27.9	334.4±31.1	302.3±25.7 (97) *
Liver	Absolute wt (g)	17.81±2.83	18.59±2.82	16.89±3.19	17.34±2.55 (97) *
	Relative wt (%)	5.16±0.72	5.39±0.70	5.05±0.85	5.74±0.69 (111) *
Brain	Absolute wt (g)	2.10±0.10	2.10±0.09	2.10±0.11	1.99±0.09 (95) *
	Relative wt (%)	0.61±0.05	0.61±0.04	0.63±0.06	0.66±0.05 (108) *
Ovaries	Absolute wt (g)	0.116±0.019	0.115±0.023	0.111±0.021	0.103±0.021 (89)
	Relative wt (%)	0.034±0.006	0.033±0.006	0.033±0.006	0.034±0.006
Uterus	Absolute wt (g)	0.715±0.253	0.571±0.117	0.841±1.027	0.567±0.136 (79) *
	Relative wt (%)	0.21±0.08	0.17±0.04 (80) *	0.25±0.28	0.19±0.05
Thymus	Absolute wt (g)	0.313±0.096	0.261±0.080	0.294±0.106	0.186±0.054 (59) *
	Relative wt (%)	0.091±0.029	0.076±0.025	0.089±0.033	0.062±0.018 (68) *

Data taken from Tables OW1K-SUM and OW2K-SUM, pp. 521-524 and 530-533, respectively, MRID 45422715.
[a] Data are given as Mean ± Standard Error.
Significantly different from control: * $p<0.05$.

B. OFFSPRING

1. **Viability and clinical signs:** Mean litter size and viability (survival) data for the F_1 and F_2 litters are given in Table 11. Mean live litter size, sex ratio at birth, and the birth, live birth, viability, and lactation indices were all similar between the treated and control groups of both generations. However, a treatment-related effect on the numbers of stillbirths was noted. There were statistically significant increases in the incidences of stillbirths in the F_1 mid- and high-dose groups, and an increased incidence of litters with stillbirths in the F_1 high-dose group (2 x 2 Chi-Square test performed by reviewer). The trend continued in the F_2 generation, with statistically significant increases in the incidences of stillbirths in the mid-

and high-dose groups, although the litter incidences of stillbirths were not affected in the F_2 generation. The provided historical control data did not include sufficient detail to fully evaluate the significance of these increases. The historical control had a mean of 0.5 still births per study, with a maximum of 3.7% in one study. This was the same incidence seen in the high dose from the F_2 generation. When the data was considered in its entirety, the stillbirths seen in the mid and high doses from both F_1 and F_2 generations was considered to be treatment related. Whole litter losses were noted only in the high-dose groups and included 2 F_1 litters (one by LD 4 from the F_0 dam that was found dead post-partum and the other by LD 7) and one F_2 litter (by LD 14).

No treatment-related clinical signs were observed in offspring during lactation of either generation.

There were no treatment-related effects on the anogenital distances of the F_2 male or female pups. The mean anogenital distances for the control, low-, mid-, and high-dose groups were 4.1, 4.0, 4.1, and 4.0 mm, respectively, for F_2 males and 2.0, 2.1, 2.1, and 2.1 mm, respectively, for F_2 females.

2. **Body weight:** Selected mean pup absolute body weight data are given in Table 12. As compared to their respective controls, mean absolute body weights of F_1 high-dose male and female pups were significantly decreased from LD 4 through the end of weaning, and mean absolute body weights of F_2 high-dose male and female pups were significantly decreased from LD 7 through the end of weaning. Pup body weight gains (Table 13) of high-dose males and females of both generations were significantly decreased throughout lactation (F_1 combined sexes: 67-76% of controls, $p<0.01$; F_2 combined sexes: 72-81% of controls, $p<0.05$ or $p<0.01$). The body weight gains of F_1 mid-dose pups of both sexes were slightly decreased during LD 7-14 (90% of controls for both sexes and 89% of controls for combined sexes; $p<0.05$).

3. **Sexual maturation (F_1):** Data pertaining to pup sexual maturation are reported in Table 14. There were treatment-related effects noted on the times to balanopreputial separation and vaginal opening, both of which were significantly increased in the high-dose pups. The statistically significant increase in the mid-dose group's time to balanopreputial separation was considered to be within the normal range according to the study report, which stated that results within ±5% of concurrent controls were considered normal for the particular rodent strain at the testing facility. However, the increased time to balanopreputial separation and vaginal opening occurred at doses which demonstrated other evidence of toxicity including decreased thymus weights. The altered sperm morphology and decreased motility may coincide with the delayed sexual maturation in males to suggest an effect of treatment on male hormone homeostasis. Taken together the noted effects on sexual maturation were considered to be treatment related and adverse.

TABLE 11. Litter parameters for F_1 and F_2 litters [a]

Observation	Treatment level (ppm) Control	150	500	2500
F_1 Litter				
Mean implantation sites	14.5±0.65	15.3±0.33	14.8±0.38	14.1±0.41
Number of viable litters	24	29	28	29
Number born live	325	399	392	377
Number (%) born dead	0 (0)	3 (0.75)	6 (1.5) *	9 (2.3) **
Number (%) of litters with stillbirths	0 (0)	3 (10.3)	3 (10.7)	6 (20.7) *
Sex Ratio Day 0 (% ♂)	48.4±3.25	53.8±2.25	50.8±2.53	48.8±2.43
# Deaths or "missing" Days 0-4	3	2	4	30 b
# Deaths or "missing" Days 4-21	1	1	5	2
Mean litter size Day 0	13.5±0.7	13.8±0.5	14.0±0.5	13.0±0.4
Day 4 c	13.4	13.7	13.9	12.4 e
Day 4 d	7.7	8.0	7.9	7.6
Day 7	7.7	8.0	7.9	7.9 e
Day 14	7.7	8.0	7.9	7.9
Day 21	7.6	8.0	7.8	7.9
Birth index	89.8±3.54	90.5±1.99	94.5±1.50	94.0±1.47
Live birth index	100.0±0	99.3±0.41	98.4±1.13	97.9±0.86
Viability index	99.2±0.43	99.4±0.63	98.7±0.85	92.4±4.58
Lactation index	99.5±0.52	99.6±0.43	97.8±1.82	96.0±3.58
F_2 Litter				
Mean implantation sites	15.0±0.56	14.4±0.82	13.9±0.78	14.0±0.62
Number of viable litters	23	25	20	28
Number born live	315	339	253	339
Number (%) born dead	1 (0.32)	3 (0.88)	8 (3.1) **	13 (3.7) **
Number (%) of litters with stillbirths	1 (4.3)	3 (12.0)	5 (25.0)	4 (14.3)
Sex Ratio Day 0 (% ♂)	53.9±2.56	47.9±3.36	50.0±2.35	48.7±2.37
# Deaths or "missing" Days 0-4	6	8	3	10
# Deaths or "missing" Days 4-21	1	3	4	8
Mean litter size Day 0	13.7±0.6	13.6±0.8	12.7 f	12.1±0.7
Day 4 [b]	13.4	13.2	12.5	11.8
Day 4 [c]	7.9	7.6	7.7	7.6
Day 7	7.9	7.6	7.6	7.6
Day 14	7.9	7.5	7.5	7.6 g
Day 21	7.8	7.5	7.5	7.6
Birth index	91.8±1.60	93.4±1.38	87.4±5.11	87.8±3.04
Live birth index	99.8±0.24	99.1±0.48	97.4±1.27	96.5±1.63
Viability index	98.0±1.03	98.1±0.87	98.8±0.68	97.0±0.88
Lactation index	99.5±0.54	98.5±0.83	97.4±1.50	96.0±3.58

Data taken from Tables 5 and 17 and Appendix V, pp. 62-63, 106-109, and 197-212, respectively, MRID 45422715.

[a] Data are given as Mean ± Standard Error, where applicable.

[b] Includes total litter loss of 14 pups from a dam that was found dead post-partum.

[c] Before standardization (culling)

[d] After standardization (culling)

[e] On LD 4, n = 28, and on LD 7 and thereafter, n = 27.

[f] Calculated by reviewer, due to a typographical error in Appendix V; not subjected to statistical analysis.

[g] On LD 14 and 21, n = 27.

Significantly different from control: * $p<0.05$, ** $p<0.01$; calculated by reviewer, using a 2 x 2 Chi-Square test.

TABLE 12. Mean pup body weights (g) [a]

	0 ppm	150 ppm	500 ppm	2500 ppm	0 ppm	150 ppm	500 ppm	2500 ppm
	F_1 Pups - males and females combined				F_2 Pups - males and females combined			
0	6.5±0.14	6.5±0.10	6.5±0.14	6.0±0.11 (92) b *	6.4±0.12	6.6±0.12	6.6±0.14	6.2±0.10
4 c	10.0±0.23	10.0±0.16	9.9±0.26	8.4±0.26 (84) **	10.0±0.27	10.3±0.28	10.3±0.32	9.1±0.24
4 d	10.1±0.23	10.0±0.16	9.8±0.26	8.4±0.26 (83) **	9.9±0.27	10.3±0.28	10.2±0.33	9.1±0.24
7	16.0±0.30	15.8±0.23	15.7±0.32	13.1±0.23 (82) **	15.9±0.36	16.3±0.36	16.1±0.39	13.9±0.30 (87) **
14	31.6±0.58	30.6±0.36	29.7±0.59	24.5±0.45 (78) **	31.6±0.55	32.1±0.56	31.8±0.60	26.6±0.41 (84) **
21	49.2±0.94	48.2±0.61	46.3±0.92	36.4±0.73 (74) **	49.2±0.98	50.0±0.98	49.9±1.25	39.1±0.77 (79) **
	F_1 Pups - males				F_2 Pups - male			
0	6.6±0.09	6.7±0.10	6.6±0.14	6.2±0.12 (94) *	6.6±0.12	6.8±0.14	6.8±0.15	6.5±0.11
4 c	10.2±0.21	10.3±0.16	10.0±0.25	8.8±0.19 (86) **	10.2±0.29	10.6±0.32	10.6±0.36	9.4±0.26
4 d	10.2±0.22	10.3±0.17	10.0±0.26	8.8±0.19 (86) **	10.2±0.30	10.6±0.32	10.5±0.37	9.4±0.25
7	16.2±0.28	16.2±0.23	16.0±0.33	13.4±0.24 (83) **	16.4±0.40	16.9±0.41	16.5±0.43	14.3±0.31 (87) **
21	49.8±0.93	49.6±0.66	47.2±1.09	37.0±0.72 (74) **	50.7±1.05	51.2±1.02	51.1±1.37	40.0±0.76 (79) **
	F_1 Pups - females				F_2 Pups - female			
0	6.2±0.14	6.3±0.10	6.3±0.14	5.8±0.11	6.2±0.12	6.4±0.12	6.5±0.13	6.0±0.10
4 c	9.8±0.23	9.7±0.16	9.7±0.27	8.2±0.26 (84) **	9.7±0.26	9.9±0.28	10.0±0.27	8.9±0.23
4 d	9.8±0.24	9.8±0.15	9.7±0.28	8.2±0.26 (84) **	9.6±0.26	9.9±0.28	9.9±0.29	8.9±0.24
7	15.5±0.32	15.4±0.24	15.4±0.33	12.8±0.25 (83) **	15.4±0.36	15.7±0.39	15.7±0.37	13.5±0.31 (88) **
21	48.2±0.98	46.8±0.63	45.5±0.83	35.8±0.80 (74) **	47.7±1.01	48.6±1.04	48.4±1.22	38.3±0.81 (80) **

Data taken from Table 15, pp. 92-97, MRID 45422715.

[a] Data are given as Mean ± Standard Error.

[b] Numbers in parentheses equal percent of control; calculated by reviewer.

[c] Before standardization (culling)

[d] After standardization (culling)

Significantly different from control: * $p<0.05$, ** $p<0.01$.

HED Records Center Series 361 Science Reviews - File R107689 - Page 530 of 721

TABLE 13. Mean pup body weight gains (g) [a]

Lactation day	0 ppm	150 ppm	500 ppm	2500 ppm	0 ppm	150 ppm	500 ppm	2500 ppm
	F_1 Pups - males and females combined				F_2 Pups - males and females combined			
0-4	3.6±0.16	3.5±0.09	3.4±0.15	2.4±0.19 (67)b **	3.6±0.18	3.7±0.20	3.6±0.20	2.9±0.17 (81) *
4-7	5.9±0.13	5.7±0.13	5.9±0.13	4.5±0.12 (76) **	6.0±0.16	5.9±0.16	5.9±0.15	4.8±0.13 (80) **
7-14	15.7±0.38	14.9±0.25	14.0±0.51 (89) *	11.5±0.31 (73) **	15.7±0.32	15.8±0.33	15.7±0.26	12.5±0.31 (80) **
14-21	17.6±0.49	17.6±0.36	16.7±0.52	11.8±0.37 (67) **	17.6±0.49	18.0±0.57	18.0±0.71	12.6±0.51 (72) **
	F_1 Pups - males				F_2 Pups - male			
0-4	3.6±0.17	3.6±0.10	3.4±0.14	2.6±0.11 (72) **	3.6±0.20	3.8±0.23	3.8±0.23	2.9±0.17 (81) *
4-7	6.1±0.14	5.9±0.12	6.0±0.15	4.6±0.12 (75) **	6.2±0.17	6.2±0.15	6.0±0.16	4.9±0.13 (79) **
7-14	15.7±0.35	15.2±0.27	14.1±0.53 (90) *	11.6±0.30 (74) **	15.9±0.34	16.0±0.34	15.80.27	12.8±0.32 (81) **
14-21	17.8±0.60	18.2±0.39	17.1±0.68	12.0±0.40 (67) **	18.4±0.52	18.4±0.62	18.7±0.78	12.8±0.54 (70) **
	F_1 Pups - females				F_2 Pups - female			
0-4	3.5±0.16	3.5±0.09	3.4±0.16	2.4±0.19 (67) **	3.5±0.16	3.5±0.19	3.5±0.16	2.8±0.17 (80) *
4-7	5.7±0.14	5.6±0.15	5.8±0.11	4.4±0.14 (76) **	5.8±0.16	5.7±0.18	5.8±0.18	4.7±0.14 (81) **
7-14	15.4±0.40	14.6±0.23	13.8±0.51 (90) *	11.3±0.33 (73) **	15.5±0.34	15.7±0.37	15.5±0.34	12.3±0.33 (79) **
21	17.3±0.50	16.8±0.39	16.3±0.46	11.7±0.39 (67) **	16.8±0.51	17.2±0.57	17.2±0.65	12.3±0.49 (73) **

Data taken from Table 16, pp. 98-104, MRID 45422715.

[a] Data are given as Mean ± Standard Error.

[b] Numbers in parentheses equal percent of control; calculated by reviewer.

Significantly different from control: * $p<0.05$, ** $p<0.01$.

516

HED Records Center Series 361 Science Reviews - File R107689 - Page 531 of 721

TABLE 14. Sexual maturation of F_1 male and female pups				
Observation	Treatment level (ppm)			
	Control	150	500	2500
Balano-preputial separation				
Age (days) [a]	41.2±0.27	41.9±0.25	42.5±0.30 (103) b **	47.9±0.53 (116) **
Mean body weight (g) [c]	190.1±17.6	198.7±12.8	191.9±19.4	189.7±15.4
Vaginal opening				
Age (days) [a]	32.4±0.31	32.2±0.32	32.1±0.46	34.7±0.45 (107) **
Mean body weight (g) [c]	104.6±13.0	104.4±10.6	98.8±10.6	90.6±14.3 (87)

Data taken from Table 18, p. 111, MRID 45422715.
[a] Data are given as Mean ± Standard Error.
[b] Number in parentheses equals percent of control; calculated by reviewer.
[c] Data are given as Mean ± Standard Deviation.
Significantly different from control: ** $p<0.01$.

4. Offspring postmortem results:

a) **Organ weights:** Selected absolute and relative (to body) organ weight data are given in Table 15. Decreased absolute thymus weights were noted in F_1 and F_2 high-dose pups of both sexes and in F_1 mid-dose pups of both sexes as compared to their respective controls. These decreases are most likely due to stress-induced thymic atrophy and are considered treatment-related. The spleen weights (relative and absolute) were reduced ($p<0.01$) in females of high dose in the F_1 generation and in males and females of high dose in the F_2 generation. These effects were also considered to be treatment related. The remaining differences were most likely attributable to the decreased weaning weights of the pups, rather than the result of test article toxicity on the organs themselves.

b) **Pathology:**

1) **Macroscopic examination:** There were no treatment-related abnormal gross necropsy findings noted in pups from either generation.

2) **Microscopic examination:** Offspring organs, tissues, and lesions were not submitted for microscopic examination.

TABLE 15. Selected absolute and relative (to body) organ weights for F_1 and F_2 pups [a]

Observation		Treatment level (ppm)			
		Control	150	500	2500
F_1 Male Pups					
Brain	Absolute wt (g)	1.50±0.01	1.52±0.01	1.50±0.02	1.42±0.01 (95) b **
	Relative wt (%)	3.08±0.07	3.11±0.05	3.26±0.08	3.86±0.07 (125) **
Thymus	Absolute wt (g)	0.231±0.007	0.226±0.007	0.201±0.009 (87) *	0.164±0.007 (71) **
	Relative wt (%)	0.472±0.015	0.460±0.015	0.425±0.012	0.440±0.015
Spleen	Absolute wt (g)	0.205±0.006	0.210±0.007	0.208±0.011	0.144±0.007 (70) **
	Relative wt (%)	0.415±0.009	0.423±0.010	0.442±0.018	0.385±0.011
F_1 Female Pups					
Brain	Absolute wt (g)	1.49±0.01	1.45±0.02	1.47±0.01	1.37±0.02 (92) **
	Relative wt (%)	3.15±0.06	3.20±0.06	3.21±0.06	3.84±0.09 (122) **
Thymus	Absolute wt (g)	0.240±0.008	0.218±0.007	0.215±0.008 (90)	0.172±0.008 (72) **
	Relative wt (%)	0.504±0.013	0.477±0.013	0.462±0.012	0.477±0.017
Spleen	Absolute wt (g)	0.210±0.005	0.206±0.008	0.211±0.010	0.136±0.007 (65) **
	Relative wt (%)	0.443±0.008	0.448±0.012	0.453±0.016	0.374±0.012 (84) **
F_2 Male Pups					
Brain	Absolute wt (g)	1.54±0.02	1.57±0.02	1.55±0.02	1.47±0.02 (96) **
	Relative wt (%)	3.09±0.06	3.08±0.04	3.07±0.07	3.67±0.07 (119) **
Thymus	Absolute wt (g)	0.251±0.010	0.238±0.009	0.234±0.009	0.187±0.009 (75) **
	Relative wt (%)	0.499±0.016	0.467±0.016	0.457±0.011	0.457±0.016
Spleen	Absolute wt (g)	0.234±0.010	0.239±0.011	0.227±0.010	0.161±0.009 (69) **
	Relative wt (%)	0.463±0.011	0.465±0.015	0.443±0.013	0.391±0.017 (84) **
F_2 Female Pups					
Brain	Absolute wt (g)	1.47±0.02	1.47±0.02	1.50±0.02	1.41±0.01
	Relative wt (%)	3.17±0.11	3.11±0.07	3.10±0.08	3.70±0.07 (116) **
Thymus	Absolute wt (g)	0.251±0.009	0.221±0.009	0.246±0.009	0.192±0.009 (76) **
	Relative wt (%)	0.548±0.033	0.461±0.017	0.502±0.012	0.497±0.018
Spleen	Absolute wt (g)	0.229±0.009	0.222±0.009	0.233±0.011	0.160±0.009 (70) **
	Relative wt (%)	0.492±0.021	0.459±0.012	0.479±0.021	0.410±0.017 (83) **

Data taken from Table OW4-SUM, pp. 535-538, MRID 45422715.
[a] Data are given as Mean ± Standard Error.
[b] Numbers in parentheses equal percent of control; calculated by reviewer.
Significantly different from control: * $p<0.05$, ** $p<0.01$.

518

III. DISCUSSION AND CONCLUSIONS:

A. INVESTIGATORS' CONCLUSIONS:

The study author concluded that the NOEL/NOAEL for maternal toxicity was 150 ppm, based on decreased body weight during lactation. The NOEL/NOAEL for offspring toxicity was 150 ppm, based on decreased pup body weight and the subsequent effect on preputial separation, and the NOEL/NOAEL for reproductive effects was 2500 ppm based on the absence of any reproductive effects at any dietary concentration.

B. REVIEWER COMMENTS: Parental systemic toxicity was evident at 2500 ppm as decreased absolute body weights and body weight gains in males of both generations during pre-mating, while females of both generations had decreased absolute body weights during pre-mating, gestation, and lactation and decreased body weight gains during pre-mating and gestation. Although food efficiency values were not provided, the concurrent increased food consumption by these animals is consistent with decreased food efficiency. Decreased absolute and/or relative thymus weights in these groups may be attributable to thymic atrophy secondary to either poor body condition or stress; however, thymuses were not examined microscopically. There was no evidence of parental systemic toxicity at the low- or mid-dose dietary concentrations. This conclusion differs from that of the study author. The above mentioned body weight decrease of mid-dose females during lactation (7% less than controls on LD 14, F_0 generation only) was both transient and of insufficient magnitude to be considered biologically relevant. **Therefore, the parental systemic toxicity LOAEL is 2500 ppm (163.4 mg/kg bw/day in males, 188.8 mg/kg bw/day in females), based on decreased absolute body weights and body weight gains, with decreased absolute and relative thymus weights in both sexes. The parental systemic NOAEL is 500 ppm (31.2 mg/kg bw/day in males, 36.8 mg/kg bw/day in females).**

Offspring toxicity was evident in the mid-dose F_1 pups and high-dose F_1 and F_2 pups as decreased body weight gains and decreased absolute thymus and spleen weights. In addition, the reduced weight gain by the high-dose pups resulted in lower absolute body weights for both generations. Lower weight gains by the high-dose pups of both generations occurred throughout lactation, whereas decreased body weight gains by the mid-dose F_1 pups occurred when the pups would have begun to eat the treated diets. Delayed sexual maturation of F_1 offspring was noted in the mid- and high-dose males and the high-dose females. A dose-related increased number of stillbirths was noted in the mid- and high-dose groups of both generations. A similar increase in the incidence of stillbirths was noted in a developmental neurotoxicity study (Hoberman, A.M. (2000.) at similar dose levels, which supports the conclusion that these findings were treatment related. **Therefore, the offspring toxicity LOAEL is 500 ppm (31.2 mg/kg bw/day in males, 36.8 mg/kg bw/day in females), based on decreased body weight gains, delayed sexual maturation (males), and decreased absolute thymus weights in F_1 pups of both sexes and an increase in stillbirths in both generations. The offspring toxicity NOAEL is 150 ppm (9.8 mg/kg bw/day in males, 11.5 mg/kg bw/day in females).**

Reproductive toxicity was evident at 2500 ppm as decreased percentages of progressively motile sperm in high-dose F_0 males, and decreased percentages of motile and progressively motile

sperm in high-dose F_1 males and corresponding increases in the percent of sperm with detached heads noted in both generations. **Therefore, the reproductive toxicity LOAEL for males is 2500 ppm (163.4 mg/kg bw/day) in males, based on decreased sperm motility and increased number of sperm with detached heads in both generations, and the reproductive toxicity NOAEL for males is 500 ppm (31.2 mg/kg bw/day). The reproductive toxicity LOAEL for females is not identified, and the reproductive toxicity NOAEL for females is 2500 ppm (188.8 mg/kg bw/day).**

C. **STUDY DEFICIENCIES:** The following major deficiency was noted:

Results of the homogeneity and concentration analyses of the diets indicated that there was significant variance between the nominal and actual dietary concentrations, with 13/33 of the samples analyzed for concentration differing from target by greater than ±15%. By itself, this is not sufficient reason to classify the study as unacceptable; however, it must be noted that significant variation in the analytical dietary concentrations at all dose levels make the actual dosages to the animals uncertain.

APPENDIX: Reproduction and Fertility Effects Study - Rat; Range-finding

TEST MATERIAL (PURITY): Tl-435 (Clothianidin; 95.2-96.0% a.i., lot no. 30037120)

CITATION: Astroff, A. (2000) A pilot reproductive toxicity study with Tl-435 in the Sprague-Dawley rat. Bayer Corporation, Agriculture Division, Toxicology Department, 17745 South Metcalf, Stilwell, Kansas 66085-9104. Laboratory report number 108035, February 15, 2000. MRID 45422714. Unpublished.

In a 1-generation reproduction and fertility effects study (MRID 45422714) Tl-435 (Clothianidin; 95.2-96.0% a.i., lot no. 30037120) was administered to 20 Sprague-Dawley rats/sex/dose in the diet at dose levels of 0, 50, 100, 500, and 1000 ppm. Pre-mating doses were 0, 2.9, 5.8, 29.1, and 58.9 mg/kg/day, respectively, for F_0 males, 0, 3.4, 6.6, 34.2, and 68.6 mg/kg/day, respectively, for F_0 females. Parental animals were administered the test or control diets for 8 weeks prior to mating, throughout mating, gestation, and lactation, and until necropsy, and a single litter was produced.

At the dose levels tested, there were no treatment-related effects on parental mortality, clinical signs, absolute body weights, body weight gains, or food consumption during pre-mating, gestation, or lactation, gross pathology, or absolute or relative organ weights of the liver, kidney, spleen, testes, or ovaries. There were also no treatment-related effects on litter size, pup body weights, sex ratios, or viability, clinical signs exhibited by the pups, or gross pathology from pup necropsies. There were no treatment-related effects on reproductive parameters, including mating, fertility, and gestation indices, the number of estrous cycles, length of the estrous cycle, days to insemination, gestation length, or the total number of implantations.

Dose selection for the range-finding reproduction study were based on the results of a subchronic study in which Crl:CD BR rats were treated with the test material at dietary concentrations of 0, 100, 250, 1250, or 2500 ppm for 13 weeks (Huntington Life Sciences Ltd., Study Number TDA 194/962814). However, as no treatment-related effects were observed in the range-finding study, it was concluded that the main study would have to include a dietary concentration greater than 1000 ppm.

DATA FOR ENTRY INTO ISIS

Reproductive Study - rats (870.3800)

PC code	MRID	Study	Species	Duration	Route	Admin	Dose range mg/kg/day	Doses mg/kg/day	NOAEL mg/kg/day	LOAEL mg/kg/day	Target organ	Comments
044309	45422714, 45422715, 45422716	reproductive	rats	2 generations	oral	diet	♂ 9.8-163.4 ♀ 11.5-188.8	♂ 0, 9.8, 31.2, 163.4 ♀ 0, 11.5, 36.8, 188.8	♂ 31.2 ♀ 36.8	♂ 163.4 ♀ 188.8	Body weight decreased, decreased thymus weights	Parental/ systemic
044309	45422714, 45422715, 45422716	reproductive	rats	2 generations	oral	diet	♂ 9.8-163.4 ♀ 11.5-188.8	♂ 0, 9.8, 31.2, 163.4 ♀ 0, 11.5, 36.8, 188.8	♂ 9.8 ♀ 11.5	♂ 31.2 ♀ 36.8	Body weight gain decreased, delayed sexual maturation, decreased thymus weights, increased stillbirths	Offspring
044309	45422714, 45422715, 45422716	reproductive	rats	2 generations	oral	diet	♂ 9.8-163.4 ♀ 11.5-188.8	♂ 0, 9.8, 31.2, 163.4 ♀ 0, 11.5, 36.8, 188.8	♂ 31.2 ♀ ≥188.8	♂ 163.4 ♀ not identified	Decreased sperm motility in both generations	Reproductive

HED Records Center Series 361 Science Reviews - File R107689 - Page 537 of 721

DATA EVALUATION RECORD

TI-435 (CLOTHIANIDIN)

Study Type: §81-8a, Neurotoxicity Screening Battery in Rats

Work Assignment No. 4-02-167B amend. I (MRIDs 45422801 and 45422802)

Prepared for

Health Effects Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
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Prepared by

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Disclaimer

This Data Evaluation Record may have been altered by the Health Effects Division subsequent to signing by Dynamac Corporation personnel.

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Registration Action Branch 3, Health Effects Division (7509C) Date 1/6/05

TXR#: 0050321

DATA EVALUATION RECORD

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

PC CODE: 044309 **DP BARCODE:** D282578
SUBMISSION NO.: S614462

TEST MATERIAL (PURITY): TI-435 (Clothianidin; ≥95.2% a.i.)

SYNONYMS: (E)-1-(2-chloro-5-thiazolylmethyl)-3-methyl-2-nitroguanidine

CITATION: Sheets, L.P., D.M. Cain, and B.P. Stuart (2000). An Acute Oral Neurotoxicity Screening Study with Technical Grade TI-435 in Fischer 344 Rats. Bayer Corp., Agriculture Division Toxicology, Stilwell, KS. Laboratory Study No: 97-412-OH, October 12, 2000. MRID 45422801. Unpublished.

Sheets, L.P. and R.G. Gilmore (2000). A Special Acute Oral Neurotoxicity Study to Establish a No-Observed-Effect-Level with Technical Grade TI-435 in Fischer 344 Rats. Bayer Corp., Agriculture Division Toxicology, Stilwell, KS. Laboratory Study Nos: 00-N12-BA and 99-N12-BA, November 8, 2000. MRID 45422802. Unpublished.

SPONSOR: Takeda Chemical Industries, Ltd., Agro Company, 2-13-10 Nihonbashi, Chuo-Ku, Toyko, Japan

EXECUTIVE SUMMARY: In an acute oral neurotoxicity study (MRID 45422801), TI-435 (Clothianidin; >=95.2% a.i., Batch # 30037120) in 0.5% methylcellulose/0.4% Tween 80 solution was administered in a single dose by gavage (10 mL/kg) to fasted Fischer 344 rats (12/sex/group) at doses of 0, 100, 200, or 400 mg/kg. The time of peak effect was estimated to be 4 hours post-dosing. After two weeks, 6 animals/sex/group were perfused *in situ* for neurohistological examination and tissues from the control and high-dose groups were examined microscopically. Functional observational battery (FOB) and motor activity were evaluated during Week -1 and on Days 0 (approximately 4 hours post-dosing), 7, and 14. Acceptable positive control data were provided that verify the ability of the observers of the performing lab to conduct the FOB and to assess motor activity, neurotoxicity and behavioral effects.

No treatment-related deaths occurred. Body weights, body weight gains, gross pathology, histopathology, and brain weights were unaffected by treatment.

The following treatment-related FOB effects (# affected/12 vs 0/12 controls, unless otherwise noted) were noted on Day 0: (i) **home cage**; slight to moderate tremors at 400 mg/kg (8 males;

11 females) and decreased activity at 200 (1 male) and 400 (8 males; 11 females) mg/kg; (ii) **open field**; slight to moderate tremors at 400 mg/kg (11 each sex), decreased arousal (sluggish, with some exploratory movement to minimal movement) at 100 (4 treated vs 1 control in males), 200 (5 treated vs 1 control in males; 3 females), and 400 (9 treated vs 1 control in males; 11 females) mg/kg, and slightly uncoordinated gait in one 400 mg/kg male; (iii) **reflex/physiologic**; pupil response (pin point) at 200 (1 male) and 400 (8 males; 9 females) mg/kg, righting reflex (slightly uncoordinated to landing on side) at 200 (2 males) and 400 (2 males; 4 treated vs 1 control in females), and body temperature was decreased ($p \leq 0.05$) in both sexes at 200 (decr 1.3-1.7°C) and 400 (decr 3.7°C) mg/kg. Additionally, clinical signs were observed at 400 mg/kg on Day 0 and included tremors, decreased activity, and ataxia in both sexes.

Total motor activity was decreased (NS) compared to controls at 100 (23%, males only), 200 (43-59%), and 400 (72-81%) mg/kg with decreases ($p<0.05$) observed during intervals 1-3 at 100 (32-41%, males only), 200 (38-80%), and 400 (77-97%) mg/kg. Total locomotor activity was similarly decreased (NS) at 100 (37%, males only), 200 (45-62%), and 400 (83-88%) mg/kg with decreases ($p<0.05$) observed during intervals 1-2 at 100 (40-50% males only), 200 (44-78%), and 400 (81-94%) mg/kg.

The LOAEL for this study is 100 mg/kg based on FOB effects (decreased arousal) and decreased motor and locomotor activity on Day 0 in males. The NOAEL was not established. Neurotoxicity was evidenced by FOB effects (decreased arousal) and decreased motor and locomotor activity on Day 0 in males.

The submitted study is classified as **acceptable/guideline** in conjunction with the supplemental acute neurotoxicity study (MRID 45422802; executive summary below with study summary provided in Appendix III of this DER). Together these studies satisfy the requirements (OPPTS 870.6200a; OECD 424) for an acute neurotoxicity screening battery in rats.

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

Special Acute Neurotoxicity Study (to establish a No-Observed-Effect-Level of TI-435 in male Fischer 344 rats)

In this acute neurotoxicity study (MRID 45422802), TI-435 (Clothianidin; ≥95.3% a.i., Batch # 30037120) in 0.5% methylcellulose/0.4% Tween 80 solution was administered in a single dose by gavage (10 mL/kg) to fasted male Fischer 344 rats (12/dose) at doses of 0, 20, 40, or 60 mg/kg. Functional observational battery (FOB) and motor activity were evaluated during week -1 and on Day 0 (approximately 4 hours post-dosing). Dose analyses indicated that nominal doses were achieved.

No unscheduled deaths occurred during the study. Clinical signs, FOB, and motor activity were unaffected by treatment.

The LOAEL was not observed. The NOAEL is 60 mg/kg.

525

The submitted study is classified as **acceptable/guideline** in conjunction with the acute neurotoxicity study (MRID 45422801). Together these studies satisfy the requirements (OPPTS 870.6200a; OECD 424) for an acute neurotoxicity screening battery in rats.

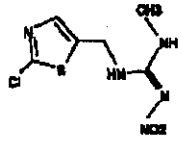
Therefore, LOAEL for the combined studies is 100 mg/kg based on FOB effects (decreased arousal) and decreased motor and locomotor activity on Day 0 in males. The NOAEL is 60 mg/kg.

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

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: TI-435 (Clothianidin)

Description:	pale yellow powder
Lot/Batch #:	300371200
Purity:	≥95.2% a.i.
Compound Stability:	It was stated that the compound was stable in the vehicle after refrigeration for up to 7 days.
CAS # of TGAI:	210880-92-5
Structure:	

2. Vehicle: 0.5% methylcellulose/0.4% Tween 80 in distilled water.

3. Test animals:

Species:	Rat	
Strain:	Fischer 344 CDF (F-344)/BR	
Age/Wt at study initiation:	9 weeks/166-171 g males and 116-118 g females	
Source:	SASCO Inc., Portage MI	
Housing:	Individually in suspended, stainless steel cages.	
Diet:	Purina Mills Rodent Lab Chow 5001-4 *ad libitum* except for overnight prior to dosing and during neurobehavioral evaluations.	
Water:	Municipal tap water *ad libitum* except for overnight prior to dosing and during neurobehavioral evaluations.	
Environmental conditions:	**Temperature:**	17.8-25.6 °C
	Humidity:	30-70 %
	Air changes:	not reported
	Photoperiod:	12 hrs dark/12 hrs light
Acclimation period:	At least 6 days.	

B. STUDY DESIGN

1. In life dates - Start: November 3, 1997 End: November 21, 1997

2. Animal assignment and treatment: Animals were randomly assigned (stratified by body weight) to the test groups noted in Table 1. Following an overnight fast, rats were given a single gavage dose (10 mL/kg) of TI-435 in 0.5% methylcellulose/0.4% Tween 80, and observed for up to 14 days. In a range-finding study (97-912-OE), 5 Fischer 344 rats/sex/dose were treated with the test material at 250, 500, or 1000 mg/kg, and were then subjected to a detailed clinical examination at 0.5, 1, 2, 3, 4, and 24 hours post-dosing and once daily thereafter to establish the time of peak-effect. At 1000 mg/kg, 2/5 males and 5/5 females died. At 500 mg/kg, signs of

toxicity (decreased activity and tremors) were observed. Additionally, 1/5 females died at two days post-dosing. It was stated that clinical signs became apparent beginning at 3 hours post-dosing, with additional effects at 4 hours post-dosing. Signs of toxicity persisted in the survivors for up to 72 hours post-dosing. Based on these results, the time of peak-effect was estimated to be 4 hours post-dosing and the doses indicated in Table 1 were selected for the current study.

Table 1. Study design. [a]

Experimental Parameter	Dose (mg/kg bw)[b]			
	0	100	200	400
Total number of animals	12/sex	12/sex	12/sex	12/sex
Behavioral Testing (FOB, Motor Activity)	12/sex	12/sex	12/sex	12/sex
Neuropathology	6/sex	6/sex	6/sex	6/sex

a Data obtained from Study Report pages 18 and 19.
b Actual doses were 0, 102, 213, and 373 mg/kg, respectively.

3. Test substance preparation and analysis: It was stated that the homogeneity and stability of TI-435 in the vehicle was verified at 5 and 50 mg/mL, and each dose mixture in the current study was analyzed for actual concentration. The data from these analyses (Bayer report # 108503) were not provided with the current study; however, it was stated that the test material was shown to be stable for up to 7 days of refrigerated storage. It was stated that the dose formulations (0, 100, 200, and 400 mg/kg) were between 93.2-106% of nominal in both sexes, and that the analytically determined doses were 0, 102, 213, and 373 mg/kg, respectively.

Results

Homogeneity Analysis: (as % coefficient of variation)
5 mg/mL = 5.1%
50 mg/mL = 2.6%

Stability Analysis: Data not provided

Concentration Analysis: Data not provided

Based on the data provided, the reviewers feel that the mixing procedure was adequate and that the variation between nominal and actual dosage to the animals was acceptable.

4. Statistics: The data were analyzed using the following statistical methods:

Parameter	Statistical Method
FOB continuous data Motor and Locomotor activity (total session data)	Repeated-Measures ANOVA followed by a one-way ANOVA followed by Dunnett's.
Categorical FOB data	General Linear Modeling (GLM) and Categorical Modeling (CATMOD), with post-hoc comparisons using Dunnett's and analysis of contrasts, respectively.
Motor and Locomotor activity (interval data)	Two-way Repeated-Measures ANOVA followed by a Repeated-Measures ANOVA followed by a one-way ANOVA followed by Dunnett's.
Terminal body weight Brain weight	Bartlett's test for homogeneity of variances. Homogeneous data were further analyzed using ANOVA followed by Dunnett's. Non-homogeneous data were analyzed using the non-parametric Kruskal-Wallis test followed by a Mann-Whitney U test.

Significance levels were defined at $p \leq 0.05$, with the exception of the Bartlett's test ($p \leq 0.001$).

C. METHODS/OBSERVATIONS

1. Mortality and clinical observations: Animals were observed at least once daily for mortality and moribundity. Detailed physical observations were performed daily.

2. Body weight: Animals were weighed weekly as a component of the FOB.

3. Food consumption: Food consumption was not recorded.

4. Cholinesterase determination: Cholinesterase activity was not determined.

5. Neurobehavioral assessment

a. Functional Observational Battery (FOB): All animals were subject to a FOB at Days -7, 0 (approximately 4 hours post-dosing), 7, and 14. The scoring criteria for the FOB are included in Appendix I of this DER (the reviewers noted errors in the alphanumeric system used for the FOB criteria; however, there does not appear to be any missing data). The technicians who conducted the FOB were "blind" as to the animals treatment groups and observations for all animals were performed by the same person throughout the study, with a second person performing the measurements (e.g., grip strength and foot splay). On the day of FOB and motor activity testing, the animals were transferred to the testing room and allowed to acclimate for at least 30 minutes prior to testing. Sets of 8 animals were individually evaluated during the FOB. The following CHECKED (X) parameters were examined:

	HOME CAGE OBSERVATIONS		HANDLING OBSERVATIONS Continued		OPEN FIELD OBSERVATIONS
X	Posture*	X	Fur appearance / staining		Mobility
X	Piloerection*	X	Palpebral closure*	X	Rearing+
X	Convulsions (Clonic/Tonic)*	X	Eye prominence (exophthalmia)*	X	Arousal*
X	Tremors*	X	Muscle tone*	X	Convulsions (Clonic/Tonic)*
X	Gait abnormalities			X	Rearing
	Palpebral closure*		**REFLEX/PHYSIOLOGIC OBSERVATIONS**	X	Respiratory rate+
X	Vocalization	X	Approach response+	X	Urination / defecation*
X	Other (activity/nutation/reactivity)*	X	Touch response+	X	Vocalization
		X	Auditory response*	X	Posture*
	HANDLING OBSERVATIONS	X	Tail pinch response*	X	Gait abnormalities*
X	Ease of removal	X	Pupil response*	X	Bizarre/stereotypic behavior*
X	Reaction to handing*	X	Pupil size	X	Piloerection*
X	Lacrimation*	X	Grip strength*		
X	Salivation*	X	Landing foot splay*		
X	Nasal discharge	X	Righting reflex+		
X	Staining (nasal/oral/perianal/urine)	X	Body temperature+		
		X	Body weight*		

*Required parameters; +Recommended parameters

Testing was staggered over a two day period for each sex. Males and females were tested on separate days and the open-fields and mazes were cleaned between intervals to reduce the residual scent from the other sex.

b. Motor activity: Approximately 30 minutes after the last animal was evaluated in the FOB, all animals were individually placed in separate figure-eight mazes for motor activity measurement. Motor and locomotor activity were measured during a 90 minute session divided into 10 minute intervals. Motor activity was measured as the number of beam interruptions that occurred during the session. Locomotor activity was measured by eliminating consecutive counts for a given beam. Habituation was evaluated as a decrement in activity during the test session. A Universal Maze Monitoring System (Columbus Instruments, Columbus, OH) was used for automated data collection. Broad spectrum background noise [approximately 70 dB(A)] was generated during the observation period via a white noise generator (Model S81-02, Coulbourn Instruments) and amplifier (Model S82-24, Coulbourn Instruments). The light intensity (100±70 lux) was verified daily using a digital light meter. The order of testing and assignment to mazes was done in semi-random order such that no animal was tested more than once in the same maze.

6. Sacrifice and pathology: All animals were subject to gross necropsy. The first 6 rats/sex/dose were anesthetized using pentobarbital (50 mg/kg i.p.), and perfused via the left ventricle with a sodium nitrite flush followed by *in situ* fixation with Universal fixative [4% (w/v) glutaraldehyde and 4% (w/v) EM-grade formaldehyde] in phosphate buffer. The entire brain and spinal cord, both eyes (with optic nerves) and selected peripheral nerves (sciatic, tibial, and sural), the gasserian ganglion, gastrocnemius muscle, and physical identifier were post-fixed in 10% buffered formalin. The brain was weighed upon removal and prior to post-fixation. All other surviving animals were sacrificed via CO_2 asphyxiation without perfusion or collection of tissues.

Brain and spinal cord tissues were embedded in paraffin. Three sections were collected from each level, and stained with hematoxylin-eosin, luxol fast blue-cresyl echt violet, and Sevier-Munger silver, respectively. The peripheral nerves (sciatic, tibial, and sural) were embedded in epoxy plastic resin, sectioned, and stained with toluidine blue. All remaining tissues were embedded in glycol methacrylate, sectioned, and stained with a modified Lee's stain. The following CHECKED (X) tissues from the control and high-dose animals, as well as, all gross lesions were examined microscopically:

	CENTRAL NERVOUS SYSTEM		PERIPHERAL NERVOUS SYSTEM
	BRAIN		**SCIATIC NERVE**
X	Olfactory bulbs	X	Sciatic Notch
X	Cerebral cortex		
X	Caudate putamen/Globus pallidus		
X	Hippocampus		**OTHER**
X	Thalamus	X	Sural Nerve
X	Hypothalamus	X	Tibial Nerve
X	Midbrain (tectum, tegmentum, and peduncles)	X	Sciatic Nerve
X	Cerebellum	X	Lumbar dorsal root ganglion
X	Medulla oblongata	X	Lumbar dorsal root fibers
	SPINAL CORD	X	Lumbar ventral root fibers
X	Cervical swelling	X	Cervical dorsal root ganglion
X	Lumbar swelling	X	Cervical dorsal root fibers
X	Thoracic swelling	X	Cervical ventral root fibers
X	Cauda Equina		
	OTHER		
X	Gasserian Ganglion		
X	Optic nerves		
X	Eyes		
X	Gastrocnemius muscle		

7. **Positive Controls:** Summaries were provided for four neurotoxicity studies (MRIDs 42770301 and 43656301) performed to generate positive control data and validate the procedures and observers of the performing lab to conduct the FOB and to assess motor activity, neurotoxicity, behavioral effects, and neuropathological lesions. The submitted positive control studies were performed between 6-7 years prior to the current study. Triadimefon (200 mg/kg, gavage, single dose) induced increased motor activity, while chlorpromazine (2 mg/kg, i.p., single dose) caused decreased motor activity. Clinical and/or functional effects produced by acrylamide (25 or 50 mg/kg, i.p., 3 times/week) included clinical signs (ataxia, urine stain, oral stain, and piloerection), decreased body weight gain and absolute brain weight, muscle fasciculations, tremors, ataxia, decreased rearing, erect tail, hindlimbs dragging, impaired air righting reflex, and increased hindlimb foot splay. Neuropathologial effects included mild to marked neuronal degeneration in the sciatic, tibial, and sural nerves, and axonal degeneration in the spinal cord. Trimethyltin (12 mg/kg, i.p., single dose) caused mortality, tremors, increased reactivity, convulsions, and ataxia. Neuropathological effects included neuronal necrosis (olfactory tract, piriform cortex, and hippocampus), chromatolysis of large neuronal soma (pons, medulla oblongata, spinal cord, and dorsal root and gasserian ganglion. Carbaryl (15 or 30 mg/kg, i.p., single dose) caused salivation, lacrimation, staining (nasal, oral, perianal, and urine), gait abnormalities, impaired air righting reflex, decreases in approach, startle, touch, and tail pinch responses, muscle fasciculations, tremors, clonic chewing movements, decreased arousal, and decreased rearing.

It should be noted that the triadimefon and chlorpromazine study (MRID 43656301) was performed using Fischer 344 rats, while the remaining studies (MRID 42770301) were performed using Sprague-Dawley rats. However, the reviewers believe that this minor deficiency does not affect the validity of this positive control data.

II. RESULTS

A. OBSERVATIONS

1. **Clinical signs:** On Day 0, the following treatment-related clinical signs (# of animals affected) were observed at 400 mg/kg (vs 0/12 controls; Table 2): (i) tremors, 12/12 males and 11/12 females; (ii) decreased activity, 10/12 males and 11/12 females; and (iii) ataxia, 2/12 males and 1/12 females. All other signs observed on Day 0 were considered unrelated to treatment because they occurred at a low incidence and/or were also observed in the control animals. All animals recovered by Day 2 post-dosing. No treatment-related clinical signs were observed in either sex at ≤200 mg/kg.

Table 2. Clinical signs observed on Day 0 in rats treated with TI-435 once via gavage. [a]

Observation	Dose (mg/kg)							
	0	100	200	400	0	100	200	400
	Males				Females			
Tremors	0	0	0	12	0	0	0	11
Decreased activity	0	0	0	10	0	0	0	11
Ataxia	0	0	0	2	0	0	0	1

a Data obtained from Study Report Table 1, page 29; n=12.

2. Mortality: No animals died prior to scheduled sacrifice.

B. BODY WEIGHT AND BODY WEIGHT GAIN: Body weights and overall (Days 0-14) body weight gains were similar to controls at all doses throughout the study (Table 3).

Table 3. Mean (±SD) body weights and body weight gains (g) in rats treated with TI-435 once via gavage. [a]

Observation	Dose (mg/kg)							
	0	100	200	400	0	100	200	400
	Males				Females			
Body weight (Day 0)	168±9	166±11	168±7	171±11	116±6	116±7	118±6	118±5
Body weight (Day 14)	226±14	221±17	226±10	226±13	151±9	152±7	152±7	149±5
Overall Weight Gain (Day 0-14)[b]	58	55	58	55	35	36	34	31

a Data obtained from Study Report Table 3, pages 103-109; n=12.
b Calculated by reviewers using data contained within the table.

C. FOOD CONSUMPTION: Food consumption was not measured.

D. CHOLINESTERASE DETERMINATION: Cholinesterase activity was not determined.

E. NEUROBEHAVIORAL RESULTS

1. FOB findings: At 400 mg/kg, the following treatment-related effects (# affected/12 treated vs 0/12 controls, unless otherwise noted) were noted on Day 0 (Table 4): (i) home cage; slight to moderate tremors (8 males; 11 females) and decreased activity (8 males; 11 females); (ii) open field; slight to moderate tremors (11 males; 11 females), decreased arousal (sluggish, with some exploratory movement to minimal movement; 9 treated vs 1 control in males; 11 females), and slightly uncoordinated gait (1 male); and (iii) reflex/physiologic; pupil response (pin point; 8

males; 9 females), righting reflex (slightly uncoordinated to landing on side; 2 males; 4 treated vs 1 control in females), and decreased body temperature (↓3.7°C, males and females, $p \leq 0.05$).

At 200 mg/kg, the following effects were noted at Day 0: (i) home cage, decreased activity (1 male); (ii) open field; decreased arousal (sluggish, with some exploratory movement to minimal movement; 5 treated vs 1 control in males; 3 females); and (iii) reflex/physiologic; pupil response (pin point; 1 male), righting reflex (slightly uncoordinated; 2 males), and body temperature was decreased ($p \leq 0.05$) in males (↓1.7°C) and females (↓1.3°C).

At 100 mg/kg, decreased arousal (sluggish, with some exploratory movement to minimal movement) was observed in 4 males during the open field observation at Day 0. This finding was considered related to treatment as motor activity was similarly decreased in the figure eight maze at this dose. The increase ($p \leq 0.05$) in rearing (↑133%) observed in females during the open field was considered unrelated to treatment because it was not dose-dependent. All animals appeared normal by Day 7 post-dosing.

Table 4. FOB results (# of affected animals) on Day 0 (approximately 4 hours post-dosing) in rats treated with TI-435 once via gavage. [a]

Observation		Dose (mg/kg)			
		0	100	200	400
Males					
Home cage					
Tremors	Slight	0	0	0	7
	Moderate	0	0	0	1
	Total	0	0	0	8
Decreased activity		0	0	1	8
Open field					
Tremors	Slight	0	0	0	10
	Moderate	0	0	0	1
	Total	0	0	0	11
Arousal	Sluggish with some exploratory movement	1	3	4	9
	Sluggish with minimal movement	0	1	1	0
	Total	1	4	5	9
Slightly uncoordinated gait		0	0	0	1
Rearing [b]		1.3±1.8	1.1±1.6	1.1±1.4	1.0±1.2
Reflex/physiologic					
Pupil response (pin point)		0	0	1	8
Righting reflex	Slightly uncoordinated	0	0	2	2
Body temperature (°C)[b]		36.5±0.5	36.1±0.6	34.8±1.0* (↓5)	32.8±1.2* (↓10)

(table continues next page)

Observation		Dose (mg/kg)			
		0	100	200	400
Females					
Home cage					
Tremors	Slight	0	0	0	9
	Moderate	0	0	0	2
	Total	0	0	0	11
Decreased activity		0	0	0	11
Open field					
Tremors	Slight	0	0	0	6
	Moderate	0	0	0	5
	Total	0	0	0	11
Arousal	Sluggish with some exploratory movement	0	0	2	9
	Sluggish with minimal movement	0	0	1	2
	Total	0	0	3	11
Slightly uncoordinated gait		0	0	0	0
Rearing [b]		1.8±1.6	4.2±3.3* (↑133)	2.3±2.2	1.0±1.4
Reflex/physiologic					
Pupil response (pin point)		0	0	0	9
Righting reflex	Slightly uncoordinated	1	1	0	3
	Landing on side	0	0	0	1
	Total	1	1	0	4
Body temperature (°C)[b]		36.4±0.4	36.3±0.7	35.1±0.5* (↓4)	32.7±1.3* (↓10)

a Data obtained from Study Report, Table 2, pages 30-101; n=12.
b Numbers are mean±SD. Numbers presented parenthetically represent percent difference from control (calculated by reviewers).
* Significantly different from controls at $p \leq 0.05$

2. Motor activity: Total motor activity was decreased (not statistically significant [NS]) on Day 0 in the males at 100 (↓23%), 200 (↓59%), and 400 (↓81%) mg/kg and in females at 200 (↓43%) and 400 (↓72%) mg/kg (Table 5a). In addition, on Day 0 motor activity was decreased ($p \leq 0.05$) during the following intervals (Table 5b): (i) interval 1, in the males at 100 (↓32%), 200 (↓63%),

and 400 (↓89%) mg/kg and in the females at 200 (↓39%) and 400 (↓77%) mg/kg; (ii) interval 2, in the males at 100 (↓41%, NS), 200 (↓80%), and 400 (↓97%) mg/kg and in the females at 200 (↓63%) and 400 (↓89%) mg/kg; and (iii) interval 3, in the males at 200 (↓38%, NS) and 400 (↓77%, NS) mg/kg and in the females at 200 (↓68%, NS) and 400 (↓82%) mg/kg. Total locomotor activity was decreased on Day 0 in the males at 100 (↓37%), 200 (↓62%), and 400 (↓88%) mg/kg and in females at 200 (↓45%) and 400 (↓83%) mg/kg (Table 6a). Additionally, Day 0 locomotor activity was decreased ($p \leq 0.05$) during the following intervals (Table 6b): (i) interval 1, in the males at 100 (↓40%), 200 (↓67%), and 400 (↓90%) mg/kg and in the females at 200 (↓44%) and 400 (↓81%) mg/kg; and (ii) interval 2, in the males at 100 (↓50%), 200 (↓78%), and 400 (↓94%) mg/kg and in the females at 200 (↓67%) and 400 (↓90%) mg/kg. Habituation was not affected by treatment. All motor and locomotor activity counts were similar to controls on Days 7 and 14. Complete interval data for motor and locomotor activity are presented in Appendix II of this DER.

Table 5a. Mean (±SD) motor activity (total activity counts for session) in rats treated with TI-435 once via gavage. [a]

Days	Dose (mg/kg)							
	0	100	200	400	0	100	200	400
	Male				Female			
-7	473±156	434±111	505±144	389±96	844±174	941±251	867±260	843±297
0	256±44	196±98 (↓23)	106±41 (↓59)	48±44 (↓81)	332±146	358±196	188±84 (↓43)	94±59 (↓72)
7	507±227	484±210	580±185	437±143	798±228	930±315	893±302	914+279
14	556±209	573±179	611±155	540±235	973±252	932±273	973±211	908±370

a Data obtained from Study Report, Table 5, pages 112-113; n=12. Numbers presented parenthetically represent percent difference from control (calculated by reviewers).

Table 5b. Mean (±SD) motor activity counts at selected intervals on Day 0 in rats treated with TI-435 once via gavage. [a]

Interval	Dose (mg/kg)							
	0	100	200	400	0	100	200	400
	Males				Females			
1	161±48	110±35* (↓32)	60±31* (↓63)	17±11* (↓89)	164±30	166±51	100±47* (↓39)	37±25* (↓77)
2	59±30	35±36 (↓41)	12±14* (↓80)	2±4* (↓97)	79±50	87±55	29±34* (↓63)	9±14* (↓89)
3	13±22	10±19	8±9 (↓38)	3±9 (↓77)	38±42	43±38	12±20 (↓68)	7±9* (↓82)

a Data obtained from Study Report, Table 7, pages 116-123; n=12. Numbers presented parenthetically represent percent difference from control (calculated by reviewers).
* Significantly different from controls at $p \leq 0.05$

Table 6a. Mean (±SD) locomotor activity (total activity counts for session) in rats treated with TI-435 once via gavage. [a]

Days	Dose (mg/kg)							
	0	100	200	400	0	100	200	400
	Male				Female			
-7	200±55	170±54	189±35	168±28	305±71	337±108	326±111	314±134
0	86±13	54±24 (↓37)	33±12 (↓62)	10±7 (↓88)	95±42	94±60	52±20 (↓45)	16±17 (↓83)
7	187±69	175±78	192±61	166±50	294±99	335±121	322±113	335±105
14	206±82	204±67	209±45	189±71	334±122	314±89	324±70	319±144

a Data obtained from Study Report, Table 6, pages 114-115; n=12. Numbers presented parenthetically represent percent difference from control (calculated by reviewers).

Table 6b. Mean (±SD) locomotor activity counts at selected intervals on Day 0 in rats treated with TI-435 once via gavage. [a]

Interval	Dose (mg/kg)							
	0	100	200	400	0	100	200	400
	Males				Females			
1	63±13	38±13* (↓40)	21±10* (↓67)	6±4* (↓90)	54±12	45±17	30±10* (↓44)	10±10* (↓81)
2	18±9	9±11* (↓50)	4±5* (↓78)	1±2* (↓94)	21±15	23±18	7±8* (↓67)	2±4* (↓90)

a Data obtained from Study Report, Table 8, pages 124-131; n=12. Numbers presented parenthetically represent percent difference from control (calculated by reviewers).
* Significantly different from controls at $p \leq 0.05$

F. SACRIFICE AND PATHOLOGY

1. **Gross pathology:** No treatment-related gross lesions were observed.

2. **Brain weight:** Absolute and relative (to body) brain weights were similar to controls (Table 7).

Table 7. Mean (±SD) absolute (g) and relative (to body) brain weights in rats treated with TI-435 once via gavage. [a]

Parameter	Dose (mg/kg)			
	0	100	200	400
Males				
Terminal Body weight	222.0±13.2	226.7±14.5	227.6±12.1	226.7±12.5
Absolute Brain weight	1.719±0.047	1.711±0.061	1.753±0.047	1.702±0.036
Relative (to body) Brain weight (%)	0.776±0.031	0.756±0.036	0.771±0.030	0.752±0.034
Females				
Terminal Body weight	150.5±8.0	151.3±9.5	154.2±7.9	152.5±3.5
Absolute Brain weight	1.667±0.028	1.709±0.048	1.719±0.034	1.684±0.032
Relative (to body) Brain weight (%)	1.111±0.070	1.133±0.063	1.117±0.060	1.104±0.014

a Data obtained from Study Report Table OW1K-SUM, pages 369-370; n=6.

3. **Neuropathology:** No treatment-related histopathological changes were observed. Incidental, minimal microscopic findings were observed in the control and 400 mg/kg groups, and were limited to dilated ventricles in the forebrain, nerve fiber degeneration in the cerebellum, tibial nerve, and sciatic nerve, and myelin vacuolation of the sciatic nerve. These findings were typically observed in 1/6 animals and were equally distributed between males and females.

III. DISCUSSION and CONCLUSIONS

A. INVESTIGATORS' CONCLUSIONS: In this study, the investigators concluded that TI-435, administered to fasted Fischer 344 rats in a single dose by gavage at doses of 0, 100, 200, or 400 mg/kg, induced clinical signs of toxicity (tremors, decreased activity, and ataxia) at 400 mg/kg in both sexes. Additionally, FOB effects and decreases in motor and locomotor activity were observed in the males at ≥100 mg/kg and in females at ≥200 mg/kg. The NOAEL was not established. The LOAEL was 100 mg/kg based on the FOB effects (decreased arousal) and decreased motor and locomotor activity on Day 0 in the males.

B. REVIEWER COMMENTS: At 400 mg/kg, clinical signs observed on Day 0 included tremors, decreased activity, and ataxia in both sexes. The following treatment-related effects (# affected/12 treated vs 0/12 controls, unless otherwise noted) were noted on Day 0: (i) home cage; slight to moderate tremors (8 males; 11 females) and decreased activity (8 males; 11 females); (ii) open field; slight to moderate tremors (11 males; 11 females), decreased arousal (sluggish, with some exploratory movement to minimal movement; 9 treated vs 1 control in males; 11 females), and slightly uncoordinated gait (1 male); and (iii) reflex/physiologic; pupil

response (pin point; 8 males; 9 females), righting reflex (slightly uncoordinated to landing on side; 2 males; 4 treated vs 1 control in females), and decreased body temperature (↓3.7°C, males and females, $p \leq 0.05$). Total motor activity on Day 0 was decreased (↓72-81%, NS) with decreases ($p \leq 0.05$) observed during intervals 1-3 (↓77-97%). Total locomotor activity was similarly decreased in both sexes (↓83-88%, NS) with decreases ($p \leq 0.05$) observed during intervals 1-2 (↓81-94%).

At 200 mg/kg, the following effects were noted on Day 0: (i) home cage, decreased activity (1 male); (ii) open field; decreased arousal (sluggish, with some exploratory movement to minimal movement; 5 treated vs 1 control in males; 3 females); and (iii) reflex/physiologic; pupil response (pin point; 1 male), righting reflex (slightly uncoordinated; 2 males), and body temperature was decreased ($p \leq 0.05$) in males (↓1.7°C) and females (↓1.3°C). Total motor activity was decreased on Day 0 in both sexes (↓43-59%, NS) with decreases ($p \leq 0.05$ or NS) observed during intervals 1-3 (↓38-80%). Total locomotor activity was similarly decreased in both sexes (↓45-62%, NS) with decreases ($p \leq 0.05$) observed during intervals 1-2 (↓44-78%).

At 100 mg/kg, decreased arousal (sluggish, with some exploratory movement to minimal movement) was observed in 4 males during the open field observation on Day 0. This finding was considered related to treatment as total motor activity was similarly decreased in males (↓23%, NS) with decreases ($p \leq 0.05$ or NS) observed during intervals 1-2 (↓32-41%). Total locomotor activity was similarly decreased in males (↓37%, NS) with decreases ($p \leq 0.05$) observed during intervals 1-2 (↓40-50%).

The LOAEL for this study is 100 mg/kg based on FOB effects (decreased arousal) and decreased motor and locomotor activity on Day 0 in males. The NOAEL was not established.

Neurotoxicity was evidenced by FOB effects (decreased arousal) and decreased motor and locomotor activity on Day 0 in males.

It should be noted as well that 7/10 animals died after a dose of 1000 mg/kg bw in the range finding study. This is interesting to note, since the acute oral toxicity study (Gardner, J.R. (1997) TI 435 Acute Oral Toxicity Study in the Rat. Covance Laboratories. Harrogate, North Yorkshire, England. Laboratory report number 586/120-1032) reported a LD_{50} of greater than 5000 mg/kg bw. The different vehicle selection between these two studies (0.5% methylcellulose/0.4% Tween 80 was used for the range finding in the current neurotoxicity study while a 5% aqueous solution of arabic gum was used for the acute oral toxicity study) may account for the difference in toxicity reported. The use of these vehicles should be noted in other studies in the database.

The submitted study is classified as **acceptable/guideline** in conjunction with the supplemental acute neurotoxicity study (MRID 45422802; summarized in Appendix III of this DER). Together these studies satisfy the requirements (OPPTS 870.6200a; OECD 424) for an acute neurotoxicity screening battery in rats.

C. STUDY DEFICIENCIES: None

DATA FOR ENTRY INTO ISIS

Acute Neurotoxicity Study - rats (870.6200a)

PC code	MRID #	Study type	Species	Duration	Route	Dosing method	Dose range mg/kg/day	Doses tested mg/kg/day	NOAEL mg/kg/day	LOAEL mg/kg/day	Target organ(s)	Comments
044309	45422801 45422802	acute neurotox	rats	14 days	oral	gavage	100-400	0, 100, 200, 400	Not established	100	FOB, Motor activity	

147

HED Records Center Series 361 Science Reviews - File R107689 - Page 556 of 721

APPENDIX I

Introduction:

This SOP is used with the "second generation" (i.e., Version 2) of the FOB data collection software.
SOP E-200 is the SOP that is used with the original version of this software.

The purpose of the FOB is to use noninvasive procedures to detect gross functional effects in animals
resulting from chemical exposure and to quantify behavioral and neurologic effects. The FOB should be
conducted blind (i.e., the observer is not told which treatment the animal has received), using standardized procedures to minimize observer variability. Variations in the test conditions should be
minimized and not systematically related to treatment.

Study Conduct:

The following procedures that constitute this FOB are based on the battery described by *Moser (1989). Enter the results directly into the SAS FOB Computer Program, or when the FOB Program is
inaccessible, use TOX FORM 286. For detailed instructions on the use of this Program see the "FOB
Data Collection Program User Reference". For reference, the OBSERVER is the person who performs
home cage and open field observations, including the approach, touch, auditory and tail pinch responses. The MEASURER performs all measurements, including the pupil assessments and the righting reflex. The RECORDER records results for the observer and the measurer.

Perform the following tests in the order in which they are listed. Remove feeders at least 30 minutes
prior to observation in order to improve visibility for home cage observations and to facilitate removal of animals from the home cage. Document feeder removal in the LOGFIL program of DATATOX or on an appropriate data sheet.

Indicate '-' if normal or not present.

Score intensity of the observation as follows:
1) Slight (barely perceptible or infrequent)
2) Moderate to Severe

Record colors as follows:
C = Clear
W = White
R = Red
Y = Yellow
B = Brown
G = Green

0 = Other (list color)

I. Home Cage Observations - After observation of posture, these are performed with the cage pulled out from the rack for an unobstructed view of the rat.

A. Posture
1) standing normally
2) rearing
3) sitting or lying in a normal position
4) sitting or lying with head in abnormal position (e.g., resting against the floor or wall)
5) lying flattened, limbs may be spread out
6) lying on side and limbs in the air
7) hunched back
8-11) other

B. Piloerection - indicate '+' if present; '-' if normal

C. Involuntary motor movements (score if present)

Clonic

1) repetitive "chewing" movements of mouth and jaw
2) muscle fasciculations
3) tremors
4) myoclonic jerks
5) explosive jumping into the air with all feet leaving the surface
6) convulsions
7) wet dog shakes
8) writhing

Tonic

1) contraction of extensors such that limbs are rigid and extended
2) head and body rigidly arching backward (opisthotonos)
3) head and body rigidly flexed forward (emprosthotonos)
4) convulsions

D. Gait abnormalities (score if present)
1) incoordination; excessive swaying, rocking or lurching
2) hindlimbs dragging, showing exaggerated or overcompensated movements or are splayed
3) hindfeet pointing outward from body (approximately 90°)
4) forelimbs dragging, extended or unable to support weight
5) walking on tiptoes
6) stilted
7) body dragging or flattened against surface
8-10) other

Scores: 1) Slight (barely perceptible or infrequent)
2) Moderate or Severe

E. Vocalizations - indicate '+' if spontaneous (i.e., without stimuli);

'-' if absent

F. Other
1) decreased activity
2) repetitive head bobbing (nutation)
3) increased reactivity
4-8) other

II. Observations During Handling

A. Ease of removing the rat from its cage
1) minimal resistance
2) minimal resistance with vocalizations
3) rearing, often following investigator's hand, with or without vocalizations
4) avoiding investigator's hand, with or without vocalizations
5) attempting to attack investigator's hand, with or without vocalizations

B. Reaction to being handled
1) minimal resistance, without vocalizations
2) minimal resistance, with vocalizations
3) rigid in hand, with or without vocalizations
4) squirming, twisting or attempting to bite, with or without vocalizations

C. Muscle tone (if abnormal)
1) flaccid
2) rigid

D. Palpebral closure (if abnormal)
1) slightly drooped
2) about half -closed
3) completely closed

G. Lacrimation - indicate '+' if present; '-' if none. If present also indicate color and score.

H. Salivation - indicate '+' if present; '-' if none. If present also indicate color and score.

K. Nasal Discharge - indicate '+' if present; '-' if none. If present also indicate color and score.

Scores: 1) Slight (barely perceptible or infrequent)
2) Moderate or Severe

Colors: C = Clear W = White R = Red
Y = Yellow B = Brown G = Green or Other

I. Stains (if present, indicate color and score)

1) lacrimal
2) nasal
3) oral
4) perianal
5) urine

J. Other (if present)

1) alopecia
2) bite marks
3) broken teeth/malocclusion
4) dehydration
5) emaciation
6) exophthalmia
7) missing toe nail(s)
8) cool-to-touch
9-12) other

III. Open Field Observations - The rat is placed in the center of a flat surface with a perimeter barrier, such as a cart, for 2 minutes. During this time, the number of rears is counted and other observations are made.

A. Piloerection - indicate' +' if present; '-' if absent.

B. Respiratory abnormalities (score if present):

1) rapid, shallow
2) labored
3) gasping, mouth breathing
4) audible sound (rales)

C. Posture:

1) standing normally
2) rearing
3) sitting or lying in a normal position
4) sitting or lying with head in abnormal position (e.g., resting against the floor or wall)
5) lying flattened, limbs may be spread out
6) lying on side and limbs in the air
7) hunched back
8-11) other

D. Involuntary motor movements (score if present):

Clonic

1) repetitive "chewing" movements of mouth and jaw
2) muscle fasciculations
3) tremors
4) myoclonic jerks
5) explosive jumping into the air with all feet leaving the surface
6) convulsions
7) wet dog shakes
8) writhing

Scores: 1) Slight (barely perceptible or infrequent)
2) Moderate or Severe

Colors: C = Clear W = White R = Red
Y = Yellow B = Brown G = Green or Other

Tonic

1) contraction of extensors such that limbs are rigid and extended
2) head and body rigidly arching backward (opisthotonos)
3) head and body rigidly flexed forward (emprosthotonos)
4) convulsions

E. Stereotypy (excessive or repetitive behavior; if present):

1) circling
2) head bobbing (nutation)
3) head weaving
4) pacing
5) stereotypic grooming

F. Bizarre behavior (if present):

1) flopping
2) retropulsion
3) self-mutilation
4) tail held erect (>45°)
5) writhing

G. Gait abnormalities (score if present) Note, if the rat did not move during the 2-minute observation period, it may be gently prodded in order to observe the gait.

1) incoordination; excessive swaying, rocking or lurching
2) hindlimbs dragging, showing exaggerated or overcompensated movements or are splayed
3) hindfeet pointing outward from body (approximately 90 degrees)
4) forelimbs dragging, extended or unable to support weight
5) walking on tiptoes
6) stilted
7) body dragging or flattened against surface
8-10) other

H. Vocalizations - indicate '+' if spontaneous (i.e., without stimuli);
'-' if absent.

I. Arousal (level of unprovoked activity and alertness; if abnormal):

1) inactive or unresponsive
2) sluggish, minimal head or body movement
3) sluggish, some exploratory movements
4) tense, agitated, sudden movements or freezing for short periods
5) hyperactive, attacking, bouts of sudden movements or freezing

Scores: 1) Slight (barely perceptible or infrequent)
2) Moderate or Severe

J. Rearing - Defined as each time both front feet come completely off the surface, although the rat does not necessarily have to raise itself up (i.e., this is a measure of the ability of the rat to place its weight on its haunches). Includes when the rat uses the perimeter barrier as support.

K. Other (1-5)

Excretion - at the end of the 2-minute period, measure:

L. Defecation - count the number of boluses. Record '0' if diarrhea is present or 'P' if partially formed.

M. Urination - count the number of pools. If urinating while moving, count dribbles as one pool. Record 'E' if polyuria (excessive volume).

IV. Reflex/Physiologic observations and measurements

Stimulus reactivity - Performed while the rat is sitting on the cart surface following open field observations.

A. Approach response - Approach the rat head-on with the end of a blunt object, such as a pencil, then hold the object approximately 3 cm from the face for approximately 4 seconds.

1) no reaction
2) slight response - e.g., ear flick, rat slowly approaching and sniffing or turning away
3) more energetic response than 2)
4) rat freezing and/or having muscle contractions
5) exaggerated reaction - jumping, biting or attacking

B. Touch response - Coming in from the side, touch rump gently with a blunt object.

1) no reaction
2) slight response-e.g., earflick, turning or walking away, with or without vocalization
3) more energetic response than 2)
4) rat freezing and/or having muscle contractions
5) exaggerated reaction - jumping, biting or attacking

C. Auditory response - Position clicker approximately 5 cm above the back of the rat and make a sudden sound.

1) no reaction
2) slight reaction, some evidence that the noise was heard
3) more energetic response than 2) .
4) rat freezing and/or having muscle contractions
5) exaggerated reaction - jumping, biting or attacking

D. Tail pinch response - metal forceps are used to squeeze the tail approximately 2-3 cm from the tip.

1) no reaction
2) slight response-e.g., earflick, turning or walking away with or without vocalization
3) more energetic response than 2)

4) rat freezing and/or having muscle contractions
5) exaggerated reaction - jumping, biting or attacking

E. Pupil size - Assess both eyes under standard lighting conditions.
1) constricted (pin-point) - Normal under these conditions.
2) dilated
(Enter 'NP' if not performed.)

F. Pupil response - Assess both eyes after adaptation in a darkened enclosure. Assess the reaction of both pupils to dark adaptation and the reaction of both pupils to the beam of a penlight as it is brought in from the side of the rat's head.
1) Pupils are dilated and then constrict in response to the penlight.
2) Pupils are dilated and show no response to the penlight.
3) Pupils are constricted (pin-point).
4) Other
(Enter 'NP' if not performed.)

E. Righting reflex - The rat is held supine, then dropped from approximately 30 cm. If the rat is severely affected, this test may not be performed.
1) normal, rat landing on feet or sternal position
2) slightly uncoordinated
3) landing on side
4) landing on back
5) test not performed

F. Grip strength - Conduct the test according to SOP E-201.
(Enter 'NP' if not performed.)

H. Body weight (g)
(Enter 'NP' if not performed.)

I. Body temperature - The colonic temperature (DC) is taken with a thermistor allowed to stabilize
before reading. Refer to SOP E-202 for details regarding thermistor use and maintenance.
(Enter 'NP' if not performed.)

G. Landing foot splay - The fourth (outermost) foot pad of each hind foot is dotted with water-based
ink. The rat is dropped three times from a prone position, approximately 30 cm onto paper and the ink spots where the feet land for each trial are marked. The distance (mm) between the middle of the ink spots for each trial are measured. A tolerance of +2 mm in measurement is considered acceptable. This test may not be performed due to condition of animal.
(Enter 'NP' if not performed.)

Enter free form text for any additional observations or explanations as needed.

APPENDIX II

THE FOLLOWING ATTACHMENT IS NOT AVAILABLE ELECTRONICALLY
SEE THE FILE COPY

BAYER CORPORATION - TOXICOLOGY

DATE: 21OCT98
TIME: 9:53:13

Table 7
TECHNICAL GRADE TI-435
Summary Interval Motor Activity for Male Rats, Pretreatment
Study Number 97-412-OH

Group	Interval 1	Interval 2	Interval 3	Interval 4	Interval 5	Interval 6	Interval 7	Interval 8	Interval 9
0 MG/KG	208 ± 54	149 ± 68	61 ± 62	33 ± 41	2 ± 8	0 ± 0	1 ± 1	3 ± 6	17 ± 34
100 MG/KG	215 ± 34	119 ± 49	59 ± 44	16 ± 25	16 ± 25	3 ± 7	1 ± 2	4 ± 11	1 ± 4
200 MG/KG	244 ± 69	142 ± 47	63 ± 60	18 ± 27	24 ± 29	7 ± 14	0 ± 0	4 ± 15	2 ± 7
400 MG/KG	199 ± 53	112 ± 44	45 ± 44	17 ± 23	14 ± 25	2 ± 6	0 ± 1	0 ± 0	0 ± 0

*Significantly different from control ($p \leq 0.05$, ANOVA)
Mean ± S.D for 1:30:00 (hh:mm:ss) Test Session, in 10 - Minute Intervals
Nominal Day 0 = 03NOV97
Number of Rats/Group: 12/0 MG/KG 12/100 MG/KG 12/200 MG/KG 12/400 MG/KG

HED Records Center Series 361 Science Reviews - File R107689 - Page 566 of 721

BAYER CORPORATION - TOXICOLOGY

DATE: 21OCT98
TIME: 9:53:13

Table 7
TECHNICAL GRADE TI-435
Summary Interval Motor Activity for Male Rats, Day 0
Study Number 97-412-OH

Group	Interval 1	Interval 2	Interval 3	Interval 4	Interval 5	Interval 6	Interval 7	Interval 8	Interval 9
0 MG/KG	161 ± 48	59 ± 30	13 ± 22	1 ± 2	4 ± 8	3 ± 10	4 ± 10	6 ± 10	4 ± 6
100 MG/KG	110* ± 35	35 ± 36	10 ± 19	9 ± 15	5 ± 13	6 ± 19	5 ± 15	8 ± 14	8 ± 18
200 MG/KG	60* ± 31	12* ± 14	8 ± 9	2 ± 7	2 ± 4	7 ± 8	3 ± 9	2 ± 6	10 ± 17
400 MG/KG	17* ± 11	2* ± 4	3 ± 9	7 ± 10	3 ± 7	3 ± 6	6 ± 7	5 ± 9	4 ± 6

552

*Significantly different from control ($p \leq 0.05$, ANOVA)
Mean ± S.D for 1:30:00 (hh:mm:ss) Test Session, in 10 - Minute Intervals
Nominal Day 0 = 03NOV97
Number of Rats/Group: 12/0 MG/KG 12/100 MG/KG 12/200 MG/KG 12/400 MG/KG

HED Records Center Series 361 Science Reviews - File R107689 - Page 567 of 721

BAYER CORPORATION - TOXICOLOGY

DATE: 21OCT98
TIME: 9:53:13

Table 7
TECHNICAL GRADE TI-435
Summary Interval Motor Activity for Male Rats, Day 7
Study Number 97-412-OH

Group	Interval 1	Interval 2	Interval 3	Interval 4	Interval 5	Interval 6	Interval 7	Interval 8	Interval 9
0 MG/KG	236 ± 72	146 ± 69	66 ± 51	27 ± 30	17 ± 34	7 ± 12	3 ± 10	5 ± 13	1 ± 3
100 MG/KG	227 ± 56	126 ± 35	71 ± 67	24 ± 26	9 ± 25	8 ± 21	8 ± 28	8 ± 14	3 ± 9
200 MG/KG	238 ± 46	151 ± 40	95 ± 75	46 ± 44	30 ± 33	10 ± 27	7 ± 24	1 ± 3	1 ± 3
400 MG/KG	206 ± 55	133 ± 45	62 ± 55	18 ± 25	12 ± 19	5 ± 9	0 ± 0	0 ± 1	1 ± 1

5553

*Significantly different from control ($p \leq 0.05$, ANOVA)
Mean ± S.D for 1:30:00 (hh:mm:ss) Test Session, in 10 - Minute Intervals
Nominal Day 0 = 03NOV97
Number of Rats/Group: 12/0 MG/KG 12/100 MG/KG 12/200 MG/KG 12/400 MG/KG

HED Records Center Series 361 Science Reviews - File R107689 - Page 568 of 721

BAYER CORPORATION - TOXICOLOGY

DATE: 21OCT98
TIME: 9:53:13

Table 7
TECHNICAL GRADE TI-435
Summary Interval Motor Activity for Male Rats, Day 14
Study Number 97-412-OH

Group	Interval 1	Interval 2	Interval 3	Interval 4	Interval 5	Interval 6	Interval 7	Interval 8	Interval 9
0 MG/KG	244 ± 72	152 ± 60	73 ± 47	43 ± 51	26 ± 35	11 ± 24	7 ± 23	7 ± 24	3 ± 6
100 MG/KG	258 ± 57	156 ± 58	71 ± 45	48 ± 40	19 ± 25	10 ± 24	2 ± 4	0 ± 1	8 ± 15
200 MG/KG	260 ± 57	156 ± 50	75 ± 43	69 ± 32	23 ± 27	13 ± 19	10 ± 15	4 ± 12	2 ± 3
400 MG/KG	210 ± 46	137 ± 44	80 ± 55	56 ± 56	20 ± 45	23 ± 58	5 ± 11	0 ± 0	9 ± 26

*Significantly different from control ($p \leq 0.05$, ANOVA)
Mean ± S.D for 1:30:00 (hh:mm:ss) Test Session, in 10 - Minute Intervals
Nominal Day 0 = 03NOV97
Number of Rats/Group: 12/0 MG/KG 12/100 MG/KG 12/200 MG/KG 12/400 MG/KG

HED Records Center Series 361 Science Reviews - File R107689 - Page 569 of 721

BAYER CORPORATION - TOXICOLOGY

DATE: 22OCT98
TIME: 8:30:44

Table 7
TECHNICAL GRADE TI-435
Summary Interval Motor Activity for Female Rats, Pretreatment
Study Number 97-412-OH

Group	Interval 1	Interval 2	Interval 3	Interval 4	Interval 5	Interval 6	Interval 7	Interval 8	Interval 9
0 MG/KG	296 ± 31	209 ± 55	173 ± 77	89 ± 53	33 ± 34	25 ± 51	10 ± 29	7 ± 17	1 ± 2
100 MG/KG	300 ± 35	237 ± 52	198 ± 67	110 ± 55	46 ± 66	36 ± 55	13 ± 22	0 ± 1	0 ± 1
200 MG/KG	301 ± 59	218 ± 50	181 ± 62	114 ± 79	41 ± 46	9 ± 18	0 ± 1	3 ± 8	1 ± 2
400 MG/KG	300 ± 40	225 ± 53	159 ± 76	95 ± 77	25 ± 38	19 ± 59	17 ± 60	3 ± 9	0 ± 0

551
97

*Significantly different from control ($p \leq 0.05$, ANOVA)
Mean ± S.D for 1:30:00 (hh:mm:ss) Test Session, in 10 - Minute Intervals
Nominal Day 0 = 05NOV97
Number of Rats/Group: 12/0 MG/KG 12/100 MG/KG 12/200 MG/KG 12/400 MG/KG

HED Records Center Series 361 Science Reviews - File R107689 - Page 570 of 721

BAYER CORPORATION - TOXICOLOGY

DATE: 22OCT98
TIME: 8:30:45

Table 7
TECHNICAL GRADE TI-435
Summary Interval Motor Activity for Female Rats, Day 0
Study Number 97-412-OH

Group	Interval 1	Interval 2	Interval 3	Interval 4	Interval 5	Interval 6	Interval 7	Interval 8	Interval 9
0 MG/KG	164 ± 30	79 ± 50	38 ± 42	15 ± 30	13 ± 26	6 ± 17	6 ± 9	7 ± 16	4 ± 8
100 MG/KG	166 ± 51	87 ± 55	43 ± 38	16 ± 33	10 ± 19	11 ± 18	12 ± 25	4 ± 12	8 ± 17
200 MG/KG	100* ± 47	29* ± 34	12 ± 20	8 ± 11	6 ± 8	9 ± 15	4 ± 7	14 ± 21	6 ± 12
400 MG/KG	37* ± 25	9* ± 14	7* ± 9	10 ± 9	6 ± 8	4 ± 6	10 ± 18	5 ± 5	5 ± 11

*Significantly different from control ($p \leq 0.05$, ANOVA)
Mean ± S.D for 1:30:00 (hh:mm:ss) Test Session, in 10 - Minute Intervals
Nominal Day 0 = 05NOV97
Number of Rats/Group: 12/0 MG/KG 12/100 MG/KG 12/200 MG/KG 12/400 MG/KG

HED Records Center Series 361 Science Reviews - File R107689 - Page 571 of 721

BAYER CORPORATION - TOXICOLOGY

DATE: 22OCT98
TIME: 8:30:45

Table 7
TECHNICAL GRADE TI-435
Summary Interval Motor Activity for Female Rats, Day 7
Study Number 97-412-OH

Group	Interval 1	Interval 2	Interval 3	Interval 4	Interval 5	Interval 6	Interval 7	Interval 8	Interval 9
0 MG/KG	266 ± 52	198 ± 49	139 ± 77	92 ± 78	47 ± 49	38 ± 63	18 ± 40	0 ± 1	0 ± 1
100 MG/KG	279 ± 45	212 ± 42	141 ± 85	147 ± 69	80 ± 77	40 ± 60	22 ± 44	10 ± 24	1 ± 2
200 MG/KG	297 ± 58	215 ± 58	174 ± 71	108 ± 67	50 ± 57	26 ± 49	5 ± 12	18 ± 55	0 ± 1
400 MG/KG	286 ± 31	236 ± 52	182 ± 69	116 ± 90	39 ± 65	24 ± 54	8 ± 23	5 ± 11	18 ± 41

*Significantly different from control ($p<0.05$, ANOVA)
Mean ± S.D for 1:30:00 (hh:mm:ss) Test Session, in 10 - Minute Intervals
Nominal Day 0 = 05NOV97
Number of Rats/Group: 12/0 MG/KG 12/100 MG/KG 12/200 MG/KG 12/400 MG/KG

HED Records Center Series 361 Science Reviews - File R107689 - Page 572 of 721

BAYER CORPORATION - TOXICOLOGY

DATE: 22OCT98
TIME: 8:30:45

Table 7
TECHNICAL GRADE TI-435
Summary Interval Motor Activity for Female Rats, Day 14
Study Number 97-412-OH

Group	Interval 1	Interval 2	Interval 3	Interval 4	Interval 5	Interval 6	Interval 7	Interval 8	Interval 9
0 MG/KG	278 ± 56	213 ± 41	175 ± 50	121 ± 63	67 ± 61	43 ± 43	32 ± 50	13 ± 31	1 ± 4
100 MG/KG	289 ± 51	219 ± 30	167 ± 41	117 ± 67	72 ± 81	38 ± 64	15 ± 29	11 ± 26	1 ± 4
200 MG/KG	298 ± 47	216 ± 61	170 ± 56	118 ± 50	78 ± 77	31 ± 36	25 ± 51	19 ± 34	18 ± 39
400 MG/KG	292 ± 54	204 ± 48	154 ± 53	96 ± 68	81 ± 88	34 ± 55	29 ± 69	12 ± 28	6 ± 19

*Significantly different from control ($p \leq 0.05$, ANOVA)
Mean ± S.D for 1:30:00 (hh:mm:ss) Test Session, in 10 - Minute Intervals
Nominal Day 0 = 05NOV97
Number of Rats/Group: 12/0 MG/KG 12/100 MG/KG 12/200 MG/KG 12/400 MG/KG

HED Records Center Series 361 Science Reviews - File R107689 - Page 573 of 721

BAYER CORPORATION - TOXICOLOGY

DATE: 21OCT98
TIME: 9:53:13

Table 8
TECHNICAL GRADE TI-435
Summary Interval Locomotor Activity for Male Rats, Pretreatment
Study Number 97-412-OH

Group	Interval 1	Interval 2	Interval 3	Interval 4	Interval 5	Interval 6	Interval 7	Interval 8	Interval 9
0 MG/KG	104 ± 24	57 ± 27	21 ± 22	13 ± 16	0 ± 1	0 ± 0	0 ± 0	1 ± 1	4 ± 11
100 MG/KG	98 ± 18	41 ± 20	18 ± 14	5 ± 10	6 ± 11	1 ± 2	0 ± 0	2 ± 5	0 ± 0
200 MG/KG	105 ± 18	46 ± 11	17 ± 16	7 ± 10	9 ± 13	2 ± 4	0 ± 0	2 ± 8	0 ± 1
400 MG/KG	99 ± 10	40 ± 14	16 ± 16	7 ± 9	6 ± 10	1 ± 3	0 ± 0	0 ± 0	0 ± 0

538

*Significantly different from control ($p \leq 0.05$, ANOVA)
Mean ± S.D for 1:30:00 (hh:mm:ss) Test Session in 10 - Minute Intervals
Nominal Day 0 = 03NOV97
Number of Rats/Group: 12/0 MG/KG 12/100 MG/KG 12/200 MG/KG 12/400 MG/KG

HED Records Center Series 361 Science Reviews - File R107689 - Page 574 of 721

BAYER CORPORATION - TOXICOLOGY

DATE: 21OCT98
TIME: 9:53:13

Table 8
TECHNICAL GRADE TI-435
Summary Interval Locomotor Activity for Male Rats, Day 0
Study Number 97-412-OH

Group	Interval 1	Interval 2	Interval 3	Interval 4	Interval 5	Interval 6	Interval 7	Interval 8	Interval 9
0 MG/KG	63 ± 13	18 ± 9	2 ± 6	0 ± 1	1 ± 3	1 ± 3	1 ± 2	1 ± 2	0 ± 0
100 MG/KG	38* ± 13	9* ± 11	2 ± 6	1 ± 2	0 ± 0	1 ± 2	0 ± 0	0 ± 0	3 ± 9
200 MG/KG	21* ± 10	4* ± 5	3 ± 4	1 ± 2	0 ± 1	2 ± 3	0 ± 1	0 ± 1	2 ± 4
400 MG/KG	6* ± 4	1* ± 2	0 ± 1	1 ± 2	0 ± 1	0 ± 0	1 ± 2	0 ± 1	1 ± 2

560

*Significantly different from control ($p \leq 0.05$, ANOVA)
Mean ± S.D for 1:30:00 (hh:mm:ss) Test Session in 10 - Minute Intervals
Nominal Day 0 = 03NOV97
Number of Rats/Group: 12/0 MG/KG 12/100 MG/KG 12/200 MG/KG 12/400 MG/KG

HED Records Center Series 361 Science Reviews - File R107689 - Page 575 of 721

BAYER CORPORATION - TOXICOLOGY

DATE: 21OCT98
TIME: 9:53:13

Table 8
TECHNICAL GRADE TI-435
Summary Interval Locomotor Activity for Male Rats, Day 7
Study Number 97-412-OH

Group	Interval 1	Interval 2	Interval 3	Interval 4	Interval 5	Interval 6	Interval 7	Interval 8	Interval 9
0 MG/KG	99 ± 28	52 ± 25	21 ± 16	7 ± 9	5 ± 9	2 ± 4	0 ± 0	1 ± 4	0 ± 0
100 MG/KG	95 ± 23	39 ± 15	21 ± 25	8 ± 10	3 ± 9	3 ± 9	3 ± 11	2 ± 5	1 ± 3
200 MG/KG	88 ± 21	49 ± 14	30 ± 26	14 ± 14	7 ± 11	2 ± 8	2 ± 7	0 ± 1	0 ± 0
400 MG/KG	90 ± 21	45 ± 18	21 ± 20	6 ± 9	4 ± 7	1 ± 2	0 ± 0	0 ± 0	0 ± 0

561

*Significantly different from control ($p \leq 0.05$, ANOVA)
Mean ± S.D for 1:30:00 (hh:mm:ss) Test Session in 10 - Minute Intervals
Nominal Day 0 = 03NOV97
Number of Rats/Group: 12/0 MG/KG 12/100 MG/KG 12/200 MG/KG 12/400 MG/KG

HED Records Center Series 361 Science Reviews - File R107689 - Page 576 of 721

BAYER CORPORATION - TOXICOLOGY

DATE: 21OCT98
TIME: 9:53:14

Table 8
TECHNICAL GRADE TI-435
Summary Interval Locomotor Activity for Male Rats, Day 14
Study Number 97-412-OH

Group	Interval 1	Interval 2	Interval 3	Interval 4	Interval 5	Interval 6	Interval 7	Interval 8	Interval 9
0 MG/KG	103 ± 29	53 ± 24	21 ± 15	15 ± 19	6 ± 13	4 ± 10	2 ± 6	2 ± 7	1 ± 2
100 MG/KG	105 ± 25	54 ± 20	21 ± 16	15 ± 14	4 ± 6	3 ± 7	0 ± 0	0 ± 0	2 ± 5
200 MG/KG	100 ± 22	52 ± 14	26 ± 15	20 ± 15	5 ± 7	3 ± 6	1 ± 3	1 ± 4	1 ± 2
400 MG/KG	87 ± 14	51 ± 16	24 ± 20	13 ± 17	3 ± 10	8 ± 22	0 ± 0	0 ± 0	2 ± 8

5762

*Significantly different from control ($p<0.05$, ANOVA)
Mean ± S.D for 1:30:00 (hh:mm:ss) Test Session in 10 - Minute Intervals
Nominal Day 0 = 03NOV97
Number of Rats/Group: 12/0 MG/KG 12/100 MG/KG 12/200 MG/KG 12/400 MG/KG

HED Records Center Series 361 Science Reviews - File R107689 - Page 577 of 721

BAYER CORPORATION - TOXICOLOGY

DATE: 22OCT98
TIME: 8:30:45

Table 8
TECHNICAL GRADE TI-435
Summary Interval Locomotor Activity for Female Rats, Pretreatment
Study Number 97-412-OH

Group	Interval 1	Interval 2	Interval 3	Interval 4	Interval 5	Interval 6	Interval 7	Interval 8	Interval 9
0 MG/KG	116 ± 12	73 ± 22	62 ± 35	27 ± 17	10 ± 11	10 ± 22	4 ± 11	2 ± 6	0 ± 0
100 MG/KG	116 ± 13	83 ± 24	66 ± 31	40 ± 25	17 ± 29	12 ± 21	3 ± 7	0 ± 0	0 ± 0
200 MG/KG	125 ± 19	83 ± 32	66 ± 31	38 ± 30	12 ± 15	3 ± 6	0 ± 0	0 ± 0	0 ± 0
400 MG/KG	115 ± 19	77 ± 25	61 ± 34	36 ± 33	8 ± 13	9 ± 28	7 ± 23	1 ± 3	0 ± 0

*Significantly different from control ($p \leq 0.05$, ANOVA)
Mean ± S.D for 1:30:00 (hh:mm:ss) Test Session in 10 - Minute Intervals
Nominal Day 0 = 05NOV97
Number of Rats/Group: 12/0 MG/KG 12/100 MG/KG 12/200 MG/KG 12/400 MG/KG

HED Records Center Series 361 Science Reviews - File R107689 - Page 578 of 721

BAYER CORPORATION - TOXICOLOGY

DATE: 22OCT98
TIME: 8:30:45

Table 8
TECHNICAL GRADE TI-435
Summary Interval Locomotor Activity for Female Rats, Day 0
Study Number 97-412-OH

Group	Interval 1	Interval 2	Interval 3	Interval 4	Interval 5	Interval 6	Interval 7	Interval 8	Interval 9
0 MG/KG	54 ± 12	21 ± 15	8 ± 10	4 ± 8	3 ± 7	1 ± 4	1 ± 3	1 ± 3	1 ± 3
100 MG/KG	45 ± 17	23 ± 18	10 ± 12	5 ± 11	2 ± 5	3 ± 6	2 ± 5	1 ± 4	1 ± 4
200 MG/KG	30* ± 10	7* ± 8	4 ± 5	2 ± 3	1 ± 2	3 ± 6	0 ± 1	4 ± 6	1 ± 3
400 MG/KG	10* ± 10	2* ± 4	1 ± 1	1 ± 2	1 ± 2	0 ± 0	1 ± 1	1 ± 2	0 ± 1

*Significantly different from control ($p \leq 0.05$, ANOVA)
Mean ± S.D for 1:30:00 (hh:mm:ss) Test Session in 10 - Minute Intervals
Nominal Day 0 = 05NOV97
Number of Rats/Group: 12/0 MG/KG 12/100 MG/KG 12/200 MG/KG 12/400 MG/KG

HED Records Center Series 361 Science Reviews - File R107689 - Page 579 of 721

BAYER CORPORATION - TOXICOLOGY

DATE: 22OCT98
TIME: 8:30:45

Table 8
TECHNICAL GRADE TI-435
Summary Interval Locomotor Activity for Female Rats, Day 7
Study Number 97-412-OH

Group	Interval 1	Interval 2	Interval 3	Interval 4	Interval 5	Interval 6	Interval 7	Interval 8	Interval 9
0 MG/KG	110 ± 30	70 ± 20	47 ± 32	32 ± 29	16 ± 18	14 ± 23	5 ± 13	0 ± 0	0 ± 0
100 MG/KG	113 ± 23	79 ± 21	47 ± 34	52 ± 29	23 ± 26	14 ± 21	6 ± 13	2 ± 6	0 ± 0
200 MG/KG	118 ± 26	75 ± 25	61 ± 33	39 ± 25	15 ± 21	9 ± 22	0 ± 1	4 ± 13	0 ± 0
400 MG/KG	120 ± 19	86 ± 24	63 ± 28	38 ± 35	12 ± 24	7 ± 16	1 ± 5	1 ± 1	7 ± 16

565

*Significantly different from control ($p \leq 0.05$, ANOVA)
Mean ± S.D for 1:30:00 (hh:mm:ss) Test Session in 10 - Minute Intervals
Nominal Day 0 = 05NOV97
Number of Rats/Group: 12/0 MG/KG 12/100 MG/KG 12/200 MG/KG 12/400 MG/KG

HED Records Center Series 361 Science Reviews - File R107689 - Page 580 of 721

BAYER CORPORATION - TOXICOLOGY

DATE: 22OCT98
TIME: 8:30:46

Table 8
TECHNICAL GRADE TI-435
Summary Interval Locomotor Activity for Female Rats, Day 14
Study Number 97-412-OH

Group	Interval 1	Interval 2	Interval 3	Interval 4	Interval 5	Interval 6	Interval 7	Interval 8	Interval 9
0 MG/KG	114 ± 27	75 ± 20	56 ± 23	41 ± 27	20 ± 22	13 ± 17	11 ± 18	4 ± 8	0 ± 0
100 MG/KG	114 ± 19	77 ± 18	53 ± 16	35 ± 23	22 ± 28	9 ± 14	3 ± 6	2 ± 7	0 ± 0
200 MG/KG	115 ± 21	75 ± 24	56 ± 20	31 ± 16	23 ± 24	8 ± 11	5 ± 9	6 ± 12	5 ± 12
400 MG/KG	117 ± 36	72 ± 25	48 ± 19	33 ± 27	26 ± 27	12 ± 19	8 ± 21	3 ± 9	2 ± 8

*Significantly different from control ($p \leq 0.05$, ANOVA)
Mean ± S.D for 1:30:00 (hh:mm:ss) Test Session in 10 - Minute Intervals
Nominal Day 0 = 05NOV97
Number of Rats/Group: 12/0 MG/KG 12/100 MG/KG 12/200 MG/KG 12/400 MG/KG

HED Records Center Series 361 Science Reviews - File R107689 - Page 581 of 721

APPENDIX III

A Special Acute Neurotoxicity Study (to establish a No-Observed-Effect-Level of TI-435 in male Fischer 344 rats)

In this acute neurotoxicity study (MRID 45422802), TI-435 (Clothianidin; ≥95.3% a.i., Batch # 30037120) in 0.5% methylcellulose/0.4% Tween 80 solution was administered in a single dose by gavage (10 mL/kg) to fasted male Fischer 344 rats (12/dose) at doses of 0, 20, 40, or 60 mg/kg. Functional observational battery (FOB) and motor activity were evaluated during week -1 and on Day 0 (approximately 4 hours post-dosing). Dose analyses indicated that nominal doses were achieved.

No unscheduled deaths occurred during the study. Clinical signs, FOB, and motor activity were unaffected by treatment.

The LOAEL was not observed. The NOAEL is 60 mg/kg.

The submitted study is classified as **acceptable/guideline** in conjunction with the acute neurotoxicity study (MRID 45422801). Together these studies satisfy the requirements (OPPTS 870.6200a; OECD 424) for an acute neurotoxicity screening battery in rats.

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

PMRA/EPA Reviewers: Scott Hancock/Pamela Hurley Signature: Pamela Hurley
EPA Work Assignment Manager:Ghazi Dannan, Ph.D. Signature: Ghazi Dannan
Registration Action Branch 3, Health Effects Division (7509C) Date 12/10/04

TXR#: 0050321

DATA EVALUATION RECORD

STUDY TYPE: Subchronic Neurotoxicity- Feeding Study in Rats, OPPTS 870.6200b [§82-7]; No OECD guideline.

PC CODE: 044309 **DP BARCODE:** D282578
SUBMISSION NO.: S614462

TEST MATERIAL (PURITY): TI-435 (Clothianidin; ≥95.3% a.i.)

SYNONYMS: (E)-1-(2-chloro-5-thiazolylmethyl)-3-methyl-2-nitroguanidine

CITATION: Sheets, L.P., D.M. Cain, and B.P. Stuart (2000). A Subchronic Neurotoxicity Screening Study with Technical Grade TI-435 in Fischer 344 Rats. Bayer Corp., Agriculture Division Toxicology, Stilwell, KS. Laboratory Study No: 97-472-OM, October 12, 2000. MRID 45422803. Unpublished.

Moore, K.D. and L.S. Shelton (1999). The Homogeneity, Room Temperature and Freezer Stability of TI 435 in Rodent Ration. Bayer Corp., Agriculture Division Toxicology, Stilwell, KS. Laboratory Study No: 97-872-NQ, January 4, 1999. MRID 45422825. Unpublished.

SPONSOR: Takeda Chemical Industries, Ltd., 2-13-10 Nihonbashi, Chuo-Ku, Toyko, Japan

EXECUTIVE SUMMARY: In a subchronic neurotoxicity study (MRID 45422803), TI-435 (clothianidin; >=95.3% a.i., Batch # 30037120) was administered in the diet for 13 weeks to 12 Fischer 344 rats/sex/group at doses of 0, 150, 1000, or 3000 ppm (equivalent to 0/0, 9.2/10.6, 60.0/71.0, or 177.0/200.1 mg/kg/day in males and females respectively). At termination, 6 animals/sex/group were perfused *in situ* and tissues from the control and 3000 ppm groups were examined microscopically. Functional observational battery (FOB) and motor activity were evaluated during Weeks -1 (prior to dosing), 4, 8, and 13. Acceptable positive control data were provided that verified the ability of the observers of the performing lab to conduct the FOB and to assess motor activity, neurotoxicity and behavioral effects.

No treatment-related mortalities were observed. Clinical signs, ophthalmoscopic observations, FOB, motor activity, absolute brain weights, gross necropsy, and neurohistology were unaffected by treatment.

569

At 3000 ppm, food consumption was decreased ($p<=0.05$) throughout most of the study (5-20%). These findings corresponded to the decreased overall body weight gains (11-13%). Body weights were decreased compared to controls ($p<=0.05$) in the males during Weeks 10-12 (4-5%), and in females during Weeks 8 and 13 (4% each).

The LOAEL is 3000 ppm (equivalent to 177.0/200.1 mg/kg [M/F]) based on slightly decreased food consumption, body weights, and body weight gains. The NOAEL is 1000 ppm (equivalent to 60.0/71.0 mg/kg [M/F]).

There was no evidence of neurotoxicity at any dose tested.

The submitted study is classified as **acceptable/guideline** and satisfies the requirements (OPPTS 870.6200b) for a subchronic neurotoxicity screening battery in rats.

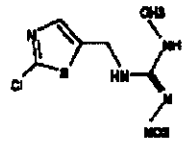
COMPLIANCE: Signed and dated GLP, Quality Assurance, and Data Confidentiality statements were provided.

570

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: TI-435 (Clothianidin)

Description:	pale yellow powder
Lot/Batch #:	300371200
Purity:	≥95.3% a.i.
Compound Stability:	The test material was stable in the diet for at least 14 days at room temperature.
CAS # of TGAI:	210880-92-5
★★★ **Structure:**	

2. Vehicle: Diet

3. Test animals:

Species:	Rat
Strain:	Fischer 344 CDF (F-344)/BR
Age/Wt at study initiation:	9 weeks/185.3-191.2 g males and 126.0-127.0 g females
Source:	SASCO Inc., Portage MI
Housing:	Individually in suspended, stainless steel cages.
Diet:	Purina Mills Rodent Lab Chow 5001-4, *ad libitum*, except during FOB and motor activity evaluations.
Water:	Municipal tap water *ad libitum* except for overnight prior to dosing and during neurobehavioral evaluations.
Environmental conditions:	**Temperature:** 18-26°C **Humidity:** 30-70 % **Air changes:** not reported **Photoperiod:** 12 hrs dark/12 hrs light
Acclimation period:	At least 6 days.

B. STUDY DESIGN

1. In life dates: Start: March 9, 1998 End: June 11, 1998

2. Animal assignment and treatment: Animals were randomly assigned (stratified by body weight) to the test groups noted in Table 1. Test substance was administered in the diet for up to 13 weeks. The dose levels in Table 1 were chosen based on the results of a three-week range-finding study (Bayer #97-972-NH) in which Fischer 344 rats were exposed to TI-435 in the diet at doses of 0, 100, 250, 1250, 2500, or 3750 ppm. Body weights were reduced in the males at 2500 (↓7%) ppm and in both sexes at 3750 (↓8-11%) ppm. Additionally, food consumption was decreased in both sexes at 1250 (↓11-12%), 2500 (↓21-25%), and 3750 (↓27-34%) ppm.

571

Table 1. Study design. [a]

Dose (ppm)	Mean Chemical Intake (mg/kg/day) [M/F]	Total # of Animals	Behavioral Testing (FOB, Motor Activity)	Neuropathology
0	0/0	12/sex	12/sex	6/sex
150	9.2/10.6	12/sex	12/sex	6/sex
1000	60.0/71.0	12/sex	12/sex	6/sex
3000	177.0/200.1	12/sex	12/sex	6/sex

a Data obtained from Study Report pages 17 and 36 (MRID 45422803).

3. Test substance preparation and analysis: Test diets were prepared weekly by mixing appropriate amounts of test substance with Purina Certified Rodent Diet #5001-4 and were stored refrigerated (approximately 6°C) until use. It was stated that homogeneity and stability were tested by analysis of 50 and 5000 ppm samples that bracketed the range of test concentrations; however, the referenced data report (Bayer #108690) was not provided. A suitable study was provided (MRID 45422825) utilizing the same batch of the test material. During the study, samples of all test diets from Weeks 1, 2, 3, 7, 11, and 14 were analyzed for concentration; however, the concentration data were not provided.

Results

Homogeneity Analysis: (as % coefficient of variation)
50 ppm = 9.8%
5000 ppm = 8.6%

Stability Analysis: (as % of Day 0)
Room temperature after 14 days
50 ppm = 103%
5000 ppm = 96.7%
Freezer temperature after 14 days
50 ppm = 90.5%
5000 ppm = 95.2%

Concentration Analysis: It was stated that the actual concentrations averaged 89.0-89.7% of nominal (no further information was provided).

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

572

4. Statistics: The data were analyzed using the following statistical methods:

Parameter	Statistical Method
FOB continuous data Motor and Locomotor activity (total session data)	Repeated-Measures ANOVA followed by a one-way ANOVA followed by Dunnett's.
Categorical FOB data	General Linear Modeling (GLM) and Categorical Modeling (CATMOD), with post-hoc comparisons using Dunnett's and analysis of contrasts, respectively.
Motor and Locomotor activity (interval data)	Two-way Repeated-Measures ANOVA followed by a Repeated-Measures ANOVA followed by a one-way ANOVA followed by Dunnett's.
Terminal body weight Brain weight	Bartlett's test for homogeneity of variances. Homogeneous data were further analyzed using ANOVA followed by Dunnett's. Non-homogeneous data were analyzed using the non-parametric Kruskal-Wallis test followed by a Mann-Whitney U test.

Significance levels were defined at $p \leq 0.05$, with the exception of the Bartlett's test ($p \leq 0.001$).

C. METHODS

1. Mortality and clinical observations: Animals were observed twice daily (once daily on weekends and holidays) for mortality and moribundity. Detailed physical examinations were performed weekly.

2. Body weight: All animals were weighed prior to the study and weekly thereafter. Additionally, perfused animals were weighed at termination for terminal body weight measurement.

3. Food consumption and mean chemical intake: Food consumption was recorded weekly and presented as g/animal/day. The average daily intake of the chemical (mg/kg/day) was calculated using the following formula: (µg of active ingredient/g feed/1000) X (feed consumed (g/kg bw/day)

4. Ophthalmoscopic examinations: The eyes of all animals were examined prior to placement in dose groups and at Week 12. The pupillary reflex was tested with a penlight, and then a mydriatic agent was applied to each eye. After mydriasis, the eye lids, conjunctiva, cornea, aqueous humor, and lens were examined with a slit lamp microscope, and the vitreous humor, retina, choroid, and optic disc were examined using an indirect ophthalmoscope and a condensing lens.

5. Neurobehavioral assessment

a. Functional Observational Battery (FOB): All animals were subject to a FOB at Weeks -1, 4, 8, and 13. The scoring criteria for the FOB are included in an Appendix to this DER (the

reviewers noted errors in the alphanumeric system used for the FOB criteria; however, there does not appear to be any missing data). The technicians who conducted the FOB were "blind" as to the animals treatment groups and observations for all animals were performed by the same person throughout the study, with a second person performing the measurements (e.g., grip strength and foot splay). On the day prior to FOB and motor activity testing, the animals were transferred to the testing room and allowed to acclimate with minimal disturbance until testing the following day. Sets of 8 animals were individually evaluated during the FOB. The following CHECKED (X) parameters were examined:

	HOME CAGE OBSERVATIONS		HANDLING OBSERVATIONS Continued		OPEN FIELD OBSERVATIONS
X	Posture*	X	Fur appearance / staining		Mobility
X	Piloerection*	X	Palpebral closure*	X	Rearing+
X	Convulsions (Clonic/Tonic)*	X	Eye prominence (exophthalmia)*	X	Arousal*
X	Tremors*	X	Muscle tone*	X	Convulsions (Clonic/Tonic)*
X	Gait abnormalities			X	Rearing
	Palpebral closure*		**REFLEX/PHYSIOLOGIC OBSERVATIONS**	X	Respiratory rate+
X	Vocalization	X	Approach response+	X	Urination / defecation*
X	Other (activity/nutation/reactivity)*	X	Touch response+	X	Vocalization
		X	Auditory response*	X	Posture*
	HANDLING OBSERVATIONS	X	Tail pinch response*	X	Gait abnormalities*
X	Ease of removal	X	Pupil response*	X	Bizarre/stereotypic behavior*
X	Reaction to handing*	X	Pupil size	X	Piloerection*
X	Lacrimation*	X	Grip strength*		
X	Salivation*	X	Landing foot splay*		
X	Nasal discharge	X	Righting reflex+		
X	Staining (nasal/oral/perianal/urine)	X	Body temperature+		
		X	Body weight*		

*Required parameters; +Recommended parameters

For each test week, testing was staggered over a two day period for each sex. Males and females were tested on separate days and the open-fields and mazes were cleaned between intervals to reduce the residual scent from the other sex.

b. Motor activity: Approximately 1 to 2.5 hours after the last animal was evaluated in the FOB, all eight animals were individually placed in separate figure-eight mazes for motor activity measurement. Motor and locomotor activity were measured during a 90 minute session with 10 minute intervals. Motor activity was measured as the number of beam interruptions that occurred during the session. Locomotor activity was measured by eliminating consecutive counts for a given beam. Habituation was evaluated as a decrement in activity during the test session. A Universal Maze Monitoring System (Columbus Instruments, Columbus, OH) was used for automated data collection. Broad spectrum background noise [approximately 70±2 dB(A)] was

generated during the observation period via a white noise generator (Model S81-02, Coulbourn Instruments) and amplifier (Model S82-24, Coulbourn Instruments). The light intensity (100±70 lux) was verified daily using a digital light meter. The order of testing and assignment to mazes was done in semi-random order such that no animal was tested more than once in the same maze.

6. Sacrifice and pathology: All animals were subject to gross necropsy. The first 6 rats/sex/dose were anesthetized using sodium pentobarbital (50 mg/kg i.p.), and perfused via the left ventricle with a sodium nitrite flush followed by *in situ* fixation with Universal fixative [4% (w/v) glutaraldehyde and 4% (w/v) EM-grade formaldehyde) in phosphate buffer. The entire brain and spinal cord, both eyes (with optic nerves) and selected peripheral nerves (sciatic, tibial, and sural), the gasserian ganglion and gastrocnemius muscle were post-fixed in 10% buffered formalin. The brain was weighed upon removal and prior to post-fixation. All other surviving animals were sacrificed via CO_2 asphyxiation without perfusion or collection of tissues. Brain and spinal cord tissues were embedded in paraffin. Three sections were collected from each level, and stained with hematoxylin-eosin, luxol fast blue-cresyl violet, and Sevier-Munger stains, respectively. The peripheral nerves (sciatic, tibial, and sural) were embedded in epoxy plastic resin, sectioned (1 μm), and stained with toluidine blue. All remaining tissues were embedded in glycol methacrylate, sectioned (2-3 μm), and stained with a modified Lee's stain. The following CHECKED (X) tissues from the control and high-dose animals, as well as all gross lesions were examined microscopically:

	CENTRAL NERVOUS SYSTEM		PERIPHERAL NERVOUS SYSTEM
	BRAIN		**SCIATIC NERVE**
X	Olfactory bulbs	X	Sciatic Notch
X	Cerebral cortex		
X	Caudate putamen/Globus pallidus		
X	Hippocampus		**OTHER**
X	Thalamus	X	Sural Nerve
X	Hypothalamus	X	Tibial Nerve
X	Midbrain (tectum, tegmentum, and peduncles)	X	Sciatic Nerve
X	Cerebellum	X	Lumbar dorsal root ganglion
X	Medulla oblongata	X	Lumbar dorsal root fibers
	SPINAL CORD	X	Lumbar ventral root fibers
X	Cervical swelling	X	Cervical dorsal root ganglion
X	Lumbar swelling	X	Cervical dorsal root fibers
X	Thoracic swelling	X	Cervical ventral root fibers
X	Cauda Equina		
	OTHER		
X	Gasserian Ganglion		

7. Positive controls: Summaries were provided for four neurotoxicity studies (MRIDs 42770301 and 43656301) performed to generate positive control data and validate the procedures and observers of the performing lab to conduct the FOB and to assess motor activity, neurotoxicity, behavioral effects, and neuropathological lesions. The submitted positive control studies were performed between 6-7 years prior to the current study. Triadimefon (200 mg/kg,

gavage, single dose) induced increased motor activity, while chlorpromazine (2 mg/kg, i.p., single dose) caused decreased motor activity. Clinical and/or functional effects produced by acrylamide (25 or 50 mg/kg, i.p., 3 times/week) included clinical signs (ataxia, urine stain, oral stain, and piloerection), decreased body weight gain and absolute brain weight, muscle fasciculations, tremors, ataxia, decreased rearing, erect tail, hindlimbs dragging, impaired air righting reflex, and increased hindlimb foot splay. Neuropathologial effects included mild to marked neuronal degeneration in the sciatic, tibial, and sural nerves, and axonal degeneration in the spinal cord. Trimethyltin (12 mg/kg, i.p., single dose) caused mortality, tremors, increased reactivity, convulsions, and ataxia. Neuropathological effects included neuronal necrosis (olfactory tract, piriform cortex, and hippocampus), chromatolysis of large neuronal soma (pons, medulla oblongata, spinal cord, and dorsal root and gasserian ganglion. Carbaryl (15 or 30 mg/kg, i.p., single dose) caused salivation, lacrimation, staining (nasal, oral, perianal, and urine), gait abnormalities, impaired air righting reflex, decreases in approach, startle, touch, and tail pinch responses, muscle fasciculations, tremors, clonic chewing movements, decreased arousal, and decreased rearing.

It should be noted that the triadimefon and chlorpromazine study (MRID 43656301) was performed using Fischer 344 rats, while the remaining studies (MRID 42770301) were performed using Sprague-Dawley rats. However, the reviewers believe that this minor deficiency does not affect the validity of this positive control data.

II. RESULTS

A. OBSERVATIONS

1. **Clinical signs:** No treatment-related clinical signs were observed at any dose level.

2. **Mortality:** One 3000 ppm male (#OM3001) died on Day 4 and exhibited decreased activity, ataxia, and convulsions prior to death. No gross pathological evidence indicating the cause of death was observed; therefore, this mortality was considered incidental.

B. BODY WEIGHT AND BODY WEIGHT GAIN: At 3000 ppm, body weights were decreased compared to controls ($p \leq 0.05$) in the males during Weeks 10-12 (↓4-5%), and in females during Weeks 8 and 13 (↓4% each, Table 2). Additionally, overall body weight gain (Weeks 0-13, calculated by reviewers) was decreased compared to controls in the males (↓11%) and females (↓13%). Body weights and body weight gains were similar to controls at ≤1000 ppm. Additionally, body weight was decreased ($p \leq 0.05$) during the FOB on Day 14 in the 3000 ppm males (↓4%).

Table 2. Mean (±SD) body weights and body weight gains (g) in rats exposed to TI-435 for up to 13 weeks. [a]

Week	Dose (ppm)			
	0	150	1000	3000
Males				
0	187.0±7.8	185.3±13.5	191.2±7.8	188.6±6.9
10	317±10.1	320.3±12.0	323.3±9.1	300.8±20.1* (↓5)
12	323.7±12.9	329.4±14.2	329.3±9.9	310.6±14.5* (↓4)
13	335.2±10.4	341.3±13.6	336.1±23.2	320.9±14.8
Overall (0-13) Weight Gain (g) [b]	148.2	156.0	144.9	132.3 (↓11)
Females				
0	126.7±6.2	126.5±4.9	126.0±6.6	127.0±7.0
8	173.7±5.1	176.5±6.8	173.3±5.8	167.3±6.5* (↓4)
9	177.5±7.1	182.2±6.8	178.5±6.4	175.3±7.7
13	186.7±7.7	191.0±6.4	186.5±7.2	179.1±7.7* (↓4)
Overall (0-13) Weight Gain (g) [b]	60.0	64.5	60.5	52.1 (↓13)

a Data obtained from Study Report Table 2, page 32-33 (MRID 45422803); n=12, except for the 3000 ppm males n=11 beginning on Week 1. Numbers presented parenthetically represent percent difference from control (calculated by reviewers).

b Calculated by reviewers.

* Statistically different from the control at $p \leq 0.05$.

C. **FOOD CONSUMPTION AND MEAN CHEMICAL INTAKE**: At 3000 ppm, food consumption was decreased ($p \leq 0.05$) in the males during Weeks 1-5 (↓5-17%) and 8-13 (↓5-9%), and in females throughout the study except Week 9 (↓5-20%, Table 3). These findings corresponded to the decreased body weight gains observed in the 3000 ppm males and females. No treatment-related differences in food consumption were noted at ≤1000 ppm. The slight decrease ($p \leq 0.05$) noted in the 150 ppm males during Week 1 was considered to be incidental. Mean chemical intake (mg/kg/day) is presented in Table 1.

Table 3. Mean (±SD) food consumption (g/animal/day) in rats exposed to TI-435 for up to 13 weeks.[a]

Week	Dose (ppm)			
	0	150	1000	3000
Males				
1	18.12±0.77	17.38±0.96* (↓4)	17.99±0.51	15.01±0.48* (↓17)
2	18.28±0.43	18.11±0.87	18.20±0.52	16.84±0.79* (↓8)
9	18.76±1.01	18.80±0.93	18.64±0.56	17.75±0.97* (↓5)
10	19.24±0.88	19.47±0.99	19.15±0.65	17.60±1.42* (↓9)
13	19.14±0.58	18.99±0.82	18.20±2.73	17.94±1.11* (↓6)
Females				
1	12.40±0.52	12.14±0.58	11.85±0.87	9.87±0.65* (↓20)
2	12.57±0.50	12.48±0.85	12.43±0.60	11.18±0.55* (↓11)
7	13.10±0.69	13.25±0.46	13.36±0.52	12.31±0.67* (↓6)
10	13.84±0.73	13.81±0.65	13.94±0.60	13.08±0.88* (↓5)
13	12.83±0.57	13.04±0.52	12.86±0.59	11.84±0.72* (↓8)

a Data obtained from Study Report Table 3, page 34-35 (MRID 45422803); n=12, except for the 3000 ppm males n=11 beginning on Week 1. Numbers presented parenthetically represent percent difference from control (calculated by reviewers).

* Statistically different from the control at $p \leq 0.05$.

D. OPHTHALMOSCOPIC EXAMINATIONS: No treatment-related ophthalmoscopic effects were observed at any dose.

E. NEUROBEHAVIORAL RESULTS

1. FOB findings: FOB findings were similar between treated and control groups throughout the study. Grip strength and landing foot splay were also similar between groups.

2. Motor activity: Total motor (Table 4) and locomotor (Table 5) activities were similar to controls at all doses throughout the study. Habituation was not affected by treatment at any dose.

Table 4. Mean (±SD) motor activity (total activity counts for session) in rats treated with TI-435 for up to 13 weeks. [a]

Time	Dose (ppm)							
	0	150	1000	3000	0	150	1000	3000
	Male				Female			
Pretreatment	290±59	302±53	333±84	348±94	393±86	361±84	346±131	450±149
Week 4	311±69	375±122	341±81	325±65	501±183	487±141	527±202	442±152
Week 8	312±60	391±70	346±85	302±44	477±156	529±122	589±261	562±172
Week 13	255±87	311±79	328±87	278±83	487±180	531±190	563±269	541±210

a Data obtained from Study Report, Table 7, pages 117-118 (MRID 45422803); n=12, except for the 3000 ppm males n=11.

Table 5. Mean (±SD) locomotor activity (total activity counts for session) in rats treated with TI-435 for up to 13 weeks. [a]

Time	Dose (ppm)							
	0	150	1000	3000	0	150	1000	3000
	Male				Female			
Pretreatment	183±34	191±21	208±53	207±45	242±47	239±65	223±86	275±102
Week 4	174±37	198±48	198±42	182±28	282±121	301±83	315±144	252±87
Week 8	167±22	201±52	182±42	164±27	256±85	296±72	313±136	310±100
Week 13	137±48	162±47	173±54	154±56	261±103	304±115	301±147	296±116

a Data obtained from Study Report, Table 8, pages 119-120 (MRID 45422803); n=12, except for the 3000 ppm males n=11.

F. SACRIFICE AND PATHOLOGY

1. **Gross pathology:** No treatment-related gross lesions were observed at any dose.

2. **Brain weight:** Absolute brain weight was similar to controls at all doses (Table 6). Relative (to body) brain weight was increased ($p \leq 0.05$) in the 3000 ppm males (↑ 10%) and females (↑ 7%), and was likely due to the decreased terminal body weights in the males (↓ 6%, not statistically significant) and females (↓ 6%, $p \leq 0.05$).

Table 6. Mean (±SD) absolute (g) and relative (to body) brain weights in rats treated with TI-435 for up to 13 weeks. [a]

Parameter	Dose (ppm)			
	0	150	1000	3000
Males				
Terminal Body weight	335.0±14.8	339.6±16.2	332.3±27.0	315.9±10.8 (↓6)
Absolute Brain weight	1.815±0.052	1.877±0.084	1.891±0.027	1.876±0.027
Relative (to body) Brain weight (%)	0.542±0.023	0.553±0.020	0.572±0.045	0.595±0.022* (↑10)
Females				
Terminal Body weight	190.9±6.2	194.4±7.6	185.9±5.4	180.3±7.9* (↓6)
Absolute Brain weight	1.763±0.054	1.759±0.038	1.764±0.030	1.779±0.026
Relative (to body) Brain weight (%)	0.924±0.040	0.906±0.040	0.950±0.018	0.988±0.035* (↑7)

a Data obtained from Study Report Table OW1K-SUM, pages 382-383 (MRID 45422803); n=6. Numbers presented parenthetically represent percent difference from control (calculated by reviewers).

* Statistically different from the control at $p \leq 0.05$.

3. Neuropathology: No treatment-related histopathological changes were observed. Non-treatment related microscopic findings were observed in the control and 3000 ppm groups, and were limited to the following: (i) hemorrhage, gliosis, and/or degeneration/necrosis in the pons (1/6 control males); (ii) optic nerve degeneration (1/6 control males); (iii) retina degeneration (1/6 control males and 1/6 each control and 3000 ppm females); (iv) a cyst on the lumbar spinal cord (1/6 3000 ppm females vs 0/6 controls); and (v) perivascular eosinophilic hyalin deposits in the optic nerve (1/6 control females).

III. DISCUSSION and CONCLUSIONS

A. INVESTIGATORS' CONCLUSIONS: The investigators concluded that TI-435 induced decreases ($p \leq 0.05$) in food consumption in the 3000 ppm males and females throughout most of the study, and as a result, decreases in body weight were also observed in both sexes at this dose. No evidence of neurotoxicity was observed at any dose tested. The NOAEL was 1000 ppm.

B. REVIEWER COMMENTS: At 3000 ppm, food consumption was decreased ($p \leq 0.05$) in the males during Weeks 1-5 (↓5-17%) and 8-13 (↓5-9%), and in females during Weeks 1-8 (↓6-20%) and 10-13 (↓5-8%). These findings correspond to the decreased overall body weight gains (calculated by reviewers) observed in the 3000 ppm males (↓11%) and females (↓13%). Body weights were decreased compared to controls ($p \leq 0.05$) in the males during Weeks 10-12 (↓4-5%), and in females during Weeks 8 and 13 (↓4% each). No treatment-related effects were observed at ≤1000 ppm.

The LOAEL is 3000 ppm (equivalent to 177.0/200.1 mg/kg [M/F]) based on slightly decreased food consumption, body weights, and body weight gains. The NOAEL is 1000 ppm (equivalent to 60.0/71.0 mg/kg [M/F]).

There was no evidence of neurotoxicity at any dose tested.

The submitted study is classified as **acceptable/guideline** and satisfies the requirements (OPPTS 870.6200b) for a subchronic neurotoxicity screening battery in rats.

C. STUDY DEFICIENCIES: None

DATA FOR ENTRY INTO ISIS

Subchronic Neurotoxicity Study - rats (870.6200b)

PC code	MRID #	Study type	Species	Duration	Route	Dosing method	Dose range mg/kg/day	Doses tested mg/kg/day	NOAEL mg/kg/day	LOAEL mg/kg/day	Endpoint(s)	Comments
044309	45422803 45422825	subchr neurotox	rats	90 days	oral	diet	9.2-200.1	0/0, 9.2/10.6, 60.0/71.0, 177.0/200.1 [M/F]	60.0	177.0	Decr FC, BW, BWG	Toxicity

HED Records Center Series 361 Science Reviews - File R107689 - Page 597 of 721

APPENDIX

Introduction:

This SOP is used with the "second generation" (i.e., Version 2) of the FOB data collection software.
SOP E-200 is the SOP that is used with the original version of this software.

The purpose of the FOB is to use noninvasive procedures to detect gross functional effects in animals
resulting from chemical exposure and to quantify behavioral and neurologic effects. The FOB should be conducted blind (i.e., the observer is not told which treatment the animal has received), using
standardized procedures to minimize observer variability. Variations in the test conditions should be
minimized and not systematically related to treatment.

Study Conduct:

The following procedures that constitute this FOB are based on the battery described by *Moser (1989). Enter the results directly into the SAS FOB Computer Program, or when the FOB Program is
inaccessible, use TOX FORM 286. For detailed instructions on the use of this Program see the "FOB
Data Collection Program User Reference". For reference, the OBSERVER is the person who performs home cage and open field observations, including the approach, touch, auditory and tail pinch responses. The MEASURER performs all measurements, including the pupil assessments and the righting reflex. The RECORDER records results for the observer and the measurer.

Perform the following tests in the order in which they are listed. Remove feeders at least 30 minutes
prior to observation in order to improve visibility for home cage observations and to facilitate removal of animals from the home cage. Document feeder removal in the LOGFIL program of DATATOX or on an appropriate data sheet.

Indicate '-' if normal or not present.

Score intensity of the observation as follows:
1) Slight (barely perceptible or infrequent)
2) Moderate to Severe

Record colors as follows:
C = Clear
W = White
R = Red
Y = Yellow
B = Brown
G = Green

0 = Other (list color)

I. Home Cage Observations - After observation of posture, these are performed with the cage pulled out from the rack for an unobstructed view of the rat.

A. Posture
1) standing normally
2) rearing
3) sitting or lying in a normal position
4) sitting or lying with head in abnormal position (e.g., resting against the floor or wall)
5) lying flattened, limbs may be spread out
6) lying on side and limbs in the air
7) hunched back
8-11) other

B. Piloerection - indicate '+' if present; '-' if normal

C. Involuntary motor movements (score if present)

Clonic
1) repetitive "chewing" movements of mouth and jaw
2) muscle fasciculations
3) tremors
4) myoclonic jerks
5) explosive jumping into the air with all feet leaving the surface
6) convulsions
7) wet dog shakes
8) writhing

Tonic
1) contraction of extensors such that limbs are rigid and extended
2) head and body rigidly arching backward (opisthotonos)
3) head and body rigidly flexed forward (emprosthotonos)
4) convulsions

D. Gait abnormalities (score if present)
1) incoordination; excessive swaying, rocking or lurching
2) hindlimbs dragging, showing exaggerated or overcompensated movements or are splayed
3) hindfeet pointing outward from body (approximately 90°)
4) forelimbs dragging, extended or unable to support weight
5) walking on tiptoes
6) stilted
7) body dragging or flattened against surface
8-10) other

Scores: 1) Slight (barely perceptible or infrequent)
2) Moderate or Severe

E. Vocalizations - indicate '+' if spontaneous (i.e., without stimuli);
'-' if absent

F. Other
1) decreased activity
2) repetitive head bobbing (nutation)
3) increased reactivity
4-8) other

II. Observations During Handling

A. Ease of removing the rat from its cage
1) minimal resistance
2) minimal resistance with vocalizations
3) rearing, often following investigator's hand, with or without vocalizations
4) avoiding investigator's hand, with or without vocalizations
5) attempting to attack investigator's hand, with or without vocalizations

B. Reaction to being handled
1) minimal resistance, without vocalizations
2) minimal resistance, with vocalizations
3) rigid in hand, with or without vocalizations
4) squirming, twisting or attempting to bite, with or without vocalizations

C. Muscle tone (if abnormal)
1) flaccid
2) rigid

D. Palpebral closure (if abnormal)
1) slightly drooped
2) about half -closed
3) completely closed

G. Lacrimation - indicate '+' if present; '-' if none. If present also indicate color and score.

H. Salivation - indicate '+' if present; '-' if none. If present also indicate color and score.

K. Nasal Discharge - indicate '+' if present; '-' if none. If present also indicate color and score.

Scores: 1) Slight (barely perceptible or infrequent)
2) Moderate or Severe

Colors: C = Clear W = White R = Red
Y = Yellow B = Brown G = Green or Other

I. Stains (if present, indicate color and score)
1) lacrimal
2) nasal
3) oral
4) perianal
5) urine

J. Other (if present)
1) alopecia
2) bite marks
3) broken teeth/malocclusion
4) dehydration
5) emaciation
6) exophthalmia
7) missing toe nail(s)
8) cool-to-touch
9-12) other

III. Open Field Observations - The rat is placed in the center of a flat surface with a perimeter barrier, such as a cart, for 2 minutes. During this time, the number of rears is counted and other observations are made.

A. Piloerection - indicate' +' if present; '-' if absent.

B. Respiratory abnormalities (score if present):
1) rapid, shallow
2) labored
3) gasping, mouth breathing
4) audible sound (rales)

C. Posture:
1) standing normally
2) rearing
3) sitting or lying in a normal position
4) sitting or lying with head in abnormal position (e.g., resting against the floor or wall)
5) lying flattened, limbs may be spread out
6) lying on side and limbs in the air
7) hunched back
8-11) other

D. Involuntary motor movements (score if present):
Clonic
1) repetitive "chewing" movements of mouth and jaw
2) muscle fasciculations
3) tremors
4) myoclonic jerks
5) explosive jumping into the air with all feet leaving the surface
6) convulsions
7) wet dog shakes
8) writhing

Scores: 1) Slight (barely perceptible or infrequent)
2) Moderate or Severe

Colors: C = Clear W = White R = Red
Y = Yellow B = Brown G = Green or Other

Tonic
1) contraction of extensors such that limbs are rigid and extended
2) head and body rigidly arching backward (opisthotonos)
3) head and body rigidly flexed forward (emprosthotonos)
4) convulsions

E. Stereotypy (excessive or repetitive behavior; if present):
1) circling
2) head bobbing (nutation)
3) head weaving
4) pacing
5) stereotypic grooming

F. Bizarre behavior (if present):
1) flopping
2) retropulsion
3) self-mutilation
4) tail held erect (>45°)
5) writhing

G. Gait abnormalities (score if present) Note, if the rat did not move during the 2-minute observation period, it may be gently prodded in order to observe the gait.
1) incoordination; excessive swaying, rocking or lurching
2) hindlimbs dragging, showing exaggerated or overcompensated movements or are splayed
3) hindfeet pointing outward from body (approximately 90 degrees)
4) forelimbs dragging, extended or unable to support weight
5) walking on tiptoes
6) stilted
7) body dragging or flattened against surface
8-10) other

H. Vocalizations - indicate '+' if spontaneous (i.e., without stimuli);
'-' if absent.

I. Arousal (level of unprovoked activity and alertness; if abnormal):
1) inactive or unresponsive
2) sluggish, minimal head or body movement
3) sluggish, some exploratory movements
4) tense, agitated, sudden movements or freezing for short periods

5) hyperactive, attacking, bouts of sudden movements or freezing

Scores: 1) Slight (barely perceptible or infrequent)
2) Moderate or Severe

J. Rearing - Defined as each time both front feet come completely off the surface, although the rat does not necessarily have to raise itself up (i.e., this is a measure of the ability of the rat to place its weight on its haunches). Includes when the rat uses the perimeter barrier as support.

K. Other (1-5)

Excretion - at the end of the 2-minute period, measure:

L. Defecation - count the number of boluses. Record '0' if diarrhea is present or 'P' if partially formed.

M. Urination - count the number of pools. If urinating while moving, count dribbles as one pool. Record 'E' if polyuria (excessive volume).

IV. Reflex/Physiologic observations and measurements

Stimulus reactivity - Performed while the rat is sitting on the cart surface following open field observations.

A. Approach response - Approach the rat head-on with the end of a blunt object, such as a pencil, then hold the object approximately 3 cm from the face for approximately 4 seconds.
1) no reaction
2) slight response - e.g., ear flick, rat slowly approaching and sniffing or turning away
3) more energetic response than 2)
4) rat freezing and/or having muscle contractions
5) exaggerated reaction - jumping, biting or attacking

B. Touch response - Coming in from the side, touch rump gently with a blunt object.
1) no reaction
2) slight response-e.g., earflick, turning or walking away, with or without vocalization
3) more energetic response than 2)
4) rat freezing and/or having muscle contractions
5) exaggerated reaction - jumping, biting or attacking

C. Auditory response - Position clicker approximately 5 cm above the back of the rat and make a sudden sound.
1) no reaction
2) slight reaction, some evidence that the noise was heard
3) more energetic response than 2) .
4) rat freezing and/or having muscle contractions

5) exaggerated reaction - jumping, biting or attacking

D. Tail pinch response - metal forceps are used to squeeze the tail approximately 2-3 cm from the tip.

1) no reaction
2) slight response-e.g., earflick, turning or walking away with or without vocalization
3) more energetic response than 2)
4) rat freezing and/or having muscle contractions
5) exaggerated reaction - jumping, biting or attacking

E. Pupil size - Assess both eyes under standard lighting conditions.

1) constricted (pin-point) - Normal under these conditions.
2) dilated
(Enter 'NP' if not performed.)

F. Pupil response - Assess both eyes after adaptation in a darkened enclosure. Assess the reaction of both pupils to dark adaptation and the reaction of both pupils to the beam of a penlight as it is brought in from the side of the rat's head.

1) Pupils are dilated and then constrict in response to the penlight.
2) Pupils are dilated and show no response to the penlight.
3) Pupils are constricted (pin-point).
4) Other
(Enter 'NP' if not performed.)

E. Righting reflex - The rat is held supine, then dropped from approximately 30 cm. If the rat is severely affected, this test may not be performed.

1) normal, rat landing on feet or sternal position
2) slightly uncoordinated
3) landing on side
4) landing on back
5) test not performed

F. Grip strength - Conduct the test according to SOP E-201.
(Enter 'NP' if not performed.)

H. Body weight (g)
(Enter 'NP' if not performed.)

I. Body temperature - The colonic temperature (DC) is taken with a thermistor allowed to stabilize
before reading. Refer to SOP E-202 for details regarding thermistor use and maintenance.
(Enter 'NP' if not performed.)

G. Landing foot splay - The fourth (outermost) foot pad of each hind foot is dotted with water-based

ink. The rat is dropped three times from a prone position, approximately 30 cm onto paper and the ink spots where the feet land for each trial are marked. The distance (mm) between the middle of the ink spots for each trial are measured. A tolerance of +2 mm in measurement is considered acceptable. This test may not be performed due to condition of animal.
(Enter 'NP' if not performed.)

Enter free form text for any additional observations or explanations as needed.

DATA EVALUATION RECORD

CLOTHIANIDIN/044309

STUDY TYPE: METABOLISM AND PHARMACOKINETICS - RAT
[OPPTS: 870.7485 (§85-1)]
MRID 45422805 (main study), 45422806

Prepared for

Health Effects Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
1921 Jefferson Davis Highway
Arlington, VA 22202

Prepared by

Toxicology and Hasard Assessment Group
Life Sciences Division
Oak Ridge National Laboratory
Oak Ridge, TN 37831
Task Order No. 02-44

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DATA EVALUATION RECORD
TXR#: 0050321

STUDY TYPE: Metabolism and Pharmacokinetics- Rat; [OPPTS 870.7485 (§85-1)]; OECD 417.

PC CODE: 044309

DP BARCODE: D282578
SUBMISSION NO.: S614462

TEST MATERIAL (PURITY): Clothianidin [TI 435] (99.8%)

SYNONYMS: [nitroimino-^{14}C]-TI-435; [thiazolyl-2- ^{14}C]-TI435; (E)-1-(2-chloro-1,3-thiazol-5-ylmethyl)-3-methyl-2-nitroguanidine; clothianidin

CITATION: Weber, E. (2000). [Nitroimino-^{14}C]- and [thiazolyl-2-^{14}C]-TI-435 toxicokinetic behavior and metabolism in the rat including whole body autoradiography. Report No. MR 348/00, Bayer AG, Agrochemical Division, Development Department, Institute for Metabolism Research and Residue Analysis, D-51368 Leverkusen-Bayerwerk, Federal Republic of Germany. October 11, 2000. Study No. MO1819065. MRID 45422805, Unpublished.

Ohta, K. and K. Mikata. (2000). Determination of metabolites of TI-435 in major tissues of rats. Takeda Chemical Industries, Ltd., Agricultural Research Laboratories, T2-10, Nihonbashi 2-chome, Chuoku, Tokyo 103-8668, Japan. December 20, 2000. Report No. 110286. MRID 45422806, Unpublished.

SPONSOR: Takeda Chemical Industries, Ltd., Agro Company, Tokyo, Japan (MRID 45422805).

Bayer Corporation, Agriculture Division, Research and Development Department, P.O. Box 4913, Kansas City, MO 64120-0013 (MRID 45422806).

EXECUTIVE SUMMARY: In a study conducted to examine the metabolism and disposition of TI-435 (clothianidin), male and female Sprague-Dawley rats were given a single 2.5 or 250 mg/kg bw oral dose, or a 14-day repeated dose (25 mg/kg/day). Reported in MRID 45422805, groups of male and female rats were subjected to the dosing regimens above using [nitroimino-^{14}C]-TI435 (lot nos. 11553/11 and 11553/17; >99% radiochemical purity; >98% chemical purity) or [thiazolyl-2-^{14}C]-TI-435 (lot no. 11649/2, >99% radiochemical purity, >99% chemical purity) and nonlabeled test article (batch no. M00157, 99.8% chemical purity). Excretion, tissue distribution, pharmacokinetic parameters, and metabolite profiles were determined. In MRID 45422806 metabolite profiles in major tissues were assessed in male Wistar rats given a single 5 mg/kg bw dose of [nitroimino-^{14}C]-TI-435 (96.7% radiochemical purity).

593

There were no biologically significant treatment-related effects noted during the course of the study. Overall recovery of administered radioactivity was an acceptable 95-100%. TI-435 was readily absorbed and excreted within 96 hours following a single 2.5 mg/kg bw or repeated oral dose of 25 mg/kg bw, but at a dose of 250 mg/kg, absorption became biphasic and was saturated. Following single or multiple oral low doses (2.5 and 25mg/kg bw, respectively) of TI-435, urinary excretion accounted for 89.2-94.6% of the administered radioactivity suggesting that a multiple exposure regimen did not affect the absorption/excretion processes. Urinary excretion was unaffected following a single 250 mg/kg dose. Excretion via the feces accounted for the remainder of the administered radioactivity in all treatment groups (3.8-8.6%). Quantitative whole body radioautography and time-course plasma studies revealed rapid absorption and distribution of administered radioactivity to all organs and tissues followed by rapid excretion with reduction to background levels in most tissues and organs within 24 hours. Gender-related differences in plasma kinetics were indicated by the somewhat greater rate of absorption and elimination in females. Excretory patterns did not exhibit gender-related variability but reflected the delayed absorption in the high-dose group. Because tissue burdens were very low, neither TI-435 nor its metabolites appear to undergo significant sequestration.

Both urinary and fecal metabolites were quantified by HPLC, TLC and most were identified using HPLC and TLC and HPLC/MS in conjunction with known standards. The metabolites identified (primarily oxidative demethylation products and cleavage products of the nitrogen-carbon bond between the nitroimino and thiazolyl moieties) were consistent with Phase I processes. Extraction efficiencies appeared to be excellent and most components in all of the matrices examined (urine, feces, and tissues) were adequately quantified and characterized. The available data, based upon studies using both the nitroimino- and the thiazolyl-2-labeled TI-435, affirmed the metabolism pathway proposed by the investigators.

These metabolism studies (MRID 45422805 and 45422806) are, collectively. **Acceptable/ Guideline** and satisfy the requirements for a Metabolism and Pharmacokinetics Study [OPPTS 870.7485 (§85-1)]. The studies were properly designed, conducted and reported.

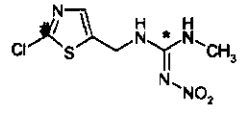
COMPLIANCE: Signed GLP, Quality Assurance, and Confidentiality Claim statements were provided in all of the study reports.

I. MATERIALS AND METHODS:

A. MATERIALS:

1. Test compound:

Radiolabeled test material:	[Nitroimino-^{14}C]-TI-435 [Thiazolyl-2-^{14}C]-TI-435
Radiochemical purity	>99% >99%
Chemical purity	>98% >99%
Specific Activity	3.78 MBq/mg 3.84 MBq/mg
Lot/Batch #:	11553/11 and 11553/17 11649/2
Non-radiolabelled test material:	TI-435
Description:	Colorless powder
Lot/Batch #:	M00157
Purity:	99.8%
Contaminants:	Not reported
CAS # of TGAI:	210880-92-5
Structure:	



2. Vehicle and/or positive control:

For the absorption, distribution, metabolite profile, and excretion experiments, a mixture of labeled and unlabeled TI-435 mixture was suspended in 0.5% aqueous tragacanth and homogenized (MRID 45422805). The dosing solution for the experiment to determine the metabolite profile of major tissues was prepared by dissolving labeled and unlabeled test material in dimethylformamide (5% of dose volume) and suspending in it corn oil (MRID 45422806).

3. Test animals:

Species:	Rat (male and female); MRID 45422805 Rat (male); MRID 45422806
Strain:	Sprague-Dawley Crl: CD BR Wistar
Age/weight at study initiation:	7-8 weeks (males and females); 196-228 g (males), 207-213 g (females) 7 weeks; ~210 g
Source:	Charles River, 97633 Sulzfeld, Germany CLEA Japan, Inc.
Housing:	Housed individually in plastic cages during acclimation and in Makrolon® metabolism cages during experiment (MRID 45422805). Housing not described for MRID 45422806.
Diet:	Altromin 1324 standard rat diet (Fa. Altrogge, D-32791 Lage/Lippe, Germany); *ad libitum* (MRID 45422805). Diet for MRID 45422806 not described; assumed *ad libitum.*
Water:	*Ad libitum* *Ad libitum*

595

Environmental conditions:	**Temperature:**	18-25°C; Not described for MRID 45422806
	Humidity:	27-65%; Not described for MRID 45422806
	Air changes:	10-15/hr; Not described for MRID 45422806
	Photoperiod:	12 hr/12 hr; Not described for MRID 45422806
Acclimation period:	A quarantine period of seven days was specified for both studies	

4. **Preparation of dosing solutions:** For the absorption, distribution, metabolism, and excretion experiment (MRID 45422805), the dose material was prepared one day prior to administration. The radiolabeled material, dissolved in acetonitrile was mixed with dry, non-labeled test article. The solution was dried under nitrogen, suspended in 0.5% aqueous tragacanth and homogenized by ultrasonication at ~70°C. Dosing solutions were stirred overnight at room temperature and heated to ~70°C for about 10 minutes prior to administration. Stability was confirmed by HPLC. For metabolite profile determination in major tissues (MRID 45422806), [nitroimino- ^{14}C]-TI-435 was diluted with unlabeled test material, dissolved in dimethylformamide (5% of dose volume) and suspended in corn oil (Wako Pure Chemicals Industries, Ltd.).

B. STUDY DESIGN AND METHODS:

1. **Group arrangements:** Animals were assigned to the experimental groups based upon body weights. The groups were established to minimize standard deviation in group mean body weight for each group.

TABLE 1. Experimental design: metabolism and disposition of [nitroimino - ^{14}C]- and [thiazolyl-2-^{14}C]-TI-435 in rats

Exp. No.	Dose (mg/kg)	Number/ sex	Remarks
1 Pilot test	2.5	4♂	Analysis of expired air, urine, feces, and carcass at terminal sacrifice
2	2.5 2.5	4♂ 4♀	Absorption, distribution, metabolism study; microplasma collection at 5, 10, 20, and 40 min. and at 1, 1.5, 2, 3, 4, 6, 8, 24, 48, and 72 hrs. Tissues at terminal sacrifice.
3	250	4♂	Absorption, distribution, metabolism study; microplasma collection at 5,10, 20, and 40 min. and at 1, 1.5, 2, 3, 4, 6, 8, 24, 48, and 72 hrs. Tissues at terminal sacrifice.
4 (repeat oral dose)	25	4♂	14-day non-labeled TI-435 followed by single dose of [nitroimino-^{14}C]-TI-435 on Day 15; terminal sacrifice at 72hrs.
5 (autoradiography)	5	6♂	Whole body autoradiography at 1, 4, 8, 24, 48, and 72 hrs.
6 (thiazolyl label)	2.5	4♂	Absorption, distribution, metabolism study; microplasma collection at 5,10, 20, and 40 min. and at 1, 1.5, 2, 3, 4, 6, 8, 24, 48, and 72 hrs. Tissues at terminal sacrifice.
7 (thiazolyl label)	25	4♂	Absorption, excretion, and additional metabolite study
8	5	3♂	Determination of metabolic profiles in blood, heart, kidney, liver, lung and spleen at 4 hours (MRID 45422806)

Information taken from pp. 7, 11, 12, 16, 17, 47, 53, and 54 MRID 45422805 and p. 4, MRID 45422806.

2. **Dosing and sample collection:**

Expired air: Expired air from the pilot experiment group was collected at intervals of 0-4, 4-8, 8-24, 24-48, and 48-72 hours in traps (ethanolamine/ethanol 1:1 for trapping of carbon dioxide and organic volatiles). The flow rate of air through the metabolism cages was ~3000 mL/min.

596

Urine/cage wash: Urine was collected from rats in the pilot test and Groups 2, 3, 4, and 6 at 4, 8, 24, 48, and 72 hours after dosing. Urine was collected over dry ice and washings were combined with respective urine samples. Cage washes per se were not performed.

Feces: Feces were collected from rats in the pilot test and Groups 2, 3, 4, and 6 at 24-hour intervals to termination. Fecal samples were lyophilized, weighed and homogenized.

Tissue/organs: The following organs/tissues were collected from rats in Groups 2, 3, and 6 at terminal sacrifice: brain, heart, liver, kidneys, lungs, spleen, muscle (site not specified), gastrointestinal tract, testes, bone (femur), skin, and carcass. Blood samples were collected at 5, 10, 20, and 40 minutes and at 1, 1.5, 2, 3, 4, 6, 8, 24, 48, and 72 hours. Blood samples were separated into erythrocytes and microplasma fractions by centrifugation. For the repetitive dose study (Group 4), the previously noted tissues and blood samples were collected following the Day-15 dose of radiolabeled TI-435. Whole-body autoradiography (radioluminography) was performed on animals of Group 5 at 1, 4, 8, 24, 48, and 72 hours following dosing. Animals in Group 8 were sacrificed 4 hours after administration of labeled test material and blood, heart, kidney, liver, lung and spleen were collected for determination of metabolic profiles. For Group 1, only the carcass was saved for radioassay. The skin, gastrointestinal tract (GIT), residual carcass, organs, and tissues taken from Groups 2, 3, and 6 at sacrifice were weighed, lyophilized, and weighed again; then homogenized and aliquots were taken for radioanalysis by combustion. Small organs and tissues (adrenals, thyroid, ovaries, renal fat and uterus) were solubilized for liquid scintillation counting using a Beckman BTS-450 tissue solubilizer.

3. **Analytical techniques:**

Liquid scintillation counting (LSC): LSC was conducted using a Philips PW 4700, Beckman LS 6000LL, or LKB/Wallac 1210 Spectral counter with external standardization. Liquid samples were added to scintillant and counted directly. Combusted samples were collected in Carbosorb and aliquots added to scintillant for radioassay. Fecal samples were homogenized in distilled water, solubilized, and decolorized prior to mixing with scintillant for counting. As required, tissue samples were minced, homogenized, solubilized and decolorized prior to mixing with scintillant.

High performance liquid chromatography (HPLC): HPLC was performed with a Hewlett Packard 1050 chromatograph using several column/solvent systems that were adequately described in the study report. Aliquots of pooled urine samples were directly injected for HPLC co-chromatography analysis without further treatment. Pooled feces from Groups 1 and 6 were extracted with methanol or methanol/water (1:1) and partitioned into hexane. Aliquots of the extracts were injected for HPLC co-chromatography. Wet residues, separated by centrifugation, were combusted and assayed by LSC and added to values obtained from extracts for materials balance of the radioactivity recovered in feces.

Thin-layer chromatography (TLC): TLC was performed on amino plates and silica gel plates to separate polar fractions of metabolites following pre-fractionation with HPLC. Amino plates were developed two-dimensionally using acetonitrile/water/trifluoro acetic acid; 90:8:2 (v/v/v) in the first direction and acetonitrile in the second. Silica gel plates were

developed in the first direction using trichloromethane/methanol/ammonia (5%)/water; 80:60:6:2 (v/v/v/v) and n-butanol/acetic acid/water/acetone; 60:15:15:10 (v/v/v/v) in the second direction. Tissues and organs from Group 8 animals were extracted with methanol and/or 50% methanol and two dimensional TLC co-chromatography was performed on silica gel (60 F_{254}) plates. Chromatograms were developed in the first direction with chloroform/methanol (8:1, v/v) or chloroform/methanol (7:3, v/v) and in the second direction using chloroform/acetone (1:1, v/v) or chloroform/acetone/methanol/formic acid (2:2:1:1, v/v/v/v). Radioactivity in separated spots was scraped from the developed plates and counted by LSC.

Mass spectroscopy (MS): As required, various metabolites were identified by mass spectroscopy by comparison to spectra of authentic standards using a TSQ 7000 Finnigan mass spectrograph. Radioactivity in fractions were detected by HPLC/MS

Whole-body autoradiography (Radioluminography: Animals from Group 5 were analyzed for absorbed radioactivity in organs and tissues by quantitative whole-body autoradiography at 1, 4, 8, 24, 48, and 72 hours post-dosing with [nitroimino-^{14}C]-TI-435. After sacrifice, animals were fixed in position using a template, frozen and imbedded in carboxymethylcellulose. Sections (50 µm) were lyophilized and exposed to imaging plates. Exposed plates were scanned using a Fuji BAS 5000 image analyzer with color printout. Background colors were established by performing the procedure on one rat orally administered non-labeled test material and sacrificed after 4 hours. Digital images of the autoradiograms were quantitatively assessed for distribution of radioactivity in various organs/tissues. Proper allowances were made for shrinkage, self absorption, tissue thickness, density, and water loss.

4. **Statistics**: Data were listed and calculations performed using a dedicated software program (ISOLAB) generated for the study protocol. Outliers were removed prior to listing of values using the outlier test by Nalimov and not used in calculation of means and coefficients of variations.. Statistical analysis was not performed on data for Group 8 (MRID 45422806).

II. RESULTS:

Preliminary experiment: Results of the preliminary experiments showed that radioactivity in expired air over a 24-hour period was 0.02% of the administered dose. Therefore, expired air was not collected in subsequent experiments. There were no deaths or morbidity observed for rats in the preliminary experiments.

A. **PHARMACOKINETIC STUDIES:** For experiments in the main study (single and repeat doses), recovery of radioactivity administered as [nitroimino-^{14}C]-TI-435 was an acceptable 95.6-99.8%. A general accounting of recovered radioactivity following dosing with [nitroimino-^{14}C]-TI-435 or [thiazolyl-2-^{14}C]-TI-435 is summarized in Table 2. Radioactivity inventory following dosing with [thiazolyl-2-^{14}C]-TI-435 also exhibited acceptable overall recovery (97.6%).

TABLE 2. Recovery of radioactivity (% of administered dose) from rats at 72 hours following oral administration of [nitroimino-^{14}C]- or [thiazolyl-2-^{14}C]-TI-435

	Dose group					
	2.5 mg/kg [nitroimino-^{14}C]-TI-435	2.5 mg/kg [nitroimino-^{14}C]-TI-435	2.5 mg/kg [nitroimino-^{14}C]-TI-435	250 mg/kg [nitroimino-^{14}C]-TI-435	25 mg/kg [nitroimino-^{14}C]-TI-435	2.5 mg/kg [thiazolyl-2-^{14}C]-TI-435
	Single dose males	Single dose males	Single dose females	Single dose males	Repeat dose males	Single dose males
Expired air	0.02	–	–	–	–	–
Tissues/organs	0.12	0.12	0.09	0.16	0.09	0.25
GI tract	0.02	0.01	0.01	0.02	–	0.07
Carcass	0.13	0.11	0.08	0.16	0.10	0.25
Urine	91.48	89.10	94.56	90.47	92.99	89.25
Feces	6.89	6.27	3.29	8.58	6.60	7.79
Total	98.66	95.61	98.03	99.39	99.78	97.61

Data obtained from Table 4, p. 50, MRID 45422805.

Absorption/excretion/kinetics:

Absorption: Absorption can be implied from urine, carcass, and tissue burden data collected in the absorption/distribution/metabolism/excretion (ADME) study (MRID 45422805). Based on urinary excretion data, absorption following a single low dose or single high dose, respectively, was approximately 89.1-94.6% and 90.5%. Low-dose female rats exhibited somewhat greater absorption (94.6%) than did male rats (89.1-91.5%) but rates of absorption were similar. Results from the 14-day repetitive dose study (93.0%) showed an excretion profile similar to that of the single low-dose or single high-dose groups and therefore, similar implied absorption. Time-course data for plasma radioactivity indicated rapid absorption as shown by T_{max} values of 1.4-2.1 hours for the low dose rats and 2.7 hours for rats in repeat dose group. Plasma C_{max} values were approximately 10-fold greater and proportional in the repeat-dose group (25 mg/kg bw) than in the low dose (2.5 mg/kg bw) groups. Short to very short half lives of absorption (0.004-.05 hours) were calculated in the pharmacokinetic model. There did not appear to be a difference in absorption of the [nitroimino-^{14}C]-labeled test material compared to the [thiazolyl-2-^{14}C]-labeled test compound.

Excretion: Time-course excretion data are summarized in Tables 3 and 4. Data generated by the ADME study (MRID 45422805) revealed that most of the administered dose is excreted in the urine; 89.1-91.5% for the low dose-group and 90.5% for the high-dose group. Greater than 90% (94.5-99.0%) of the urinary excretion of administered radioactivity occurred within 24 hours for the low-dose rats and 98.2% within 48 hours for the high-dose rats. Fecal excretion of the absorbed dose is relatively rapid and essentially complete within 24 hours for low-dose rats and 48 hours for high dose rats. There were no biologically relevant gender-related quantitative difference in excretion profiles. Excretion time-course was somewhat more prolonged in the high-dose group relative to the low-dose group. Time-courses for urinary and fecal excretion in the repetitive low-dose group were similar to those of the single low-dose group. Similar to the preceding experiment, excretion of radioactivity following administration of [thiazolyl-2-^{14}C]- TI-435 was primarily via the urine (89.2% over 72 hours)

599

with the remainder (7.8%) excreted in the feces. Greater than 97% of the excretion following a single dose of the thiazolyl-2-labeled test article occurred within 24 hours of dosing.

TABLE 3. Time-course of urinary excretion (% of administered dose) in rats orally administered [nitroimino-^{14}C]- or [thiazolyl-2-^{14}C]-TI-435

Time (hrs)	Dose group					
	2.5 mg/kg [nitroimino-^{14}C]-TI-435	2.5 mg/kg [nitroimino-^{14}C]-TI-435	2.5 mg/kg [nitroimino-^{14}C]-TI-435	250 mg/kg [nitroimino-^{14}C]-TI-435	25 mg/kg [nitroimino-^{14}C]-TI-435	2.5 mg/kg [thiazolyl-2-^{14}C]-TI-435
	Single dose males	Single dose males	Single dose females	Single dose males	Repeat dose males	Single dose males
0-4[a]	27.12	34.90	43.09	3.01	16.32	32.65
4-8	26.85	36.72	35.11	18.30	40.30	33.82
8-24	36.63	16.59	15.12	35.86	34.71	21.38
24-48	0.64	0.59	0.89	31.69	1.24	1.03
48-72	0.24	0.30	0.35	1.61	0.42	0.37
Total	91.48	89.10	94.56	90.47	92.99	89.25

Data taken from Table 2, p. 48, MRID 45422805.
[a]Interval recovery values calculated from cumulative recovery values by reviewer.

TABLE 4. Time-course of fecal excretion (% of administered dose) in rats orally administered [nitroimino-^{14}C]- or [thiazolyl-2-^{14}C]-TI-435

Time (hrs)	Dose group					
	2.5 mg/kg [nitroimino-^{14}C]-TI-435	2.5 mg/kg [nitroimino-^{14}C]-TI-435	2.5 mg/kg [nitroimino-^{14}C]-TI-435	250 mg/kg [nitroimino-^{14}C]-TI-435	25 mg/kg [nitroimino-^{14}C]-TI-435	2.5 mg/kg [thiazolyl-2-^{14}C]-TI-435
	Single dose males	Single dose males	Single dose females	Single dose males	Repeat dose males	Single dose males
0-24[a]	6.56	5.99	2.90	3.49	6.18	7.02
24-48	0.24	0.19	0.30	4.33	0.17	0.32
48-72	0.09	0.09	0.09	0.76	0.25	0.45
Total	6.89	6.27	3.29	8.58	6.60	7.79

Data taken from Table 2, p. 48, MRID 45422805.
[a]Interval recovery values calculated from cumulative recovery values by reviewer.

Plasma pharmacokinetics: The time-course data for plasma radioactivity in rats following single oral doses at 2.5 mg/kg bw, a single oral dose at 250 mg/kg bw, or repeat oral doses of 25 mg/kg bw of TI-435 are shown in Table 5 and kinetic indices are shown in Table 6. Following peak plasma concentration at 2 hours for the low-dose group, 4 hours for the repeat mid-dose group, and 8 hours for the high-dose group, radioactivity is rapidly eliminated from the plasma compartment. However, the broad range of high concentration of radioactivity observed between 1 and 32 hours post-dosing and two peaks in the plasma curve at 6 and 32 hours, indicates a biphasic absorption for high-dose rats. Gender-related differences in plasma kinetics suggested a somewhat greater rate of absorption and plasma clearance by female rats. Plasma pharmacokinetic parameters for the repetitive low-dose group were similar to those of the single low-dose group although AUC was expectedly greater.

TABLE 5. Mean plasma radioactivity (expressed as µg eq/g) in rats following oral administration of [nitroimino-^{14}C]- or [thiazolyl-2-^{14}C]-TI-435

Time (hrs)	Dose group				
	2.5 mg/kg [nitroimino-^{14}C]-TI-435	2.5 mg/kg [nitroimino-^{14}C]-TI-435	250 mg/kg [nitroimino-^{14}C]-TI-435	25 mg/kg [nitroimino-^{14}C]-TI-435	2.5 mg/kg [thiazolyl-2-^{14}C]-TI-435
	Single dose *males*	Single dose *females*	Single dose *females*	Repeat dose *males*	Single dose *males*
0.08	0.129 (0.046)[a]	0.101 (0.039)	3.105 (0.012)	1.031 (0.043)	0.099 (0.040)
0.16	0.363 (0.128)	0.356 (0.137)	9.082 (0.036)	2.921 (0.121)	0.370 (0.148)
0.33	0.870 (0.308)	0.679 (0.261)	18.020 (0.072)	5.088 (0.211)	0.536 (0.216)
0.66	1.470 (0.520)	1.100 (0.422)	33.340 (0.133)	8.137 (0.338)	0.860 (0.346)
1	1.742 (0.616)	1.225 (0.469)	45.580 (0.181)	10.710 (0.444)	1.133 (0.456)
1.5	1.829 (0.647)	1.311 (0.503)	57.510 (0.228)	12.372 (0.513)	1.262 (0.508)
2	1.716 (0.607)	1.283 (0.492)	63.820 (0.253)	12.555 (0.521)	1.255 (0.505)
3	1.411 (0.499)	1.022 (0.392)	71.340 (0.282)	14.350 (0.595)	1.133 (0.456)
4	1.143 (0.404)	0.728 (0.279)	74.340 (0.294)	15.115 (0.626)	1.024 (0.412)
6	0.628 (0.222)	0.431 (0.166)	79.490 0.314)	10.995 (0.455)	0.707 (0.285)
8	0.358 (0.127)	0.247 (0.095)	78.470 (0.310)	7.325 (0.304)	0.446 (0.179)
24	0.014 (0.005)	0.012 (0.005)	23.870 (0.095)	0.122 (0.005)	0.028 (0.011)
32	0.006 (0.002)	0.012 (0.005)	47.810 (0.190)	0.065 (0.003)	0.019 (0.008)
48	0.005 (0.002)	0.007 (0.003)	1.483 (0.006)	0.042 (0.002)	0.013 (0.005)
56	0.006 (0.002)	0.006 (0.002)	0.623 (0.003)	0.039 0.002)	0.014 (0.006)
72	0.007 (0.003)	0.005 (0.002)	0.360 (0.001)	0.029 (0.001)	0.010 (0.004)

Data obtained from Tables 6, p. 52, MRID 45422805.
[a]( ) = Dose normalized concentration.

TABLE 6. Plasma pharmacokinetic parameters for orally administered [nitroimino-^{14}C]- or [thiazolyl-2-^{14}C]-TI-435					
Dose (mg/kg)	C_{max} (µg eq./g)	T_{max} (hrs)	$t_{1/2}$ (hrs)	Renal excretion	AUC (µg eq–hr/g)
[Nitroimino-^{14}C]-TI-435					
2.5 mg/kg single oral Male Female	 1.82 1.29	 1.50 1.35	 0.513 0.270	 89.10 94.56	 10.3 7.28
25 mg/kg repeat oral Male	 15.0	 2.70	 0.0220	 92.99	 116.0
[Thiazolyl-2-^{14}C]-TI-435					
2.5 mg/kg, single oral Male	 1.27	 2.08	 0.00364	 89.25	 10.2

Data taken from Table 7, p. 53, MRID 45422805.

B. TISSUE DISTRIBUTION: The distribution of the administered radioactivity from the gastrointestinal tract into various tissues and organs, following oral administration, was followed by time-course plasma studies and quantitative whole body autoradiography. Within one hour after administration of 5 mg/kg bw [nitroimino-^{14}C]-TI-435, the radioactivity was rapidly distributed to all organs and tissues as shown in Table 7. The radioactivity appears to rapidly dissipate after 4 hours, has nearly dissipated from all tissues by 8 hours and has reached background concentrations in most tissues and organs at 24 hours post-treatment. The autoradiogram for the rat sacrificed one hour after dosing indicated highest concentrations of radioactivity in the stomach, urinary bladder, kidney, liver and blood, indicating rapid absorption and distribution of label and serves as an early indicator of renal excretion. The nasal mucosa also exhibited an affinity for the radiolabel at one hour. At 4 hours, the distribution pattern was similar to that at 1 hr except for increased concentration of radioactivity in the intestinal contents indicating initiation of fecal excretion. Radioactivity also increased in the eye (vitreal body) at this time. At 8 hrs the distribution pattern was similar to that at 4 hours except ~55% of the radioactivity had been excreted at this time. At 24 hrs, ~98% of excretion had occurred with most of the remaining radioactivity concentrated in the intestinal tract and feces as well as the nasal mucosa, the vitreous body of the eye, the liver and kidneys. At 48 and 72 hours radioactivity levels were very low in all tissues and organs with detectable radioactivity remaining only in the kidney, adrenal, liver, parts of the intestine, feces, nasal mucosa and the eye. There was no evidence of significant bioaccumulation in any tissue or organ at 72 hours post-dosing.

TABLE 7. Total radioactive residue (µg/g) in organs and tissues in male rats following oral administration of 5 mg/kg [nitroimino-^{14C}]-TI-435 as determined by quantitative whole-body-autoradiography

Organ/Tissue	Total radioactive residues in organs and tissues (µg/g)					
	1 hr	4 hr	8 hr	24 hr	48 hr	72 hr
Adrenal	2.943	1.925	0.641	0.025	0.010	0.010
Blood	3.000	1.140	0.376	0.010	0.004	0.005
Bone marrow	0.908	0.687	0.240	0.004	0.001	0.001
Brain	0.338	0.250	0.116	0.002	0.000	0.000
Brown fat	1.854	1.330	0.331	0.018	0.004	0.004
Eye (vitreal body)	0.009	0.550	0.324	0.112	0.048	0.020
Heart	1.993	1.174	0.428	0.007	0.002	0.001
Kidney (renal cortex)	4.075	2.472	0.778	0.020	0.009	0.008
Kidney (renal medula)	2.892	4.389	0.870	0.019	0.004	0.002
Kidney (whole)	3.721	3.587	0.864	0.023	0.008	0.006
Liver	3.424	2.229	0.821	0.065	0.026	0.021
Lung	0.492	0.148	0.215	0.006	0.001	0.001
Muscle	1.877	1.132	0.392	0.006	0.001	0.001
Nasal mucosa	5.641	3.816	4.306	0.072	0.040	0.022
Pituitary gland	1.878	1.082	0.354	0.006	–	–
Pineal body	1.348	0.878	0.318	0.007	–	–
Salivary gland	2.215	1.497	0.544	0.008	0.002	0.002
Skin	2.239	1.337	0.556	0.013	0.004	0.003
Spinal marrow	0.276	0.205	0.105	0.002	0.000	0.000
Spleen	1.532	0.959	0.316	0.006	0.002	0.001
Testes	0.656	0.533	0.211	0.004	0.001	0.000
Thymus	1.370	0.861	0.282	0.004	0.001	0.001
Thyroid	2.340	1.531	0.472	0.006	0.003	0.001
Tongue	2.231	1.333	0.398	0.007	0.002	0.001
Urinary bladder	5.998	6.169	5.987	–	0.018	0.002

Data obtained from Table 8, p. 54, MRID 45422805.

C. METABOLITE CHARACTERIZATION STUDIES:

Based upon HPLC and TLC analyses with known standards, most of the metabolite fractions were identified as follows. Those metabolites found at ≥ 1% of the dose administered are denoted by asterisks:

NTG*	Nitroquanidine
MNG*	Methylnitroguanidine
MG	Methylguanidine
TZG	BN0215F (isolated and identified in separate study)
TMG*	Thiazolylmethylguanidine
TZU	Thiazolylurea
THMN	NI-UK-4
TZMU	Thiazolylmethylurea
TZNG*	Thiazolylnitroguanidine
ACT*	5-Aminomethyl-2-chlorothiazole
CTCA	2-Chlorotiazole-5-carboxylic acid
MTCA*	2-Methylthiothiazole-5-carboxylic acid
Urea	Urea
TI-435*	Parent; (E)-1-(2-chloro-1,3-thiazol-5-ylmethyl)-3-methyl-2-nitroguanidine

Urine: Eleven radioactive components (including an unidentified fraction and the parent compound) were detected in the urine of rats receiving [nitroimido-^{14}C]-TI-435, although six were detected at trace levels or at levels representing no greater than 1.0% of the administered dose (Table 8). Parent compound was the most prevalent component in any dose group 54.6-73.5% of administered) and for both males and females. Other major metabolites found in composite urine samples were TZNG (7-12.5%), MNG (7.8-13.2%), and NTG (1.4-3.9% of administered). Females tended to exhibit less overall metabolism than males and less of each metabolite was present, however no qualitative differences were noted. Only minor quantitative differences were noted for the high dose group indicating that enzymatic sites of metabolism probably were not saturated at the high dose. No qualitative or quantitative differences of biological importance were observed for the repetitive dose group compared to the other groups of male rats.

TABLE 8. Metabolite profile (% of administered radioactivity) in urine of rats following oral dosing with [nitroimino-^{14}C]-TI-435 or [thiazolyl-5-^{14}C]-TI-435

Metabolite	Dose group				
	2.5 mg/kg [nitroimino-^{14}C]-TI-435	2.5 mg/kg [nitroimino-^{14}C]-TI-435	250 mg/kg [nitroimino-^{14}C]-TI-435	25 mg/kg [nitroimino-^{14}C]-TI-435	2.5 mg/kg [thiazolyl-2-^{14}C]-TI-435
	Single dose males	Single dose females	Single dose males	Repeat dose males	Single dose males
	0-24 hrs	0-24hrs	0-48 hrs	0-24 hrs	0-24 hrs
TI-435 (parent)	54.65	73.49	59.98	66.53	59.83
ACT	ND	ND	ND	ND	1.02
CTCA	ND	ND	ND	ND	0.89
MTCA	ND	ND	ND	ND	8.52
Urea	0.25	0.13	0.06	0.09	ND
NTG	3.92	1.42	3.49	1.92	ND
MNG	13.21	7.75	9.46	8.78	ND
MG	0.45	0.30	0.30	0.24	ND
TMG	0.11	0.13	0.19	0.16	0.20
TZU	0.52	0.49	0.64	0.67	0.21
THMN	0.11	0.12	0.09	0.17	ND
TZMU	0.16	0.10	0.29	0.06	0.19
TZNG	11.25	7.01	12.48	10.18	10.44
Unidentified	3.58	2.38	1.86	2.48	6.55
Total	88.20	93.32	88.85	91.30	87.85

Data from Tables 10-14, pp. 56-60, MRID 45422805.
ND: Not detected.

The urinary metabolite profile for rats given [thiazolyl-2-^{14}C]-TI-435 included nine metabolites, five of which were considered minor metabolites (~1% or less of administered radioactivity). Major metabolites detected were parent compound (59.8%), TZNG (10.4%), and MTCA (8.52%). MTCA, ACT, and CTCA were not found in urine of rats administered the [nitroimino^{14}C] labeled test material whereas urea, NTG, MNG, MG, and THMN were not found in urine of rats administered the [thiazolyl-2-^{14}C] labeled test material.

Feces: Nine components (including parent, a polar fraction, and unidentified components) were detected in fecal samples from rats given a single oral dose of [nitroimino-^{14}C]-TI-435 (Table 9). Parent compound was not the prevalent component extracted from feces (0.5-0.9% of administered) and fecal metabolites account for only 2.1-4.0% of the administered dose. The major metabolite isolated from feces was TMG 0.6-1.4%. Similar to urine metabolite profiles, there were no significant qualitative differences between males and female but marked quantitative differences were observed. Likewise metabolism was less in low-dose females than in males.

TABLE 9. Metabolite profile (% of administered radioactivity) in feces of rats following oral dosing with [nitroimino-^{14}C]-TI-435 or [thiazolyl-5-^{14}C]-TI-435

Metabolite	Dose group		
	2.5 mg/kg [nitroimino-^{14}C]-TI-435	2.5 mg/kg [nitroimino-^{14}C]-TI-435	2.5 mg/kg [thiazolyl-2-^{14}C]-TI-435
	Single dose males	Single dose females	Single dose males
	0-24 hrs	0-48 hrs	0-72 hrs
TI-435 (parent)	0.90	0.53	1.51
Polar HPLC fraction (Urea, NTG, MNG, MG)	0.60	0.34	ND
ACT	ND	ND	0.28
CTCA	ND	ND	0.06
MTCA	ND	ND	0.02
TZG	0.36	0.19	0.54
TMG	1.44	0.60	2.17
TZU	0.12	0.07	0.20
TZMU	0.10	0.03	ND
TZNG	0.14	0.06	0.18
Unidentified	0.29	0.24	0.41
Total	3.95	2.06	5.37

Data from Tables 10, 11, and 14, pp. 56, 57, and 60, MRID 45422805.
ND: Not detected

The metabolite profile in feces of rats given [thiazolyl-2-^{14}C]-TI435 consisted of nine components The metabolite profile was qualitatively slightly more complex relative to that of the groups receiving [nitroimino-^{14}C]-TI-435; six components plus parent compound, a polar fraction, and an unidentified fraction were detected. Similar to rats receiving the nitroimino- labeled test article, metabolite TMG was the most prevalent component (2.2% of the administered dose) of the fecal profile and was accompanied by higher level of parent compound (1.5% of the administered dose compared to 0.5-0.9%).

Plasma: Metabolite profiles were not obtained for plasma.

Tissues: In a related study (MRID 45422806), [nitroimino-^{14}C]-TI-435 was administered orally to 3 male Wistar rats at 5 mg/kg bw. At 4 hours post-dose, the animals were sacrificed and metabolite profiles were determined in blood, heart, kidney, liver lung, and spleen. The extractable radioactivity in these major tissues ranged from 91.6-99.7% of the total radioactive residue (TRR) in each tissue. Parent compound accounted for 47.1-89.2% of the TRR in the tissues examined; more parent compound being metabolized by the liver than any other tissue studied. Qualitative and quantitative recoveries were similar for all tissues except the liver, where a higher level of metabolism as well as a wider range of metabolites were observed. TZNG and MNG were major metabolites in all tissues (3.1-6.1% and 2.0-6.1% of TRR, respectively). TMG and MG were major metabolites detected only in the liver (24.3 and 7.4%, respectively). The data indicate that absorbed TI-435 is metabolized primarily in the liver explains the presence of this compound in the feces. This study also verifies the rapid absorption, distribution and dissipation indicated by the data obtained in MRID 45422805.

606

TABLE 10. Metabolite profiles (% of total radioactive residue in various tissues) for rats following a single 5 mg/kg oral dose of [nitroimino-^{14}C]-TI-435

Metabolite	Blood	Heart	Kidney	Liver	Lung	Spleen
ET-751 (parent)	86.1	88.7	87.3	47.1	89.0	89.2
TZMU	0.1	ND	ND	0.4	0.7	0.3
TZNG	3.1	5.9	6.1	4.6	4.0	4.2
MNG	6.1	4.8	5.2	2.0	5.7	6.0
TMG	ND	ND	ND	24.3	ND	ND
MG	ND	ND	ND	7.4	ND	ND
Unknown	ND	ND	ND	0.3	ND	ND
Origin	ND	ND	ND	5.5	ND	ND
Extractable	95.4	99.4	98.6	91.6	99.4	99.7
Unextractable	4.6	0.6	1.4	8.4	0.6	0.3
Total	100.0	100.0	100.0	100.0	100.0	100.0

Data from Table 2, p. 7, MRID 45422806.
ND: Not detected

III. DISCUSSION AND CONCLUSIONS:

A. **INVESTIGATORS' CONCLUSIONS**: Overall recovery of radioactivity among the various experimental groups ranged from 95.6-99.8 %. Approximately 90% of the administered dose of TI-435 was absorbed at 2.5, 25, or 250 mg/kg bw. Following oral administration, TI-435 was rapidly absorbed and excretion was nearly complete in 72 hours. At sacrifice, tissue residues of the administered radioactivity were low (0.09-0.33%) with the highest concentrations found in the liver and kidneys. Quantitative whole body autoradiography was used to demonstrate rapid absorption and distribution of TI-435 to all tissues and organs. The highest concentrations of radioactivity were found in the urinary bladder, kidney, liver, and the nasal mucosa. Initial high concentrations in the organs of excretion indicated rapid excretion following absorption. Radioactivity dropped to non-detectable levels within 72 hours of dosing with no signs of bioaccumulation in tissues and organs. Urine was the major route of excretion (89.1-91.5% of administered dose) with >90% of the administered dose excreted via this route with 24 hours. Feces accounted for only 3.3-7.8% of the administered dose. Excretion data did not indicate biologically important, gender-related quantitative differences in excretion profiles but did suggest delayed absorption at the high dose.

Metabolite profiles for urine, feces and tissues indicated parent compound was consistently found in the highest concentrations of all metabolites profiled; accounting for more than half of the administered dose (55-74%). The metabolites were primarily oxidative demethylation products with TZNG, MNG, NTG, and MCTA being the major metabolites found in urine. The main metabolite in feces was TMG. Minor metabolites were detected at < 2% each of the administered dose and amounted to < 4% of the total administered dose. Metabolic profiles were similar for all groups administered [nitroimino-^{14}C]-TI-435. The major product of oxidative demethylation was TZNG. Metabolic profiles for rats administered [thiazolyl-2-^{14}C]-TI-435 indicated cleavage of the nitrogen-carbon bond between the thiazolyl-methyl position and the nitroimino moiety. The nitroimino moiety appeared to result in formation of MNG and NTG whereas the thiazolyl moiety forms CTCA and MTCA. A minor additional product is ACT. Other minor metabolites of the nitroimino moiety were the urea compounds, TZMU and TZU, the guanidines TMG and TZG, and THMN. The study authors proposed

the metabolic scheme depicted in Figure 1 for the metabolism of TI-435. An additional study, in which metabolic profiles were determined for major tissues and organs 4 hours post-dosing, indicated rapid uptake of TI-435 by tissues and metabolism primarily in the liver resulting in rapid excretion of administered radioactivity. The metabolites TMG and MG were detected only in the liver and explains the presence of these compounds in the feces.

B. REVIEWER COMMENTS: Two studies were conducted to examine the metabolism and disposition of TI-435 (clothianidin) in male and female Sprague-Dawley rats (MRID 45422805) and male Wistar rats (MRID 45422806) following a single 2.5, 5, or 250 mg/kg bw oral dose, or a 14-day repeated dose (5 mg/kg/day). Rats were subjected to the dosing regimens above using [nitroimido-^{14}C]-TI-435 (lot nos. 11553/11 and 11553/17; >99% radiochemical purity; >98% chemical purity) or [thiazolyl-2-^{14}C]-TI-435 (lot no. 11649/2; >99% radiochemical purity, >99% chemical purity) and nonlabeled test article (lot no. M00157, 99.8% chemical purity). Excretion, tissue distribution, pharmacokinetic, and metabolite profiles were determined. In MRID 45422806, metabolite profiles were assessed in major tissues and organs of male rats given a single 5 mg/kg dose of [nitroimido-^{14}C]-TI-435 (lot no. and radiochemical and chemical purity not stated) and sacrificed after 4 hours post-dosing.

These were well-designed and conducted studies that adequately described the metabolism and disposition of orally administered TI-435 (clothianidin) in male and female rats. There were no biologically significant treatment-related effects noted during the course of the study. The data supported the contention that TI-435 is readily absorbed and rapidly excreted within 72 hours following a single or repeated oral dose of 2.5 mg/kg or 25 mg/kg bw but at a dose of 250 mg/kg, absorption becomes biphasic and may be approaching saturation. Following single low (2.5 mg/kg) doses or multiple oral low doses (5 mg/kg) of TI-435, urinary excretion accounted for 89.2-94.6% of the administered radioactivity suggesting that a multiple exposure regimen did not affect the absorption/excretion processes. Excretion via the feces accounted for the remainder of the administered radioactivity in all treatment groups (3.3-8.6%). Quantitative whole body radioautography and time-course plasma studies revealed rapid absorption and distribution of administered radioactivity to all organs and tissues followed by rapid excretion with reduction to background levels in most tissues and organs within 24 hours. Gender related differences in plasma kinetics were indicated by the somewhat greater rate of elimination in females than males which correlates with the somewhat higher rate observed for absorption in female rats. Excretory patterns did not exhibit gender-related variability, but suggest near-saturation of absorption at the high dose. Based upon tissue burden data, neither TI-435 nor its metabolites appear to undergo any significant sequestration in most tissues. With the exception of transiently higher levels in the liver, kidneys, intestines and urinary bladder during the elimination phase, and an unexplained affinity of nasal mucosa for TI-435 or its radiolabeled metabolites during the absorption and distribution phase, radioactivity concentrations in any given tissue consistently represented considerably less than 1% of the administered dose within 24 hours of administration of TI-435..

Both urinary and fecal metabolites were quantified by HPLC, TLC and most were identified using HPLC, TLC, and HPLC/MS in conjunction with known standards. The metabolites identified (oxidative demethylation products and cleavage of the nitrogen-carbon bond

608

between the nitroimino and thiazolyl moieties) were consistent with Phase I processes. Extraction efficiencies appeared to be excellent and most components in all of the matrices examined (urine, feces, and tissues) were adequately quantified and characterized. The available data, based upon studies using both the nitroimino- and the thiazolyl-2-labeled TI-435, affirmed the metabolism pathway (Figure 1) proposed by the investigators.

These metabolism studies (MRID 45422805 and 45422806 are, collectively, **Acceptable/ Guideline** and satisfy the requirements for a Metabolism and Pharmacokinetics Study [OPPTS 870.7485 (§85-1)]. The studies were properly designed, conducted and reported. The reports provided data that adequately described the metabolism and disposition of TI-435 (clothianidin) in male and female rats following both a low (2.5 mg/kg) and high (250 mg/kg) single oral dose and a 14-day repeated (25 mg/kg) oral dose regimen in male rats. Data were also obtained for alternate label positions. Metabolic profiles in tissues were determined in male Wistar rats administered a 5 mg/kg bw oral dose and indicated the liver to be the major organ of metabolism of TI-435 and the kidney to be the major organ excretion of TI-435 and its metabolites.

C. **STUDY DEFICIENCIES:** There were no deficiencies noted in the main study. For MRID 45422806, the labeled and unlabeled test materials were incompletely characterized and the conditions of animal handling and diets were not adequately described. The outcome of the experiments were not compromised, however, by these omissions.

APPENDIX
(MRID NO. 45422805 (main study, 45422806)

THE FOLLOWING ATTACHMENTS ARE NOT AVAILABLE ELECTRONICALLY. SEE THE FILE COPY

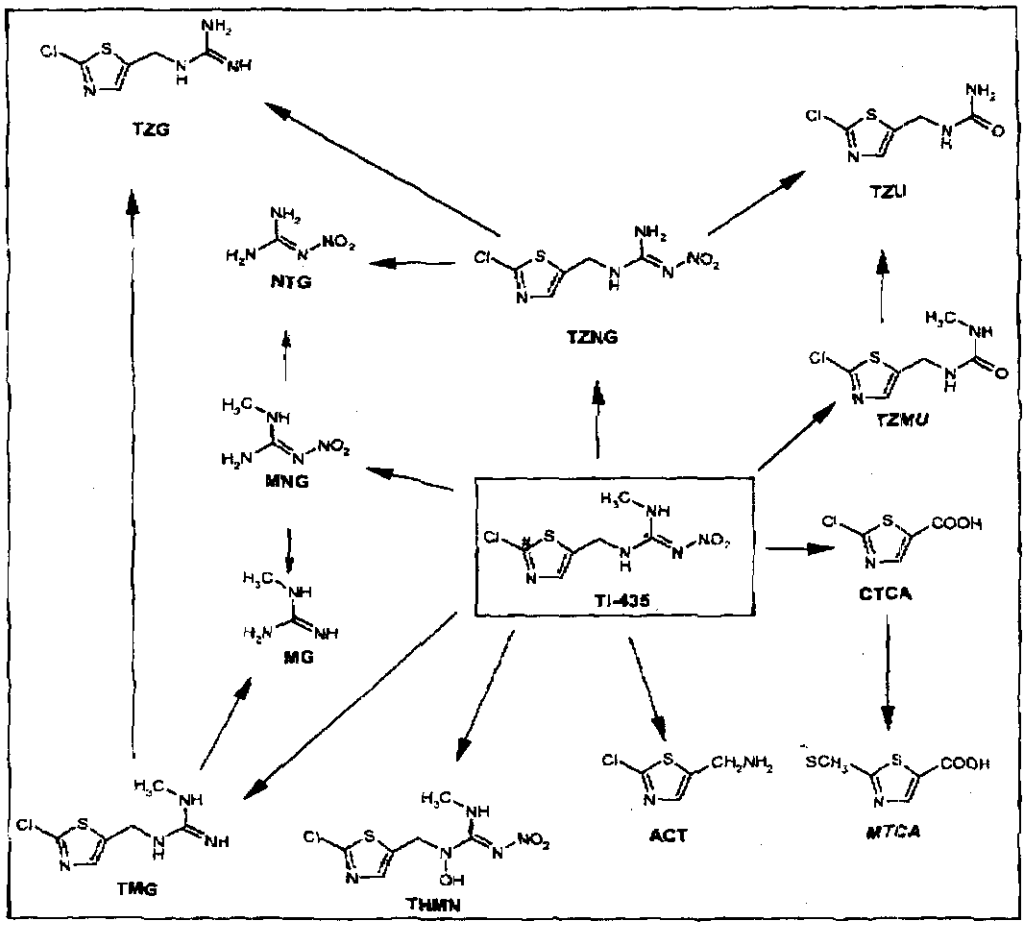


Figure 1. Proposed metabolic pathway of TI-435 in the rat.
Taken from Figure 66, p. 133 of MRID 45422805.

DATA EVALUATION RECORD

CLOTHIANIDIN/044309

STUDY TYPE: METABOLISM AND PHARMACOKINETICS - MOUSE
[OPPTS: 870.7485 (§85-1)]
MRID 45422807

Prepared for

Health Effects Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
1921 Jefferson Davis Highway
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Prepared by

Toxicology and Hazard Assessment Group
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DATA EVALUATION RECORD
TXR#: 0050321

STUDY TYPE: Metabolism - Mouse; [OPPTS 870.7485 (§85-1)]; OECD 417.

PC CODE: 044309

DP BARCODE: D282578
SUBMISSION NO.: S614462

TEST MATERIAL (PURITY): Clothianidin [TI-435]

SYNONYMS: [nitroimino-^{14}C]-TI-435; (E)-1-(2-chloro-1,3-thiazol-5-ylmethyl0-3-methyl-2-nitroguanidine); clothianidin

CITATION: Mikata, K., H. Nagasaki, and K. Ohta. 2000. Absorption, distribution, metabolism and excretion of TI-435 in mice. Report No. 110491, Takeda Chemical Industries, Ltd., Agricultural Research Laboratories, 12-10, Nihonbashi 2-chome, Chuo-ku, Tokyo 1003-8668, Japan. 29 November 2000. Study No. DTOX063. MRID 45422807. Unpublished.

SPONSOR: Bayer Corporation, Agriculture Division, Research and Development Department, P.O. Box 4913, Kansas City MO 64120-0013.

EXECUTIVE SUMMARY: This study (MRID 45422807) was conducted to examine the absorption, excretion, tissue distribution and metabolism of [nitroimino- ^{14}C]-TI-435 (96.7% radiochemical purity) in male and female ICR mice following a single 5 mg/kg oral dose.

There were no biologically significant treatment-related effects or mortality noted during the course of the study. Of the administered radioactivity, 98.7-99.2% was recovered. TI-435 was readily absorbed and excreted within 168 hours following a single oral dose of 5 mg/kg body weight. Urine was the major route of excretion of TI-435, accounting for 92.4-93.7% of the administered radioactivity. Feces accounted for 5.0-6.8% of the administered radioactivity. Within 24 hours, 89.0-91.7% of the administered radioactivity was excreted in the urine and 4.9-6.2% was excreted in the feces. Residual radioactivity in any given tissue at 168 hours post-dose was considerably less than 1% of the administered dose. Therefore, neither TI-435 nor its metabolites appeared to exhibit potential for bioaccumulation. Excretory patterns did not exhibit gender-related variability.

Both urinary and fecal metabolites were identified using TLC and radioautography in conjunction with known standards and were quantified by TLC/LSC. The major metabolites in both urine and feces were the parent compound (TI-435) and TZNG [N-(2-chlorothiazol-5-ylmethyl)-N′-nitroguanidine] which resulted from N-demethylation of TI-435. Extraction

efficiencies were excellent and most components in the urine and feces were adequately quantified and characterized. Based on the data from the oral administration of [nitroimino-^{14}C]-TI-435, the metabolism pathway proposed by the investigators was supported.

This metabolism study (MRID 45422007) is **Acceptable** because it provides supplementary information to the requirements for a Tier I Metabolism and Pharmacokinetics Study. Although, this study does not satisfy the guideline requirements for a Metabolism and Pharmacokinetics study [OPPTS 870.7485 (§85-1)] in the mouse.

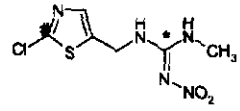
COMPLIANCE: Signed GLP, Quality Assurance, and Confidentiality Claim statements were provided in the study report.

I. MATERIALS AND METHODS

A. MATERIALS:

1. Test compound:

Radiolabeled test material:	[nitroimino-^{14}C] TI-435
Radiochemical purity	96.7% determined by HPLC-UV
Specific Activity	0.58 MBq/mg
Lot/Batch #:	Not provided
Non-radiolabelled test material:	ET-751
Description:	Not provided
Lot/Batch #:	Not provided
Purity:	Not provided
Contaminants:	Not reported
CAS # of TGAI:	210880-92-5
Structure:	



* ^{14}C labeled position

2. Vehicle and/or positive control: The dosing solution was prepared by dissolving the test material in dimethylformamide (5% of dose volume) and suspending in corn oil (Wako Pure Chemicals Industries, Inc.).

3. Test animals:

Species:	Mouse	
Strain:	ICR	
Age/weight at study initiation:	7 weeks; mean body weight: 34.4 g (males), 27.7 g (females)	
Source:	Charles River Japan	
Housing:	Housed individually in metabolism cages during experiment	
Diet:	Not provided; assumed ad libitum	
Water:	Not provided; assumed ad libitum	
Environmental conditions:	**Temperature:**	Not specified
	Humidity:	Not specified
	Air changes:	Not specified
	Photoperiod:	Not specified
Acclimation period:	14 days	

4. Preparation of dosing solutions: The dosing solution was prepared by dissolving [nitroimino-^{14}C]-TI-435 in 5% of dosage volume of dimethylformamide and suspending in corn oil to final volume. Homogeneity was not confirmed.

B. STUDY DESIGN AND METHODS:

1. Group arrangements: Groups of 5 males and 5 females were administered the test dose (5mg/kg bw) for the absorption, distribution, excretion and metabolic profile studies. Urine and feces were collected at 24-hr intervals and tissues and organs were collected from 3 males and 3 females at terminal sacrifice (168 hrs). Group assignment was random.

2. **Dosing and sample collection:** Five males and five females were administered a single dose, 5 mg/kg bw, of [nitroimino-^{14}C]-TI-435 by gavage. Mice were housed individually in metabolic cages for collection of urine and feces.

   Expired air: Expired air was not sampled.

   Urine/feces/cage wash: Urine and feces were collected at 24-hr intervals until study termination at 168 hours. Collection of cage washes and sample storage were not described.

   Tissue/organs: The following organs/tissues were collected from 3 male and 3 female mice at termination (168 hrs): caecum, hair, blood, brain, thyroid, heart, liver, lungs, adrenal glands, spleen, pancreas, fat (site not specified), muscle (site not specified), stomach, intestine, testes, ovaries, uterus, skin, sciatic nerve, spinal cord and kidney.

3. **Analytical techniques:**

   Sample preparation: Urine was vacuum evaporated with the addition of methanol and ethanol. Precipitated insoluble matter was separated by filtration. The ethanol-soluble fraction was concentrated under a vacuum and two-dimensional thin-layer chromatography (TLC) was carried out with synthetic standards. The ethanol-insoluble fraction of urine was dissolved in water, added to liquid scintillator A and radioactivity was measured by liquid scintillation counting (LSC). Blood was hemolyzed by the addition of water followed by sodium hydroxide solution, hydrogen peroxide solution, acetic acid and liquid scintillator A to be measured by LSC. Feces was extracted with 50% methanol, then 100% methanol and the residue was separated by centrifugation. The supernatant was concentrated and treated the same as the ethanol-soluble fraction of urine undergoing two-dimensional TLC using synthetic standards. The ethanol-insoluble fractions of the fecal samples were homogenized in distilled water, air dried and combusted before being measured by LSC. Similarly, tissues (whole or portions) were air dried and combusted before LSC measurements. Combustions were carried out using an Aloka ASC-113 automatic sample combuster.

   Liquid scintillation counting (LSC): LSC was conducted using a Beckman LS-6000TA liquid scintillation spectrometer. Blood and urine samples were added to scintillator A which is a toluene-nonion system. Fecal and tissue samples were combusted and the generated $^{14}CO_2$ was trapped in Packard Carbo-Sorb E and counted in liquid scintillator B which is a toluene system like scintillator A without the addition of nonion NS-210. Counting efficiency for LSC was determined using the external radioactive standard method.

   Thin-layer chromatography (TLC): TLC was performed using silica gel G60 and the following solvent systems: A) chloroform/methanol (8:1, V/V); B) chloroform/acetone (1:1, V/V); C) chloroform/methanol (7:3, V/V); and D) chloroform/acetone/methanol/formic acid (2:2:1:1, V/V/V/V). Following development, silica gel plates were attached to an imaging plate and autoradiograms (ARG) produced using a BAS 2000 bio-imaging analyzer. Radioactive spots detected by TLC-ARG were scraped from the plates and counted by LSC. Authentic standards on plates were detected with UV light.

4. **Statistics:** Group means were calculated and presented. There were no statistical comparisons performed among the treatment groups.

II. RESULTS:

A. PHARMACOKINETIC STUDIES: Recovery of administered radioactivity was an acceptable 98.7-99.2%. A general accounting of recovered radioactivity following dosing with [nitroimino-^{14}C]-TI-435 is summarized in Table 1.

TABLE 1. Recovery of radioactivity (% of administered dose) from mice at 168 hours following oral administration of [nitroimino-^{14}C]-TI-435

Compartment	5 mg/kg bw Single Dose	
	Males	Females
Tissues	ND	ND
Urine	92.4	93.7
Feces	6.8	5.0
Total	99.2	98.7

Data obtained from Tables 2 and 3, pp. 10-11, MRID 45422807.
ND: below reliable detection limit.

1. Absorption/excretion/kinetics:

Absorption: Extensive absorption can be implied from urine data given in Table 1. Based on the data following a single low dose (5 mg/kg bw), ~92-93% of [nitroimino- 14C - TI-435 was absorbed. Time-course data for excretion (Table 2) indicates rapid and nearly complete absorption within 48 hrs following dose administration.

Excretion Time-course excretion data are summarized in Table 2. This data indicates that most of the administered dose is excreted in the urine (92.4-93.7%) within 168 hours post-dosing. Excretion via feces accounted for only 5.0-6.8% of the administered dose in the same time period. Greater than 95% (95.6% males; 96.6% females) of the radioactivity excreted into urine occurred within 24 hours (89.0-91.7% of total recovered radioactivity). Likewise, 91.2-96.0% of the radioactivity excreted in feces occurred within 24 hours of dosing (4.9-6.2 % of total recovered radioactivity). Urine was the major route of excretion. There did not appear to be any biologically significant gender-related differences in excretion rates. Biliary excretion was not assessed.

TABLE 2. Time-course of urinary and fecal excretion (% of administered dose) in mice orally administered [nitroimino-^{14}C]-TI-435 at 5 mg/kg bw

Time (hours)	Urinary Excretion[a]		Fecal Excretion[a]	
	Males	Females	Males	Females
0-24	88.3	90.5	6.2	4.8
24-48	2.3	1.4	0.4	0.2
48-72	0.9	0.4	0.1	0.0
72-96	0.4	0.4	0.0	0.0
96-120	0.3	0.3	0.0	0.0
120-144	0.1	0.2	0.1	0.0

TABLE 2. Time-course of urinary and fecal excretion (% of administered dose) in mice orally administered [nitroimino-^{14}C]-TI-435 at 5 mg/kg bw				
Time (hours)	Urinary Excretion[a]		Fecal Excretion[a]	
	Males	Females	Males	Females
144-168	0.1	0.4	0.0	0.0
Total	92.4	93.7	6.8	5.0

Data taken from Table 2, p. 10, MRID 45422807.
[a]Interval recovery values calculated from cumulative recovery values by reviewer.

Plasma pharmacokinetics: Plasma pharmacokinetics were not evaluated in the study (MRID 45422807).

2. **Tissue distribution:** Tissue burdens (expressed as percent administered radioactivity) were negligible (0.01-0.02%) in all tissues and organs sampled at sacrifice. Residual radioactivity and the % distribution of radioactivity are summarized in Table 3.

TABLE 3. Residual radioactivity and the % distribution of radioactivity in tissues of male and female mice after a single oral administration (5 mg/kg) of [nitroimino-^{14}C]-TI-435 after 168 hours. [(a)]				
Tissue	^{14}C - Concentration [(b)]		% of ^{14}C dosed	
	Male	Female	Male	Female
Adrenal	0.02**	<0.01**	<0.01**	<0.01**
Blood	0.02	<0.01**	0.02	<0.01**
Brain	<0.01**	<0.01**	<0.01**	<0.01**
Caecum	<0.01**	<0.01**	<0.01**	<0.01**
Fat	<0.01**	<0.01**	<0.01**	<0.01**
Hair	<0.01**	0.02**	<0.01**	<0.01**
Heart	<0.01**	<0.01**	<0.01**	<0.01**
Intestine	<0.01**	<0.01**	<0.01**	<0.01**
Kidney	<0.01**	<0.01**	<0.01**	<0.01**
Liver	0.02	0.02	0.02	0.01
Lung	<0.01**	<0.01**	<0.01**	<0.01**
Muscle	<0.01**	<0.01**	<0.01**	
Ovary		<0.01**		<0.01**
Pancreas	<0.01**	<0.01**	<0.01**	<0.01**
Sciatic Nerve	<0.01**	<0.01**	<0.01**	<0.01**
Skin	<0.01**	<0.01**	<0.01**	<0.01**
Spinal cord	<0.01**	<0.01**	N/A	N/A
Spleen	<0.01**	<0.01**	<0.01**	<0.01**

TABLE 3. Residual radioactivity and the % distribution of radioactivity in tissues of male and female mice after a single oral administration (5 mg/kg) of [nitroimino-^{14}C]-TI-435 after 168 hours. [a]				
Tissue	^{14}C - Concentration [b]		% of ^{14}C dosed	
	Male	Female	Male	Female
Stomach	<0.01**	<0.01**	<0.01**	<0.01**
Testis	<0.01**		<0.01**	
Thyroid	<0.01**	<0.01**	<0.01**	<0.01**
Uterus		<0.01**		<0.01**

Data taken from Table 3, p. 11, MIRD 45422807.
(a) Values represent the mean of 3 mice. Results derived from data ≤ 100 (*) and ≤ 50 (**) dpm above background.
(b) Results were shown as µg equivalent of TI-435/g wet tissue.
N/A - Not analyzed

B. METABOLITE CHARACTERIZATION STUDIES:

Based upon TLC analyses with known standards, most of the prevalent metabolite fractions from urine and feces were identified as follows:

MM-A (E)-1-(2-chloro-1,3-thiazol-5-ylmethyl)-3-methyl-2-nitroguanidine; TI-435
MM-B N-(2-chlorothiazol-5-ylmethyl)-N′-nitroguanidine; TZNG
MM-C N-methyl-N′-nitroguanidine; MNG
MM-D Nitroguanidine; NTG
MM-E N-(2-chlorothiazol-5-ylmethyl)-N′-methylguanidine; TMG

Urine: Five radioactive components (including the origin) were detected in the urine of male and female mice receiving [nitroimino-^{14}C]-TI-435 (Table 3). Metabolites MM-A and MM-B (parent and TZNG, respectively) were the most prevalent components representing 36.8-38.3% and 29.2-29.3% of the recovered administered radioactivity. Lesser amounts of MM-C (MNG) and MM-D (NTG) were also identified in urine. Approximately 6% of the recovered administered radioactivity remained at the TLC origin. No biologically significant gender-related quantitative differences were observed.

Feces: Eight components (including parent compound) were detected in fecal samples from mice given a single oral dose of [nitroimino-^{14}C]-TI-435 (Table 4). Most of the recovered ethanol-soluble radioactivity from feces was found as metabolites MM-A (parent), MM-B (TZNG) and MM-E (TMG) comprising 1.4-1.5, 0.9-1.3, and 0.8-1.0% of the administered radioactivity, respectively. Metabolites MM-C (MNG), MM-D (NTG), and the unknowns MM-F, MM-G, and MM-H each comprised <1.0% of the recovered administered radioactivity. Ethanol-insoluble residues (unextractables) accounted for 0.8-1.0% of the administered radioactivity. There were no notable gender-related differences.

Plasma and Bile: Plasma and bile were not evaluated for the presence of radioactive metabolites of [nitroimino-^{14}C]-TI-435.

TABLE 4. Metabolite profile (% of administered radioactivity) in urine and feces of mice following oral dosing with 5 mg/kg bw [nitroimino-^{14}C]-TI-435				
Metabolite	Urine		Feces	
	Males	Females	Males	Females
MM-A TI-435 (parent)	36.8	38.3	1.5	1.4
MM-B TZNG	29.2	30.2	1.3	0.9

TABLE 4. *Metabolite profile* (% of administered radioactivity) in urine and feces of mice following oral dosing with 5 mg/kg bw [nitroimino-^{14}C]-TI-435

Metabolite	Urine		Feces	
	Males	Females	Males	Females
MM-C MNG	8.7	9.0	0.2	0.2
MM-D NTG	11.3	10.7	0.4	0.2
MM-E TMG	ND	ND	1.0	0.8
MM-F Unknown	ND	ND	0.3	0.2
MM-G Unknown	ND	ND	0.4	0.3
MM-H Unknown	ND	ND	0.7	0.2
Origin	6.4	5.5	ND	ND
Unextractable	ND	ND	1.0	0.8
Total	92.4	93.7	6.8	5.0

Data from Table 4 & 5, p. 12, MRID 45422807.
ND: not detected

III. DISCUSSION AND CONCLUSIONS:

A. INVESTIGATORS' CONCLUSIONS: Overall recovery of radioactivity was approximately 99%. Following oral administration of 5 mg/kg bw [nitroimino-^{14}C]-TI-435, 98.7-99.2% of the administered radioactivity was excreted in the urine and feces within 168 hours. The majority (92.4-93.7%) of [nitroimino-^{14}C]-TI-435, was excreted in the urine and most of the radioactivity (94.5-95.3%) was excreted within 24 hours. Only 5.0-6.8% of the administered radioactivity was recovered in feces indicating urine was the major excretory route. There were no biologically relevant gender-related differences in excretion profiles or rates of absorption/excretion. Tissue distribution burden data showed no significant accumulation of radioactivity (<0.01-0.02%) and no potential for bioaccumulation of the test article or its metabolites at the dose tested..

Metabolites MM-A, MM-B, MM-C, MM-D, and MM-E were found in the ethanol-soluble fraction of urine and feces and were identified by two-dimensional TLC co-chromatography as TI-435, TZNG, MNG, NTG, and TMG, respectively. Five spots (including the origin) were detected in the urine of male and female mice. TI-435 constituted 36.8-38.3% of the administered radioactivity; TZNG, 29.2-30.2%; MNG 8.7-9.0%; NTG, 10.7-11.3%; and 5.5-6.0% remained at the origin. Eight spots were detected in feces of male and female mice. TI-435 constituted 1.4-1.5% of the administered radioactivity; TZNG, 0.9-1.3%; TMG, 0.8-1.0%; and NTG, and unknown metabolites (MM-F, MM-G, and MM-H) were <1%. Approximately 0.8-1.0% of the administered radioactivity was recovered as unextractable residues. The study authors proposed the following reactions: demethylation of TI-435 yielding TZNG, with cleavage of the nitrogen-carbon bond between nitroguanidine and the thiazolylmethyl group of TZNG to produce NTG; cleavage of the nitrogen-carbon bond between nitroquanidine and the thiazolylmethyl group of TI-435 to form MNG with subsequent demethylation of MNG to produce NTG; and dinitration of TI-435 to form MNG.

B: REVIEWER COMMENTS: One study (MRID 45422807) was conducted to examine the metabolism and disposition of TI-435 (clothianidin) in male and female ICR mice following a single 5 mg/kg bw oral dose of [nitroimino-^{14}C]-TI-435 (radiochemical purity 96.7%). Excretion, tissue distribution, and metabolite profiles were determined.

Although nonguideline, this study provides supplemental information on the metabolism and disposition of orally administered TI-435 (clothianidin) in male and female mice. Total recovery of administered radioactivity was an acceptable 98.7-99.2 %. There were no biologically significant treatment-related effects noted and no deaths occurred during the course of the study. The data supported the contention that TI-435 is readily absorbed and excreted within 168 hours following a single oral dose of 5 mg/kg bw. The urine was the major route of excretion of TI-435 with 92.4-93.7% of the recovered administered radioactivity recovered in 168 hours; the remainder was recovered in the feces (5.0-6.8%). Within 24 hours, urine accounted for 89.0-91.7% of the administered radioactivity excreted within 24 hours and feces accounted for 4.9-6.2% during the same period. Residual radioactivity in any given tissue at sacrifice was considerably less than 1% of the administered dose. Therefore, neither TI-435 nor its metabolites appeared to exhibit potential for bioaccumulation.

628

Both urinary and fecal metabolites were identified using TLC and radioautography in conjunction with known standards and were quantified by TLC/LSC. Extraction efficiencies were excellent and most components in the urine and feces were adequately quantified and characterized. Based on the data from oral administration of [nitroimino-^{14}C]-TI-435, the metabolism pathway (attach Figure 1, page 9, MRID 45422807) proposed by the investigators was supported.

C. **STUDY DEFICIENCIES:** Justification for the election of the dose level was not included within the study. Also, the characterization of the dosing compound was lacking and chemical purity was not stated. The conditions for animal housing, diet and sample collection were not adequately described. These deficiencies did not adversely impact the quality of this study.

DATA EVALUATION RECORD

TI-435 (CLOTHIANIDIN)

Study Type: §82-1b, Subchronic Oral Toxicity Study in Dogs

Work Assignment No. 4-02-167D-Amendment (formerly 4-01-167D)
MRIDs 45422810, 45422808, and 45422811

Prepared for

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Disclaimer

This Data Evaluation Record may have been altered by the Health Effects Division subsequent to signing by Dynamac Corporation personnel.

PMRA/EPA Reviewers: Scott Hancock and Pamela Hurley Signature:
EPA Work Assignment Manager:Ghazi Dannan, Ph.D. Signature:
Registration Action Branch 3, Health Effects Division (7509C) Date 12/10/04

Template version 11/01

TXR#: 0050321

DATA EVALUATION RECORD

STUDY TYPE: 90-Day Oral Toxicity [feeding] - dog; OPPTS 870.3150 [§82-1b]; OECD 409.

PC CODE: 004309 **DP BARCODE:** D282578
SUBMISSION NO.: S614462

TEST MATERIAL (PURITY): TI-435 (Clothianidin; 95.2-95.6% a.i.)

SYNONYMS: (E)-1-(2-chloro-1,3-thiazol-5-ylmethyl)-3-methyl-2-nitroguanidine

CITATION: Bernier, L. (2000). 13-week Dietary Toxicity Study with TI 435 in Dogs. Covance Laboratories Inc., Vienna, VA. Laboratory Project Id: Covance 6155-111, March 14, 2000. MRID 45422810. Unpublished.

Moore, M.R. (2000). 4-week Dietary Toxicity Study with TI-435 in Dogs. Covance Laboratories Inc., Vienna, VA. Laboratory Project Id: Covance 6155-106, February 1, 2000. MRID 45422808. Unpublished.

Moore, M.R. (1998). Palatability Pilot Study for Dietary Concentrations of TI 435 in Dogs. Covance Laboratories Inc., Vienna, VA. Laboratory Project Id: Covance 6155-107, May 1, 1998. MRID 45422811. Unpublished.

SPONSOR: Takeda Chemical Industries Ltd., Agro Company, 13-10, Nihonbashi 2-chome, Chuo-ku, Tokyo, Japan

EXECUTIVE SUMMARY: In a subchronic oral toxicity study (MRIDs 45422810, 45422808, and 45422811), TI-435 (clothianidin; 95.2-95.6% a.i., Lot #30037120) was administered to 4 beagle dogs/sex/group in the diet at dose levels of 0, 325, 650, 1500, or 2250 ppm (equivalent to 0/0, 9.2/9.6, 19.3/21.2, 40.9/42.1, and 58.2/61.8 mg/kg bw/day in males and females respectively) for 13 weeks. Food efficiency was not determined.

There were no effects of treatment on mortality, neurological evaluations, food consumption, ophthalmology, urinalysis, organ weights, gross pathology, or histopathology. In the males, increased incidence and frequency of thinness was observed at 1500 and 2250 ppm. Males dosed with 1500 ppm gained 0.4 +/- 1.74 kg treated vs 1.1 +/- 0.26 controls. One male in this group (animal #G34993) lost 20% body weight over the course of the study. At 2250 ppm, males displayed decreases in body weights throughout treatment (decr 4-10%; not significant) and in overall (weeks 1-13) body weight gains (0.3 +/- 0.69 kg treated vs 1.1 +/- 0.26 controls; $p<=0.05$). Additionally, males displayed anaemia (1/4 at 1500 ppm and 2/4 at 2250 ppm). These treatment related effects were observed in a pilot study, whereby dogs fed a diet with a slightly higher concentration of test substance (2500 ppm) in the 4-week study suffered mortality, decreased food consumption, body weight gains, erythrocytes, and leukocytes in addition to bone marrow hypocellularity, lymphoid depletion, and changes in clinical chemistry. Taken together the hematological effects seen in the two highest doses were considered to be adverse in male animals. In the females in the current study at week 13, decreases ($p<=0.05$) were observed in leukocytes (decr. 52%), specifically, segmented neutrophils (decr. 61%), and monocytes (decr. 75%). Additionally in the females, decreases ($p<=0.05$) were observed in alanine aminotransferase (decr. 65-72%) and albumin (decr. 8-13%) at weeks 5 and 13 and in total protein (decr. 17%) at week 13. The decreases in ALT were of equivocal toxicological significance.

The only treatment-related findings at 1500 were decreased ($p<=0.05$) alanine aminotransferase (ALT) at weeks 5 and 13 (decr 53-55%) and albumin at week 13 (decr 11%) in the females. These effects were not considered to be adverse to the health of the animals. There were no effects of treatment at 325 or 650 ppm.

The LOAEL for this study is 1500 ppm in males and 2250 ppm in females (equivalent to 40.9/61.8 mg/kg/day in the males/females) based on thinness, decreased body weight, body weight gains, and anaemia in one male and on decreased white blood cells, albumin, and total protein in the females. The NOAEL is 650 ppm in males and 1500 ppm in females (equivalent to 19.3/42.1 mg/kg/day in the males/females).

The submitted study is classified as **acceptable/guideline** and satisfies the guideline requirements for a 90-day oral toxicity study in the dog (OPPTS 870.3150; OECD 409).

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

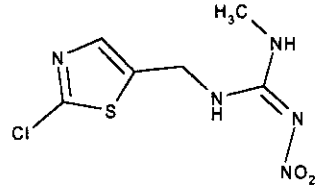
I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

TI-435 (Clothianidin)

Description: Pale-yellow powder
Lot/Batch #: 30037120
Purity: 95.2-95.6% a.i.
Compound Stability: Stable in the diet for 14 days at room temperature
CAS #: 210880-92-5
Structure:



2. Vehicle: Diet

3. Test animals:

Species:	Dog
Strain:	Beagle
Age/weight at study initiation:	Approximately 5-6 months/8.5-11.4 kg, males; 7.3-9.2 kg, females
Source:	Covance Research Products, Inc., Cumberland, VA
Housing:	Individually, in elevated, stainless steel cages
Diet:	Certified Canine Diet® Meal #5007 (PMI® Nutrition International), *ad libitum*, except during overnight fasting prior to blood and urine sampling
Water:	Tap water, *ad libitum*
Environmental conditions:	**Temperature:** 21.3-27.6°C **Humidity:** 30.1-48.5% **Air changes:** ≥10 per hour **Photoperiod:** 12 hours light/12 hours dark
Acclimation period:	At least 2 weeks

B. STUDY DESIGN

1. In life dates - Start: January 16, 1998 End: April 21, 1998

2. Animal assignment: Animals were randomly assigned, stratified by weight, to the test groups presented in Table 1. At the start of the study, animals were within ±20% of the mean weight for each sex.

Table 1. Study design [a]

Test Group	Conc. in Diet (ppm)	Mean Dose to Animal (mg/kg/day) M/F	# Males	# Females
Control	0	0/0	4	4
Low	325	9.2/9.6	4	4
Mid	650	19.3/21.2	4	4
Mid-High	1500	40.9/42.1	4	4
High	2250	58.2/61.8	4	4

a Data were obtained from page 18 and Table 6 on page 67 of the study report (MRID 45422810).

3. **Dose selection rationale** - Based upon the results of a 4-week range-finding study (MRID 45422808) and palatability pilot study (MRID 45422811) submitted with the 90-day study, the doses summarized in Table 1 were selected for the 90-day study. See Appendices in this DER for details of the pilot and 4-week studies.

4. **Treatment preparation, administration, and analysis** - Test diets were prepared weekly by first mixing the required amount of test material with approximately 200 g of diet to make a pre-mix. This pre-mix was then diluted with additional diet and mixed to achieve the desired dietary concentrations. Stability for up to 14 days at room temperature and homogeneity (top, middle, and bottom) were verified for the 50, 325, and 2500 ppm diets prior to treatment. Concentration analyses were determined for each dose level at weeks 1 and 13. All samples were analyzed in duplicate.

Results - Homogeneity Analysis (mean ± SD % of nominal; % SD):

50 ppm: 97.8 ± 1.08; 1.10
325 ppm: 96.2 ± 1.09; 1.13
2500 ppm: 93.5 ± 0.758; 0.811

Stability Analysis (range as % of nominal after 14 days at room temperature):

50 ppm: 92.3-92.4
325 ppm: 93.1
2500 ppm: 90.1-90.9

Concentration Analysis (range as % of nominal):

325 ppm: 96.2-97.5
650 ppm: 93.2-97.1
1500 ppm: 92.8-96.5
2250 ppm: 92.3-95.4

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

5. **Statistics** - The following statistical procedures were employed:

Parameter	Statistical Test
Body weights, body weight gains, food consumption, clinical pathology (except hemolysis, cellular morphology gradings, and routine urinalysis data), and organ weights	Bartlett's test or Levene's test for homogeneity of variances. If variances were heterogeneous, data were rank-transformed. If transformation did not achieve homogeneity of variances, the rank-transformed data were still analyzed. Analysis of variance (ANOVA) was then followed with Dunnett's test or pair-wise t-test, as necessary.

The levels denoting significance were $p \leq 0.05$ and $p \leq 0.01$ for each statistical comparison. In general, the statistical methods were considered appropriate.

C. METHODS

1. Observations

a. Cageside observations - Animals were checked twice daily for mortality and moribundity and once daily for clinical signs of toxicity.

b. Clinical examinations - Detailed physical examinations were performed weekly during the treatment period.

c. Neurological evaluations - Dogs were examined for pupillary reflex, gait, extensor thrust reflex, placement reflex, righting reflex, auditory startle reflex, patellar reflex, and pain perception prior to treatment and during weeks 1, 5, and 13.

2. Body weight and body weight gain - Each dog was weighed prior to treatment, weekly throughout the study, and at termination. Group mean body weight gains were reported for each week during the study and for the overall (weeks 0-13) study.

3. Food consumption, food efficiency, and compound intake - Food consumption (kg) was recorded weekly for each dog. Group mean achieved test substance intake (mg/kg/day) was calculated for each week and for the overall (weeks 1-13) study using the nominal dose and the body weight and food consumption data. Food efficiency was not calculated.

4. Ophthalmoscopic examination - The eyes of each animal were examined by indirect ophthalmoscopy after dilation with a mydriatic agent prior to treatment and during week 13.

5. Hematology & clinical chemistry - Blood was collected from the jugular vein of each dog prior to treatment and during weeks 5 and 13. An additional blood sample was collected from each dog during week 9 for complete blood count and differential only. Animals were fasted

(food only) overnight prior to blood sampling. The CHECKED (X) parameters were examined.

a. Hematology

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

* Recommended for 90-day oral non-rodent studies based on Guideline 870.3150

b. Clinical Chemistry

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

* Recommended for 90-day oral non-rodent studies based on Guideline 870.3150

6. Urinalysis - Urine was collected overnight from each dog prior to treatment and during weeks 5 and 13. Food was withheld from animals during overnight urine collection. The CHECKED (X) parameters were examined.

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

* Recommended for 90-day oral non-rodent studies based on Guideline 870.3150

7. **Sacrifice and pathology** - After 13 weeks of treatment, all animals were fasted (food only) overnight, euthanized by exsanguination under sodium thiopental anesthesia, and were subjected to gross necropsy. The CHECKED (X) tissues were collected, preserved in 10% neutral-buffered formalin, embedded in paraffin, sectioned, stained with hematoxylin and eosin, and examined microscopically. In addition, the (XX) organs were weighed.

	DIGESTIVE SYSTEM		CARDIOVASC./HEMAT.		NEUROLOGIC
	Tongue	X	Aorta, thoracic*	XX	Brain*+
X	Salivary glands*	XX	Heart*+	X	Peripheral nerve*
X	Esophagus*	X	Bone marrow*	X	Spinal cord (3 levels)*
X	Stomach*	X	Lymph nodes*	X	Pituitary*
X	Duodenum*	XX	Spleen*+	X	Eyes (optic nerve)*
X	Jejunum*	XX	Thymus*+		GLANDULAR
X	Ileum*			XX	Adrenal gland*+
X	Cecum*		UROGENITAL		Lacrimal gland
X	Colon*	XX	Kidneys*+	XX	Parathyroid*+
X	Rectum*	X	Urinary bladder*	XX	Thyroid*+
XX[a]	Liver*+	XX	Testes*+		OTHER
XX[a]	Gall bladder*+	XX	Epididymides*+	X	Bone (sternum and/or femur)
X	Pancreas*	X	Prostate*	X	Skeletal muscle
	RESPIRATORY	XX	Ovaries*+	X	Skin*
X	Trachea*	XX	Uterus*+	X	All gross lesions and masses*
XX	Lung*	X	Mammary gland (females)*		
	Nose*	X	Vagina (and cervix)		
	Pharynx*				
	Larynx*				

a Liver and drained gall bladder were weighed together.
* Recommended for 90-day oral non-rodent studies based on Guideline 870.3150
\+ Organ weight required for non-rodent studies.

II. RESULTS

A. OBSERVATIONS

1. **Clinical signs of toxicity** - Increased incidence and frequency of thinness was observed in the 1500 and 2250 ppm males (Table 2). Thinness was also noted in the females; however, the observations in this sex were unrelated to dose in incidence and frequency and were uncorroborated by decreases in body weights and body weight gains. All other clinical observations were minimal in incidence and frequency and were not attributed to treatment.

Table 2. Number of thin animals observed (mean # of animal days)[a] in dogs treated with TI-435 in the diet for up to 13 weeks[b]

Dose (ppm)				
0	325	650	1500	2250
Males				
0 (0)	0 (0)	0 (0)	2 (4.5)	2 (5.0)
Females				
1 (3.0)	0 (0)	0 (0)	1 (1.0)	3 (3.3)

a Data are presented as the number of affected animals (mean number of animal days). Mean number of animal days were calculated by adding the total number of days observed per group and dividing that sum by the number of animals in which the finding was noted.

b Data were obtained from Table 2A on page 48 and Appendix 2A on pages 169-172 and 175-188 of the study report (MRID 45422810); n=4.

2. **Mortality** - There were no unscheduled deaths during the study.

3. **Neurological Evaluations** - Reflexes (pupillary, extensor thrust, placement, righting, auditory startle, and patellar), gait, and pain perception were normal in all dogs at all time points tested.

B. BODY WEIGHT AND WEIGHT GAIN: Body weights were decreased (↓4-10%; not significant) in the 2250 ppm males throughout treatment (Table 3). Overall (weeks 1-13) body weight gains were decreased in these animals (0.3 ± 0.69 kg treated vs 1.1 ± 0.26 controls; $p \leq 0.05$). Males dosed with 1500 ppm gained 0.4 ± 1.74 kg treated vs 1.1 ± 0.26 controls. One male in this group (animal #G34993) lost 20% body weight over the course of the study. This animal displayed other signs of toxicity (anaemia, and thin body) and as such the body weight effect was considered to be treatment related. Body weights and body weight gains of the females were comparable to controls throughout the study.

Table 3. Mean (± SD) body weights and overall body weight gains (kg) in dogs treated with TI-435 in the diet for up to 13 weeks[a]

Study Week	Dose (ppm)				
	0	325	650	1500	2250
Males					
1	10.0 ± 0.97	9.4 ± 0.57	9.8 ± 0.19	9.8 ± 0.59	9.9 ± 1.00
2	10.2 ± 1.06	9.7 ± 0.70	10.1 ± 0.26	10.0 ± 0.33	9.8 ± 0.85 (↓4)
8	10.9 ± 1.20	10.7 ± 0.59	11.4 ± 0.77	10.9 ± 0.52	10.1 ± 1.00 (↓7)
13	11.1 ± 1.21	11.5 ± 0.91	12.2 ± 0.51	10.2 ± 1.28 (↓7)	10.2 ± 0.93 (↓8)
Overall (1-13) gain	1.1 ± 0.26	2.1 ± 0.34*	2.5 ± 0.34*	0.4 ± 1.74 (↓55)	0.3 ± 0.69*(↓72)
Females					
1	7.9 ± 0.44	8.1 ± 0.78	8.4 ± 0.48	8.2 ± 0.68	8.3 ± 0.45
2	8.0 ± 0.53	8.4 ± 0.74	8.8 ± 0.44	8.5 ± 0.64	8.2 ± 0.21
8	8.4 ± 1.10	8.7 ± 0.52	9.6 ± 0.60	9.2 ± 0.76	8.6 ± 0.38
13	8.9 ± 0.95	9.0 ± 0.76	10.0 ± 0.68	9.1 ± 0.70	9.0 ± 0.38
Overall (1-13) gain	1.0 ± 0.57	0.9 ± 0.61	1.6 ± 0.31	0.9 ± 0.69 (↓10)	0.7 ± 0.41 (↓30)

a Data were obtained from Tables 3 and 4 on pages 55-60 of the study report (MRID 45422810); n=4. Percent difference from controls, calculated by the reviewers, is included in parentheses.

* Significantly different from the control group at $p \leq 0.05$

C. FOOD CONSUMPTION

1. Food consumption - There were no treatment-related changes in food consumption in males or females. At 650 ppm, weekly group mean food consumption was increased ($p \leq 0.05$) in the males at week 9 (↑50%) and in the females at weeks 2 and 11 (↑31-47%); however, these increases were not adverse or dose-related.

2. Compound consumption - Group mean compound intake values (mg/kg/day) for the overall study are reported in Table 1.

3. Food efficiency - Food efficiency was not determined.

D. OPHTHALMOSCOPIC EXAMINATION: There were no treatment-related ophthalmological findings. A focal retinal fold was present in the right eye of a 650 ppm female; however, this lesion was present prior to dosing. There were no other visible lesions in the eyes of the males or females of any dose group.

E. BLOOD ANALYSES

1. Hematology - Hematocrit, red blood cells and hemoglobin were decreased in the 1500 and 2250 ppm males. The mean corpuscular hemoglobin concentration (MCHC) was increased (↑3%; $p \leq 0.05$) at week 13 in high dose males as well(Table 4). Although the decrease in red blood cell parameters did not reach levels of significance in the 1500 ppm dose, the reductions (hematocrit (↓14%), red blood cells (↓11%) and hemoglobin (↓13%) met the criteria for anaemia (≥10% decrease). The high dose males had reduced red blood cell parameters from control values (hematocrit (↓12%*), red blood cells (↓8%) and hemoglobin (↓9.2%). Two of the high dose males were considered anaemic by the reviewer, with greater than 10% reductions in all three red blood cell parameters. Taken together the hematological effects seen in the two highest doses were considered to be adverse in male animals.

A similar reduction in red blood cell parameters was seen in the 1500 ppm females (hematocrit (↓10%*), red blood cells (↓8%*) and hemoglobin (↓7*%), however high dose females did not display similar decreases in red blood cells. In the 2250 ppm females at week 13, decreases ($p \leq 0.05$) were observed in leukocytes (↓52%), specifically segmented neutrophils (↓61%), and monocytes (↓75%). There were no other hematological findings in the males or females that could be attributed to treatment. Based on these results, adverse hematological effects were seen in males dosed at 1500 and 2250 ppm and in females dosed at 2250 ppm.

Table 4. Selected mean (± SD) hematological findings in dogs treated with TI-435 in the diet for up to 13 weeks[a]

Hematological Parameter	Study week	Dose (ppm)				
		0	325	650	1500	2250
Males						
Hematocrit (%)	-1	45.0 ± 2.56	43.4 ± 2.32	42.0 ± 1.59	43.4 ± 1.73	45.7 ± 3.35
	5	42.9 ± 3.19	42.5 ± 2.25	41.0 ± 2.90	41.3 ± 1.49	41.5 ± 2.22
	13	43.1 ± 1.29	45.1 ± 2.02	41.9 ± 1.78	37.3 ± 8.66 (↓14)	40.3 ± 1.07* (↓12)
RBC (MI/UL)	-1	6.69±0.37	6.39±0.37	6.45±0.05	6.47±0.30	6.88±0.53
	5	6.48±0.54	6.3±0.33	6.29±0.39	6.19±0.29	6.38±0.37
	13	6.68±0.45	6.75±0.37	6.53±0.33	5.77±1.33 (↓11)	6.33±0.22 (↓8)
Hemoglobin (g/dL)	-1	14.7±.09	14.3±0.9	13.8±0.5	14.3±0.6	15.2±1.1
	5	14.3±1.2	14.2±0.7	13.6±0.9	13.8±0.5	14.0±0.7
	13	14.4±0.4	15.2±0.7	14.1±0.6	12.5±3.0(↓13)	13.8±0.4 (↓9.2)
MCHC (g/dL) [b]	-1	32.6 ± 0.57	33.0 ± 0.40	32.9 ± 0.42	33.1 ± 0.10	33.2 ± 0.10
	5	33.3 ± 0.49	33.4 ± 0.45	33.2 ± 0.31	33.4 ± 0.22	33.7 ± 0.35
	13	33.4 ± 0.58	33.6 ± 0.10	33.7 ± 0.32	33.5 ± 0.39	34.3 ± 0.29* (↑3)
Females						
Hematocrit (%)	-1	41.4± 0.8	44.4±2.9	40.3±4.2	41.3±1.9	42.5±1.1
	5	43.7 ±3.5	45±2.5	44.7±3.9	42.5±2.5	42.2±1.2
	13	42.1±1.8	44.6±1.1	43.1±1.9	37.2±1.9*(↓10)	40.7±1.4
RBC (MI/UL)	-1	6.16±0.2	6.51±0.4	6.11±0.4	6.2±0.3	6.39±0.2
	5	6.52±0.7	6.66±0.5	6.76±0.5	6.44±0.3	6.48±0.2
	13	6.29±0.2	6.63±0.3	6.58±0.2	5.71±0.3*(↓8)	6.35±0.3
Hemoglobin (g/dL)	-1	13.7±0.2	14.6±1.0	13.3±1.3	13.6±0.6	14.1±0.3
	5	14.7±0.9	15.2±0.8	15±1.4	14.3±0.8	14.2±0.6
	13	14.2±0.6	15.1±0.4	14.4±0.6	12.7±0.6*(↓7)	13.8±0.2
Leukocytes (10^3/L)	-1	13.3 ± 4.34	13.0 ± 3.41	15.8 ± 4.36	15.1 ± 6.39	15.7 ± 3.69
	5	11.4 ± 3.26	10.0 ± 3.05	8.6 ± 0.81	9.4 ± 0.98	12.9 ± 17.02
	13	9.9 ± 1.75	8.8 ± 2.14	8.5 ± 1.39	9.3 ± 1.07	4.8 ± 3.24* (↓52)
Segmented Neutrophils (10^3/L)	-1	9.1 ± 3.88	8.9 ± 2.84	10.8 ± 3.28	10.2 ± 4.91	11.1 ± 3.76
	5	7.0 ± 2.15	5.8 ± 2.01	5.1 ± 0.93	5.6 ± 1.07	9.0 ± 13.96
	13	6.2 ± 1.28	4.7 ± 0.93	5.0 ± 1.09	5.6 ± 1.06	2.4 ± 2.58* (↓61)
Monocytes (10^3/L)	-1	0.6 ± 0.42	0.8 ± 0.17	1.3 ± 0.61	1.1 ± 0.71	1.3 ± 1.06
	5	0.6 ± 0.22	0.5 ± 0.22	0.4 ± 0.24	0.5 ± 0.10	1.1 ± 2.12
	13	0.4 ± 0.10	0.4 ± 0.14	0.5 ± 0.16	0.4 ± 0.10	0.1 ± 0.15** (↓75)

a Data were obtained from Table 7 on pages 70-79 of the study report (MRID 45422810); n=4. Percent difference from controls, calculated by the reviewers, is included in parentheses.
b MCHC = Mean corpuscular hemoglobin concentration.
* Significantly different from the control group at $p \leq 0.05$.
** Significantly different from the control group at $p \leq 0.01$.

2. Clinical chemistry - Decreases ($p \leq 0.05$) were observed in the following clinical chemistry parameters in the females (Table 5): (i) alanine aminotransferase at 1500 (↓53-55%) and 2250 (↓65-72%) ppm at weeks 5 and 13; (ii) total protein at 2250 ppm at week 13 (↓17%); and (iii)

albumin at 1500 ppm at week 13 (↓11%) and at 2250 ppm at weeks 5 and 13 (↓8-13%). There were no other changes in clinical chemistry that could be attributed to treatment.

Table 5. Selected mean (± SD) clinical chemistry findings in female dogs treated with TI-435 in the diet for up to 13 weeks.[a]

Clinical Chemistry Parameter	Study Week	Dose (ppm)				
		0	325	650	1500	2250
Alanine aminotransferase (U/L)	-1	23 ± 4.9	29 ± 7.0	29 ± 3.6	23 ± 8.2	22 ± 1.3
	5	47 ± 18.5	39 ± 7.3	39 ± 14.3	22 ± 5.9* (↓53)	13 ± 3.0** (↓72)
	13	40 ± 7.4	43 ± 8.7	34 ± 11.3	18 ± 9.7* (↓55)	14 ± 7.2** (↓65)
Total protein (g/dL)	-1	5.5 ± 0.43	5.5 ± 0.33	5.0 ± 0.29	6.3 ± 0.46* (↑15)	5.3 ± 0.26
	5	6.0 ± 0.17	5.7 ± 0.38	5.5 ± 0.16	5.5 ± 0.51	5.3 ± 0.53
	13	5.9 ± 0.53	5.7 ± 0.22	5.5 ± 0.24	5.4 ± 0.22	4.9 ± 0.42* (↓17)
Albumin (g/dL)	-1	3.4 ± 0.21	3.5 ± 0.12	3.2 ± 0.27	3.4 ± 0.21	3.3 ± 0.21
	5	3.8 ± 0.32	3.8 ± 0.15	3.7 ± 0.18	3.5 ± 0.26	3.3 ± 0.31* (↓13)
	13	3.8 ± 0.21	3.8 ± 0.19	3.8 ± 0.22	3.4 ± 0.19* (↓11)	3.5 ± 0.13* (↓8)

a Data were obtained from Table 8 on pages 85 and 87 of the study report; n=4. Percent difference from controls, calculated by the reviewers, is included in parentheses.
* Significantly different from the control group at $p \leq 0.05$.
** Significantly different from the control group at $p \leq 0.01$.

F. URINALYSIS: There were no treatment-related effects on urinalysis.

G. SACRIFICE AND PATHOLOGY

1. Organ weight - There were no treatment-related effects on organ weights. Relative (to body) kidney weights were increased (↑25%; $p \leq 0.05$) in the 1500 ppm males; however, this finding was unrelated to treatment because it was not dose-related and because the absolute kidney weights in these animals were comparable to controls. All other organ weights in the treated males and females were comparable to controls.

2. Gross pathology - There were no treatment-related macroscopic findings. One 2250 ppm female had a small thymus; however, a similar incidence of this observation was noted in the control males. There were no other gross observations that could be attributed to treatment.

3. Microscopic pathology - There were no treatment-related microscopic findings. Lymphoid necrosis was observed in the ileum, cecum, colon, and rectum of a 2250 ppm female (#G35006); however, the necrosis was only minimal to slight in severity and present in a single animal. All other microscopic findings were either unrelated to dose or were minimal in incidence and/or severity.

III. DISCUSSION and CONCLUSIONS

A. INVESTIGATORS' CONCLUSIONS: It was concluded that the LOAEL was 1500 ppm based on increased incidence of thinness and subtle decreases in albumin, total protein, and alanine aminotransferase in dogs fed the 1500 or 2250 ppm diets. Additionally at 2250 ppm, initial decreases were noted in food consumption. Decreases were also observed in body weight gain (males) and leukocytes at this dose. Other findings possibly related to treatment included: (i) decreased thrombocytes noted in a single 1500 ppm male (#G34993) at weeks 5, 9, and 13 and a single 2250 ppm male (#G34999) at weeks 9 and 13; and (ii) anemia at week 13 and panleukopenia at weeks 9 and 13 in the same 1500 ppm male (#G34993). The NOAEL was 650 ppm.

B. REVIEWER COMMENTS: In the males, increased incidence and frequency of thinness was observed at 1500 and 2250 ppm. Males dosed with 1500 ppm gained 0.4 ± 1.74 kg treated vs 1.1 ± 0.26 controls. One male in this group (animal #G34993) lost 20% body weight over the course of the study. This animal displayed other signs of toxicity (anaemia, and thin body) and as such the body weight effect was considered to be treatment related. At 2250 ppm, this clinical finding was corroborated by decreases in body weights throughout treatment (↓4-10%; not significant) and in overall (weeks 1-13) body weight gains (0.3 ± 0.69 kg treated vs 1.1 ± 0.26 controls; $p \leq 0.05$). Additionally at this dose, two of the high dose males were considered anaemic by the reviewer, with greater than 10% reductions in all three red blood cell parameters. Mean corpuscular hemoglobin concentration (MCHC) was increased (↑3%; $p \leq 0.05$) at week 13 as well. These treatment related effects were observed in a pilot study, whereby dogs fed a diet with a slightly higher concentration of test substance (2500 ppm) in the 4-week study suffered mortality, decreased food consumption, body weight gains, erythrocytes, and leukocytes in addition to bone marrow hypocellularity, lymphoid depletion, and changes in clinical chemistry. Taken together the hematological effects seen in the two highest doses were considered to be adverse in male animals.

In the females, decreases ($p \leq 0.05$) were observed in leukocytes (↓52%), specifically, segmented neutrophils (↓61%), and monocytes (↓75%) at 2250 ppm at week 13. Alanine aminotransferase was decreased ($p \leq 0.05$) at 1500 (↓53-55%) and 2250 (↓65-72%) ppm at weeks 5 and 13; these decreases were of equivocal toxicological significance. Total protein was decreased ($p \leq 0.05$) at 2250 ppm at week 13 (↓17%). Albumin was decreased ($p \leq 0.05$) at 1500 ppm at week 13 (↓11%) and at 2250 ppm at weeks 5 and 13 (↓8-13%).

The LOAEL for this study is 1500 ppm in males and 2250 ppm in females (equivalent to 40.9/61.8 mg/kg/day in the males/females) based on thinness, decreased body weight, body weight gains, and anaemia in one male and on decreased white blood cells, albumin, and total protein in the females. The NOAEL is 650 ppm in males and 1500 ppm in females (equivalent to 19.3/42.1 mg/kg/day in the males/females).

The submitted study is classified as **acceptable/guideline** and satisfies the guideline requirements for a subchronic oral toxicity study in the dog (OPPTS 870.3150; OECD 409).

635

C. **STUDY DEFICIENCIES:** The following minor deficiencies were noted, but do not change the conclusions of this DER:

- Sorbitol dehydrogenase was not measured.
- The nose, larynx, and pharynx were not examined microscopically at sacrifice.

DATA FOR ENTRY INTO ISIS

Subchronic Oral Study - dogs (870.3150)

PC code	MRID #	Study type	Species	Duration	Route	Dosing method	Dose range mg/kg/day	Doses tested mg/kg/day	NOAEL mg/kg/day	LOAEL mg/kg/day	Target organ(s)	Comments
044309	45422810 45422808 45422811	subchronic	dog	90 days	oral	diet	9.2-61.8	0/0, 9.2/9.6, 19.3/21.2, 40.9/42.1, 58.2/61.8 M/F	40.9	58.2	decr. BW, BWG, hematology, clinical chemistry	

637

HED Records Center Series 361 Science Reviews - File R107689 - Page 652 of 721

APPENDICES

APPENDIX I

Four-Week Dietary Toxicity Study with TI-435 in Dogs

Since this is a range-finding study, only a summary is provided in order to confirm the adequacy of the dose selection rationale used in subsequent studies.

In this non-guideline oral toxicity study (MRID 45422808), TI-435 (clothianidin; 95.2-95.4% a.i.; Lot# 30037120) was administered in the diet to 3 beagle dogs/sex/group at nominal doses of 0, 1250, 2500, or 5000 ppm (equivalent to 0/0, 34.3/35.8, 36.9/53.5, 62.4/57.4 mg/kg/day for the males/females) for up to 4 weeks.

Beginning in week 3, a total of eight unscheduled deaths occurred at 2500 and 5000 ppm: (i) on day 18, one 5000 ppm male was found dead and one 2500 ppm male was sacrificed *in extremis*; (ii) on day 19, one male and one female at 5000 ppm were sacrificed *in extremis*; (iii) on day 22, the remaining 3 animals in the 5000 ppm group were sacrificed; and (iv) on day 32, one 2500 ppm female was sacrificed *in extremis*. Scheduled sacrifice occurred on day 32 for the males and day 33 for the females. Total food consumption was decreased (↓18-59%) at 2500 and 5000 ppm. Body weight gains were decreased in these animals: (i) -0.7 kg in the 2500 ppm males vs 0.8 kg in controls; (ii) 0.1 kg in the 2500 ppm females vs 0.7 kg in controls; and (iii) individual cumulative body weight losses ranging from 1.0 to 1.7 kg in the 5000 ppm group at the beginning of week 4. Thus, all surviving animals in this group were terminated in week 4.

Dose-dependent decreases (not significant) were observed in the following hematological parameters, generally during weeks 3 and 4: (i) leukocytes in the 2500 ppm males (↓35-47%) and females (↓31-45%) and in the 5000 ppm males (↓31-56%) and females (↓73-77%); (ii) segmented neutrophils in the 2500 ppm males (↓42-58%) and females (↓25-54%) and in the 5000 ppm males (↓37-60%) and females (↓91-93%); (iii) lymphocytes in the 2500 ppm males (↓22%; week 3 only) and females (↓20-26%; weeks 1, 3, and 4) and 5000 ppm males (↓53%; week 3 only) and females (↓32-44%; weeks 1, 3, and 4); and (iv) erythrocytes, hemoglobin, and hematocrit in the 5000 ppm males (↓5-9%). Generalized hypocellularity and congestion/hemorrhage were observed in the bone marrow of these animals. Lymphoid depletion of the spleen, ileum, thymus, and mesenteric lymph nodes was also observed in these animals.

Several clinical chemistry parameters were also decreased during weeks 3 and 4: (i) glucose in the 5000 ppm males (↓16-17%); (ii) alanine aminotransferase in the 1250 ppm females (↓42-45%), 2500 ppm males (↓54-57%) and females (↓63-73%), and 5000 ppm males (↓28-57%) and females (↓13-53%); (iii) total protein in the 5000 ppm males (↓8-14%) and 2500 and 5000 ppm females (↓8% each; week 3 only); (iv) albumin in the 5000 ppm males (↓18-30%) and 2500 (↓13% each week) and 5000 ppm females (13%; week 4 only); (v) albumin/globulin ratio in the 5000 ppm males (↓27-40%) and 2500 and 5000 ppm females (↓29-34%; week 4 only). Additionally in the 5000 ppm males at weeks 3 and 4, increases were observed in alkaline phosphatase (↑88-158%) and total cholesterol (↑85-127%).

The LOAEL for this study is 2500 ppm (equivalent to 36.9/53.5 mg/kg/day in M/F) based on mortality, decreases in food consumption, body weight gains, erythrocytes, and leukocytes and on bone marrow hypocellularity, lymphoid depletion, and changes in clinical chemistry. The NOAEL is 1250 ppm (equivalent to 34.3/35.8 mg/kg/day in M/F). Based upon the results of this 4-week range-finding study, the doses summarized in Table 1 were selected for the 13-week study.

The submitted study is classified as an **acceptable/non-guideline** study and satisfies the requirements for which it was intended as a dose range-finding study.

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

APPENDIX II

Palatability Pilot Study for Dietary Concentrations of TI-435 in Dogs

Since this is a pilot study, only a summary is provided in order to confirm the adequacy of the dose selection rationale used in the 4-week range-finding study.

In this non-guideline oral toxicity study (MRID 45422811), TI-435 (clothianidin; 95.2% a.i.; Lot# 30037120) was administered in the diet to 2 female beagle dogs/group (control and treated). Dogs in the treated group were given diets containing concentrations of 3000 ppm (days 1 through 3), 4000 ppm (days 4 through 8), and 5000 ppm (days 9 through 11), while dogs in the control group were given the basal diet for days 1 through 11.

Food consumption was dose-dependently decreased at 3000 (↓35%), 4000 (↓57%), and 5000 (↓63%) ppm. Body weight changes averaged -0.85 kg in the treated group compared to +0.25 kg in the control group during administration of the 3000 and 4000 ppm diets and -0.25 kg in the treated group compared to -0.10 kg in the control group during administration of the 5000 ppm diet. However, it could not be determined within the time frame of the current study whether improved acceptance of diet concentrations up to 5000 ppm would occur with time in a longer term study. Therefore, the maximum dietary concentration of 5000 ppm was selected for the 4-week range-finding study.

The submitted study is classified as an **acceptable/non-guideline** study and satisfies the requirements for which it was intended as a dose range-finding study.

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

PMRA ARLA

for Reviewer: Scott hancock , Date May 28 /02
Pamela M Hurley

STUDY TYPE: Primary Dermal Irritation -Rabbits; OPPTS 870.2500 [§81-5]: OECD 404.

TEST MATERIAL (PURITY): TI-435-CCMT-Adduct (99.4%)

SYNONYMS: Clothianidin technical

CITATION: Leuschner, P.J. (1999). Acute Skin Irritation Test (Patch Test) of TI-435-CCMT-Adduct in Rabbits. LPT Laboratory of Pharmacology and Toxicology. Harnburg, Germany. Laboratory report number 9300/391/95, November 18, 1999. Unpublished. MRID 45422813.

SPONSOR: Bayer AG, Department of Toxicology, Wuppertal, Germany.

EXECUTIVE SUMMARY: In a primary dermal irritation study, 3 male, young adult, Himalayan Rabbits were dermally exposed to 0.5g of TI-435-CCMT-Adduct (99.4%) in a water paste for 4 hours; applied to approx 6 cm^2 of the lower back of each rabbit. Animals then were observed for 3 days. Irritation was scored by the method of Draize.

The test substance TI-435-CCMT-Adduct (99.4%) produced no dermal irritation or signs of toxicity during the course of the observation period. Erythema and Edema scores were 0 for all animals, for all times tested, therefore the MAS score for 24, 48, and 72 hours was 0. There were no clinical signs of toxicity or ill health in any rabbit during the observation period.

This substance is **non-irritating to the skin**. [EPA Toxicity Category IV].

This study is classified as acceptable and satisfies the guideline requirement for a primary dermal irritation study (81-5; OECD 404) in the Rabbit.

COMPLIANCE: Signed and dated GLP, Quality Assurance, and No Data Confidentiality statements were provided.

I. MATERIALS AND METHODS

A. MATERIALS:

1. **Test Material:** TI-435-CCMT-Adduct (99.4%)
   Description: Solid
   Lot/Batch #: 17007/99
   Purity: 99.4 % a.i.

2. **Vehicle and/or positive control:** 0.5g of test substance was made into a paste using 0.1 ml deionized water.

3. **Test animals:**

Species:	Rabbit	
Strain:	Himalayan	
Age/weight at treatment:	5.5 months old/ 2.4 to 2.6 kg	
Source:	LPT Laboratory of Pharmacology and Toxicology KG	
Housing:	Individually in wire mesh cages	
Diet:	Altromin 2023, GmbH, D-32791 Lage/Lippe, *ad libitum*	
Water:	Tap Water *ad libitum*	
Environmental conditions:	**Temperature:**	20 ± 3°C
	Humidity:	50 ± 20%
	Air changes:	Not notedr
	Photoperiod:	12hrs dark/ 12hrs light
Acclimation period:	20 days	

B. STUDY DESIGN and METHODS:

1. **In life dates** - Start: November 4, 1999 End: November 7, 1999

2. **Animal assignment and treatment** - Animals (3 male) were given a single 500 mg dose of TI-435-CCMT-Adduct (99.4%) dermally (shaved, intact, moistened skin) to a 6 cm^2 area, for a 4 hour period. The area was covered with a gauze patch, which was held in contact with the skin by means of a semi-occlusive dressing. After removal of the wrap, animals were observed at 1, 24, 48 and 72 hours following testing. Irritation was scored according to the Draize scale.

II. RESULTS AND DISCUSSION:

A. The test substance TI-435-CCMT-Adduct produced no dermal irritation or signs of toxicity during the course of the observation period. Erythema and Edema scores were 0 for all animals, for all times tested, therefor the calculated Mean Average Score (MAS) score for 24, 48 and 72 hours post-dosing was found to be 0. This substance is non-irritating to the skin and no label comments are required. There were no clinical signs of toxicity or ill health in any rabbit during the observation period.

Conclusions: A single semi-occluded application of TI-435-CCMT-Adduct to intact rabbit skin for four hours did not elicit any irritation reactions.

Table 1: Individual and Mean Animal Skin Irritation Scores According to the Draize Scheme

Time	Erythema				Oedema			
Rabbit Number	1	2	3	Mean	1	2	3	Mean
1 h	0	0	0	0	0	0	0	0
24 h	0	0	0	0	0	0	0	0
48 h	0	0	0	0	0	0	0	0
72 h	0	0	0	0	0	0	0	0
Mean of 3 time points (24, 48, 72 h)	0	0	0	0	0	0	0	0
Day when score = 0	0	0	0	0	0	0	0	0

MAS = 0
MIS = 0

B. Deficiencies - None noted.

PMRA ARLA

for Reviewer: Scott Hancock , Date May 28/02

Pamela Hurley

TXR No. 0050321

STUDY TYPE: Dermal Sensitization - Guinea Pig; OPPTS 870.2600 [§81-6]; OECD 406.

TEST MATERIAL (PURITY): TI-435-Triazan (95.6%)

SYNONYMS: Clothianidin Intermediate

CITATION: Vohr, H.W. (2000). TI-435-Triazan Study for the Skin Sensitization Effect in Guinea Pigs (Guinea Pig Maximization Test According to Magnusson and Kligman).Bayer AG, Department of Toxicology, Wuppertal, Germany. Laboratory report number T9069608, August 23, 2000. Unpublished. MRID 45422821

SPONSOR: Takeda Chemical Industries Ltd. Agro Company, Chuo-ku, Tokyo, Japan.

EXECUTIVE SUMMARY: In a dermal sensitization study with TI-435-Triazan in polyethylene glycol 400, young adult female Hartley Guinea pigs were tested using the method of Magnusson-Kligman Maximization.

No clinical observations of ill health or toxicity were noted during the study for either the main study or positive control groups. After the intradermal induction the animals in the control group and in the test item group showed strong effects up to encrustation at the injection sites of the first induction. Challenge with a 12.5% solution of the test material resulted in a positive sensitization reaction in 60% of the animals tested, while control animals displayed no signs of dermal irritation. Based on the results of the study, TI-435-Triazan was considered to be a dermal sensitizer.

In this study, TI-435-Triazan (95.6%) **is a dermal sensitizer.**

This study is classified as acceptable and satisfies the guideline requirement for a dermal sensitization study (81-6; OECD 406) in the Guinea Pig.

COMPLIANCE: Signed and dated GLP, Quality Assurance, and No Data Confidentiality statements were provided.

I. MATERIALS AND METHODS

A. MATERIALS:

1. **Test Material:** TI-435-Triazan (95.6%)

Description:	White Crystalline Solid
Lot/Batch #:	17003/99
Purity:	95.6 % a.i.
Cas #	141856-57-7

2. **Vehicle and/or positive control:** Polyethylene glycol 400

3. **Test animals:**

Species:	Guinea Pig	
Strain:	Hsd:Poc:DH, female	
Age/weight at start:	3-5 weeks old/ 309-365g	
Source:	Harlan Winkelmann GmbH Laboratory Animal Breeders, Borchen	
Housing:	5 per polypropylene cage.	
Diet:	Altromin 3020 Maintenance Diet for Guinea Pigs, Altromin GmbH, Lage ad libitum	
Water:	Tap Water *ad libitum*	
Environmental conditions:	**Temperature:**	19-25°C
	Humidity:	40-60%
	Air changes:	10/ hour
	Photoperiod:	12hrs dark/ 12hrs light
Acclimation period:	7 days	

B. STUDY DESIGN and METHODS:

1. **In life dates** - Start: April 25, 2000 End: May 19, 2000

2. **Animal assignment and treatment** - The Magnusson-Kligman Maximization Test was used to assess the sensitization potential of the test chemical TI-435-Triazan. The dose levels used for induction and challenge were determined from a preliminary screen, whereby the dose which caused moderate irritation was used for induction and the highest non-irritating concentration was chosen for the challenge dose. Guinea pigs were observed daily for clinical signs of reaction to treatment. Animals were weighed on day 1 and day 24, following the completion of the challenge phase.

Induction:
Intradermal Injection:
The dorsum of each test and control guinea pig was clipped on the day before treatment commenced. The denuded area was confirmed to be free from injury prior to injections. 10 animals were given 0.1 ml injections of the test substance (2.5% w/v) dissolved in PEG 400 with/without Freund's Complete Adjuvant (FCA), or FCA in physiological saline. Irritation was recorded by group on day 2. Vehicle controls were handled and prepared similar to the test group, however test material was not included in the injections.

Topical Application:
On day 7, animals were clipped, while on day 8 the area of skin including the intradermal injection sites

were subject to application of a 2 x 4 cm patch treated with approximately 0.5 mL of 25% m/m TI-435-Triazan in PEG 400 (test animals) or PEG 400 alone (control group). Occlusion of the treated skin was effected by successive layers of aluminium foil and Oraband adhesive tape. The patches and dressings remained in place for 48 hours. Irritation or other dermal changes at the sites of occluded topical application were recorded by group on Day 11.

Challenge Stage:
The dorsal region and the left flank of the animals were shorn one day prior to the challenge. During the challenge a hypoallergenic patch loaded with 0.5 mL of a 12% test item formulation was placed on the left flank (caudal) of the animals of the test item group and the control group and held securely in place on the skin with a ORABAND@ self- adhesive tape for 24 hours. A patch loaded only with the vehicle was placed also on the left flank (cranial) as control. Dermal responses to challenge were assessed approximately 24 and 48 hours after removal of the chambers. Responses to challenge were recorded individually.

Data Interpretation:
Dermal reactions to challenge among test animals were compared with those of the control animals. The response of each test animal was classified as positive or negative according to the following system:

The criterion for existence of sensitization was taken to be the occurrence of skin reactions in the test item group animals at a higher incidence and greater intensity than in the control animals. A substance is interpreted to be sensitizing if by comparison with the control group 30 % or more of the test group animals reacted positive.

II. RESULTS AND DISCUSSION:
No clinical observations of ill health or toxicity were noted during the study for either the main study or positive control groups. The body weights of animals in the treatment group were similar to control animals at the end of the study period.

A. <u>Induction reactions and duration</u> -
Injection:
At 48 hours following injection control animals showed red or white wheal with surrounding encrustation. Animals in the test group showed red wheal with red surrounding and encrustation.

Topical:
No irritation reactions were apparent among the test animals following topical application of 25%m/m TI-435-Triazan.

B. <u>Challenge reactions and duration</u> -
Naive control challenged with 12.5% TI-435-Triazan displayed no dermal irritation at 48 and 72 hours post-application. Test animals had incidences of 6/10 and 2/10 of erythema reaction to challenge with 12.5% TI-435-Triazan at 48 and 72 hours post-application respectively.

<u>Conclusion:</u>

"After the intradermal induction the animals in the control group and in the test item group showed strong effects up to encrustation at the injection sites of the first induction.

The challenge with the 12% test item formulation led to skin effects (grade 1) in 6 of 10 animals (60%) in the test item group and no skin effects were seen in the control group animals.

In summary, by comparing the results in the treatment group and in the control group under the conditions of the maximization test and with respect to the evaluation criteria, the test item therefore exhibits a skin-sensitization potential." The reviewer agrees with the study author, in that the test material demonstrates sensitization potential under the conditions of the study.

E. Deficiencies - The individual animal irritation scores for the induction phase of the experiment were not provided in the study report. The range finding study demonstrated only slight irritation due to dermal application of a 50% solution of the test material. Similarly, the range finding study for the challenge phase was only tested up to a 12.5% concentration, which was non-irritating. Based on these results, the test material should have been tested at a higher concentration for the main study. However, since the study demonstrated a positive sensitization reaction, these deficiencies did not negatively impact on the interpretation of the data.

DATA EVALUATION RECORD

CLOTHIANIDIN (TI435)/044309

STUDY TYPE: NON-GUIDELINE (Neurotoxicity and Pharmacology)
Rat, mouse (*in vivo*); guinea pig (*in vitro*)
MRID 45422823

Prepared for

Health Effects Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
1921 Jefferson Davis Highway
Arlington, VA 22202

Prepared by

Toxicology and Hazard Assessment Group
Life Sciences Division
Oak Ridge National Laboratory
Oak Ridge, TN 37831
Task Order No. 02-44G

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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.

PMRA/EPA Reviewers: Scott Hancock and Pamela Hurley **Signature:** Pamela M Hurley
EPA Work Assignment Manager:Ghazi Dannan, Ph.D. **Signature:** Ghazi Dannan
Registration Action Branch 3, Health Effects Division (7509C) Date 12/10/04 Template version 11/01

DATA EVALUATION RECORD
TXR#: 0050321

STUDY TYPE: Non-Guideline Neurotoxicity - Rats, mice (*in vivo*); guinea pig (*in vitro*)

PC CODE: 044309 **DP BARCODE:** D282578
SUBMISSION NO.: S614462

TEST MATERIAL (PURITY): TI-435 (Clothianidin) (95.5%)

SYNONYMS: (E)-1-(2-Chloro-5-thiazolylmethyl)-3-methyl-2-nitroguanidine

CITATION: Unakami, S. (2000). Pharmacological studies on TI-435. Kashima Laboratory, Mitsubishi Chemical Safety Institute, Ltd., 14 Sunayama, Hasaki-machi, Kashima-gun, Ibaraki, Japan. Laboratory Project ID Study No. 9L668, Bayer Corporation Agriculture Division Report No. 110231, January 20, 2000. MRID45422823. Unpublished.

SPONSOR: Takeda Chemical Industries, Ltd., Agro Company, 13-10 Nihonbashi 2-chome, Chuo-ku, Tokyo 103, Japan.

EXECUTIVE SUMMARY: In a non-guideline study that addressed some aspects of neurotoxicity and pharmacology (MRID 45422823), groups of three male CD-1 mice were tested for acute neurobehavioral effects following single gavage doses of TI-435 (95.5% a.i.; vehicle, 5% arabic gum) at doses of 0 (5% arabic gum), 12.5, 25, 50, 100, 200, or 400 mg/kg bw. Clinical signs and mortality were monitored from 0.5 hours to 24 hours post-treatment. The following tests were also conducted with groups of eight or ten male CD-1 mice: prolongation of hexobarbital-induced sleeping time following doses of 25, 75, or 225 mg/kg bw; induction of tonic flexor and tonic extensor convulsions with sub-threshold electroshock following doses of 6.25, 12.5, 25, 75, or 225 mg/kg bw; synergistic effect of pentylenetetrazol on convulsions following doses of 25, 75, or 225 mg/kg bw; effect on intestinal transport following doses of 25, 75, or 225 mg/kg bw; and effect on muscle strength following doses of 25, 75, or 225 mg/kg bw. Groups of six male CD rats were tested for effects on body temperature following gavage doses of 30, 100, 300, 1000, or 3000 mg/kg bw. In other tests, groups of four male CD rats were tested for effects on blood pressure and heart rate following doses of 300, 1000, or 3000 mg/kg bw, and groups of six male CD rats were tested for effects on prothrombin and activated thromboplastin times following doses of 300, 1000, or 3000 mg/kg bw. In an *in vitro* study, effects of agonist-induced contraction (acetylcholine, histamine, and barium) on the isolated ileum of guinea pigs was tested in the presence of 10^{-6}, 10^{-5}, or 10^{-4} mol/L of TI-435. Cholinesterase activity was not

determined. Animals were not examined grossly or microscopically for neuropathological lesions.

In the clinical and neurobehavioral assessment, no effects were observed in mice at doses of 12.5 or 25 mg/kg. At 50 mg/kg, decreased spontaneous motor activity was observed from all three mice at the 0.5-hour observation and tremors and deep respirations were observed in a single mouse at the 0.5- or 1-hour observations. All of these effects were graded slight. Additional clinical signs including decreases in reactivity, grooming, and muscle tone; prone position; staggering gait; mydriasis, and hypothermia were observed at the higher doses, with effects becoming more severe with increased dose. These signs had disappeared in the lower dose groups at the 24-hour observation, but tremor, staggering gait and a decrease in spontaneous locomotor activity were observed in one mouse in the 400 mg/kg group at the 24-hour observation. Cyanosis was observed in another mouse at 400 mg/kg prior to death at 3 hours posttreatment.

Hexobarbital-induced sleeping time was prolonged at 225 mg/kg, tonic flexor and extensor convulsions with sub-threshold shock were induced at ≥25 mg/kg, intestinal transport was suppressed at ≥75 mg/kg, and muscle strength was suppressed at ≥225 mg/kg. There was no effect of treatment on pentylenetetrazol-induced convulsions. In studies with rats, body temperature was decreased at ≥300 mg/kg. There was no effect of treatment on blood coagulation parameters or on blood pressure and heart rate in rats. Treatment with TI-435 had no dose-related effect on contractile response to various agonists in isolated guinea pig ileum preparations. A NOAEL for acute neurotoxicity (for acute reference dose consideration during subsequent risk assessment) is 25 mg/kg bw.

Based on the effects seen in this study, the LOAEL for acute neurotoxicity of TI-435 in male CD-1 mice was 50 mg/kg bw (based on transient signs of decreased spontaneous motor activity, tremors, and deep respirations), with a NOAEL of 25 mg/kg bw.

This neurotoxicity/pharmacology study is classified as **Acceptable/Non-guideline** and does *not* satisfy the guideline requirement for an acute neurotoxicity study in rats (870.6200a; OECD 424). The study was not intended to fulfill 870.6200a guidelines. Major deviations from 870.6200a guidelines include failure to use the established protocol for neurotoxicity and motor activity testing, the use of the mouse as the test species, use of a single sex, and use of only 3 mice/test group.

COMPLIANCE: Signed and dated Quality Assurance and Data Confidentiality statements were provided. The study was conducted in accordance with Japanese GLP guidelines.

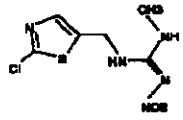
I. MATERIALS AND METHODS

A. MATERIALS:

1. Test material: TI-435 (technical)

Description:	Physical description not provided; expiration date: 2/22/02
Lot/Batch #:	Lot no. 30037120 (Takeda Chemical Industries, Ltd.)
Purity:	95.5% a.i. (HPLC)
CAS # of TGAI:	210880-92-5 (formerly 205510-53-8)

Structure:



2. Vehicle and/or positive control: Vehicle: 5% arabic gum for *in vivo* experiments; DMSO for *in vitro* experiments.

3. Test animals:

Species:	Mouse, rat, guinea pig
Strain:	*in vivo* experiments: male CD-1 (ICR) SPF mice; male CD (SD)(SPF) rats *in vitro* experiments: male Hartley (SPF) guinea pigs
Age/weight at dosing:	Mice (neurobehavioral assessment): 5 weeks/26.2-31.5 g; Mice (other studies): 5 weeks/22.7-34.2 g Rats (body temperature): 5 weeks/136.5-163.3 g Rats (blood pressure) 7 weeks/244.9-318.5 g; (prothrombin time) 5 weeks/158.6-174.1 g Guinea pigs: 6-8 weeks of age/422-448 g
Source:	Mice and rats: Charles River Japan, Inc.; guinea pigs: Japan Slc Co. Ltd.
Housing:	Mice: 4-5/cage in polycarbonate cages Rats: 1-2/cage in hanging type stainless steel cages Guinea pigs: 2-3/cage in hanging-type aluminum cages
Diet:	Mice and rats: MF pelleted diet (Oriental Yeast Co., Ltd)., *ad libitum* Guinea pigs: LRC4 pelleted diet (Oriental Yeast Co., Ltd), *ad libitum*
Water:	Filtered and UV irradiated tap water, *ad libitum*
Environmental conditions:	**Temperature:** 22-23°C **Humidity:** 45-71% **Air changes:** 15/hr **Photoperiod:** 12 hrs dark/12 hrs light
Acclimation period:	5-19 days
Structure:	Not available

B. STUDY DESIGN:

1. In life dates: Start: October 21, 1999; End: November 26, 1999 (various tests)

2. Animal assignment, treatment, and dose selection rationale:

a. Neurobehavioral assessment: For the *in vivo* neurotoxicity study, male mice were randomly assigned to the test groups noted in Table 1. Mice were administered a single oral dose (by gavage) of the test material in 5% arabic gum. An overnight fast was not

mentioned in this part of the study. Mice were observed prior to and at 0.5, 1, 3, 6, and 24 hours after oral dosing.
Dose selection for the acute neurotoxicity study in mice was based on a range-finding study in which 3 mice/group were administered the test material at doses of 200, 280, 390, 550, and 770 mg/kg bw. Clinical signs and mortality were monitored at 0.5, 1, 2, 4, 6, 8, and 12 hours, and daily for 7 days after administration. The following results were reported: 200 mg/kg: decreased locomotor activity, prone posture, and deep respirations; 280 mg/kg: all of the signs observed at 200 mg/kg plus staggering gait; 300 mg/kg: all of the signs observed at 200 ppm plus tremors. At 550 and 700 mg/kg, additional signs including decreased body temperature and cyanosis were observed; mortalities in the 550 and 700 mg/kg dose grous were 1/3 and 2/3, respectively, at 4 hours to 1 day post-treatment. No toxic signs or mortalities were observed in any group during days 2-7 after treatment. From these results, a high-dose of 400 mg/kg and lower doses of 50, 100, and 200 mg/kg were chosen for the main study. As clinical signs were observed at all of the selected doses for the main study, additional dose groups of 12.5 and 25 mg/kg bw were added following start of the main study. Survivors were sacrificed with carbon dioxide and a necropsy was not performed. Neurotoxicity tests are further described in Section C.5.a.

In a range-finding study, 3 rats/group were administered the test material at single gavage doses of 1750, 2280, 2960, 3850, or 5000 mg/kg bw. Observations were performed at the same time periods as in the mouse range-finding study. Clinical signs ranged from decreases in motor activity and body temperature and closed eyes at 1750 mg/kg to cyanosis, convulsion and death at 3850 mg/kg. From these results, the high dose was set at 3000 mg/kg; lower doses were selected by reducing 3000 mg/kg by a factor of 3, to 1000 and 300 mg/kg. In the main neurotoxicity study, groups of six 5-week-old rats were administered 300, 1000, or 3000 mg/kg in 5% arabic gum by gavage (Table 1). Rectal temperature was measured at 0.5, 1, 3, and 6 hours post administration. As body temperature was affected at all doses selected for the main study, additional groups of 6 rats/group were administered doses of 0, 30, or 100 mg/kg in 5% arabic gum by gavage. Rectal temperatures were checked at the above times.

TABLE 1. Study design for neurotoxicity main studies							
Species/Experimental Parameter	Dose group (mg/kg bw)						
	Control	12.5	25	50	100	200	400
Male Mice							
Clinical signs, activity	3	3	3	3	3	3	3
Species/Experimental Parameter	Dose group (mg/kg bw)						
	Control		30	100	300	1000	3000
Male Rats							
Body temperature	6		6	6	6	6	6

Five additional neurotoxicity/pharmacology studies were performed with mice.

b. **Anesthetic effect:** Groups of 8 male mice were administered 25, 75, or 225 mg/kg bw of the test material in 5% arabic gum by gavage. At 1 hour after administration, 80 mg/kg of hexobarbital was injected intraperitoneally. The sleeping mice were placed on a pad heated to 37°C and the time between disappearance to recovery of the righting reflex was monitored.

c. **Effect of sub-threshold electroshocks:** Groups of 10 male mice were administered the test material in 5% arabic gum by gavage at doses of 0, 25, 75, and 225 mg/kg bw. At 1 hour post-administration, electrodes were attached to the cornea of each mouse and an electric shock of 8.0 mA was delivered for 0.6 seconds (pulse duration: 5 msec; stimulation interval: 10 msec; instrument: MK-800 Muromachi Kikai Co., Ltd.). Appearance of tonic flexor and tonic extensor convulsions were monitored. As only positive results were observed at these doses, lower doses of 6.25 and 12.5 mg/kg bw, as well as a control group were also tested.

d. **Stimulation by pentylenetetrazol:** Groups of 10 male mice were administered gavage doses of 0, 25, 75, or 225 mg/kg bw in 5% arabic gum. After 1 hour, pentylenetetrazol at 55 mg/kg was injected subcutaneously. The appearance of clonic and tonic extensor convulsions was monitored for 30 minutes.

e. **Effect on gastrointestinal transport:** Following a 19-hour fast, 8 mice/group were administered the test material at 0, 25, 75, or 225 mg/kg in 5% arabic gum. After 1 hour, a 5% charcoal suspension in 5% arabic gum at a volume of 0.2 mL/animal was administered orally. At 0.5 hours following the charcoal administration, the animals were sacrificed by cervical dislocation and the gastric tube was isolated. The transfer rate of charcoal (%) was measured as (the length from the pylorus to the farthest point reached by the charcoal/total intestine length) x 100.

f. **Effect on muscle strength:** Groups of 8 mice were administered 0, 25, 75, or 225 mg/kg in 5% arabic gum. Prior to and at 1, 3, and 6 hours post-treatment, mice were tested on their ability to grasp a horizontal wire with their hind paws within 10 seconds of placing their fore paws on the wire. Only those mice that had successfully completed the test pre-exposure were treated and retested.

Two additional studies were performed with rats.

g. **Effect on blood pressure and heart rate:** Groups of four 7-week-old rats were administered 0, 300, 1000, or 3000 mg/kg in 5% arabic gum. Blood pressure was determined prior to and at 0.5, 1, 3, and 6 hours after administration. Blood pressure was measured by plethysmography by inserting the tail into the cuff of the instrument. Heart rate was monitored at the same time. Because effects were seen at these doses, additional groups of 4 rats were administered the arabic gum vehicle or 100 mg/kg and blood pressure and heart rate were monitored at the above times.

h. **Effect on blood coagulation:** Groups of six 5-week-old rats were administered 0, 300, 1000, or 3000 mg/kg in 5% arabic gum. One hour later blood samples were taken from the inferior vena cava under ether anesthesia and tested for prothrombin and activated thromboplastin times.

One *in vitro* study was performed with 6- to 8-week-old guinea pigs.

i. **Effect on muscle contraction:** Sections of guinea pig ileum were isolated and bathed in Krebs solution. Acetylcholine (10^{-6} mol/L), histamine (3 x 10^{-6} mol/L), and barium (10^{-3} mol/L) were used as contractile agonists. Once the preparations were stabilized, DMSO followed by the test material at 10^{-6}, 10^{-5}, and 10^{-4} as final concentrations (4 replicates of each) were applied in succession. Each treatment was for 5 minutes, prior to and following the agonist treatment. Contraction was measured with an isotonic transducer (NEC San-ei, Inc.).

3. **Test substance preparation and analysis:** Arabic gum was dissolved in water to produce a 5% solution; all arabic gum solutions were used within two weeks of preparation. For the *in vivo* experiments, appropriate amounts of the test substance were suspended in the 5% arabic gum solution to make dosing solutions of 0.625 to 500 mg/mL. The dose volume was 10 mL/kg. No further details were given. For the *in vitro* experiment, solutions of 10^{-3}, 10^{-2}, and 10^{-1} mol/L solutions were prepared. Application solutions were reduced to 10^{-6}, 10^{-5}, and 10^{-4} mol/L (details not provided) and applied 4 times to the isolated guinea pig ileum in a volume of 0.02 mL. All dosing solutions were prepared on the day of administration. Stability, concentration, and homogeneity of the dosing solutions were not verified.

4. **Statistics:** Qualitative data were presented as incidences, usually with severity ratings. Incidences were analyzed by the chi-square test. Quantitative data were expressed as mean ± standard deviation. Groups were tested for homogeneity of variance with Bartlett's test. When the variance was homogenous, the data were analyzed by a one-way analysis of

variance. When differences were significant, the data were analyzed by Dunnett's mean multiple comparison test. For non-homogeneity of variances, the Kruskal-Wallis test was applied. For groups with significant differences, the data were analyzed by Dunnett's mean multiple comparison test. For homogeneity of variance between two groups, the F test was performed. When the variance was homogenous, the data were analyzed by the Students t-test; when the variances was not homogenous, the data were analyzed by Aspin-Welch's t-test. Significance was flagged at the 0.05 level. The Reviewer considers the analyses used to be appropriate.

C. METHODS/OBSERVATIONS:

1. **Mortality and clinical observations:** In the neurobehavioral assessment with male mice, the animals were observed at 0.5, 1, 3, and 6 hours and 1 day after treatment for mortality and morbidity. At these times, a neurobehavioral assessment was also performed (see FOB below). Additional clinical observations were not mentioned. After the observations, animals were sacrificed by carbon dioxide asphyxiation.

2. **Body weight:** Animals were not weighed.

3. **Food consumption:** Food consumption was not monitored.

4. **Cholinesterase determination:** Cholinesterase activity was not determined.

5. **Neurobehavioral assessment:**

   a. **Functional observational battery (FOB):** An FOB according to OPPTS 8706200a guidelines was not utilized. Behavior of male mice was monitored according to Irwin's multiple observation method (Animal and Clinical Pharmacological Techniques in Drug Evaluation, J.H. Nodine and P.E. Siegler, eds., Year Book Medical Publishers, Chicago, 1964, pp. 36-54). Observations were performed at 0.5, 1, 3, and 6 hours and 1 day after administration of the test substance. The observational and behavioral endpoints are listed in the table below. The severity of each sign was graded on a 5-point scale of no change, slight, moderate, marked, and extreme. Results were reported as slight (+), moderate (++), or severe (+++). No information on the place of observations, environmental conditions, technicians used for the observations, duration of the observations, or equipment used was given. Because the place(s) of observation was not mentioned, the observations were arbitrarily assigned to the sections of the neurotoxicity guideline table below.

The CHECKED (X) parameters were examined.

X	HOME CAGE OBSERVATIONS	X	HANDLING OBSERVATIONS	X	OPEN FIELD OBSERVATIONS
X	Posture*	X	Reactivity*	X	Mobility
	Biting	X	Lacrimation* / chromodacryorrhea		Rearing+
X	Convulsions*	X	Salivation*	X	Arousal/ general activity level*
X	Tremors*	X	Piloerection*		Convulsions*
	Abnormal Movements*		Fur appearance		Tremors*
X	Palpebral closure*		Palpebral closure*		Abnormal movements*
X	Faeces consistency	X	Respiratory rate+	X	Urination / defecation*
X	Straub tail		Red/crusty deposits*	X	Grooming
	SENSORY OBSERVATIONS	X	Mucous membranes /eye /skin colour	X	Gait abnormalities / posture*
	Approach response+	X	Eye prominence*	X	Gait score*
X	Touch response+	X	Muscle tone*	X	Bizarre / stereotypic behavior*
X	Startle response*				Backing
X	Pain response*				Time to first step
X	Pupil response*			X	Vocalizations
	Eyeblink response		**PHYSIOLOGICAL OBSERVATIONS**		**NEUROMUSCULAR OBSERVATIONS**
	Forelimb extension		Body weight*		Hindlimb extensor strength
	Hindlimb extension	X	Body temperature+		Forelimb grip strength*
	Air righting reflex+			X	Hindlimb grip strength*
	Olfactory orientation				Landing foot splay*
X	Pinna reflex		**OTHER OBSERVATIONS**		Rotarod performance

*Required parameters; +Recommended parameters

b. **Locomotor activity:** Locomotor Activity was evaluated as part of the general behavior observations above; no mechanized system was used.

6. **Sacrifice and pathology:** Animals were sacrificed by carbon dioxide asphyxiation. No gross observations or microscopic observations of the tissues/organs were performed. The organs were not weighed.

7. **Positive controls:** Positive control data were not supplied.

II. RESULTS

A. OBSERVATIONS:

1. **Clinical signs:** Clinical signs other than those recorded during the Irwin screening were not described. Therefore, all clinical signs are listed under neurobehavioral results below.

2. **Mortality:** One of three mice administered 400 mg/kg bw in the neurotoxicity study died; death occurred by the 3-hour observation.

B. BODY WEIGHT AND BODY WEIGHT GAIN: Body weight and body weight gain were not monitored.

C. FOOD CONSUMPTION: Food consumption was not monitored.

D. CHOLINESTERASE ACTIVITIES: Cholinesterase activities were not measured.

E. NEUROBEHAVIORAL RESULTS:

1. **FOB Findings:** An FOB according to OPPTS 8706200a guidelines was not conducted. No clinical signs were observed in the 12.5 or 25 mg/kg bw dose groups at any time (Table 2). Clinical signs increased in severity with increasing dose and also increased in severity with time. For example, slight tremors were observed in the 50 and 100 mg/kg dose groups at the 0.5 hour (50 mg/kg) and 0.5 and 1 hour observations (100 mg/kg). In the 200 mg/kg dose group, tremors were observed over a longer period of time (0.5 to 3 hours) and became more severe, attaining a severity rating of moderate in 1/3 animals. In the 400 mg/kg group, slight to moderate tremors were observed in all 3 mice at two or more observation times. In the 400 mg/kg group, decreased motor activity, tremors, deep respirations, decreased grooming, decreased reactivity, prone position, decrease in muscle tone, hypothermia, and cyanosis attained severity ratings of moderate over the 1-day observation period. Staggering gait and mydriasis progressed to severe for 3/3 and 2/3 mice, respectively. With only three mice/group, statistical evaluations were not conducted.

TABLE 2. Neurobehavioral assessment of mice (clinical signs, activity)

Observation[a]	Dose level (mg/kg bw)						
	Control	12.5	25	50	100	200	400
Decreased spontaneous motor activity	0/3	0/3	0/3	3/3 (+)	3/3 (+, ++)	3/3 (+, ++)	3/3 (+, ++)
Tremor	0/3	0/3	0/3	1/3 (+)	1/3 (+)	3/3 (+, ++)	3/3 (+, ++)
Deep respiration	0/3	0/3	0/3	1/3 (+)	3/3 (+)	2/3 (+)	3/3 (+, ++)
Decreased grooming	0/3	0/3	0/3	0/3	3/3 (+)	3/3 (++)	3/3 (++)
Decreased reactivity	0/3	0/3	0/3	0/3	1/3 (+)	3/3 (+)	3/3 (++)
Prone position	0/3	0/3	0/3	0/3	1/3 (+)	3/3 (+)	3/3 (+, ++)
Staggering gait	0/3	0/3	0/3	0/3	1/3 (+)	2/3 (+)	3/3 (+, ++, +++)
Decrease in muscle tone	0/3	0/3	0/3	0/3	1/3 (+)	3/3 (+, ++)	3/3 (++)
Mydriasis	0/3	0/3	0/3	0/3	2/3 (+, ++)	2/3 (+)	2/3 (+, ++, +++)
Hypothermia	0/3	0/3	0/3	0/3	3/3 (+)	3/3 (+)	3/3 (+, ++)
Cyanosis	0/3	0/3	0/3	0/3	0/3	0/3	1/3 (++)[b]

Data were extracted from Table 3-1, pp. 30 and 31, MRID 45422823.
n = 3 for all groups.
Values represent incidences [symbols in parenthesis are severity ratings: + = slight, ++ = moderate, +++ = severe (more than one rating refers to successive observation times from 0.5 hours to 1 day)].
[a]Pretest observations were not conducted.
[b]This mouse also demonstrated Straub tail and inhibition of pinna reflex and corneal reflex at 1 hour.

2. **Motor activity:** Motor activity according to OPPTS 870.6200a guidelines was not assessed.

F. OTHER TESTS:

1. **Anesthetic Effect (mice):** Doses of 25 and 75 mg/kg had no effect on hexobarbital-induced sleeping time in mice. At 225 mg/kg, 2 of the 8 treated animals died; the sleeping time was significantly prolonged in the surviving 6 mice (51.3 minutes compared with a control value of 31.4 minutes; $p<0.05$).

2. **Effect of electroshocks (mice):** Incidences of tonic flexor or tonic extensor convulsion for the control and 6.25, 12.5, 25, 75, 225 mg/kg dose groups were 1/10, 3/10, 2/10, 8/10, 10/10, and 10/10, respectively. Incidences in the 25, 75, and 225 mg/kg dose group were significantly different from the control group at $p<0.05$.

3. **Stimulation by pentylenetetrazol (mice):** Incidences of clonic convulsion induced by pentylenetetrazol in the control and 25, 75, and 225 dose groups were 2/10, 2/10, 0/10, and 0/10, respectively. Tonic extensor convulsions were not observed in any group.

4. **Effect on intestinal transport of charcoal (mice):** Intestinal transport [(% of intestine traversed/entire length of intestine) x 100] for the control, 25, 75, and 225 mg/kg groups were 66.5, 61.5, 26.3 ($p<0.05$), and 14.5% ($p<0.05$), respectively.

5. **Effect on muscle strength (mice):** There was no effect of treatment with 25 and 75 mg/kg on muscle strength at the 1-, 3-, and 6-hour observations times. Treatment with 225 mg/kg resulted in failures of 4/8, 4/8, and 2/8 mice at the 1-, 3-, and 6-hour post-treatment test times, respectively. None of these incidences were statistically significant by the χ^2 test at $p<0.05$.

6. **Effect on blood pressure and heart rate (rat):** A dose of 100 mg/kg had no effect on heart rate at any observation period. Doses of 300 and 3000 mg/kg significantly increased the heart rate at 0.5 hours after administration ($p<0.05$), but increases were not statistically significant at the other observation periods. In the 1000 mg/kg group, slight increases were observed at all of the observation times, but these increases did not attain statistical significance. Compared with the control values, systolic blood pressure was decreased only in the 1000 mg/kg group and only at the 1-hour observation period. Mean blood pressure was significantly lower in the 1000 mg/kg group at both the 1- and 6-hour observation time and in the 3000 mg/kg group at the 6-hour observation time.

7. **Effect on blood coagulation (rat):** Compared with the control group, treatment with 300, 1000, or 3000 mg/kg had no effect on the prothrombin time or activated thromboplastin time in rats.

8. **Effect on muscle contraction (guinea pigs, *in vitro*):** At 10^{-4} mol/L of the test material, barium (10^{-3} mol/L) suppressed the contractile response of isolated guinea pig ileum by 12.2% compared with the control. There were no effects of barium at the other concentrations of TI-435 or of acetylcholine or histamine on any of the preparations.

G. **SACRIFICE AND PATHOLOGY:** Animals were sacrificed by carbon dioxide asphyxiation. No gross or microscopic examinations were performed.

1. **Gross pathology:** Sacrificed animals were not examined grossly.

2. **Brain weight:** Brains were not weighed.

3. **Neuropathology:** Tissues of the nervous system were not examined microscopically.

III. DISCUSSION AND CONCLUSIONS

A. **INVESTIGATORS' CONCLUSIONS:** Regarding the neurobehavioral effects of TI-435 on mice observed within 1 day after treatment, administration of 12.5 or 25 mg/kg of TI-435 had no effect on clinical signs or general activity. At 50 mg/kg, spontaneous motor activity was decreased and tremors and deep respirations were observed. At 100 and 200 mg/kg, the

previous three signs as well as decreases in grooming, reactivity, and muscle tone; a prone position; staggering gait; mydriasis, and hypothermia were observed. Effects were slightly more severe and were observed for a longer period of time in the 200 mg/kg group compared with the 100 mg/kg group. At 400 mg/kg, all of the above signs were observed; in addition 1 of 3 mice appeared cyanotic and subsequently died. The "minimum lethal dose" was considered 400 mg/kg.

Hexobarbital in combination with the test substance had a synergistic anesthetic action such that 2/8 mice administered 225 mg TI-435 and 80 mg/kg hexobarbital died and sleeping time in the surviving mice was prolonged compared with the hexobarbital control group. No effect was seen at 25 and 75 mg/kg.

Application of a sub-threshold electric shock to mice following administration of 25, 75, or 225 mg/kg of the test substance produced convulsions; 6.25 and 12.5 mg/kg were no-effect doses. Administration of pentylenetetrazol at a sub-threshold dose of 55 mg/kg did not induce convulsions in mice following TI-435 doses of 25, 75, or 225 mg/kg.

Administration of doses of TI-435 up to 3000 mg/kg had little effect on heart rate and blood pressure of unanesthetized rats. The authors concluded that the test material had little effect on the circulatory system. In addition, the test substance had no effect on blood clotting factors in rats.

Doses of 75 and 225 mg/kg suppressed gastrointestinal transfer in mice; 25 mg/kg was a no-effect level.

T-435 had a slight suppressive effect on skeletal muscle strength in mice for up to 3 hours following administration of 225 mg/kg. Doses of 25 and 75 mg/g had no effect.

In vitro, the test material at 10^{-6} to 10^{-4} mol/L had almost no effect on the contractile response of isolated guinea pig ileum in the presence of the contractile substances, acetylcholine, histamine, and barium.

The study author concluded that TI-435 induced convulsions with sub-threshold electroshock at ≥25 mg/kg, exerted a suppressive effect on the central nervous system at ≥50 mg/kg, exerted a suppressive effect on the gastrointestinal track at ≥75 mg/kg, and exerted a slight suppressive effect on the skeletal muscular system at 225 mg/kg. There was no effect on the circulatory system or on the contractile response to various agonists in isolated guinea pig ileum preparations.

B. REVIEWER COMMENTS: In range-finding experiments, an oral gavage dose of 550 mg/kg was lethal to 1 of 3 male CD-1 mice and a dose of dose of 3850 mg/kg was lethal to 1 of 3 male CD rats. Doses of 390 and 2960 mg/kg were non-lethal to the mouse and rat, respectively, but did result in neurobehavioral signs including decreases in spontaneous motor activity, deep respirations, staggering gait, tremor, hypothermia, and prone position. In further neurobehavioral tests with male mice (Irwin's observational method), a dose of 25

mg/kg was a NOAEL for neurobehavioral signs and a dose of 50 mg/kg was a LOAEL, with transient signs of decreased spontaneous motor activity, tremor, and deep respirations. These signs were observed for up to 1 hour post-administration. TI-435 had a suppressive effect on the central nervous system as evidenced by prolonged hexobarbital sleeping time following a dose of 225 mg/kg; 75 mg/kg did not affect sleeping time. In tests with convulsion-inducing agents, a dose of 25 mg/kg induced tonic flexor and extensor convulsions in combination with a sub-threshold electric shock, but not in combination with pentylenetetrazol when TI-435 was administered at 225 mg/kg. The relevance of some of the latter tests to neurotoxicity is not well understood by the Reviewer. The test material appears to affect primarily the central nervous system as evidenced by anesthetic properties, decreased reactivity, hypothermia, tremors, and a decrease in muscle tone. The effects were transient. As noted by the Study Author, there was no effect on the circulatory system, blood pressure, or on the contractile response to various agonists in isolated guinea pig ileum preparations. The Reviewer agrees with the NOAELs and LOAELs as assigned by the Study Author to the results of the various tests.

Although the study addressed neurotoxicity, it was not intended to fulfill OPPTS 870.6200a guidelines. Because it failed to follow most of the guidelines outlined in OPPTS 870.6200a, it should be considered a non-guideline study. The modified FOB can be used as a range-finding study for neurotoxicity. This study addressed "pharmacological effects" and as such identified target areas of the nervous system.

Based on the effects seen in this study, the LOAEL for acute neurotoxicity of TI-435 in male CD-1 mice was 50 mg/kg bw (based on transient signs of decreased spontaneous motor activity, tremors, and deep respirations), with a NOAEL of 25 mg/kg bw.

C. **STUDY DEFICIENCIES:** The study failed to follow most OPPTS 870.6200a guidelines. Mice rather than rats were used as the test species in the neurobehavioral tests, although an alternate species to the rat is acceptable. Only 3 mice/group were tested in the neurobehavioral tests. Tests were conducted for only 24 hours post-treatment, based on the results of a range-finding test in which observations continued for 7 days. No positive or historical control data were included. The technicians were not described. When reviewed as a non-guideline study, there were no major deficits.

DATA FOR ENTRY INTO ISIS

Acute Neurotoxicity Study - rats (870.6200a)

PC code	MRID #	Study type	Species	Duration	Route	Dosing method	Dose range mg/kg	Doses tested mg/kg	NOAEL mg/kg	LOAEL mg/kg	Target organ(s)	Comments
044309	45422823	nonguideline	mouse	1 day	oral	gavage	12.5-400	0, 12.5, 25, 50, 100, 200, 400	25	50	nervous system	

HED Records Center Series 361 Science Reviews - File R107689 - Page 678 of 721

Primary Reviewer: Name Robert P Zendzian PhD
Title Senior Pharmacologist
Toxicology Br, HED (7509C)
Date 03/13/03

Secondary Reviewer Name Rebecca Daiss
Title Envir. Health Scientist
Br, HED (7509C)
Date 3/13/03

TXR # 0050321

DATA EVALUATION RECORD

STUDY TYPE: Dermal Absorption - Primate (monkey)

P.C. CODE: 044309 DP BARCODE: D288641 SUBMISSION NO: S630314

TEST MATERIAL (RADIOCHEMICAL PURITY):TI-435 [Clothianidin](99.3%)

SYNONYMS: TI-435 FS 600

CITATION: Sebesta, C.A. Feb 27, 2003, A Study to Determine the Dermal Absorption of TI 435 FS 600 when Administered Dermally to Male Rhesus Monkeys. Charles River Laboratories, Bayer Crop Science. Report No VCBZ-0107-03-111 MRID 45868001. Unpublished

SPONSOR: Bayer

EXECUTIVE SUMMARY: In a dermal penetration study (MRID 45868001) TI-435 [Clothianidin] as the FS 600 formulation (10% a.i.) [nitroimino- ^{14}C] TI-435) was administered to 5 male Rhesus monkeys. Test material was applied to a shaved area (4 cm x 6 cm) of skin on the back of each animal. The total dose was contained in 100 ml of test substance and was applied at a dose of 6.13 ug/cm^2. Exposure duration was 8 hours at the end of which time the application site was washed. Subjects were monitored for 120 hours. Urine and feces were collected for the exposure period and the subsequent monitoring period.

Table 1. Mean Dermal Data as Percent of a Dermal Dose of 6.13 ug/cm^2

Sample	% Recovery
Urine	0.05 (± 0.04)
Feces	0.13 (±0.21)
Cage/Pan/Chair Wash, Debris	0.06 (±0.09)
Duoderm/Dome	2.99 (±4.75)
Skin Wash	94.77 (varied with sample)
Totals	98.00 (±0.66)

Dermal absorption as the sum of urinary and fecal excretion and Cage/Pan/Chair Wash, Debris was 0.24 (± 0.11) as percent of dose. Adjustment of the direct absorption determination was not necessary because recovery from the dermal dose was >90%.

This study in the monkey was acceptable.

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

OPP does not have a guideline for human or monkey studies and does not request human or monkey studies as a matter of policy. Nor are they needed to effectively estimate dermal absorption. However, we do receive such studies performed by pesticide registrants and have developed minimal standards for acceptance. In general these studies follow the experimental design first presented by Feldman and Maibach in 1970 for studies in humans and later adapted for the monkey. In this design, a small radio labeled dose of the test chemical is administered intravenously and total urine collected until all label is excreted. This provides a percent urinary excretion of a 100% absorbed dose. Then the same individual receives a labeled dermal dose and total urine is collected. The percent of dose excreted following the intravenous dose is used to correct the percent of dose excreted following the dermal dose to calculate total dermal absorption. For example if 20% of the intravenous dose is excreted in the urine, the portion of the dermal dose excreted in the urine is 20% of the dermally absorbed dose.

Because these studies are time consuming and expensive, they are generally limited to one dose and one duration of exposure. The total dose is small and chosen to be biologically inactive. It is also limited by the amount of radioactivity that can be administered to a human at one time. Since the percent of a pesticide absorbed varies with dose (decreasing with increasing dose per unit area) and duration of exposure (increasing in a nonlinear fashion with time), the value

supplied frequently does not fit the exposure. The percent absorption from a human or monkey study can be used for risk assessment if the exposure dose per unit area is equal to or greater than the study dose per unit area and the duration of exposure is equal to or less than the duration of the study dose. In such cases the absorption error will only overestimate the risk (underestimate the MOE).

I. MATERIALS AND METHODS

portions of this section are extracted from the report and are indicated as " "

A. MATERIALS:

1. Radiolabeled Test Material

Test Substance:	[^{14}C] TI-435 formulated in FS 600 (also referred to as TI-435 FS 600 containing ^{14}C TI-435, Clothianidin, and [nitroimino- ^{14}C] TI-435)
Chemical Name: (Active Ingredient)	(*E*)-*N*-[(2-Chloro-5-thiazolyl)methyl]-*N'*-methyl-*N* " -nitroguanidine
Lot Number:	15209/1
Physical Description:	Red liquid (suspension)
Specific Activity:	117.2 uC i/mg
Concentration:	1.47 mg active ingredient/mL
Radiochemical Purity:	99.3% (HPLC)
Storage Conditions:	-70 ± 10°C
Preparation Date:	January 20, 2003
Stability:	7 days at 40°C; 97 days at ≤20°C
Date Received:	January 24, 2003
Amount Received:	3 mL (516 uCi)
Manufacturer/Supplier:	Bayer Crop Science LP

2. Nonradiolabeled Test Material:
Not Applicable see above

3. Formulation
Name. SF 600
Source; Bayer Crop Science

4. Vehicle Solvent
undiluted

5. Test animals:
Species: Rhesus Monkeys
Sex: Male
Age: Adult
Weight: 4.46 (3.20-5.53)kg

B. STUDY DESIGN AND METHOD

1. Dose Selection

The dermal absorption of TI-435 in an FS 600 formulation was evaluated using monkeys. The dermal dose level, of approximately 6.13 ug/cm^2, was selected to reflect human exposure in the field.

The study design is summarized below in Text Table 1.

Text Table 1
Study Design

Group Number	Number of Males	Treatment Administration				Route
		Test Substance	Target Dose	Target Conc.	Dose Volume	
1	5	[^{14}C]TI-435 in FS 600 formulation	147 µg (17.2 µCi)[a]	1.47 µg/µL	0.1 mL	Dermal

Conc. = Concentration
a: at 6.13 µg/cm^2 with a total skin area of 24 cm^2

2. Subject Preparation

"On the day prior to the dermal application, the animals were anesthetized (to effect) with ketamine HCl. An area of the back was shaved with clippers, with care taken to avoid irritation. The entire shaved area was gently cleaned with water and patted dry. The total shaved area was large enough to accommodate the Duoderm® patch and protective dome. "

3. Dose Preparation, Administration and Quantification

677

Preparation:

Dosing material was applied as supplied by the sponsor.

Application:

"A single dermal dose (target 147 ug at 6.13 ug/cm ^{2}) of TI-435 FS 600, containing ^{14} C TI-435, was applied to a shaved area (4 cm x 6 cm) of skin on the back of each animal. The total dose was contained in 100 ml of test substance. The animals were anesthetized and restrained on a table for dosing and were then placed in a primate chair for the 8-hour exposure period. After 8 hours, the protective dome and its securing material, and the Duoderm®) patch were removed. After subsequent dermal swabbings, the animals were placed in a metabolism cage."

"The animals were not fed on the morning of dosing feeding was delayed until approximately four hours following dose administration. The animals were anesthetized (to effect) with ketamine HCL and a Duoderm® patch was placed around the dosing site to expose and isolate a 4 cm x 6 cm dosing area. Following the placement of the Duoderm® patch, the animals were restrained on a table for dermal dose administration. The dose Solution was mixed on a stir plate and then vortexed for approximately 20-30 seconds prior to withdrawal of each dose. The dose solution was withdrawn using a 100-uL micropipet. The dose solution (100 uL) was applied by using the 100-uL micropipet and was gently spread over the entire 24-cm area, using the micropipet, The dose administration time was recorded. The dose application site was covered with a protective dome (4-inch perforated aluminum pie pan) and secured with tape. Each animal was then placed into a primate chair in an upright position immediately after securing the protective covering. The dosing micropipet was placed in a glass liquid scintillation vial and saved for analysis of residual radioactivity."

"A tray (pan) was placed under each animal to capture excreta. Pre-weighed polypropylene collection containers were used for the urine collection from the pan during the 0-4 hour and 4-8 hour post-dose periods. Any feces excreted during the 0-4 hour and 4-8 hour post-dose periods were transferred to pre-weighed polypropylene containers."

"After the 8-hour exposure period, the animals were anesthetized with ketamine HCl (to effect) and the protective dome, securing material, and the Duoderm® patch were removed and placed into separate containers (one container for the dome and a separate container for the securing material and Duoderm® patch). The surface of the test site was washed with a series of 20 soapy water swabs (1 % Ivory™ dishwashing liquid diluted with distilled water) followed by two dry cotton swabs. Each swab's stick was shortened to allow the cotton-tip to be fully submerged within sequentially numbered scintillation vials (one swab per vial). The skin was then swabbed with two cotton swabs dipped in isopropyl alcohol and further washed with four soapy water

678

swabs to assure the dose site was appropriately washed. The animals were then returned to the metabolism cages. Any urine or feces collected after the 8-hour time point, while the animals were still in the primate chairs, were added to the 8-12 hour collection containers when the animals were returned to the metabolism cages, Based on the results of the analysis of the 8 hour dermal swabbings, no additional dermal swabbing was necessary."

Animal Observation

"The animals were continuously observed throughout the restraint period, The animals were offered water at regular intervals and food after 4 hours post-dose. Additionally, the restraints Were moved/rotated and the hands and feet were massaged to promote circulation. Observations of the presence or absence of clinically relevant abnormalities were documented in the study file"

Analysis of Dose Formulation

"Four aliquots (100 uL each) of the dosing solution were collected prior to dosing and two aliquots (100 uL each) were collected post-dose for analysis. One post-dose aliquot was collected following completion of dose administration to Animal No. 1002 and the second was collected following completion of dose administration to Animal No. 1005. The weight of each aliquot was recorded and the aliquots were diluted to 25 mL with acetonitrile. Triplicate sub-aliquots (~400 uL) of each dilution were weighed, mixed with scintillation fluid, and analyzed by liquid scintillation counting. The multiple dose formulation aliquots were collected to ensure homogeneity of the formulation throughout dosing. The concentration of radioactivity in the dose formulation was determined using only aliquots that were collected while the formulation was continuously stirring. Therefore, pre-dose aliquot No. 4 and the two post-dose aliquots were used to determine the concentration of radioactivity in the dose formulation. The results of the analysis were used to calculate the dose applied to the skin of each animal."

Sample Collection

"Samples were collected at the time points indicated in Text Table 2. The post-dose samples were transferred to the Metabolism and Pharmacokinetics laboratory for processing for radioanalysis."

Urine

"Urine samples were collected using pan catch methods for up to the 8-hour time point. After the 8-hour time point, samples were collected on dry ice using a metabolism cage. The weight of each sample was recorded."

Feces

"Feces samples were collected using pan catch methods for up to the 8-hour time point. After the 8-hour collection, samples were collected using a metabolism cage. Samples were collected at room temperature and were transferred into containers at the time of collection. The weight of each sample was recorded."

Text Table 2
Sample Collection Schedule

Group	Clinical Pathology		Sample Collection (post-dose)			
	Hematology	Serum Chemistry	Urine/Feces/Cage Debris/Rinse[a]	Urine Pan Wash/Wipe	Chair Wipe	Cage Wash/Wipe
1	Prior to Treatment for Pre-Study Health Assessment		0-4, 4-8, 8-12, 12-24 hours and at 24 hour intervals up to 120 hours	4 and 8 hours	8 hours	120 hours
Volume /Timepoint	~ 0.5 mL	~ 1.8 mL				
Anticoagulant	EDTA	None				

a: Cage Debris/Rinse not collected at the 0-4 and 4-8 hour intervals.

Cage Debris/Cage Rinse

"Cage debris (mostly comprised of residual food and hair) was collected after each interim fecal collection beginning at the 8-12 hour collection interval. Following each cage debris collection, cage floors, screens and pans were rinsed with 50% reagent alcohol and the rinse was collected in the cage debris container. The weight of each sample was recorded."

Urine Pan/Screen and Primate Chair Wipe

"A urine pan/screen wash was performed at 4 hours and 8 hours post-dose using 50% reagent alcohol. The wash was collected into separate containers. Following the urine pan/screen wash, the urine pans/screens were wiped with gauze pads and the pads were collected into separate containers. Once the animals were retained to the metabolism cages at 8 hours post-dose, the primate chairs were thoroughly wiped with gauze pads soaked with isopropyl alcohol. The gauze pads were collected into separate containers. The weight of each sample was recorded."

Cage Wash/Cage Wipe

"After the final excreta collection at 120 hours, each cage was washed with 50% reagent alcohol and the wash was collected into separate containers. Following the wash, each cage was wiped with gauze pads soaked with isopropyl alcohol and the gauze pads were placed into separate containers. The weight of each sample was recorded."

Additional Samples

"Urine spills occurred during the 24-48 and 48-72 hour collection intervals for Animal No. 1001. The samples were collected onto gauze pads and the gauze pads were placed into separate containers for analysis. The weight of each sample was recorded."

Sample Storage Conditions

"The dermal swab samples were stored at 22 ± 5'C until analysis. Samples other than the aforementioned, that were not processed immediately upon receipt, were stored at -20 + 5'C, except when removed for radioanalysis. Residual samples were stored at -20 ± 5°C, until disposition."

4. <u>Samples Analyzed</u>

Urine
Feces
Cage Debris/Cage Rinse
Urine Pan/Screen and Primate Chair Wipe
Cage Wash/Cage Wipe

Additional Samples/Urine spills

II. RESULTS

Animal Observations

Feces were not produced at the 4-hour time point for Animal Nos. 1002 and 1004, the 8-hour time point for Animal No. 1004, and the 12-hour time point for all of the animals. Animal No. 1005 had soft feces (mild) noted at the 24-hour time point. There were no additional clinically relevant abnormal observations noted for the remainder of the study.

Summary Tables

Analysis of radioactivity concentration of [^{14}C]TI-435 in the dose formulation is presented in Table 1 from the report.

Quantitative dose administration is presented in Table 2 from the report.

Urinary and fecal elimination of [^{14}C]TI-435 derived radioactivity is presented in Table 3 from the report.

Total recovery and dose distribution of [^{14}C]TI-435 derived radioactivity is presented in Table 4 from the report.

Total Absorbed Dose

The mean absorption based on urinary and fecal excretion of radioactivity was 0.24%. Mean recovery of applied dose was 98.00%. Dermal absorption adjustment was not necessary because recovery from the dermal dose was >90% (98.00%).

III. DISCUSSION and CONCLUSIONS

Investigators' Conclusions:

"Dermal application of TI-435 FS 600, containing 1 4 C TI-435, resulted in recovery of a small fraction of the dose in excreta. Total mean recoveries through 120 hours post-dose were 0.05% in urine (range of 0.0 I - 0. I I %) and 0. 13% in feces. The cage debris/rinse samples contained 0.00% of the dose. The low recoveries in excreta and high percent of radioactivity at the application site suggest minimal dermal absorption of the compound and a low degree of systemic exposure to [^{14}C}TI-435-derived radioactivity. A large fraction of the total excreted radioactivity was recovered within 8 hours of dose application and the overall excretion was essentially complete by 24 hours."

"At 8 hours post-application, the aluminum dome contained a mean total of 0.24% of the applied radioactivity and the Duoderm® patch and related securing materials contained a mean total of

2.75% of the applied radioactivity. The mean total recovery in the first four dermal swabbings (soapy water swab extracts, residual extracts, and soapy water swabs) at 8 hours post-dose was 91,24% of the dosed radioactivity, the majority of which was associated with the soapy water swab extracts (80.15%). The mean total recovery in the remaining soapy water swabs at 8 hours post-dose was 3.41% of the dosed radioactivity. The dry swabs and isopropyl alcohol swabs, taken after the dermal swabbing at 8 hours postdose, contained mean totals of 0.03% and 0.09% of the dose, respectively, indicating that most of the applied radioactivity was easily removed with the soapy water Swabs."

"The mean total of excreted radioactivity was 0.24%. The mean total recovery of radioactivity from the application site area at the 8-hour sampling was 97.75%. The overall mean total recovery of radioactivity from excreta and from the application site was 98.00%, the majority of which was associated with the radioactivity recovered from the application site. The extent and pattern of excretion in this study were similar to that of the exploratory study. These data suggest that complete excretion of absorbed radioactivity occurred in this current study. Therefore, the dermal absorption of radioactivity (0.24%) can be normalized to 100% total recovery, resulting in a nominalized dermal absorption of 0.245%."

Reviewers Comments:

No problems were observed by the reviewer in this study.

Recovery of the applied dose (mass balance) was acceptable. Results need not be not adjusted for incomplete recovery of the applied dose. The majority of the administered dose was recovered from the wash of the application site (mean 94.77 % of the dose. Estimates of dermal absorption were based on the sum urine + feces + Cage/Pan/Chair Wash, Debris.

Nothing unusual was observed in the absorption pattern.

Table 1
Analysis of Radioactivity Concentration of [^{14}C]TI-435 in the Dose Formulation

Dose Formulation Aliquot No.	Diluted Dose Formulation Aliquot No.	Total dpm	Mean dpm	Formulation Aliquot (mL)	dpm/mL Formulation	Radioactivity Concentration (µCi/mL)	Concentration[a] (mg/mL)
Pre-dose 4	1	36401975	36427808	0.100	364278080	164	1.40
	2	36458925					
	3	36422525					
Post-dose 1	1	36011675	35925683	0.100	359256830	162	1.38
	2	35862700					
	3	35902675					
Post-dose 2	1	35875650	35917058	0.100	359170580	162	1.38
	2	35802125					
	3	36073400					
Mean (SD)						**163 (1.15)**	**1.39 (0.0115)**

a: Calculated based on the Sponsor-provided specific activity (117.2 µCi/mg).

Table 2
Dose Administration

Dose Route	Animal Number	Animal Weight (kg)	Dose Formulation Administered (mL)	Residual in Dosing Micropipet (µCi)	Residual in Dosing Micropipet (µg)	Dose Administered (µCi)[a]	Dose Administered (µCi/kg)	Dose Administered (µg)[b]	Dose Administered (µg/cm^2)	Dose Administered (µg/kg)
Dermal	1001	3.2605	0.100	0.398	3.40	15.9	4.88	136	5.67	41.7
	1002	4.6410	0.100	0.171	1.46	16.1	3.47	137	5.71	29.5
	1003	4.6535	0.100	0.214	1.83	16.1	3.46	137	5.71	29.4
	1004	4.2437	0.100	0.244	2.08	16.1	3.79	137	5.71	32.3
	1005	5.5286	0.100	0.163	1.39	16.1	2.91	137	5.71	24.8
	Mean (SD)					**16.1 (0.089)**	**3.70 (0.731)**	**137 (0.447)**	**5.70 (0.018)**	**31.5 (6.28)**

Note: The concentration of radioactivity was 163 µCi/mL.
The concentration of [^{14}C]TI-435 was 1.39 mg/mL.

a: µCi in dispensed dose formulation minus the residual µCi in the dosing micropipet.
b: µg in dispensed dose formulation minus the residual µg in the dosing micropipet.

Table 3
Elimination of [^{14}C]TI-435-Derived Radioactivity by Male Rhesus Monkeys Following Dermal Administration of TI-435 FS 600 Containing ^{14}C TI-435 at a Target Dose of 147 µg

Sample	Time (hours)	Percentage of Dose (%)						
		Animal Number					Mean	SD
		1001	1002	1003	1004	1005		
Urine	0-4	0.01	0.00	0.01	0.01	0.00	**0.01**	**0.01**
	4-8	0.03	0.00	0.03	0.00	0.03	**0.02**	**0.02**
	8-12	0.00	0.04	0.05	0.00	0.00	**0.02**	**0.02**
	12-24	0.00	0.00	0.02	0.00	0.00	**0.00**	**0.01**
	24-48	0.00[a]	0.00	0.00	0.00	0.00	**0.00**	**0.00**
	48-72	0.00[a]	0.00	0.00	0.00	0.00	**0.00**	**0.00**
	72-96	0.00	0.00	0.00	0.00	0.00	**0.00**	**0.00**
	96-120	0.00	0.00	0.00	0.00	0.00	**0.00**	**0.00**
	Subtotal	**0.04**	**0.04**	**0.11**	**0.01**	**0.03**	**0.05**	**0.04**
Feces	0-4	0.00	NS	0.00	NS	0.00	**0.00**	**0.00**
	4-8	0.00	SC	0.00	NS	0.00	**0.00**	**0.00**
	8-12	NS	NS	NS	NS	NS	**0.00**	**0.00**
	12-24	0.00	0.00	0.00	0.00	0.00	**0.00**	**0.00**
	24-48	0.00	0.00	0.00	0.00	0.14	**0.03**	**0.06**
	48-72	0.05	0.00	0.00	0.00	0.00	**0.01**	**0.02**
	72-96	0.00	0.00	0.00	0.00	0.00	**0.00**	**0.00**
	96-120	0.15	0.00	0.00	0.00	0.33	**0.10**	**0.15**
	Subtotal	**0.20**	**0.00**	**0.00**	**0.00**	**0.47**	**0.13**	**0.21**
Cage Debris/ Rinse	8-12	0.00	0.00	0.00	0.00	0.00	**0.00**	**0.00**
	12-24	0.00	0.00	0.00	0.00	0.00	**0.00**	**0.00**
	24-48	0.00	0.00	0.00	0.00	0.00	**0.00**	**0.00**
	48-72	0.00	0.00	0.00	0.00	0.00	**0.00**	**0.00**
	72-96	0.00	0.00	0.00	0.00	0.00	**0.00**	**0.00**
	96-120	0.00	0.00	0.00	0.00	0.00	**0.00**	**0.00**
	Subtotal	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**

NS = No sample produced.
SC = Sample combined with the 12-24 hour sample due to insufficient sample size.
a: Radioactivity in the urine spills was 0.00%.

676

Table 4
Total Recoveries of [^{14}C]TI-435-Derived Radioactivity up to 120 Hours Following Dermal Administration of TI-435 FS 600 Containing ^{14}C TI-435 to Male Rhesus Monkeys at a Target Dose of 147 µg

Recovery	Sample	Percentage of Dose (%)						
		Animal Number					Mean	SD
		1001	1002	1003	1004	1005		
Elimination	Urine	0.04	0.04	0.11	0.01	0.03	**0.05**	**0.04**
(0-120 hours)	Feces	0.20	0.00	0.00	0.00	0.47	**0.13**	**0.21**
	Cage Debris/Rinse	0.00	0.00	0.00	0.00	0.00	**0.00**	**0.00**
	Urine Pan Wash/Wipe (4 hours)	0.00	0.00	0.00	0.00	0.00	**0.00**	**0.00**
	Chair/Urine Pan Wash/Wipe (8 hours)	0.21	0.00	0.11	0.00	0.00	**0.06**	**0.09**
	Cage Wash	0.00	0.00	0.00	0.00	0.00	**0.00**	**0.00**
	Cage Wipe	0.00	0.00	0.00	0.00	0.00	**0.00**	**0.00**
	Subtotal	**0.45**	**0.04**	**0.22**	**0.01**	**0.50**	**0.24**	**0.23**
Residual								
(8 hours)	Duoderm® Patch/Securing Material	1.65	1.30	0.27	10.13	0.38	**2.75**	**4.17**
	Dome	0.00	0.00	0.00	0.37	0.83	**0.24**	**0.37**
	First Four Soapy Water Swabs[a]	92.87	94.24	92.51	83.83	92.75	**91.24**	**4.20**
	Remaining Soapy Water Swabs	3.54	2.82	3.81	3.24	3.66	**3.41**	**0.39**
	Dry Swabs	0.04	0.01	0.02	0.03	0.03	**0.03**	**0.01**
	Alcohol Swabs	0.06	0.05	0.16	0.09	0.07	**0.09**	**0.04**
	Subtotal	**98.16**	**98.42**	**96.77**	**97.69**	**97.72**	**97.75**	**0.63**
Overall Total		**98.61**	**98.46**	**96.99**	**97.70**	**98.22**	**98.00**	**0.66**

a: Percent of dose of the first four soapy water swabs for each animal represents a combination of the radioactivity from the soapy water swab extracts, residual extracts, and the actual soapy water swabs.

677

PMRA ARLA

Reviewer: Scott Hancock , Date May 26, 2002
for : Pamela Hurley

TXR No. 0050321

STUDY TYPE: Acute Oral Toxicity -*Rat.* OPPTS 870.1100; OECD 401.

TEST MATERIAL (PURITY): BN0335E2 (99% a.i.)

SYNONYMS: Metabolite of Clothianidin

CITATION: Ruddock, W.D. (2000). BN0335E2 Acute Oral Toxicity Study in the Rat. Covance Laboratories. Harrogate, North Yorkshire, England. Laboratory report number 586/229-D6144, September 5, 2000. Unpublished. MRID 45422623

SPONSOR: Takeda Chemical Industries Ltd. Agro Company, Chuo-ku, Tokyo, Japan.

EXECUTIVE SUMMARY: In an acute oral toxicity study, groups of fasted, young adult Crl:CD(SD)IGS.BR, rats (5/sex) were given a single oral dose of BN0335E2 (99% a.i.) in a 5% aqueous solution of arabic gum at a limit dose of 2000 mg/kg bw and observed for 14 days.

Treatment of animals with BN0335E2 at a limit dose resulted in no mortalities. All animals survived to the end of the 14 week observation period without obvious clinical signs, body weight changes, or macroscopic evidence of toxicity. Necropsy results also provided no indication of toxicity as a consequence of oral dosing with BN0335E2.

Oral LD_{50} Males & Females > 2000 mg/kg bw

BN0335E2 **is of LOW Toxicity** based on the LD_{50} in males and females [EPA Toxicity Category III].

This acute oral study is classified acceptable, and satisfies the guideline requirement for an acute oral study (OPPTS 870.1100; OECD 401) in the rat.

COMPLIANCE: Signed and dated GLP, Quality Assurance, and No Data Confidentiality statements were provided.

I. MATERIALS AND METHODS

A. MATERIALS:

1. **Test Material:** BN0335E2
   Description: Yellow powder
   Lot/Batch #: 30070904
   Purity: 99 % a.i.

2. **Vehicle and/or positive control:** Formulated in a 5% aqueous arabic gum solution. Animals were dosed at a 20 ml/Kg bw volume.

3. **Test animals:**

Species:	Rat	
Strain:	Crl:CD(SD)IGS.BR	
Age/weight at dosing:	6-8 weeks (♂) and 10-11 weeks old (♀)/ 257-264 g (♂) and 228-255 g (♀)	
Source:	Charles River (UK) Ltd,	
Housing:	1 - 5 / stainless steel mesh cage	
Diet:	SQC(E) Rat and Mouse Maintenance Diet No 1. *ad libitum*	
Water:	Mains water *ad libitum*	
Environmental conditions:	**Temperature:**	19 - 25 °C
	Humidity:	40 - 70 %
	Air changes:	14 / hr
	Photoperiod:	12 hrs dark/ 12 hrs light
Acclimation period:	8 - 14 days	

B. STUDY DESIGN and METHODS:

1. **In life dates** - Start: October 19, 1999 End: November 24, 1999

2. **Animal assignment and treatment** - Animals were assigned to the test groups noted in Table 1. Following an overnight fast, rats were given a single dose of BN0335E2 by gavage then observed and weighed for 14 days. Observations were recorded frequently on the day of dosing, twice daily on days 2 through day 4, and daily for the remainder of the study. Body weights were recorded on the day of dosing, day 4 or 5, day 8 and at termination on day 15. Survivors were sacrificed and a necropsy was performed on all animals.

TABLE 1. Doses, mortality/animals treated

Dose (mg/kg bw)	Males	Females	Combined
2000	0/5	0/5	0/10

3. **Statistics** - Since no animals died during the experiment, an oral LD_{50} could not be calculated.

II. RESULTS AND DISCUSSION:

A. Mortality There were no mortalities recorded during the study.

The oral LD_{50} is greater than 2000 mg/kg bw.

B. Clinical observations - None noted.

C. Body Weight - All animals gained weight in both the first and second weeks of the observation period.

D. Necropsy - There were no treatment related macroscopic signs of toxicity recorded during necropsy.

E. Reviewer's Conclusions: In agreement with the study author: "Single oral administration of BN0335E2 at a dose level of 2000 mg/kg caused no death in a group of ten fasted rats. The acute median lethal oral dose level (LD_{50}) was found to exceed 2000 mg/kg."

F. Deficiencies - None noted.

PMRA ARLA

for Reviewer: Scott Hancock , Date May 26, 2002
Pamela M Hurley

TXR No. 0050321

STUDY TYPE: Acute Oral Toxicity - *Rat* OPPTS 870.1100; OECD 401.

TEST MATERIAL (PURITY): Clothianidin metabolite TZMU

SYNONYMS: TZMU

CITATION: Ruddock, W.D. (1999). TZMU Acute Oral Toxicity Study in the Rat. Covance Laboratories. Harrogate, North Yorkshire, England. Laboratory report number 586/152-D6144, February 4, 1999. Unpublished. MRID 45422624.

SPONSOR: Takeda Chemical Industries Ltd. Agro Company, Chuo-ku, Tokyo, Japan.

EXECUTIVE SUMMARY: In an acute oral toxicity study, groups of fasted, young adult Crl:CD.BR, rats (5/sex) were given a single oral gavage dose of TZMU (99.3% a.i.) in a 5% aqueous solution of arabic gum at doses of 920, 1152, 1440, 1800 or 2250 mg/kg bw and observed for 15 days.

Deaths occurred between 4.5 hours and 2 days following dosing. Principal clinical signs of reaction to treatment were lethargy and palpebral closure seen in all animals in all groups. Other reactions to treatment included low gait, hunched posture, piloerection, ataxia, pronation, flaccidity, vasodilation and breathing irregularities which increased in incidence with increasing dose level. Onset of principal clinical signs was between ¼ and ½ hour after treatment and recovery of surviving rats was advanced by Day 2 and complete by Day 3.

Oral LD_{50} Males = 1424 mg/kg bw (95% C.I. 1104 - 1824)
Females = 1282 mg/kg bw (95% C.I.912 - 1613)
Combined = 1351 mg/kg bw (95% C.I.1145 - 1565)

TZMU **is of SLIGHT Toxicity** based on the LD_{50} in both males and females [EPA Toxicity Category III]. Labels should include a **CAUTION POISON** hazard identification along with the accompanying symbol.

This acute oral study is classified acceptable, and satisfies the guideline requirement for an acute oral study (OPPTS 870.1100; OECD 401) in the Rat.

COMPLIANCE: Signed and dated GLP, Quality Assurance, and No Data Confidentiality statements were provided.

I. MATERIALS AND METHODS

A. MATERIALS:

1 **Test Material:** TZMU

Description:	White powder
Lot/Batch #:	89066326
Purity:	99.3 % a.i.

2. **Vehicle and/or positive control:** Prepared in a 5% aqueous solution of arabic gum (dosed at 20 ml/kg bw)

3 **Test animals:**

Species:	Rat	
Strain:	Crl:CD.BR	
Age/weight at dosing:	7 - 11 weeks (♂/♀) / 239 - 347 g (♂) or 213 - 250 g (♀)	
Source:	Charles River (UK) Ltd,	
Housing:	1 - 5 / stainless steel mesh cage	
Diet:	SQC(E) Rat and Mouse Maintenance Diet No 1. *ad libitum*	
Water:	Mains water *ad libitum*	
Environmental conditions:	**Temperature:**	19 - 25 °C
	Humidity:	40 - 80 %
	Air changes:	10 / hr
	Photoperiod:	12 hrs dark/ 12 hrs light
Acclimation period:	7 - 15 days	

B. STUDY DESIGN and METHODS:

1. **In life dates** - Start: September 3, 1998 End: October 14, 1998

2. **Animal assignment and treatment** - Animals were assigned to the test groups noted in Table 1. Following an overnight fast, rats were given a single dose of TZMU by gavage then observed and weighed for 14 days. Observations were recorded frequently on the day of dosing, twice daily on days 2 through day 4, and daily for the remainder of the study. Body weights were recorded on the day of dosing, day 8 and at termination on day 15. Survivors were sacrificed and a necropsy was performed on all animals.

TABLE 1. Doses, mortality/animals treated

Dose (mg/kg bw)	Males	Females	Combined
920	37260	37260	37265
1152	37260	37319	37355
1440	37350	37350	37477
1800	37350	37319	37477
2250	37350	37380	37508

3. Statistics - The oral LD_{50}, and 95% confidence limits were calculated using a computerized version of probit analysis.

II. RESULTS AND DISCUSSION:

A. Mortality is given in Table 1. Rats were found dead following a single oral dose of TZMU among males and females dosed at 1800 mg/kg and 2250 mg/kg. Three male and two female rats were found dead and one male and two female rats were killed *in extremis* following treatment at 1440 mg/kg. Decedents amongst animals treated at 1152 mg/kg (one male and three females) were killed *in extremis*. Deaths occurred at intervals from within 4½ hours of dosing up to Day 2.

The acute median lethal oral dose (LD_{50}) and its 95% confidence limits, for combined sexes was 1351 (1145 to 1565) mg/kg, for males was 1424 (1104 to 1824) mg/kg and females 1282 (912 to 1613) mg/kg.

B. Clinical observations - Principal clinical signs of reaction to treatment were lethargy and palpebral closure seen in all animals in all groups. Other reactions to treatment included:
Low gait in males dosed at 920 mg/kg; Hunched posture, piloerection and ataxia in males and females treated at 1152 mg/kg and greater doses;-Pronation in males and females dosed at 1440 mg/kg and greater; -flaccidity in males dosed at 1440 mg/kg and females treated at 2250 mg/kg;
-vasodilatation seen in males and females treated at 2250 mg/kg; Males treated at 2250 mg/kg were unconscious and salivated after treatment and females were salivating and lachrymating;
A number of isolated cases of the above observations were seen in other treated groups. Isolated cases of dyspnoea, bradypnoea, tachypnoea, rales, sunken flanks, limp, twitching, staining of the snout, exophthalmus, chromodacryorrhoea, unkempt appearance and hypothermia were seen in treated animals. Onset of principal clinical signs was between ¼ and ½ hour after treatment and recovery of surviving rats was advanced by Day 2 and complete by Day 3.

C. Body Weight - All surviving rats achieved body weight gains during the first and second weeks of the study.

D. Necropsy - Principal necropsy findings of decedents included inflated and dark lungs, pale/mottled liver, yellow colouration to the mucosal surfaces of the stomach and impaction of the caecum. Less common findings included renal pelvic dilatation, pale kidneys, red fluid in the abdominal/thoracic cavity and small intestine, red jejunum, distension of the small intestine, yellow coloured/large mesenteric lymph nodes and uterine distension. Isolated findings included reddening of the liver, abnormal appearance of the eye, dark contents of the stomach, thickened, pale and gelatinous stomach, pale/red/yellow mucosal surface and red foci of the duodenum, clear fluid in the thoracic cavity, gaseous distension and abnormal contents of the jejunum and darkened caecum and uterus. No macroscopic changes were found at the terminal necropsy of all surviving animals on Day 15.

E. Reviewer's Conclusions: "Following a single administration of TZMU to male and female rats, the acute median lethal oral doses (LD_{50}) and its 95% confidence limits was estimated to be 1351 (1145 to 1565) mg/kg." The reviewer is in agreement with the study author.

F. Deficiencies - None noted.

PMRA ARLA

for Reviewer: Scott Hancock , Date May 26, 2002

Pamela M Hurley

TXR No. 0050321

STUDY TYPE: Acute Oral Toxicity - *Rat* OPPTS 870.1100; OECD 401.

TEST MATERIAL (PURITY): Clothianidin metabolite TZNG

SYNONYMS: TZNG

CITATION: Ruddock, W.D. (2000). TZNG Acute Oral Toxicity Study in the Rat. Covance Laboratories. Harrogate, North Yorkshire, England. Laboratory report number 586/163-D6144, January 20, 2000. Unpublished. MRID 45422626.

SPONSOR: Takeda Chemical Industries Ltd. Agro Company, Chuo-ku, Tokyo, Japan.

EXECUTIVE SUMMARY: In an acute oral toxicity study, groups of young adult Crl:CD.BR, rats (5/group) were given a single oral gavage dose of TZNG (98.6% a.i.) in a 5% aqueous solution of arabic gum at doses of 1125, 1350 and 1620 mg/kg bw or 1450 mg/kg bw for females and males respectively. Animals were observed for 15 days following dosing.

Oral LD_{50} Males > 1450 mg/kg bw
Females = 1481 mg/kg bw (95% C.I. 1257 - 1882 mg/kg bw)

Female mortalities occurred in the two highest dose groups between the 2nd and 5th days following dosing. Lethargy and palpebral closure were observed in animals in all dose groups and females in all dose groups displayed arched gait. Hunched posture and piloerection were noted in females dosed at 1350 and 1620 mg/kg bw, while piloerection was also noted in males. Some weight loss was evident in the first week following dosing, while all treated rats gained weight from Day 8 to Day 15 and gained weight overall between Day 1 and Day 15. Necropsy of males revealed small and soft testes, while female decedents had red discolouration of the lungs and stomach.

TZNG is of SLIGHT Toxicity based on the LD_{50} in females.[EPA Toxicity Category III].

This acute oral study is classified acceptable, and satisfies the guideline requirement for an acute oral study (OPPTS 870.1100; OECD 401) in the Rat.

COMPLIANCE: Signed and dated GLP, Quality Assurance, and No Data Confidentiality statements were provided.

I. MATERIALS AND METHODS

A. MATERIALS:

1. **Test Material:** TZNG
   - **Description:** White powder
   - **Lot/Batch #:** 89066524
   - **Purity:** 98.6 % a.i.

2. **Vehicle and/or positive control:** Prepared in a 5% aqueous solution of arabic gum (dosed at 20 ml/kg bw)

3. **Test animals:**
   - **Species:** Rat
   - **Strain:** Crl:CD.BR
   - **Age/weight at dosing:** 8-11 weeks (♂/♀) / 326-366 g (♂) or 218-246 g (♀)
   - **Source:** Charles River (UK) Ltd,
   - **Housing:** 1 - 5 / stainless steel mesh cage
   - **Diet:** SQC(E) Rat and Mouse Maintenance Diet No 1. *ad libitum*
   - **Water:** Mains water *ad libitum*
   - **Environmental conditions:**
     - **Temperature:** 19 - 25 °C
     - **Humidity:** 40 - 70 %
     - **Air changes:** 14 / hr
     - **Photoperiod:** 12 hrs dark/ 12 hrs light
   - **Acclimation period:** 8 - 21 days

B. STUDY DESIGN and METHODS:

1. **In life dates** - Start: February 4, 1999 End: March 23, 1999

2. **Animal assignment and treatment** - Animals were assigned to the test groups noted in Table 1. A preliminary study was conducted whereby groups of 2 female rats were dosed at 1000, 1200, 1400, 1800 and 2000 mg/kg bw. The results of this preliminary study were used for dose selection for the main study. Following an overnight fast, rats were given a single dose of TZNG by gavage then observed and weighed for 14 days. Observations were recorded frequently on the day of dosing, twice daily on days 2 through day 4, and daily for the remainder of the study. Body weights were recorded on the day of dosing, day 8 and at termination on day 15. Survivors were sacrificed and a necropsy was performed on all animals.

TABLE 1. Doses, mortality/animals treated

Dose (mg/kg bw)	Males	Females
1125	-	0/5
1350	-	1/5
1450	0/5	-
1620	-	4/5

3. **Statistics** - The oral LD_{50}, and 95% confidence limits were calculated using a computerized version of probit analysis.

II. RESULTS AND DISCUSSION:

A. Mortality is given in Table 1. Three rats were found dead on Day 3 and one on Day 4 following treatment at 1620 mg/kg. One rat dosed at 1350 mg/kg was found dead on Day 5. There were no deaths amongst rats dose at 1125 or 1450 mg/kg.

B. Clinical observations - Lethargy and palpebral closure were observed in animals in all dose groups and females in all dose groups displayed arched gait. Hunched posture and piloerection were noted in females dosed at 1350 and 1620 mg/kg bw, while piloerection was also noted in males.

C. Body Weight - Among surviving rats, some marked body weight losses were recorded between Day 1 and Day 8. All treated rats gained weight from Day 8 to Day 15 and gained weight overall between Day 1 and Day 15.

D. Necropsy - No macroscopic changes were observed at necropsy of the female rats killed on Day 15. Necropsy of males dosed at 1450 mg/kg revealed small testes affecting all rats, soft testes in two of the same animals and distension of the colon affecting one rat. Macroscopic examination of decedents treated at 1620 mg/kg included dark and red lungs, distended and pale stomach and dark foci and red area also affecting the stomach. Necropsy of one rat found dead after treatment at 1350 mg/kg revealed a small spleen and pale stomach.

E. Reviewer's Conclusions: "Following a single administration of TZNG to female rats, the acute median lethal oral dose (LD_{50}) and its 95% confidence limits were estimated to be 1481 (1257 to 1882) mg/kg. A confirmatory dose of 1450 mg/kg administered to male fasted rats, resulted in no death, indicating that males were not markedly more susceptible to the toxic effects of TZNG than were the females." The reviewer is in agreement with the study author.

F. Deficiencies - None noted

PMRA ARLA

for Reviewer: Scott Hancock , Date May 26, 2002
Pamela M Hurley

TXR No. 0050321

STUDY TYPE: Acute Oral Toxicity - Rat. OPPTS 870.1100; OECD 401.

TEST MATERIAL (PURITY): Clothianidin metabolite TMG (98.6% a.i.)

SYNONYMS: TMG

CITATION: Ruddock, W.D. (1999). TMG Acute Oral Toxicity Study in the Rat. Covance Laboratories. Harrogate, North Yorkshire, England. Laboratory report number 586/164-D6144, July 8, 1999. Unpublished. MRID 45422627.

SPONSOR: Takeda Chemical Industries Ltd. Agro Company, Chuo-ku, Tokyo, Japan.

EXECUTIVE SUMMARY: In an acute oral toxicity study, groups of young adult Crl:CD.BR, rats (5/group) were given a single oral gavage dose of TMG (98.6% a.i.) in a 5% aqueous solution of arabic gum at doses of 225, 650 and 1100 mg/kg bw for females or 550 mg/kg bw for males respectively. Animals were observed for 15 days following dosing.

Rats were found dead following treatment at 550 mg/kg and greater. Deaths occurred at intervals from within 1 hour of dosing until Day 2. Clinical signs included lethargy and palpebral closure seen in animals at all doses. Tremors, twitching, labored or rapid breathing and prone body position were noted to occur in a dose dependent manner. Clinical signs were first apparent between ¼ and four hours after treatment, while surviving animals had recovered by day 2 of the recovery period. All surviving rats gained weight during the first and second weeks of the observation period. Principal necropsy findings of decedents included dark and inflated lungs, darkened livers and distension and reddening of the jejunum. There were no treatment related macroscopic changes apparent at necropsy of animals killed on Day 15.

Oral LD_{50} Males < 550 mg/kg bw
Females = 567 mg/kg bw

TMG is of HIGH toxicity based on the LD_{50} in males. [EPA Toxicity Category II].

This acute oral study is classified acceptable, and satisfies the guideline requirement for an acute oral study (OPPTS 870.1100; OECD 401) in the Rat.

COMPLIANCE: Signed and dated GLP, Quality Assurance, and No Data Confidentiality statements were provided.

I. MATERIALS AND METHODS

A. MATERIALS:

1. **Test Material:** TMG
   - **Description:** Yellow solid
   - **Lot/Batch #:** 30063517
   - **Purity:** 96 % a.i.

2. **Vehicle and/or positive control:** Prepared in a 5% aqueous solution of arabic gum (dosed at 20 ml/kg bw)

3. **Test animals:**

Species:	Rat	
Strain:	Crl:CD.BR	
Age/weight at dosing:	8-11 weeks (♂/♀) / 323-360 g (♂) or 212-259 g (♀)	
Source:	Charles River (UK) Ltd,	
Housing:	1 - 5 / stainless steel mesh cage	
Diet:	SQC(E) Rat and Mouse Maintenance Diet No 1. *ad libitum*	
Water:	Mains water *ad libitum*	
Environmental conditions:	**Temperature:**	19 - 25 °C
	Humidity:	40 - 70 %
	Air changes:	14 / hr
	Photoperiod:	12 hrs dark/ 12 hrs light
Acclimation period:	8 - 21 days	

B. STUDY DESIGN and METHODS:

1. **In life dates** - Start: February 4, 1999 End: March 23, 1999

2. **Animal assignment and treatment** - Animals were assigned to the test groups noted in Table 1. A preliminary study was conducted whereby groups of 2 female rats were dosed at 400, 570, 800, 1120 and 2000 mg/kg bw. All rats died following treatment at 1120 or 2000mg/kg and one rat was found dead in each group treated at 570 or 800 mg/kg. Both rats survived treatment at 400 mg/kg. Deaths occurred between ¼ and 3 hours after treatment. The results of this preliminary study were used for dose selection for the main study. Following an overnight fast, rats were given a single dose of TMG by gavage then observed and weighed for 14 days. Observations were recorded frequently on the day of dosing, twice daily on days 2 through day 4, and daily for the remainder of the study. Body weights were recorded on the day of dosing, day 8 and at termination on day 15. Survivors were sacrificed and a necropsy was performed on all animals.

TABLE 1. Doses, mortality/animals treated

Dose (mg/kg bw)	Males	Females
225	-	0/5
550	3/5	-
650	-	4/5
1100	-	5/0

3. Statistics - The oral LD_{50}, and 95% confidence limits were calculated using a computerized version of probit analysis.

II. RESULTS AND DISCUSSION:

A. Mortality is given in Table 1. Rats were found dead following treatment at 550 mg/kg (three males), 650 mg/kg (four females) or 1100 mg/kg (five females). Deaths occurred at intervals from within 1 hour of dosing until Day 2. There were no deaths following treatment at 225 mg/kg.

B. Clinical observations - Principal clinical signs of reaction to treatment were lethargy and palpebral closure seen in all animals dosed at 550 mg/kg and above and a number of rats treated at 225 mg/kg. Other reactions included: Tremors, twitching, dyspnoca, tachypnoca and prone body position. The incidence of these clinical signs generally increased in a dose dependent manner. Clinical signs were first apparent between ¼ and four hours after treatment, while surviving animals had recovered by day 2 of the recovery period.

C. Body Weight - All surviving rats gained weight during the first and second weeks of the observation period.

D. Necropsy - Principal necropsy findings of decedents included dark and inflated lungs, darkened livers and distension and reddening of the jejunum. Less common findings included mottling of the lung and liver and a yellow jejunum. There were no treatment related macroscopic changes apparent at necropsy of animals killed on Day 15.

E. Reviewer's Conclusions: "Following a single administration of TMG to female rats, the acute median lethal oral doses (LD_{50}) was estimated to be 567 mg/kg. A confirmatory dose of 550 mg/kg administered to male rats resulted in three deaths which indicated that the males were not markedly more susceptible to the toxic effects of TMG than were females." Since 60% of males died following dosing at 550 mg/kg bw, it is possible that the LD_{50} for males could be below 500 mg/kg bw which would classify the compound as highly toxic. In the absence of data to rule out this possibility, such precautionary hazard classification is warranted.

F. Deficiencies - None noted.

689

PMRA ARLA

for Reviewer: Scott Hancock , Date May 26, 2002
Pamela M Hurley

TXR No. 0050321

STUDY TYPE: Acute Oral Toxicity - *[Rat]* OPPTS 870.1100; OECD 401.

TEST MATERIAL (PURITY): Clothianidin metabolite BN0230M

SYNONYMS: BN0230M (94.4% a.i.)

CITATION: Ruddock, W.D. (2000). BN0230M Acute Oral Toxicity Study in the Rat. Covance Laboratories. Harrogate, North Yorkshire, England. Laboratory report number 586/228-D6144, September 5, 2000. Unpublished. MRID 45422628.

SPONSOR: Takeda Chemical Industries Ltd. Agro Company, Chuo-ku, Tokyo, Japan.

EXECUTIVE SUMMARY: In an acute oral toxicity study, groups of young adult Crl:CD.BR, rats (5/sex) were given a single oral gavage dose of BN0230M (94.4% a.i.) in corn oil at a dose of 2000 mg/kg bw. Animals were observed for 15 days following dosing.

There were no mortalities recorded during the study. There were no signs of reaction to treatment among females. Males showed breathing changes, between ¼ and 1¾ hours of dosing. All males were overtly normal by the 3 hour observation. All rats achieved body weight gains during the first and second weeks of the study. There were no treatment related macroscopic signs of toxicity reported during the necropsy examination.

Oral LD_{50} Males and Females > 2000 mg/kg bw
No animals died.

BN0230M is of LOW toxicity based on the LD_{50} in both males and females. [EPA Toxicity Category III]

This acute oral study is classified acceptable, and satisfies the guideline requirement for an acute oral study (OPPTS 870.1100; OECD 401) in the Rat.

COMPLIANCE: Signed and dated GLP, Quality Assurance, and No Data Confidentiality statements were provided

I. MATERIALS AND METHODS

A. MATERIALS:

1. **Test Material:** BN0230M
   Description: White powder
   Lot/Batch #: 30068304
   Purity: (94.4% a.i.)

2. **Vehicle and/or positive control:** *Prepared in corn oil* (dose volume 10 ml/kg bw)

3. **Test animals:**

Species:	Rat	
Strain:	*Crl:CD.BR*	
Age/weight at dosing:	6-8 weeks (♂) 10-11 (♀) / 245-264 g (♂) or 237-263 g (♀)	
Source:	Charles River (UK) Ltd,	
Housing:	1 - 5 / stainless steel mesh cage	
Diet:	SQC(E) Rat and Mouse Maintenance Diet No 1. *ad libitum*	
Water:	Mains water *ad libitum*	
Environmental conditions:	**Temperature:**	19 - 25 °C
	Humidity:	40 - 70 %
	Air changes:	14 / hr
	Photoperiod:	12 hrs dark/ 12 hrs light
Acclimation period:	8-14 days	

B. STUDY DESIGN and METHODS:

1. **In life dates** - Start: October 19, 1999 End: November 24, 1999

2. **Animal assignment and treatment** - Animals were assigned to the test groups noted in Table 1. A preliminary study was conducted whereby groups of 2 female rats were dosed at 500, 1215 or 2000 mg/kg bw. There were no deaths recorded during the preliminary study. The results of this preliminary study were used for dose selection for the main study. Following an overnight fast, rats were given a single dose of BN0230M by gavage then observed and weighed for 14 days. Observations were recorded frequently on the day of dosing, twice daily on days 2 through day 4, and daily for the remainder of the study. Body weights were recorded on the day of dosing, day 8 and at termination on day 15. Survivors were sacrificed and a necropsy was performed on all animals.

TABLE 1. Doses, mortality/animals treated

Dose (mg/kg bw)	Males	Females
2000	0/5	0/5

3. **Statistics** -Since there were no deaths, a LD_{50} could not be calculated.

II. RESULTS AND DISCUSSION:

A. Mortality is given in Table 1. There were no mortalities recorded during the study.

B. Clinical observations - There were no signs of reaction to treatment among the females. Males showed tachypnoea between ¼ and 1¾ hours of dosing. All males were overtly normal by the 3 hour observation.

C. Body Weight - All rats achieved body weight gains during the first and second weeks of the study.

D. Necropsy - There were no treatment related macroscopic signs of toxicity reported during the necropsy examination.

E. Reviewer's Conclusions: "Single oral administration of BN0230M at a dose level of 2000 mg/kg caused no death in a group of ten fasted rats. The acute median lethal oral dose level (LD_{50}) was found to exceed 2000 mg/kg." The reviewer is in agreement with the study author.

F. Deficiencies - None noted.

Reviewer: Scott Hancock , Date May 26, 2002

TXR No. 0050321

STUDY TYPE: Acute Oral Toxicity - *[Rat]* OPPTS 870.1100; OECD 401.

TEST MATERIAL (PURITY): TI-435-CCMT-Adduct (99.4%)

SYNONYMS: Clothianidin intermediate

CITATION: Krotlinger, F. (2000) TI-435-CCMT-Adduct Study for Acute Oral Toxicity in Rats. Bayer AG, Department of Toxicology, Wuppertal, Germany. Laboratory report number T2069070, April 6, 2000. Unpublished. MRID 45422630

SPONSOR: Takeda Chemical Industries Ltd. Agro Company, Chuo-ku, Tokyo, Japan.

EXECUTIVE SUMMARY: In an acute oral toxicity study, groups of fasted, young adult, HsdCpb:WU Wistar, rats (3/sex) were given a single oral gavage dose of TI-435-CCMT-Adduct (99.4%) in 0.5% aqueous carboxymethyl cellulose at a dose of 2000 mg/kg bw and observed for 15 days.

Oral administration of TI-435-CCMT-Adduct (99.4%) resulted in no mortalities at a limit dose of 2000 mg/kg bw. Similarly there were no clinical signs of toxicity, no effects on body weight or body weight gain, and no evidence of toxicity noted during gross necropsy.

Oral LD_{50} Males and females > 2000 mg/kg bw

TI-435-CCMT-Adduct (99.4%) **is of LOW Toxicity** based on the LD_{50} in both males and females. [EPA Toxicity Category III].

This acute oral study is classified acceptable and satisfies the guideline requirement for an acute oral study (OPPTS 870.1100; OECD 401) in the rat.

COMPLIANCE: Signed and dated GLP, Quality Assurance, and No Data Confidentiality statements were provided.

I. MATERIALS AND METHODS

A. MATERIALS:

1. **Test Material:** TI-435-CCMT-Adduct (99.4%)
   Description: Beige to brownish powder
   Lot/Batch #: 17007/99
   Purity: 99.4 % a.i.
   CAS #: Not noted.

2. **Vehicle and/or positive control:** Prepared in 0.5% aqueous carboxymethyl cellulose (dosed at 10 ml/kg bw)

3. **Test animals:**

Species:	Rat	
Strain:	HsdCpb:WU Wistar	
Age/weight at dosing:	8 weeks (♂) or 9 weeks (♀) / 217-230 g (♂) or 167-175 g (♀)	
Source:	Harlan Winkelmann GmbH, Borchen, Paderborn	
Housing:	3 per polycarbonate cage prior to dosing the one per cage for the remainder of the study	
Diet:	Altromin 1324 Diet for Rats and Mice *ad libitum*	
Water:	Tap water *ad libitum*	
Environmental conditions:	**Temperature:**	20-24°C
	Humidity:	50-60 %
	Air changes:	15-20/ hr
	Photoperiod:	12 hrs dark/ 12 hrs light
Acclimation period:	5 days	

B. STUDY DESIGN and METHODS:

1. **In life dates** - Start: January 7, 2000 End: January 26, 2000

2. **Animal assignment and treatment** - The test substance was formulated in 0.5% aqueous carboxymethyl cellulose before administration. The applied formulations were well mixed by pumping the syringe several times. The test substance was administered orally, in a single dose by stomach tube to fasted male and female rats (fasted for approximately 17 hours). A volume of 10 ml/kg body weight was administered. The animals were allowed to feed approximately two hours after administration. Appearance and behaviour were recorded several times on the day of treatment, and at least once a day thereafter. Where signs occurred, the type, period and intensity (1= weak, 2 = moderate up to strong) were determined individually. The post-treatment observation period lasted for at least 14 days.

The body weights of the rats were recorded on day 1 before administration and then weekly. Additionally, all animals that died or were sacrificed were weighed. After the end of the post-treatment observation period the animals were anesthetized by inhaling diethyl ether and sacrificed. They were then subjected to a gross pathology examination, as were any animals which may have died during the study.

TABLE 1. Doses, mortality/animals treated

Dose (mg/kg bw)	Males	Females	Combined
2000	0/3	0/3	0/6

3. Statistics - Since there were no mortalities, a LD_{50} could not be determined.

II. RESULTS AND DISCUSSION:

A. Mortality is given in Table 1. No animals died during the study period.

The oral LD_{50} for both male and female rats is greater than 2000 mg/kg bw.

B. Clinical observations - There were no clinical signs of toxicity evident during the study period.

C. Body Weight - Body weight and body weight gain were unaffected by treatment during the course of the study.

D. Necropsy - There were no pathological signs of toxicity noted during the gross necropsy at the study termination.

E. Reviewer's Conclusions "Based on the present investigations, the test substance is therefore to be regarded as non-toxic." The reviewer agrees with the study author, in that the LD_{50} is >2000 mg/kg bw, and as such the test material is of low toxicity by the oral route of exposure.

F. Deficiencies - None noted.

Reviewer: Scott Hancock , Date May 26, 2002

Pamela Hurley

TXR No. 0050321

STUDY TYPE: Acute Oral Toxicity - *[Rat]* OPPTS 870.1100; OECD 401.

TEST MATERIAL (PURITY): TI-435-Hexahydropyrimidine (99.5%)

SYNONYMS: Clothianidin intermediate

CITATION: Krotlinger, F. (2000). TI-435-Hexahydropyrimidine (By-Product of Technical TI-435) Study for Acute Oral Toxicity in Rats. Bayer AG, Department of Toxicology, Wuppertal, Germany. Laboratory report number T4070025, December 13, 2000. Unpublished. MRID 45422631.

SPONSOR: Takeda Chemical Industries Ltd. Agro Company, Chuo-ku, Tokyo, Japan.

EXECUTIVE SUMMARY: In an acute oral toxicity study, groups of fasted, young adult, HsdCpb:WU Wistar, rats (3/sex) were given a single oral gavage dose of TI-435-Hexahydropyrimidine (99.5%) in 2% aqueous Cremophor EL at a dose of 2000 mg/kg bw and observed for 15 days.

Oral administration of TI-435-Hexahydropyrimidine (99.5%) resulted in no mortalities at a limit dose of 2000 mg/kg bw. Similarly there were no clinical signs of toxicity, no effects on body weight or body weight gain in males, and no evidence of toxicity noted during gross necropsy. There was a marginal decrease in body weight observed for one female on day 15 of the study period.

Oral LD_{50} Males and females > 2000 mg/kg bw

TI-435-Hexahydropyrimidine (99.5%) **is of LOW Toxicity** based on the LD_{50} in both males and females. [EPA Toxicity Category III].

This acute oral study is classified acceptable and satisfies the guideline requirement for an acute oral study (OPPTS 870.1100; OECD 401) in the rat.

COMPLIANCE: Signed and dated GLP, Quality Assurance, and No Data Confidentiality statements were provided.

I. MATERIALS AND METHODS

A. MATERIALS:

1. **Test Material:** TI-435-Hexahydropyrimidine (99.5%)
   - **Description:** White crystals
   - **Lot/Batch #:** GAW 2641
   - **Purity:** 99.5 % a.i.
   - **Molecular formula** $C_{21}H_{25}N_3$

2. **Vehicle and/or positive control:** Prepared in 2% aqueous Cremophor EL (dosed at 10 ml/kg bw)

3. **Test animals:**
   - **Species:** Rat
   - **Strain:** HsdCpb:WU Wistar
   - **Age/weight at dosing:** 8 weeks (♂) or 10 weeks (♀) / 218-233 g (♂) or 171-178 g (♀)
   - **Source:** Harlan Winkelmann GmbH, Borchen, Paderborn
   - **Housing:** 3 per polycarbonate cage
   - **Diet:** NAFAG No: 9439 Long Life W 10 (Eberle Nafag AG, Gossau) *ad libitum*
   - **Water:** Tap water *ad libitum*
   - **Environmental conditions:**
     - **Temperature:** 20-24°C
     - **Humidity:** 50-60 %
     - **Air changes:** 15-20/ hr
     - **Photoperiod:** 12 hrs dark/ 12 hrs light
   - **Acclimation period:** 5 days

B. STUDY DESIGN and METHODS:

1. **In life dates** - Start: November 6, 2000 End: November 24, 2000

2. **Animal assignment and treatment** - The test substance was formulated in 2% aqueous Cremophor EL before administration. The applied formulations were well mixed by pumping the syringe several times. The test substance was administered in a single dose by stomach tube oral to fasted male and female rats (fasted for approximately 17 hours). A volume of 10 ml/kg body weight was administered. The animals were allowed to feed approximately two hours after administration. Appearance and behaviour were recorded several times on the day of treatment, and at least once a day thereafter. Where signs occurred, the type, period and intensity (1= weak, 2 = moderate up to strong) were determined individually. The post-treatment observation period lasted for at least 14 days.

The body weights of the rats were recorded on day 1 before administration and then weekly. Additionally, all animals that died or were sacrificed were weighed. After the end of the post-treatment observation period the animals were anesthetized by inhaling diethyl ether and sacrificed. They were then subjected to a gross pathology examination, as were any animals which may have died during the study.

TABLE 1. Doses, mortality/animals treated

Dose (mg/kg bw)	Males	Females	Combined
2000	0/3	0/3	0/6

3. Statistics - Since there were no mortalities, a LD_{50} could not be determined.

II. RESULTS AND DISCUSSION:

A. Mortality is given in Table 1. No animals died during the study period.

The oral LD_{50} for both male and female rats is greater than 2000 mg/kg bw.

B. Clinical observations - There were no clinical signs of toxicity evident during the study period.

C. Body Weight - Body weight and body weight gain of male rats were unaffected by treatment during the course of the study. There was a marginal decrease in body weight observed for one female on day 15 of the study period.

D. Necropsy - There were no pathological signs of toxicity noted during the gross necropsy at the study termination.

E. Reviewer's Conclusions "Based on the present investigations, the test substance is regarded as non-toxic." The reviewer agrees with the study author, in that the LD_{50} is >2000 mg/kg bw, and as such the test material is of low toxicity by the oral route of exposure.

F. Deficiencies - None noted.

PMRA ARLA

for Reviewer: Scott Hancock , Date May 26, 2002

Pamela McHenley

TXR No. 0050321

STUDY TYPE: Acute Oral Toxicity - *[Rat]* OPPTS 870.1100; OECD 401.

TEST MATERIAL (PURITY): TI-435-Triazan (95.6%)

SYNONYMS: Clothianidin Intermediate

CITATION: Krotlinger, F. (2000) TI 435-Triazan Acute Oral Toxicity Study in Male and Female Wistar Rats. Bayer AG, Department of Toxicology, Wuppertal, Germany. Laboratory report number T5069640, July 25, 2000. Unpublished. MRID 45422632

SPONSOR: Takeda Chemical Industries Ltd. Agro Company, Chuo-ku, Tokyo, Japan.

EXECUTIVE SUMMARY: In an acute oral toxicity study, groups of fasted, young adult, HsdCpb:WU Wistar, rats (3/sex) were given a single oral gavage dose of TI-435-Triazan (95.6%) in polyethylene glycol 400 at a dose of 2000 mg/kg bw and observed for 15 days.

Oral administration of TI-435-Triazan resulted in no mortalities at a limit dose of 2000 mg/kg bw. Similarly there were no clinical signs of toxicity, no effects on body weight or body weight gain, and no evidence of toxicity noted during a gross necropsy.

Oral LD_{50} Males and females > 2000 mg/kg bw

TI-435-Triazan (95.6%) **is of LOW Toxicity** based on the LD_{50} in both males and females. [EPA Toxicity Category III].

This acute oral study is classified acceptable and satisfies the guideline requirement for an acute oral study (OPPTS 870.1100; OECD 401) in the rat.

COMPLIANCE: Signed and dated GLP, Quality Assurance, and No Data Confidentiality statements were provided.

699

I. MATERIALS AND METHODS

A. MATERIALS:

1. **Test Material:** TI-435-Triazan (95.6%)
   Description: White crystal
   Lot/Batch #: VE 97040 Pt. 17003/99
   Purity: 95.6 % a.i.
   CAS #: 141856-57-7

2. **Vehicle and/or positive control:** Prepared in PEG 400 (dosed at 5 ml/kg bw)

3. **Test animals:**

Species:	Rat	
Strain:	HsdCpb:WU Wistar	
Age/weight at dosing:	8 weeks (♂) or 11 weeks (♀) / 226-233 g (♂) or 173-179 g (♀)	
Source:	Harlan Winkelmann GmbH, Borchen, Paderborn	
Housing:	3 per polycarbonate cage	
Diet:	Altromin 1324 Diet for Rats and Mice *ad libitum*	
Water:	Tap water *ad libitum*	
Environmental conditions:	**Temperature:**	20-24°C
	Humidity:	50-60 %
	Air changes:	15-20/ hr
	Photoperiod:	12 hrs dark/ 12 hrs light
Acclimation period:	5 days	

B. STUDY DESIGN and METHODS:

1. **In life dates** - Start: May 4, 2000 End: May 24, 2000

2. **Animal assignment and treatment** - The test substance was formulated in polyethylene glycol 400 before administration. The applied formulations were well mixed by pumping the syringe several times. The test substance was administered in a single dose by stomach tube oral to fasted male and female rats (fasted for approx. 17 hours + 2 hours). A volume of 5 ml/kg body weight was administered. The animals were allowed to feed approx. two hours after administration. Appearance and behaviour were recorded several times on the day of treatment, and at least once a day thereafter. Where signs occurred, the type, period and intensity (1= weak, 2 = moderate up to strong) were determined individually. The post-treatment observation period lasted for at least 14 days.

The body weights of the rats were recorded on day 1 before administration and then weekly. Additionally, all animals that died or were sacrificed were weighed. After the end of the post-treatment observation period the animals were anesthetized by inhaling diethyl ether and sacrificed. All animals that died or were sacrificed were subjected to a gross pathology examination.

TABLE 1. Doses, mortality/animals treated

Dose (mg/kg bw)	Males	Females	Combined
2000	0/3	0/3	0/6

3. Statistics - Since there were no mortalities, a LD_{50} could not be determined.

II. RESULTS AND DISCUSSION:

A. Mortality is given in Table 1. No animals died during the study period. .

The oral LD_{50} for both male and female rats is greater than 2000 mg/kg bw.

B. Clinical observations - There were no clinical signs of toxicity evident during the study period.

C. Body Weight - Body weight and body weight gain were unaffected by treatment during the course of the study.

D. Necropsy - There were no pathological signs of toxicity noted during the gross necropsy at the study termination.

E. Reviewer's Conclusions "Based on the present investigations, the test substance is therefore to be regarded as non-toxic." The reviewer agrees with the study author, in that the LD_{50} is >2000 mg/kg bw, and as such the test material is of low toxicity by the oral route of exposure.

F. Deficiencies - None noted.

Reviewer: Scott Hancock , Date May 28/02

TXR No. 0050321

STUDY TYPE: Dermal Sensitization - Guinea Pig; OPPTS 870.2600 [§81-6]; OECD 406.

TEST MATERIAL (PURITY): TI-435-CCMT-Adduct (99.4%)

SYNONYMS: Clothianidin Intermediate

CITATION: Vohr, H.W. (2000). TI-435-CCMT-Adduct Study for the Skin Sensitization Effect in Guinea Pigs (Guinea Pig Maximization Test According to Magnusson and Kligman).Bayer AG, Department of Toxicology, Wuppertal, Germany. Laboratory report number T9069272, April 13, 2000. Unpublished. MRID 45422815

SPONSOR: Takeda Chemical Industries Ltd. Agro Company, Chuo-ku, Tokyo, Japan.

EXECUTIVE SUMMARY: In a dermal sensitization study with TI-435-CCMT-Adduct (99.4%) in polyethylene glycol 400, young adult female Hartley Guinea pigs were tested using the method of Magnusson-Kligman Maximization.

No clinical observations of ill health or toxicity were noted during the study for either the main study or positive control groups. At 48 hours following intradermal induction, control and test animals showed white wheal with red surrounding and encrustation. No irritation reactions were apparent among the animals following topical induction application of test material or vehicle control. Challenge with a 50% solution of the test material resulted in no evidence of sensitization reaction in the animals tested, while control animals displayed no signs of dermal irritation. Based on the results of the study, TI-435-CCMT-Adduct was not considered to be a dermal sensitizer.

In this study, TI-435-CCMT-Adduct (99.4%) **is not a dermal sensitizer.**

This study is classified as acceptable and satisfies the guideline requirement for a dermal sensitization study (81-6; OECD 406) in the Guinea Pig.

COMPLIANCE: Signed and dated GLP, Quality Assurance, and No Data Confidentiality statements were provided.

I. MATERIALS AND METHODS

A. MATERIALS:

1. **Test Material:** TI-435-CCMT-Adduct (99.4%)
   Description: Brownish powder
   Lot/Batch #: 17007/99
   Purity: 99.4 % a.i.

2. **Vehicle and/or positive control:** Polyethylene glycol 400

3. **Test animals:**

Species:	Guinea Pig	
Strain:	Hsd:Poc:DH, female	
Age/weight at start:	4-6 weeks old/ 314-411g	
Source:	Harlan Winkelmann GmbH Laboratory Animal Breeders, Borchen	
Housing:	5 per polypropylene cage.	
Diet:	Altromin 3020 Maintenance Diet for Guinea Pigs, Altromin GmbH, Lage ad libitum	
Water:	Tap Water *ad libitum*	
Environmental conditions:	**Temperature:**	19-25°C
	Humidity:	40-60%
	Air changes:	10/ hour
	Photoperiod:	12hrs dark/ 12hrs light
Acclimation period:	7 days	

B. STUDY DESIGN and METHODS:

1. **In life dates** - Start: January 25, 2000 End: February 18, 2000

2. **Animal assignment and treatment** - The Magnusson-Kligman Maximization Test was used to assess the sensitization potential of the test chemical TI-435-CCMT-Adduct. The dose levels used for induction and challenge were determined from a preliminary screen, whereby the dose which caused moderate irritation was used for induction and the highest non-irritating concentration was chosen for the challenge dose. Guinea pigs were observed daily for clinical signs of reaction to treatment. Animals were weighed on day 1 and day 24, following the completion of the challenge phase.

Induction:
Intradermal Injection:
The dorsum of each test and control guinea pig was clipped on the day before treatment commenced. The denuded area was confirmed to be free from injury prior to injections. 20 animals were given 0.1 ml injections of the test substance (5% w/v) dissolved in PEG 400 with/without Freund's Complete Adjuvant (FCA), or FCA in physiological saline. Irritation was recorded by group on day 2. Vehicle controls were handled and prepared similar to the test group, however test material was not included in the injections.

Topical Application:
On day 7, animals were clipped, while on day 8 the area of skin including the intradermal injection sites were subject to application of a 2 x 4 cm patch treated with approximately 0.5 mL of 50% m/m TI-435-

CCMT-Adduct in PEG 400 (test animals) or PEG 400 alone (control group). Occlusion of the treated skin was effected by successive layers of aluminium foil and Oraband adhesive tape. The patches and dressings remained in place for 48 hours. Irritation or other dermal changes at the sites of occluded topical application were recorded by group on Day 11.

Challenge Stage:
The dorsal region and the left flank of the animals were shorn one day prior to the challenge, three weeks after the intradermal induction. During the challenge a hypoallergenic patch loaded with 0.5 mL of a 50% test item formulation was placed on the left flank (caudal) of the animals of the test item group and the control group and held securely in place on the skin with a ORABAND@ self- adhesive tape for 24 hours. A patch loaded only with the vehicle was placed also on the left flank (cranial) as control. Dermal responses to challenge were assessed approximately 24 and 48 hours after removal of the patches. Responses to challenge were recorded individually.

Data Interpretation:
Dermal reactions to challenge among test animals were compared with those of the control animals. The response of each test animal was classified as positive or negative according to the following system:

The criterion for existence of sensitization was taken to be the occurrence of skin reactions in the test item group animals at a higher incidence and greater intensity than in the control animals. A substance is interpreted to be sensitizing if by comparison with the control group 30 % or more of the test group animals reacted positive.

II. RESULTS AND DISCUSSION:
No clinical observations of ill health or toxicity were noted during the study for either the main study or positive control groups. The body weights of animals in the treatment group were similar to control animals at the end of the study period.

A. <u>Induction reactions and duration</u> -
Injection:
At 48 hours following injection control and test animals showed white wheal with red surrounding and encrustation.

Topical:
No irritations reactions were apparent among the animals following topical induction application of test material or vehicle control.

B. <u>Challenge reactions and duration</u> -
Naive control and test animals challenged with 50% TI-435-CCMT-Adduct displayed no dermal irritation at 48 and 72 hours post-application.

<u>Conclusion:</u>

"After the intradermal induction the animals in the control group and in the test item group showed strong effects up to encrustation at the injection sites of the first induction.

The challenge with the 50% test item formulation led to no skin effects in the test item group and no skin

effects were seen in the control group animals.

In summary, by comparing the results in the treatment group and in the control group under the conditions of the maximization test and with respect to the evaluation criteria, the test item therefore exhibits no-skin-sensitization potential." The reviewer agrees with the study author, in that the test material demonstrates no-sensitization potential under the conditions of the study.

E. Deficiencies - The individual animal irritation scores for the induction phase of the experiment were not provided in the study report. However, since the study demonstrated no irritation or sensitization reactions following challenge using the same concentration as the induction, these deficiencies did not negatively impact on the interpretation of the data.

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