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

OFFICE OF PREVENTION, PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

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

December 13, 2007
TXR # 0054347

SUBJECT: Pyroxsulam Toxicology Data Evaluation Records
PC Code: 108702
DP Barcode: D332276

FROM: Kimberly Harper Alan C. Levy for
Registration Action Branch 2
Health Effects Division (7509P)

TO: Joanne Miller, Risk Manager (RM23)
Herbicide Branch
Registration Division (7505P)

THROUGH: Richard Loranger R. Loranger
Registration Action Branch 2
Health Effects Division (7509C)

Action Requested: The Health Effects Division (HED) was requested to review the submitted toxicology studies conducted with the new chemical, pyroxsulam (XDE-742). The Data Evaluation Records (DERs) are attached for the MRIDs listed in Table 1. The acute toxicity studies were reviewed by the Registration Division (RD).

Background: Pyroxsulam is a new herbicide belonging to the triazolopyrimidine sulfonamide class of pesticides. Dow AgroSciences is currently seeking food uses on wheat, hay and straw. Pyroxsulam is being reviewed jointly by the US, Canada and Australia; it is also under current review in the European Union.

Conclusions: The toxicology database is complete and adequate for risk assessment purposes.

Read RRC 3/18/09

EPA Reviewer: Linda L. Taylor, Ph.D. **Signature:** Linda L. Taylor
Reregistration Branch, Health Effects Division (7509P) **Date:** 01-03-08
EPA Secondary Reviewer: Kimberly Harper **Signature:** Kimberly D Harper
RAB2, Health Effects Division (7509P) **Date:** 12/10/07

Template version 02/06

TXR#: 0054347

DATA EVALUATION RECORD

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

PC CODE: 108702 **DP BARCODE:** D332276

TEST MATERIAL (PURITY): XDE-742/BAS 770 H (98% a.i.) [pyroxsulam]

CHEMICAL: N-(5,7-dimethoxy[1,2,4]triazolo[1,5-a]pyrimidin-2-yl)-2-methoxy-4-(trifluro methyl)pyridine-3-sulfonamide

SYNONYMS: X666742, XDE-742, LY-666742, BAS-770H

CITATION: Kaspers, U. (2004). XDE-742 / BAS 770H – Dermal Test Study in Wistar Rats Application for 2 Weeks. Experimental Toxicology and Ecology BASF Aktiengesellschaft, Germany. Laboratory report number 13S0298/03020, September 2, 2003 to September 25, 2003. MRID 46908353.

SPONSOR: BASF Aktiengesellschaft, Germany

EXECUTIVE SUMMARY: In a 2-week dermal toxicity study (MRID 46908353), XDE-742/BAS 770 H (98% a.i.) [pyroxsulam; Batch # E0952-52-01]was applied to the shaved skin of 3 Wistar CrlG1xBrlHan:WI rats/sex/dose at dose levels of 0 or 1000 mg/kg bw/day, 6 hours/day for 5 days/week for 2 weeks. (mg/kg bw/day hereafter referred to as mg/kg/day)

There were no compound-related effects in mortality, clinical signs, body weight, food consumption, or food efficiency. Hematology, clinical chemistry, organ weights, and gross and histologic pathology were not monitored during the study. Skin irritation was not observed. **The LOAEL is >1000 mg/kg/day, based on the lack of any effect on the parameters monitored. The NOAEL is 1000 mg/kg/day (the limit dose).**

This 2-week (range-finding study/pilot) dermal toxicity study in the rat is acceptable, (non-guideline), and it does not 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, and Data Confidentiality statements were provided. The Quality Assurance statement indicated that the study itself was not inspected;

Table 1. List of Pyroxsulam Toxicology Studies with New DERs

Study Type	MRID #	Studies included in review	Comments
870.3050 Subchronic Oral Study in Rats	46908349	n/a	Acceptable/guideline
870.3100 Subchronic Oral Study in Rats	46908350	n/a	Acceptable/guideline
870.3100 Subchronic Oral Study in Mice	46908351	n/a	Acceptable/guideline
870.3150 Subchronic Oral Study in Dogs	46908352	46908401	Acceptable/guideline
870.3200 Repeat Dermal Exposure in Rats	46908353	n/a	Accetpable/nonguideline
870.3700a Prenatal Developmental Toxicity Study in Rats	46908355	46908402	Acceptable/guideline
Nonguideline Prenatal Developmental Toxicity Study in Rabbits (range-finding)	46908415	n/a	Acceptable/nonguideline
870.3700b Prenatal Developmental Toxicity Study in Rabbits (main study)	46908354	n/a	Acceptable/guideline
870.3800 2-Generation Reproduction Study in Rats	46908404	46908403	Acceptable/guideline
870.4100b Chronic Toxicity Study in Dogs	46908405	n/a	Acceptable/guideline
870.4200a Carcinogencity Study in Mice	46908406	n/a	Acceptable/guideline
870.4300 Chronic/Carcinogenicity Study in Rats	46908407	n/a	Acceptable/guideline
870.5100 *In Vitro* Bacterial Gene Mutation	46908414	n/a	Acceptable/guideline
870.5300 *In Vitro* Mammalian CHO Gene Mutation Assay	46908408	n/a	Acceptable/guideline
870.5375 *In Vitro* Mammalian Cytogenetics	46908409	n/a	Acceptable/guideline
870.5395 *In Vivo* Mammalian Cytogenetics	46908410	n/a	Acceptable/guideline
870.5550 Unscheduled DNA Synthesis	47022001	n/a	Acceptable/guideline
Nonguideline 12-month Neurotoxicity Study in Rats	46908411	n/a	Accetpable/nonguideline
870.7485 Metabolism – Rats	46908412	n/a	Acceptable/guideline
Nonguideline Metabolism – Mice	46908413	n/a	Accetpable/nonguideline

EPA Reviewer: Kimberly Harper **Signature:** Kimberly D Harper
RAB2, Health Effects Division (7509P) **Date:** 12/10/07
EPA Secondary Reviewer: Alan Levy **Signature:** Alan C. Levy
RAB2, Health Effects Division (7509P) **Date:** 1-3-2008

Template version 02/06

TXR#: 0054347

DATA EVALUATION RECORD

STUDY TYPE: 28-Day Oral Toxicity Feeding Study - rat; OPPTS 870.3050 [§82-1a] (rodent); OECD 407

PC CODE: 108702 **DP BARCODE:** 332276

TEST MATERIAL (PURITY): XR-742 (96.7%) [N-(5,7-dimethoxy[1,2,4]triazolo[1,5-a]pyrimidin-2-yl)-2-methoxy-4-(trifluoromethyl)pyridine-3-sulfonamide]

SYNONYMS: X666742, XDE-742, pyroxsulam

CITATION: Stebbins, K.E., D.V.M. and S. J. Day, B.S. (2001). XR-742: 28-Day Dietary Toxicity Study in Fischer 344 Rats. Toxicology & Environmental Research and Consulting, The Dow Chemical Company, Midland, Michigan. Project No. 011044, 16 August 2001. MRID 46908349. Unpublished

SPONSOR: Dow AgroSciences LLC, 9330 Zionsville Road, Indianapolis, Indiana 46268

EXECUTIVE SUMMARY:

In a 28-day oral toxicity study (MRID 46908349) [XR-742 (96.7% a.i., lot# 200100558-14B, TSN102505)] was administered to 5 Fischer 344 rats/sex/dose in their diet at nominal dose levels of 0, 10, 100, 500, or 1000 mg/kg bw/day (mg/kg bw/day is alternately referred to as mg/kg/day). Animals were observed daily for clinical signs and mortality. Detailed clinical observations, body weights, and food consumption were recorded twice during the first week and weekly thereafter. Ophthalmology, hematology, clinical chemistry, urinalysis, organ weights, and gross pathology and histopathology were also examined.

There were no treatment related effects on mortality, clinical signs, or body weight and/or body weight changes throughout the treatment period. There were no effects observed in ophthalmology, hematology, clinical chemistry, urinalysis, organ weights, gross pathology, or histopathology at the end of the study.

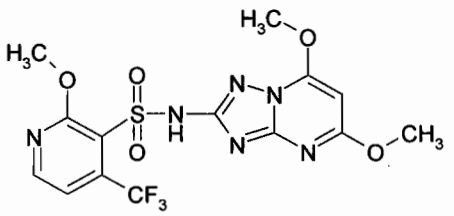
The LOAEL was not observed. The NOAEL is 1000 mg/kg/day, the limit dose.

This 28-day oral toxicity study in the rat is acceptable/NON-guideline: it is a range-finding study for the 90-day and 2-year rat studies. This does not satisfy the guideline requirement for a 90-day oral toxicity study (OPPTS 870.3100; OECD 408.)

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

I. MATERIALS AND METHODS:

A. MATERIALS:

1.	**Test Material:**	XR-742
	Description:	Powder, white
	Lot/Batch #:	Lot #200100558-14B; TSN102505
	Purity:	The purity of the compound was determined to be 96.7% XR-742 by high-performance, liquid chromatography (HPLC). Structural confirmation was performed by proton nuclear magnetic resonance.
	Compound Stability:	The stability of XR-742 in rodent feed at concentrations ranging from 0.005% to 5% over a 36-day period was determined concurrent with study conduct. The mean concentrations of the 0.005% and 5% diets were 96.2% and 101.3%, respectively, of the initial values after 36 days with a standard deviation for all of the analyses <5%.
	CAS #:	422556-08-9
	Structure	

2. **Vehicle and/or positive control:** LabDiet® Certified Rodent Diet #5002 (PMI Nutrition International)

3.	**Test animals:**		
	Species:	Rats	
	Strain:	Fischer 344	
	Age/weight at study initiation:	Animals were approximately six weeks of age at the start of the study. Body weights ranged from 115.9 to 139.5 g for males and 85.0 to 97.5 g for females at the start of the study.	
	Source:	Charles River Laboratories Inc. (Raleigh, North Carolina)	
	Housing:	Animals were housed one per cage in stainless steel cages in rooms designed to maintain adequate conditions (temperature, humidity, and photocycle). Cages with wire-mesh floors were suspended above catch pans. Cages had a feed container and a pressure activated, nipple-type watering system. Room temperature was recorded daily.	
	Feed & Water:	Animals were provided LabDiet® Certified Rodent Diet #5002 (PMI Nutrition International, St. Louis, Missouri) in meal form. Feed and municipal water were provided *ad libitum*. Drinking water obtained from the municipal water source was periodically analyzed for chemical parameters and biological contaminants by the municipal water department.	
	Environmental conditions:	**Temperature:** **Humidity:** **Air changes:** **Photoperiod:**	21.8-22.1 °C 45.6-54.3% 12-15 times/hour 12-hour light/dark
	Acclimation period:	One week prior to the start of the study.	

B. STUDY DESIGN:

1. **In life dates:** Test material administration for animals began on April 17, 2001. Rats were necropsied on May 16, 2001 (test day 30).

2. **Animal assignment:** Animals were stratified by pre-exposure body weight and then randomly assigned to treatment groups using a computer program. Animals placed on study were uniquely identified via subcutaneously implanted transponders (BioMedic Data Systems, Seaford, Delaware) which were correlated to unique alphanumeric identification numbers.

TABLE 1: Oral Toxicity Study Design for XR-742 Given to Fischer Rats in their Diets for 28-days

Test group	Nominal Dose mg/kg/day	Dose to animal mg/kg/day	# Male	# Female
1	0	0	5	5
2	10	11.9 males 11.6 females	5	5
3	100	120 males 112 females	5	5
4	500	583 males 563 females	5	5

5	1000	1165 males 1140 females	5	5

3. **Dose selection rationale:** This is a dose range-finding study and tested up to the limit dose of 1000 mg/kg/day.

4. **Diet preparation and analysis:** Diets were prepared by serially diluting a concentrated test material-feed mixture (premix) with ground feed. Premixes and diets were mixed weekly and dietary concentrations were adjusted based upon the most recent body weight and feed consumption data. Initial concentrations of test material in the diet were calculated from historical body weights and feed consumption data.

   The homogeneity of the low-dose female and the high-dose male test material-feed mixtures were determined prior to start of the study. The stability of XR-742 in rodent feed at concentrations ranging from 0.005% to 5% over a 36-day period was determined concurrent with study conduct. Analyses of all treated and control diets were conducted at the study start. The method for analyzing the test material in feed was a solvent extraction method followed by analysis using liquid chromatography (LC) and mass spectroscopy detection with internal as well as external standards.

II. Results:

Homogeneity Analysis: For the 10 mg/kg/day females, the target concentration {% (w/w)} was 0.0113 and, for the 1000 mg/kg/day males, the target concentration {% (w/w)} was 1.372. The range of concentrations for the females was 0.0106 to 0.0111, with a mean of 0.0109 and a percent relative standard deviation of 1.67. The range of concentrations for the males was 1.27 to 1.38, with a mean of 1.32 and a percent relative standard deviation of 3.69.

Stability Analysis: The mean concentrations of the 0.005% and 5% diets were 96.2% and 101.3%, respectively, of the initial values after 36 days with a standard deviation for all of the analyses of <5%.

Concentration Analysis: The actual concentrations of test material in individual diets ranged from 94 to 100% of targeted values.

5. **Statistics:** Means and standard deviations were calculated for all continuous data. All parameters examined statistically (feed consumption was addressed below) were first tested for equality of variance using Bartlett's test. If the results from Bartlett's test were significant at alpha = 0.01, then the data for the parameter may have been subjected to a transformation to obtain equality of the variances. The transformations that were examined were the common log, the inverse, and the square root, in that order. The data were reviewed and an appropriate form of the data was selected. The selected form of the data was then subjected to the appropriate parametric analysis as described below.

   In-life body weights were evaluated using a repeated measures (RM) analysis of variance (ANOVA), the multivariate approach, for time (the repeated factor), sex, and dose. In the RM-ANOVA, differences between the groups were primarily detected by the time-dose interaction.

Terminal body weight, organ weight (absolute and relative), urine specific gravity, hematologic parameters (excluding RBC indices and differential WBC counts), coagulation, and clinical chemistry parameters were evaluated using a two-way ANOVA with the factors of sex and dose. Differences between the groups were primarily detected by the dose factor.

Results for epididymides and testes weight (absolute and relative) were analyzed using a one-way ANOVA. If significant dose effects were determined in the one-way ANOVA at alpha = 0.05, then individual dose groups were compared to controls using Dunnett's test.
Feed consumption data were evaluated by Bartlett's test for equality of variances. Exploratory data analysis was performed by a parametric ANOVA and if significant at alpha = 0.05, was followed by Dunnett's test at alpha = 0.05, experiment-wise error.

Descriptive statistics only (means and standard deviations) were reported for body weight gains, RBC indices, and differential WBC counts. Statistical outliers were identified by a sequential test (alpha = 0.02), and routinely excluded from feed consumption statistics. Other outliers may have been excluded only for documented scientifically sound reasons. Detailed clinical observations (DCO) incidence scores were evaluated qualitatively.

C. METHODS:

1. Observations:

1a. Cageside observations: Twice each day a cage-side examination was conducted, and to the extent possible, the following parameters were evaluated: skin, fur, mucous membranes, respiration, nervous system function (including tremors and convulsions), animal behavior, moribundity, mortality, and the availability of feed and water.

1b. Clinical examinations: Detailed clinical observations (DCO) were conducted pre-exposure and weekly throughout the study. The DCO was conducted on all animals, at approximately the same time each examination day according to an established format. The examination included cage-side, hand-held and open-field observations that were recorded categorically or using explicitly defined scales (scored).

1c. Neurological evaluations: Neurological examinations were not performed as part of this study.

2. Body weight: All rats were weighed during the pre-exposure period, twice during the first week and weekly during the remainder of the study.

3. Food consumption and compound intake: Food consumption data were collected twice during the first week of dosing, and weekly thereafter for all animals. Feeder containers were weighed at the start and end of a measurement cycle and consumption was calculated using the following equation:

$$\text{Food consumption (g/day)} = \frac{\text{(initial weight of feed container - final weight of feed container)}}{\text{(\# of days in measurement cycle) (\# of animals per cage)}}$$

Compound Intake: Test material intake (TMI) was calculated using actual feed concentrations, body weights, and feed consumption data in the following equation:

$$TMI = \frac{(\text{feed consumption}\left(\frac{\text{g}}{\text{day}}\right) * (1000\,\text{mg/g}) * \frac{(\%\text{ of test material in feed})}{100}}{\frac{\left(\frac{\text{current BW[g]} + \text{previous BW[g]}}{2}\right)}{1000\,\text{g/kg}}}$$

4. **Ophthalmoscopic examination:** The eyes of all animals were examined by a veterinarian pre-exposure and prior to termination using indirect ophthalmoscopy. One drop of 0.5% tropicamide ophthalmic solution was instilled in each eye to produce mydriasis prior to the indirect ophthalmic examinations. Eyes were also examined by a prosector during necropsy through a moistened glass slide pressed to the cornea.

5. **Hematology and clinical chemistry:** Blood samples were collected from the orbital sinus of all fasted animals, anesthetized with CO_2, at the scheduled necropsy.
The CHECKED (X) parameters were examined.

a. **Hematology:** Blood samples for a complete blood count were mixed with ethylenediaminetetraacetic acid (EDTA). Blood smears were stained with Wright's stain and archived. Hematologic parameters were assayed using a Technicon H•1E Hematology Analyzer (Bayer Corporation, Tarrytown, New York).

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		
	(Thromboplastin time)		
	(Clotting time)		
X	(Prothrombin time)		

X = parameter examined

b. **Clinical chemistry:** Blood samples were collected in glass tubes and sera were separated from cells as soon as possible following blood collection. Serum parameters were measured using a Hitachi 914 Clinical Chemistry Analyzer (Boehringer-Mannheim, Indianapolis, Indiana).

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

X = parameter examined

6. **Urinalysis:** Urine was collected from all non-fasted animals during the week prior to necropsy by manual compression of the bladder. If an insufficient quantity of urine was collected from a particular rat, a second attempt was made as soon as possible.

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

X = parameter examined
[$]Semiquantitative analysis (Multistix® Reagent Strips, Bayer Corporation, Elkhart, Indiana on the Clinitek 200+).

7. **Sacrifice and pathology**: Fasted rats were anesthetized by the inhalation of CO_2, weighed, and blood samples were obtained from the orbital sinus. Their tracheas were exposed and clamped, and the animals were euthanized by decapitation.

A complete necropsy was conducted on all animals by a veterinary pathologist assisted by a team of trained individuals. The necropsy included an examination of the external tissues and all orifices. All visceral tissues were dissected from the carcass, examined and selected tissues were incised. Representative samples of tissues listed in the table below were collected and preserved in neutral, phosphate-buffered 10% formalin. Transponders were removed and placed in jars with the tissues.

The brain, liver, kidneys, heart, adrenals, testes, epididymides, thymus, and spleen were trimmed and weighed immediately. The ratios of organ weight to terminal body weight were calculated.

Standard histologic procedures were used to process preserved tissues from control and high-dose group animals. Paraffin embedded tissues were sectioned approximately 6 µm thick,

stained with hematoxylin and eosin and examined by a veterinary pathologist using a light microscope.

Selected histopathologic findings were graded to reflect the severity of specific lesions to evaluate: 1) the contribution of a specific lesion to the health status of an animal, 2) exacerbation of common naturally occurring lesions as a result of the test material, and 3) dose-response relationships for treatment-related effects. Very slight and slight grades were used for conditions that were altered from the normal textbook appearance of an organ/tissue, but were of minimal severity and usually with less than 25% involvement of the parenchyma. This type of change would neither be expected to significantly affect the function of the specific organ/tissue nor would it have a significant effect on the overall health of the animal. A moderate grade would have been used for conditions of sufficient severity and/or extent (up to 50% of the parenchyma) that the function of the organ/tissue may have been adversely affected but not to the point of organ failure. The health status of the animal may or may not have been affected, depending on the organ/tissue involved, but generally lesions graded as moderate would not be life threatening. A severe grade would have been used for conditions that were extensive enough to cause significant organ/tissue dysfunction or failure. This degree of change in a critical organ/tissue may have been life threatening.

	DIGESTIVE SYSTEM
X	Tongue and oral tissues
X	Salivary glands
X	Esophagus
X	Stomach
X	Duodenum
X	Jejunum
X	Ileum
X	Cecum
X	Colon
X	Rectum
XX	Liver+
	Gall bladder (not rat)
	Bile duct (rat)
X	Pancreas
	RESPIRATORY
X	Trachea
X	Lung
X	Nose
X	Pharynx
X	Larynx

	CARDIOVASC./HEMAT.
X	Aorta
XX	Heart+
X	Bone marrow
X	Lymph nodes
XX	Spleen*+
XX	Thymus*+
	UROGENITAL
XX	Kidneys+
X	Urinary bladder
XX	Testes+
XX	Epididymides+
X	Prostate
X	Seminal vesicles
X	Ovaries*+
X	Uterus+
X	Mammary gland
X	Coagulating glands
X	Vagina and cervix
X	Oviducts

	NEUROLOGIC
XX	Brain+
X	Peripheral nerve
X	Spinal cord (3 levels)
X	Pituitary
X	Eyes (optic nerve)
	GLANDULAR
XX	Adrenal gland+
X	Lacrimal gland
X	Parathyroid
X	Thyroid
	OTHER
X	Bone (sternum and/or femur)
X	Skeletal muscle
X	Skin
X	All gross lesions and masses
X	Auditory sebaceous glands
X	Mediastinal and mesenteric tissues

+ Organ weights required for rodent studies.
X = Tissues collected at necropsy.
XX = Tissues collected and weighed.

II. RESULTS:

A. OBSERVATIONS:

1. **Clinical signs of toxicity:**

The only DCO finding possibly related to treatment was urine soiling of the perineal area of females given 500 or 1000 mg/kg/day. Three females in the 500 mg/kg/day dose group (#2278, 2280, 2281) had perineal soiling on at least one occasion, beginning as early as day 4 with the last appearance on day 29. Females #2282 and 2285 in the 1000 mg/kg/day dose group had perineal soiling at least once. Perineal soiling first appeared in female 2285 on days 8 -15 and again on days 24-29.

2. **Mortality:** There was no mortality observed in the study animals.

3. **Neurological evaluations:** Not applicable.

B. **BODY WEIGHT AND WEIGHT GAIN:** There were no statistically-identified differences in the body weights of any treated groups relative to controls. Body weight gains for all treated groups of males and females were also comparable to controls.

TABLE 1. Average body weights and body weight gains during 28 days of treatment [a]

Dose rate mg/kg/day	Body weights (g±SD)				Total weight gain	
	Day 1	Day 8	Day 15	Day 30	g	% of control
0	130.1 ± 6.1	167.2 ± 5.8	201.9 ± 7.1	218.8 ± 9.2	88.7 ± 5.1	-
10	129.8 ± 6.7	167.2 ± 4.2	205.4 ± 6.9	221.5 ± 10.2	91.7 ± 12.8	103
100	130.5 ± 7.9	171.5 ± 11.2	206.9 ± 14.1	222.4 ± 17.7	91.9 ± 14.3	104
500	129.7 ± 6.4	166.4 ± 7.1	200.6 ± 10.2	217.9 ± 12.1	88.2 ± 12.1	99
1000	128.4 ± 7.8	166.5 ± 14.2	200.8 ± 17.3	213.3 ± 12.8	84.9 ± 7.8	96
Female						
0	90.9 ± 2.6	109.8 ± 3.3	143.5 ± 3.9	135.6 ± 5.7	44.7 ± 5.2	-
10	91.3 ± 3.2	110.8 ± 2.6	142.2 ± 3.6	136.5 ± 3.5	45.3 ± 6.5	101
100	91.1 ± 3.3	109.0 ± 4.4	140.5 ± 6.6	134.2 ± 6.3	43.1 ± 3.5	96
500	90.3 ± 4.2	108.1 ± 5.2	140.0 ± 6.4	132.1 ± 3.8	41.8 ± 3.1	94
1000	90.8 ± 4.7	111.4 ± 3.3	141.4 ± 4.6	133.6 ± 3.5	42.7 ± 3.3	96

[a] Data obtained from pages 45-46 in the study report. (n = 5)
* Statistically different ($p < 0.05$) from the control.
** Statistically different ($p < 0.01$) from the control.

C. **FOOD CONSUMPTION AND COMPOUND INTAKE:**

1. **Food consumption:** There were no treatment-related differences in the amount of feed consumed by any treated groups when compared to their respective controls.

2. **Compound consumption:** The targeted values for compound intake were 0, 10, 100; 500 and 1000 mg/kg/day. Male rats received time-weighted average dosages of 0, 11.9, 120, 583,

or 1165 mg/kg/day, respectively; female rats received time-weighted average dosages of 0, 11.6, 112, 563, or 1140 mg/kg/day, respectively

3. **Food efficiency:** Was not performed.

D. OPHTHALMOSCOPIC EXAMINATION: There were no treatment-related ophthalmology effects.

E. BLOOD ANALYSES:

1. **Hematology:** There were no treatment-related changes in hematologic parameters for male and female rats at any dose level.

2. **Clinical chemistry:** There were no treatment-related effects on clinical chemistry parameters of male and female rats at any dose level.

F. URINALYSIS: There were no treatment-related effects on urinalysis parameters.

G. SACRIFICE AND PATHOLOGY:

1. **Organ weight:** There were no treatment-related effects on the terminal body weights and organ weights of male and female rats at any dose level.

2. **Gross pathology:** The only possible treatment-related gross pathology observation was perineal soiling, noted at least once in three females given 500 mg/kg/day and two females given 1000 mg/kg/day. Perineal soiling was noted on multiple days in two females at 500 mg/kg/day and one female at 1000 mg/kg/day.

3. **Microscopic pathology:** There were no treatment-related histopathologic observations in males and females at any dose. All histopathologic observations were interpreted to be spontaneous alterations, unassociated with exposure to XR-742.

III. DISCUSSION AND CONCLUSIONS:

A. INVESTIGATORS' CONCLUSIONS: The only possible treatment-related effect was perineal urine soiling of females, which was seen more than once over the course of the study in two females at 500 mg/kg/day, and only one female at 1000 mg/kg/day. There were no histopathologic alterations in any dose group in either males or females.

Based on the multiple parameters evaluated in this study, the high-dose of 1000 mg/kg/day was interpreted to be the no-observed-adverse-effect level (NOAEL) for males and females.

B. REVIEWER COMMENTS:

Perineal soiling occurred in three females at 500 mg/kg/day and in two females at 1000 mg/kg/day. Perineal soiling was not seen in any of the controls or lower dose group females.

However, there were no treatment related observations noted in the individual pathology reports for these females that would indicate kidney effects. Therefore, the perineal soiling is not considered biologically significant or adverse.

The registrant, Dow Chemical Company, originally prepared this STUDY PROFILE TEMPLATE (STP) (MRID 46908548) in HED's DER format. The HED reviewers may have added minor adjustments/additions to the original STP. The conclusions of the study and assignment of its classification as determined by HED reviewers are in the Executive Summary above.

The **LOAEL was not observed**. The **NOAEL is 1000 mg/kg/day** in both males and females.

C. STUDY DEFICIENCIES:

None

EPA Reviewer: Kimberly Harper **Signature:** Kimberly D Harper
RAB2, Health Effects Division (7509C) **Date:** 12/10/07
EPA Secondary Reviewer: Alan Levy **Signature:** Alan C. Levy
RAB2, Health Effects Division (7509C) **Date:** 1-3-2008

Template version 02/06

TXR#: 0054347

DATA EVALUATION RECORD

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

PC CODE: 108702 **DP BARCODE:** D332276

TEST MATERIAL (PURITY): XDE-742 (98.0%)

SYNONYMS: X666742, XR-742, BAS-770H,

CITATION: Stebbins, K.E., D.V.M., M. D. Dryzga, B.S., K. J. Brooks, B.S., J. Thomas, D.V.M., Ph.D. (2003). XDE-742/BAS-770H: 90-DAY DIETARY TOXICITY STUDY WITH A 28-DAY RECOVERY IN FISCHER 344 RATS. Toxicology & Environmental Research and Consulting, The Dow Chemical Company, Midland, Michigan. Laboratory report number 021107, March 25, 2003. MRID 46908350. Unpublished

SPONSOR: Dow AgroSciences LLC, 9330 Zionsville Road, Indianapolis, Indiana 46268

EXECUTIVE SUMMARY: Ten male and ten female Fischer 344 rats per group were given test diets formulated to supply 0, 10, 100, or 1000 milligrams XDE-742/BAS-770H per kilogram body weight per day (mg/kg/day) for at least 90 days. Parameters evaluated were daily observations, detailed clinical observations, ophthalmologic examinations, body weight, feed consumption, hematology, clinical chemistry, urinalysis, selected organ weights, and gross and histopathologic examinations. An additional ten male and ten female rats in the control and high-dose groups were held untreated for at least 28 days following the dosing period to assess recovery from treatment-related effects.

There were no treatment-related effects on feed consumption, ophthalmologic observations, and hematologic parameters. A few males and up to 50% of females given 1000 mg/kg/day had treatment-related perineal urine soiling at various times during the study. Females given 1000 mg/kg/day had statistically identified decreases in mean body weights from test day 29 through the end of the 90-day dosing period. Males given 1000 mg/kg/day had a statistically identified lower alanine aminotransferase (ALT) value, and a statistically identified higher cholesterol concentration, that were interpreted to be treatment-related. Males and females given 1000 mg/kg/day also had a treatment-related lower concentration of protein in the urine, relative to controls. The alterations in ALT, cholesterol, and urine protein were interpreted to be of no toxicological significance. The only treatment-related change in male organ weights was a

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statistically identified higher relative liver weight for the 1000 mg/kg/day group. Females given 1000 mg/kg/day had statistically identified lower absolute heart, ovary, and thymus weights, and statistically identified higher relative kidney, liver, and brain weights. The alterations in these female organ weights were reflective of the treatment-related lower body weights at the 1000 mg/kg/day dose level. There were no treatment-related gross or histopathologic effects.

Following a 28-day recovery period, the ALT value for males given 1000 mg/kg/day was still lower than controls but not statistically identified, following the 28-day recovery period. There was complete recovery of all other treatment-related effects.

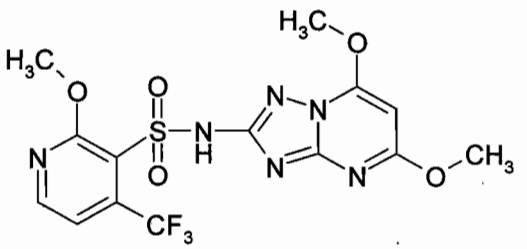
The effects observed at 1000 mg/kg/day were not considered to be toxicologically significant and, therefore, **the NOAEL for this study is 1000 mg/kg/day. A LOAEL was not observed.**

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

COMPLIANCE: Signed and dated GLP, Quality Assurance, and Data Confidentiality statements were provided. This study provides a satellite group of animals (control and high dose) to investigate the recovery of rats exposed to XDE-742.

I. MATERIALS AND METHODS:

A. MATERIALS:

1.	**Test Material:**	XDE-742/BAS-770H
	Description:	powder, white
	Lot/Batch #:	Lot #E0952-52-01; TSN103826
	Purity:	98.0% XDE-742/BAS-770H
	Compound Stability:	A previous 28 day dietary toxicity study with Fischer 344 rats (MRID 46908349) has shown XDE-742/BAS-770H to be stable for at least 36 days in the feed at concentrations ranging from 0.005% to 5%. This range spanned the diets used in this study; therefore, additional stability was not conducted.
	CAS #:	422556-08-9
		

2. **Vehicle and/or positive control:** LabDiet® Certified Rodent Diet #5002 (PMI Nutrition International, St. Louis, Missouri).

3.	**Test animals:**	
	Species:	Rats
	Strain:	Fischer 344
	Age/weight at study initiation:	Approximately 7 weeks
	Source:	Charles River Laboratories, Inc. (Raleigh, North Carolina)
	Housing:	Animals were housed one per cage in stainless steel cages after assignment to the study. Cages had wire-mesh floors that were suspended above catch pans and contained a feed container and a pressure activated nipple-type watering system. These values were within the laboratory recommended range for rats.
	Food & Water:	Animals were provided LabDiet® Certified Rodent Diet #5002 (PMI Nutrition International, St. Louis, Missouri) in meal form. Feed and municipal water were provided *ad libitum*. Drinking water obtained from the municipal water source was periodically analyzed for chemical parameters and biological contaminants by the municipal water department.

	Environmental conditions:	**Temperature:** **Humidity:** **Air changes:** **Photoperiod:**	20.5-22.4 °C 47.2-60.3% 12-15 times/hr 12 hrs dark/12 hrs light
	Acclimation period:	Approximately one week prior to the start of the study.	

B. STUDY DESIGN:

1. **In life dates:** Start: September 18, 2002; End of 90-Day Exposure: December 19 and 20 (males and females, respectively), 2002; End of 28-Day Recovery Period: January 16, 2003 (test day 121)

2. **Animal assignment:** Animals were stratified by pre-exposure body weight and then randomly assigned to treatment groups using a computer program. Animals placed on study were uniquely identified via subcutaneously implanted transponders (BioMedic Data Systems, Seaford, Delaware) which were correlated to unique alphanumeric identification numbers.

TABLE 1: Study design

Test group	Nominal Conc. (mg/kg/day)	Dose to animal (mg/kg/day)	# Male	# Female
Control*	0	0/0	20	20
Low	10	10.3/10.2	10	10
Mid	100	103/102	10	10
High*	1000	1030/1020	20	20

*Main study (N=10) and recovery group animals (N=10).

3. **Dose selection rationale: :** The high-dose level of 1000 mg/kg/day represented the limit test, as specified by several regulatory agencies for 90-day dietary toxicity studies and was chosen based on the results of a 28-day dietary study conducted with XDE-742. The remaining dose levels (10 and 100 mg/kg/day) were expected to provide dose-response data for any treatment-related effect(s) observed in the high-dose group and to ensure definition of a no-observed-effect level (NOEL).

4. **Diet preparation and analysis:** Diets were prepared by serially diluting a concentrated test material-feed mixture (premix) with ground feed. Premixes were mixed periodically throughout the study based on stability data. Diets were prepared weekly based upon the most recent body weight and feed consumption data. Initial concentrations of test material in the diet were calculated from historical body weights and feed consumption data.

Results:

Dose Confirmation and Homogeneity Analysis: The 10 mg/kg/day female and 1000 mg/kg/day male test diets (which had the lowest and highest concentrations used in the study) were determined to be homogeneous, with the relative standard deviations for all diets sampled between 1.26% and 4.59%. The concentrations of XDE-742/BAS-770H

were determined for the control, 10, 100, and 1000 mg/kg/day diets mixed on 9/9/02, 11/10/02, and 12/9/02, for male and female rats. LC-MS analysis with solvent standards incorporating an internal standard indicated 86.8 to 113% of the target concentration was obtained for each individual sample. The mean concentrations for each dose level ranged from 93.3 to 105% of targeted concentration. No test material was found in the control diets.

Stability Analysis: A previous 28 day dietary toxicity study with Fischer 344 rats (MRID 46908349) has shown XDE-742/BAS-770H to be stable for at least 36 days in the feed at concentrations ranging from 0.005% to 5%. This range spanned the diets used in this study; therefore, additional stability was not conducted.

5. **Statistics:** Means and standard deviations were calculated for all continuous data. Body weights, feed consumption, organ weights, urine volume, urine specific gravity, clinical chemistry data, coagulation, and appropriate hematologic data were evaluated by Bartlett's test (alpha = 0.01) for equality of variances. Based on the outcome of Bartlett's test, exploratory data analyses were performed by a parametric or nonparametric analysis of variance (ANOVA). If significant at alpha = 0.05, the ANOVA will be followed respectively by Dunnett's test (alpha = 0.05) or the Wilcoxon Rank-Sum test (alpha = 0.05) with a Bonferroni correction for multiple comparisons to the control. The experiment-wise alpha levels were reported for these two tests. DCO incidence scores were statistically analyzed by a z-test of proportions comparing each treated group to the control group (alpha = 0.05). Data collected at different time-points was analyzed separately. Descriptive statistics only (means and standard deviations) were reported for body weight gains, RBC indices, and differential WBC counts. Statistical outliers were identified by a sequential test (alpha = 0.02), but routinely excluded only from feed consumption calculations. Outliers may be excluded from other analyses only for documented, scientifically sound reasons.

   Because numerous measurements were statistically compared in the same group of animals, the overall false positive rate (Type I errors) was greater than the nominal alpha levels. Therefore, the final interpretation of the data considered statistical analyses along with other factors, such as dose-response relationships and whether the results were consistent with other biological and pathological findings and historical control values.

C. METHODS:

1. Observations:

1a. Cageside observations: Twice each day a cage-side examination was conducted and to the extent possible the following parameters were evaluated: skin, fur, mucous membranes, respiration, nervous system function (including tremors and convulsions), animal behavior, moribundity, mortality, and the availability of feed and water.

1b. Clinical examinations: Detailed clinical observations (DCO) were conducted at pre-exposure and weekly throughout the study. The DCO was conducted on all animals, at approximately the same time on each examination day according to an established format.

The examination included cage-side, hand-held, and open-field observations that were recorded categorically or using explicitly defined scales (scored).

2. **Body weight:** All rats were weighed pre-exposure and weekly during the remainder of the study. Body weight gains were also calculated.

3. **Food consumption and compound intake:**
4.

Feed consumption: Feed consumption data were collected at least weekly for all animals. Feed containers were weighed at the start and end of a measurement cycle and consumption was calculated using the following equation:

$$\text{Feed consumption (g/day)} = \frac{\text{(initial weight of feed container - final weight of feed container)}}{\text{(\# of days in measurement cycle)}}$$

Test Material Intake: The actual test material intake (TMI) was calculated using test material feed concentrations, body weights, and feed consumption data in the following equation:

$$TMI = \frac{(\text{feed consumption}\left(\frac{g}{day}\right)) * (1000\,mg/g) * \frac{(\%\ of\ test\ material\ in\ feed)}{100}}{\frac{\left(\frac{Current\,BW\,[g] + Previous\,BW\,[g]}{2}\right)}{1000\,g/kg}}$$

4. **Ophthalmoscopic examination:** The eyes of all animals were examined by a veterinarian pre-exposure and prior to the scheduled necropsy using indirect ophthalmoscopy. One drop of 0.5% tropicamide ophthalmic solution was instilled in each eye to produce mydriasis prior to the indirect ophthalmic examinations. Eyes were also examined by a prosector during necropsy through a moistened glass slide pressed to the cornea.

5. **Hematology and clinical chemistry:** Blood samples were collected from the orbital sinus of all fasted animals, anesthetized with CO_2, at the scheduled necropsy.

a. **Hematology:** Blood samples were mixed with ethylenediamine-tetraacetic acid (EDTA) and smears were prepared, stained with Wright's stain and archived for potential future evaluation if warranted. Hematologic parameters were assayed using a Technicon H•1E Hematology Analyzer (Bayer Corporation, Tarrytown, New York).
Coagulation: Blood samples were collected in sodium citrate tubes, centrifuged and plasma collected and assayed using an ACL9000 (Instrumentation Laboratory, Lexington, Massachusetts).

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*		
	(Thromboplastin time)		
	(Clotting time)		
X	(Prothrombin time)		

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

b. Clinical chemistry: Blood samples were collected in glass tubes and sera were separated from cells as soon as possible following blood collection. Serum parameters were measured using a Hitachi 914 Clinical Chemistry Analyzer (Boehringer-Mannheim, Indianapolis, Indiana).

	ELECTROLYTES		OTHER
X	Calcium*	X	Albumin*
X	Chloride*	X	Blood creatinine*
	Magnesium	X	Blood urea nitrogen*
X	Phosphorus*	X	Total Cholesterol*
X	Potassium*		Globulins
X	Sodium*	X	Glucose*
		X	Total bilirubin
	ENZYMES	X	Total serum protein (TP)*
X	Alkaline phosphatase (ALP)*		Triglycerides
	Cholinesterase (ChE)		Serum protein electrophores
	Creatine phosphokinase		
	Lactic acid dehydrogenase (LDH)		
X	Serum alanine amino-transferase (ALT/SGPT)*		
X	Serum aspertate amino-transferase (AST/SGOT)*		
	Gamma glutamyl transferase (GGT)		
	Glutamate dehydrogenase		

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

6. Urinalysis*: Urine was obtained from all surviving nonfasted rats during the week prior to necropsy. Animals were housed in metabolism cages and the urine collected overnight (approximately 16 hours).

Sediment (microscopic): Urine was also collected from each animal by manual compression of the urinary bladder. The urine was pooled from each group, and the microsediment was characterized microscopically.

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

* Optional for 90-day oral rodent studies

$Semiquantitative analysis (Multistix® Reagent Strips, Bayer Corporation, Elkhart, Indiana on the Clinitek 200+).

6. Sacrifice and pathology:

Necropsy: Fasted rats were anesthetized by the inhalation of CO_2, weighed, and blood samples were obtained from the orbital sinus. A complete necropsy was conducted on all animals. The necropsy included an examination of the external tissues and all orifices. All visceral tissues were dissected from the carcass, re-examined and selected tissues were incised. In addition, the brain, liver, kidneys, heart, adrenals, testes, epididymides, uterus, ovaries, thymus, and spleen were trimmed and weighed immediately. The ratios of organ weight to terminal body weight were calculated. Representative samples of tissues listed in Histopathology section were collected and preserved in neutral, phosphate-buffered 10% formalin. Transponders were removed and placed in jars with the tissues.

Histopathololgy: The sections from all preserved tissues listed below were processed by standard histologic procedures from control- and high-dose group animals. Paraffin embedded tissues were sectioned approximately 6 µm thick, stained with hematoxylin and eosin and examined by a veterinary pathologist using a light microscope. The following tissues from the remaining groups were processed and histopathologically examined: liver, kidneys, lungs, and relevant gross lesions.

	DIGESTIVE SYSTEM		CARDIOVASC./HEMAT.		NEUROLOGIC
X	Tongue	X	Aorta*	XX	Brain (multiple sections)*+
X	Oral Tissues		Tonsils	X	Periph.nerve*
X	Salivary glands*	XX	Heart*+	X	Spinal cord (3 levels)*
X	Esophagus*	X	Bone marrow*	X	Pituitary*
X	Stomach*		Lymph nodes*	X	Eyes (retina, optic nerve)*
X	Duodenum*	X	Mediastinal lymph nodes	X	Cranial nerve – optic
X	Jejunum*	X	Mesenteric lymph nodes		
X	Ileum*	XX	Spleen*+		GLANDULAR
X	Cecum*	XX	Thymus*+	XX	Adrenal gland*+
X	Colon*			X	Lacrimal gland/Harderian gland
X	Rectum*		UROGENITAL	X	Mammary gland* females
XX	Liver*+	XX	Kidneys*+	X	Thyroids* with Parathyroid
X	Pancreas*	X	Urinary bladder*	X	Auditory Sebaceous Glands
		X	Coagulating Glands		
	RESPIRATORY	X	Seminal Vesicles*		OTHER
X	Trachea*			X	Bone Including joint
X	Lung*	XX	Testes*+	X	Skeletal muscle
	Nose/Nasal				
X	Tissues/Pharynx*	XX	Epididymides*+	X	Skin
X	Larynx*	X	Prostate*		
X	Mediastinal Tissues			X	All gross lesions and masses*
X	Mesenteric Tissues	XX	Ovaries*+		
		X	Oviducts		
		XX	Uterus*+		
		X	Cervix		
		X	Vagina		

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

7. **28-Day Recovery Group:** Body weights, feed consumption, test material intake, cage-side observations, pre-study ophthalmology, and DCO's were conducted on the recovery animals

throughout the 90-day dosing period as previously described for the 90-day group. Weekly body weights, feed consumption, and daily cage-side observations were conducted on the animals throughout the 28-day recovery period. A necropsy was also conducted on these animals. Other parameters (determined for the 90-day dosing animals) determined to have treatment-related effects at the end of the dosing period were examined in the recovery animals and addressed in a protocol change/revision.

II. RESULTS:

A. OBSERVATIONS:

1. **Clinical signs of toxicity:** A few males and up to 50% of females given 1000 mg/kg/day had treatment-related perineal urine soiling. Perineal soiling was first observed in the males on Day 57 and in the females on Day 8. Perineal soiling was in observed sporadically in one of the 10 mg/kg/day females beginning on Day 57; none of the control females or females in the mid-dose group showed signs on of perineal soiling. No males in the control, low-, and mid-dose were observed to have perineal soiling. There were no other treatment-related clinical or cage-side observations noted during the study.

2. **Mortality:** There was no unscheduled mortality during the study.

B. BODY WEIGHT AND WEIGHT GAIN: Females given 1000 mg/kg/day had statistically identified lower mean body weights from test day 29 through the end of the 90-day dosing period (6.0% lower than controls at end of study) when compared with controls. There were no statistically identified differences in the body weights of males at any dose level, and in females given 10 or 100 mg/kg/day. Body weight gains for males and females given 1000 mg/kg/day were slightly lower than controls over the duration of the study. By the end of the 28-day recovery period, the mean body weights and body weight gains of males and females given 1000 mg/kg/day were comparable to controls.

TABLE 2. Average body weights and body weight gains during 90 days of treatment [a]

Dose rate [insert units]	Body weights (g±SD)				Total weight gain	
	Initial	Week 4	Week 9	Week 13	g	% of control
Male						
0 (n=20)	162.8 ± 11.1	250.1 ± 17.1	305.6 ± 19.2	330.4 ± 17.2	167.6 ± 11.6	-
10 (n=10)	162.5 ± 7.4	250.1 ± 15.6	304.8 ± 22.2	327.0 ± 24.0	164.4 ± 23.2	98.1
100 (n=10)	161.0 ± 9.8	245.5 ± 17.0	298.3 ± 24.5	322.3 ± 23.1	161.2 ± 20.7	96.2
1000 (n=20)	163.2 ± 11.3	241.1 ± 24.7	297.1 ± 25.5	320.4 ± 25.8 (↓3%)	157.1 ± 17.0	93.7
Female						
0 (n=20)	109.6 ± 4.3	157.4 ± 6.2	180.6 ± 6.7	188.9 ± 7.9	79.2 ± 5.6	-
10 (n=10)	109.8 ± 5.2	155.0 ± 8.0	177.4 ± 6.8	186.3 ± 7.4	76.6 ± 5.8	96.7
100 (n=10)	109.9 ± 4.6	151.5 ± 7.4	176.1 ± 8.1	185.8 ± 9.1	75.8 ± 5.7	95.7
1000 (n=20)	109.9 ± 3.9	150.2* ± 7.9 (↓5%)	171.2* ± 10.9 (↓5%)	177.5* ± 10.6 (↓6%)	67.6 ± 7.7	85.4

[a] Data obtained from Tables 8-9 on pages 48-55 in the study report.
* Statistically different ($p < 0.05$) from the control.

C. FOOD CONSUMPTION AND COMPOUND INTAKE:

1. **Food consumption:** There were no significant differences in the amount of food consumed by any treated groups when compared to their respective controls during the 90-day dosing period. Males and females given 1000 mg/kg/day had a statisically identified increase in food consumption during the first week of the 28-day recovery period, and had comparable food consumption to controls for the remainder of the recovery period.

2. **Compound consumption:** The targeted values for test material intake were 10, 100, and 1000 mg/kg/day. Male rats from the low-, middle-, and high-dose groups received acceptable time-weighted average doses of 10.3, 103, and 1030 mg/kg/day, respectively; female rats from the low-, middle-, and high-dose groups received acceptable time-weighted average doses of 10.2, 102, and 1020 mg/kg/day, respectively.

D. OPHTHALMOSCOPIC EXAMINATION: Ophthalmologic observations prior to study termination consisted of pale fundus, cloudy cornea, and periocular soiling. These observations occurred sporadically among the control and treated groups with no relationship to the dose of the test material.

E. BLOOD ANALYSES:

1. **Hematology:** There were no treatment-related changes in any of the hematologic parameters for male and female rats at any dose level.

2. **Clinical chemistry:** Males given 1000 mg/kg/day had a statistically identified lower ALT value, and a statistically identified higher cholesterol concentration. Both of these clinical chemistry parameters (52 u/l and 74 mg/dl, ALT and cholesterol, respectively) were slightly outside the historical control range (58-71 u/l and 51-68 mg/dl, ALT and cholesterol, respectively) from recently conducted 90-day oral toxicity studies of this laboratory (Table 3). The alterations in ALT and cholesterol were interpreted to be treatment-related, but there was no dose response relationship, and the alterations were of no toxicological significance. Toxicologically significant alterations in ALT are usually manifested by an increase, rather than a decrease, in this parameter. In addition, there were no treatment related histopathologic alterations of the liver in males or females given 1000 mg/kg/day.

   There was complete recovery of the cholesterol alteration for males given 1000 mg/kg/day for 90 days, followed by a 28-day recovery period. However, the mean ALT value of males given 1000 mg/kg/day for 90 days, followed by a 28-day recovery period, was still lower than controls but not statistically identified.

Table 3. Clinical Chemistry Effects in Male Rats Given XDE-742.BAS-770H for 90-Days

	Dose (mg/kg/day)				
Parameter	0	Historical[1]	10	100	1000
ALT (u/l)	65 (81)	58-71	54	54	52* (59)
Cholesterol (mg/dl)	54 (54)	51-68	57	58	74* (62)

Data obtained from Text Table 2 on page 27 of the study report.
() parameter values for male rats following a 28-day recovery period.
*Statistically Different from Control Mean by Dunnett's Test, Alpha = 0.05.
[1]Historical controls group mean range from eight 90-day dietary studies done since 1998.

F. URINALYSIS: There were no treatment related effects observed during urinalysis.

G. SACRIFICE AND PATHOLOGY:

1. **Organ weight:** Parameters that were statistically identified are summarized in Table 4. Males and females given 1000 mg/kg/day had lower final body weights, relative to controls (statistically identified in females only). The lower final body weight of females (but not males) given 1000 mg/kg/day was interpreted to be related to treatment, though the value for this parameter in females was only slightly outside the historical control range from recently conducted studies of this laboratory. The only treatment related change in male organ weights was a statistically identified higher relative liver weight for the 1000 mg/kg/day group (8.2% higher than controls). Females given 1000 mg/kg/day had statistically identified lower absolute heart, ovary, and thymus weights with no statistical effect on the respective relative weights, and statistically identified higher relative kidney, liver, and brain weights. The alterations in these female organ weights were reflective of the treatment-related lower body weights at the 1000 mg/kg/day dose level. There were no histopathologic correlates to any of the statistically identified organ weight changes.

	Dose (mg/kg/day)				
	0	Historical[1]	10	100	1000
Parameter	MALES				
Final Body Weight (g)	311.2 (318.8)	293.6-324.3	303.7	299.0	296.1 (314.3)
Absolute Heart (g)	0.891	nr	0.886	0.855	0.827
Relative Kidneys (g/100g bw)	0.663	nr	0.682	0.675	0.689
Relative Liver (g/100g bw)	2.725 (2.864)	2.61-2.881	2.764	2.806	2.968* (2.868)
Relative Brain (g/100g bw)	0.638	nr	0.657	0.655	0.664
Absolute Ovaries (g)	3.100	nr	3.106	3.117	3.073
Absolute Thymus (g)	0.204	nr	0.198	0.187	0.188
	FEMALES				
Final Body Weight (g)	175.7 (181.3)	163.2-183.9	173.5	171.8	162.9* (174.8)
Absolute Heart (g)	0.607 (0.627)	0.544-0.671	0.603	0.596	0.570* (0.609)
Relative Kidneys (g/100g bw)	0.707 (0.691)	0.666-0.726	0.718	0.720	0.753* (0.716)
Relative Liver (g/100g bw)	2.633 (2.587)	2.410-2.727	2.630	2.702	2.779* (2.600)
Relative Brain (g/100g bw)	1.032 (1.009)	0.957-1.103	1.040	1.059	1.108* (1.042)
Absolute Ovaries (g)	0.073 (0.060)	0.054-0.069	0.073	0.077	0.064* (0.067)
Absolute Thymus (g)	0.187 (0.159)	0.174-0.221	0.169	0.184	0.160* (0.156)

Data are from Tables 31 and 33 on pages 79-80 and 82-83 of the study report.
n=10 for all groups; nr = not reported
() = organ weights for animals following a 28-day recovery period
*Statistically Different from Control Mean by Dunnett's Test, Alpha = 0.05.
[1]Historical controls group mean range from seven 90-day dietary studies done since 1998.

Following the 28-day recovery period, the final body weight and selected organ weight values of males and females given 1000 mg/kg/day were not statistically different from controls.

2. **Gross pathology:** There were no treatment-related gross pathologic observations.

3. **Microscopic pathology:** There were no treatment-related histopathologic observations.

III. DISCUSSION AND CONCLUSIONS:

A. INVESTIGATORS' CONCLUSIONS: There were no treatment-related effects on feed consumption, ophthalmologic observations, and hematologic parameters. A few males and up to 50% of females given 1000 mg/kg/day had treatment-related perineal urine soiling at various times during the study. Females given 1000 mg/kg/day had statistically identified decreases in mean body weights from test day 29 through the end of the 90-day dosing period. Males given 1000 mg/kg/day had a statistically identified lower alanine aminotransferase (ALT) value, and a statistically identified higher cholesterol concentration, that were interpreted to be treatment-related. Males and females given 1000 mg/kg/day also had a treatment-related lower concentration of protein in the urine, relative to controls. The

alterations in ALT, cholesterol and urine protein were interpreted to be of no toxicological significance. The only treatment-related change in male organ weights was a statistically identified higher relative liver weight for the 1000 mg/kg/day group. Females given 1000 mg/kg/day had statistically identified lower absolute heart, ovary, and thymus weights, and statistically identified higher relative kidney, liver, and brain weights. The alterations in these female organ weights were reflective of the treatment-related lower body weights at the 1000 mg/kg/day dose level. There were no treatment-related gross or histopathologic effects. The ALT value for males given 1000 mg/kg/day was still lower than controls but not statistically identified, following the 28-day recovery period. There was complete recovery of all other treatment-related effects.

The effects observed at 1000 mg/kg/day were not considered to be toxicologically significant and, therefore, the NOAEL for this study.

B. REVIEWER COMMENTS: There were no treatment-related effects on mortality, feed consumption, ophthalmologic observations, and hematologic parameters. A few males and up to 50% of females given 1000 mg/kg/day had treatment-related perineal urine soiling at various times during the study. Females given 1000 mg/kg/day had statistically identified decreases in mean body weights (5-6%) from test day 29 through the end of the 90-day dosing period, with an overall 15% reduction in body weight gain compared to controls. Males given 1000 mg/kg/day had a statistically identified lower alanine aminotransferase (ALT) value, and a statistically identified higher cholesterol concentration, that were interpreted to be treatment-related. Males and females given 1000 mg/kg/day also had a treatment-related lower concentration of protein in the urine, relative to controls. The alterations in ALT, cholesterol, and urine protein were interpreted to be of no toxicological significance. The only treatment-related change in male organ weights was a statistically identified higher relative liver weight for the 1000 mg/kg/day group. Females given 1000 mg/kg/day had statistically identified lower absolute heart, ovary, and thymus weights, and statistically identified higher relative kidney, liver, and brain weights. The alterations in these female organ weights were reflective of the treatment-related lower body weights at the 1000 mg/kg/day dose level. There were no treatment-related gross or histopathologic effects.

Following a 28-day recovery period, the ALT value for males given 1000 mg/kg/day was still lower than controls but not statistically identified, following the 28-day recovery period. There was complete recovery of all other treatment-related effects.

The NOAEL for this study is 1000 mg/kg/day. A LOAEL was not observed.

C. STUDY DEFICIENCIES:

None.

EPA Reviewer: Kimberly Harper **Signature:** Kimberly D Harper
RAB2, Health Effects Division (7509P) **Date:** 12/10/07
EPA Secondary Reviewer: Alan Levy **Signature:** Alan C. Levy
RAB2, Health Effects Division (7509P) **Date:** 1-3-2008

Template version 02/06

TXR#: 0054347

DATA EVALUATION RECORD

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

PC CODE: 108702 **DP BARCODE:** 332276

TEST MATERIAL (PURITY): XDE-742/BAS-770H, (98.0%), (N-(5,7-dimethoxy[1,2,4]triazolo[1,5-a]pyrimidin-2-yl)-2-methoxy-4-(trifluoromethyl)pyridine-3-sulfonamide)

SYNONYMS: X666742, XR-742, BAS-770H

CITATION: Johnson, K.A., D.V.M., Ph.D.; K. J. Brooks, B.S.; M. D. Dryzga, B.S. (2003), XDE-742/BAS-770H: 90-DAY DIETARY TOXICITY STUDY IN CD-1 MICE. Toxicology & Environmental Research and Consulting, The Dow Chemical Company, Midland, Michigan. Laboratory report number 021106, 16 April 2003. MRID 46908351. Unpublished

SPONSOR: Dow AgroSciences LLC, 9330 Zionsville Road, Indianapolis, Indiana 46268.

EXECUTIVE SUMMARY:

Ten male and ten female CD-1 mice per group were given test diets formulated to supply 0, 10, 100, or 1000 milligrams XDE-742/BAS-770H per kilogram body weight per day (mg/kg bw/day or alternately expressed mg/kg/day) for at least 90 days. Parameters evaluated were daily observations, detailed clinical observations, ophthalmologic examinations, body weight, feed consumption, hematology, clinical chemistry, selected organ weights, gross and histopathologic examinations.

There were no treatment-related effects on body weight, feed consumption, ophthalmology, clinical observations or hematologic parameters. Females given 1000 mg/kg/day had statistically-identified increased serum cholesterol (29.9% greater than controls), which was at the high-end of the historical control range (5/10 females had cholesterol levels in excess of the historical control average). Males at 1000 mg/kg/day also had increased cholesterol (22.3%) that was not statistically identified likely due to one high dose male that had higher cholesterol levels than all the others (242 compared to <200 mg/dL). Half (5/10) of the high-dose males had cholesterol levels outside the historical control range. The only other finding was a statistically-identified increase in absolute and relative liver weights for the 1000 mg/kg/day group males (18.3% and 12.3% higher than controls, respectively). The absolute and relative liver weights of females given 1000 mg/kg/day were 7.7% and 5.0% greater than controls,

respectively, and were not statistically identified. Taken together, the increased cholesterol levels in males and females and the increased liver weights in males could indicate hepatic disease, however, there was no corroborating evidence of gross or histopathological changes in the liver. Therefore, these effects were not considered adverse.

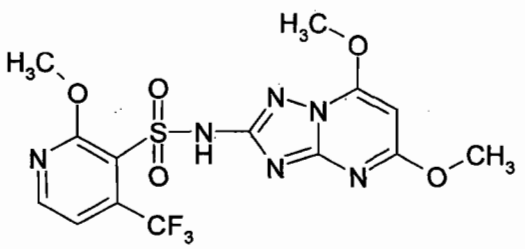
The LOAEL was not observed. The NOAEL is 1000 mg/kg/day.

This study is acceptable and satisfies the guideline requirement for a Subchronic Oral Toxicity [feeding] study in CD-1 Mice; OPPTS 870.3100 (rodent); OECD 408, EEC, Part B.26, JMAFF (Subchronic Oral Toxicity Study).

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

I. MATERIALS AND METHODS:

A. MATERIALS:

1.	**Test Material:**	XDE-742/BAS-770H
	Description:	White, powder
	Lot/Batch #:	Lot #E0952-52-01, TSN103826
	Purity:	98.0% XDE-742/BAS-770H
	Compound Stability:	A previous 28-day dietary toxicity study with Fischer 344 rats (MRID 46908349) has shown XDE-742/BAS-770H to be stable for at least 36 days in the feed at concentrations ranging from 0.005 to 5%. This range spanned the concentrations for the diets used in this study; therefore, stability was not conducted as part of this study.
	CAS #:	422556-08-9
		

2. **Vehicle and/or positive control:** LabDiet® Certified Rodent Diet #5002 in meal form (PMI Nutrition International, St. Louis, Missouri).

3.	**Test animals:**	
	Species:	Mice
	Strain:	CD-1
	Age/weight at study initiation:	Approximately seven weeks old Males 29.5 – 29.9 g; Females 23.4 – 23.8 g
	Source:	Charles River Laboratories, Inc. (Portage, Michigan)

	Housing:	Animals were housed one per cage in stainless steel cages after assignment to the study. Cages had wire-mesh floors that were suspended above catch pans and contained a feed container and a pressure activated, nipple-type watering system.	
	Food & Water:	Animals were provided LabDiet® Certified Rodent Diet #5002 (PMI Nutrition International, St. Louis, Missouri) in meal form. Feed and municipal water were provided *ad libitum*. Drinking water obtained from the municipal water source was periodically analyzed for chemical parameters and biological contaminants by the municipal water department.	
	Environmental conditions:	**Temperature:** **Humidity:** **Air changes:** **Photoperiod:**	21.0-22.3 °C 45.5-56.8% 12-15/hr 12 hrs dark/12 hrs light
	Acclimation period:	One week prior to the start of the study.	

B. STUDY DESIGN:

1. **In life dates:** Start: September 17, 2002; End: December 17, 2002 (males) and December 18, 2002 (females) after 92 and 93 days on test, respectively.

2. **Animal assignment:** Animals were stratified by pre-exposure body weight and then randomly assigned to treatment groups using a computer program. Animals placed on study were uniquely identified via subcutaneously implanted transponders (BioMedic Data Systems, Seaford, Delaware) which were correlated to unique alphanumeric identification numbers.

TABLE 1: Study design for dietary feeding study in mice.

Test group	Nominal Dose mg/kg/day	Dose to animal mg/kg/day (male/female)	# Male	# Female
Control	0	0	10	10
Low	10	10.3 / 10.2	10	10
Mid	100	102 / 103	10	10
High	1000	1030 / 1010	10	10

Data obtained from Tables 12 and 13 on pages 51-52 of the study report.

3. **Dose selection rationale:** The high-dose (limit dose) level of 1000 mg/kg/day was chosen based on results of the 28-day dietary rat study. The remaining dose levels were expected to provide dose-response data for any treatment-related effect(s) observed in the high-dose group and to ensure definition of a no-observed-effect level (NOEL).

4. **Diet preparation and analysis:** Diets were prepared by serially diluting a concentrated test material-feed mixture (premix) with ground feed. Premixes were mixed periodically throughout the study based on stability data. Diets were prepared weekly based upon the most recent body weight and feed consumption data. Initial concentrations of test material in the diet were calculated from historical body weights and feed consumption data.

The homogeneity of the low-dose female and high-dose male test material-feed mixtures was determined pre-exposure, near the middle, and at the end of the study. Aliquots were taken from multiple areas within the containers. The method for analysis of the test material in feed was a solvent extraction method followed by analysis using liquid chromatography-mass spectrometry (LC-MS) and solvent standards incorporating an internal standard.

Analyses of all dose levels, premix, and the 0 (control) mg/kg/day diet were conducted pre-exposure, near the middle, and at the end of the study. The method used for analyzing the test material in feed was as previously described.

Results: The concentrations of XDE-742/BAS-770H were determined for the control, premix, 10, 100, and 1000 mg/kg/day diets mixed on 9/8/02, 11/8/02, and 12/6/02, for male and female mice. LC-MS analysis found 85 to 110% of the target concentration for each individual sample. The mean concentrations for each dose level ranged from 93 to 103% of targeted concentration. No test material was found in the control diets. The low-dose female (10 mg/kg/day) and the high-dose male (1000 mg/kg/day) diets (which had the lowest and highest concentrations used in the study) were determined to be homogeneous, with the relative standard deviations for all diets sampled between 1.18 and 10.7%.

No additional stability analysis was performed.

5. **Statistics:** Means and standard deviations were calculated for all continuous data. Body weights, feed consumption, organ weights, clinical chemistry data, and appropriate hematologic data were evaluated by Bartlett's test (alpha = 0.01) for equality of variances. Based on the outcome of Bartlett's test, exploratory data analysis was performed by a parametric or non-parametric analysis of variance (ANOVA). If significant at alpha = 0.05, the ANOVA were followed respectively by Dunnett's test (alpha = 0.05) or the Wilcoxon Rank-Sum test (alpha = 0.05) with a Bonferroni correction for multiple comparisons to the control. The experiment-wise alpha level was reported for these two tests. Detailed clinical observations (DCO) incidence scores were statistically analyzed by a z-test of proportions comparing each treated group to the control group (alpha = 0.05). Data collected at different time-points were analyzed separately. Descriptive statistics only (means and standard deviations) were reported for body weight gains, RBC indices, and differential WBC counts. Statistical outliers were identified by a sequential test (alpha = 0.02), but routinely excluded only from feed consumption calculations. Outliers may have been excluded from other analyses only for documented, scientifically sound reasons.

Because numerous measurements were statistically compared in the same group of animals, the overall false positive rates (Type I errors) were greater than the nominal alpha levels. Therefore, the final interpretation of the data considered statistical analyses along with other factors, such as dose-response relationships and whether the results were consistent with other biological and pathological findings or historical control values.

C. METHODS:

1. **Observations:**

   1a. **Cageside observations:** Twice each day, a cage-side examination was conducted and, to the extent possible, the following parameters were evaluated: skin, fur, mucous membranes, respiration, nervous system function (including tremors and convulsions), animal behavior, moribundity, mortality, and the availability of feed and water.

   1b. **Clinical examinations:** Detailed clinical observations (DCO) were conducted at pre-exposure and weekly throughout the study. The DCO was conducted on all animals, at approximately the same time each examination day according to an established format. The examination included cage-side, hand-held and open-field observations that were recorded categorically or using explicitly defined scales (scored DCOs).

2. **Body weight:** All mice were weighed during pre-exposure and weekly during the remainder of the study. Body weight gains were also calculated.

3. **Food consumption and compound intake:** Food consumption data were collected weekly for all animals. Food containers were weighed at the start and end of a measurement cycle and consumption was calculated using the following equation:

$$\text{Food consumption (g/day)} = \frac{\text{(initial Wt. of feeder - final Wt. of feeder)}}{\text{(\# of days in measurement cycle) (\# of animals in cage)}}$$

   Test Material Intake: The actual test material intake (TMI) was calculated using test material feed concentrations, body weights and feed consumption data in the following equation:

$$TMI = \frac{(\text{feed consumption}\left(\frac{\text{g}}{\text{day}}\right)) * (1000\,\text{mg/g}) * \frac{(\%\text{ of test material in feed})}{100}}{\frac{\left(\frac{\text{current BW [g]} + \text{previous BW [g]}}{2}\right)}{1000\,\text{g/kg}}}$$

4. **Ophthalmoscopic examination:** The eyes of all animals were examined by a veterinarian pre-exposure and prior to the scheduled necropsy using indirect ophthalmoscopy. One drop of 0.5% tropicamide ophthalmic solution was instilled in each eye to produce mydriasis prior to the indirect ophthalmic examinations. Eyes were also examined by a prosector during necropsy through a moistened glass slide pressed to the cornea.

5. **Hematology and clinical chemistry:** Blood samples were collected from the orbital sinus of all non-fasted animals anesthetized with CO_2 at the scheduled necropsy.

   a. **Hematology:** Blood samples for a complete blood count were mixed with ethylenediamine-tetraacetic acid (EDTA). Blood smears were prepared, stained with Wright's stain, and archived for potential future evaluation if warranted. Hematologic parameters were assayed using a Technicon H•1E Hematology Analyzer (Bayer Corporation, Tarrytown, New York).

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*		
	(Thromboplastin time)		
	(Clotting time)		

X = parameter examined
* Recommended for 90-day oral rodent studies based on Guideline 870.3100

b. **Clinical chemistry:** Blood samples were collected in glass tubes, and serum was separated from cells as soon as possible following blood collection. Serum parameters were measured using a Hitachi 914 Clinical Chemistry Analyzer (Boehringer-Mannheim, Indianapolis, Indiana).

	ELECTROLYTES		OTHER
X	Calcium*	X	Albumin*
X	Chloride*	X	Blood creatinine*
	Magnesium	X	Blood urea nitrogen*
X	Phosphorus*	X	Total Cholesterol*
X	Potassium*		Globulins
X	Sodium*	X	Glucose*
		X	Total bilirubin
	ENZYMES	X	Total serum protein (TP)*
X	Alkaline phosphatase (ALP)*		Triglycerides
	Cholinesterase (ChE)		Serum protein electrophores
	Creatine phosphokinase		
	Lactic acid dehydrogenase (LDH)		
X	Serum alanine amino-transferase (ALT/SGPT)*		
X	Serum aspertate amino-transferase (AST/SGOT)*		
	Gamma glutamyl transferase (GGT)		
	Glutamate dehydrogenase		

X = parameters examined
* Recommended for 90-day oral rodent studies based on Guideline 870.3100

6. **Urinalysis:** Urinalysis is optional for 90-day studies and was not performed.

7. **Sacrifice and pathology**: Non-fasted mice were anesthetized by the inhalation of CO_2, weighed, and blood samples were obtained from the orbital sinus. Their tracheas were exposed and clamped, and the animals were euthanized by decapitation.

The necropsy included an examination of the external tissues and all orifices. All visceral tissues were dissected from the carcass, re-examined and selected tissues were incised. The brain, liver, kidneys, heart, adrenals, testes, epididymides, uterus, ovaries, thymus and spleen were trimmed and weighed immediately. The ratios of organ weight to terminal body weight were calculated.

Representative samples of tissues listed in the table below were collected and preserved in neutral, phosphate-buffered 10% formalin. Transponders were removed and placed in jars with the tissues. All tissues from the control and high-dose groups were examined. In addition the liver, kidneys, lungs, and relevant gross lesions were examined from the low- and mid-dose groups.

	DIGESTIVE SYSTEM		CARDIOVASC./HEMAT.		NEUROLOGIC
X	Tongue	X	Aorta*	XX	Brain (multiple sections)*+
X	Oral Tissues	X	Tonsils	X	Periph.nerve*
X	Salivary glands*	XX	Heart*+	X	Spinal cord (3 levels)*
X	Esophagus*	X	Bone marrow*	X	Pituitary*
X	Stomach*	X	Lymph nodes*	X	Eyes (retina, optic nerve)*
X	Duodenum*	X	Mediastinal lymph nodes	X	Cranial nerve – optic
X	Jejunum*	X	Mesenteric lymph nodes		
X	Ileum*	XX	Spleen*+		**GLANDULAR**
X	Cecum*	XX	Thymus*+	XX	Adrenal gland*+
X	Colon*			X	Lacrimal gland/Harderian gland
X	Rectum*		**UROGENITAL**	X	Mammary gland* females
XX	Liver*+ with Gallbladder* (mice)	XX	Kidneys*+	X	Thyroids* with Parathyroid*
X	Pancreas* (1)	X	Urinary bladder* (1)		Auditory Sebaceous Glands – collected but not examined
		X	Coagulating Glands		
	RESPIRATORY	X	Seminal Vesicles*		**OTHER**
X	Trachea*			X	Bone Including joint
X	Lung*	XX	Testes*+	X	Skeletal muscle
X	Nose/Nasal Tissues/Pharynx*	XX	Epididymides*+	X	Skin
X	Larynx*	X	Prostate*		
X	Mediastinal Tissues			X	All gross lesions and masses*
X	Mesenteric Tissues	XX	Ovaries*+		
		X	Oviducts		
		XX	Uterus*+		
		X	Cervix		
		X	Vagina		

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

II. RESULTS:

A. OBSERVATIONS:

1. Clinical signs of toxicity:

Cage-side Observations: There were no treatment-related clinical or cage-side observations noted during the study.

Detailed Clinical Observations: There were no treatment-related, detailed clinical observations noted during the study.

2. Mortality: All mice survived the 13-week dosing period.

B. BODY WEIGHT AND WEIGHT GAIN: Final body weights were slightly increased in the high-dose males and females (↑5% and ↑3% compared to control, respectively). Body weight gains for male and female mice were slightly higher for the 1000 mg/kg/day dose level (125-126% of the control value). The slight increase in final body weights in the high dose is not considered biologically significant or treatment-related.

TABLE 2. Average body weights and body weight gains during 90 days of treatment [a]

Dose rate mg/kg/day	Body weights (g±SD)				Total weight gain	
	Initial (Day 0)	Week 1	Week 7	Week 13	g	% of control
Male						
0	29.8 ± 1.8	31.5 ± 2.2	36.0 ± 1.9	37.7 ± 2.3	7.8 ± 1.8	-
10	29.5 ± 2.0	31.0 ± 2.2	34.9 ± 2.6	37.1 ± 2.8	7.6 ± 2.0	97
100	29.7 ± 2.1	31.1 ± 2.1	35.6 ± 2.4	38.0 ± 3.0	8.3 ± 2.7	106
1000	29.9 ± 2.1	31.9 ± 2.3	36.9 ± 3.5	39.7 ± 4.3 (↑5)	9.8 ± 3.1	126
Female						
0	23.8 ± 0.9	24.8 ± 1.6	28.3 ± 2.0	29.1 ± 1.5	5.3 ± 1.2	-
10	23.7 ± 1.0	24.9 ± 1.0	28.0 ± 1.4	28.8 ± 1.3	5.1 ± 0.9	96
100	23.6 ± 1.9	24.8 ± 1.6	28.1 ± 2.2	29.4 ± 2.8	5.8 ± 1.2	109
1000	23.4 ± 1.3	24.9 ± 1.5	28.7 ± 1.9	29.9 ± 2.3 (↑3)	6.6 ± 1.4	125

[a] Data obtained from Tables 8 and 9 on pages43-48 of the study report.

C. FOOD CONSUMPTION AND COMPOUND INTAKE:

1. **Food consumption:** Males given 1000 mg/kg/day had sporadic, statistically identified increases in food consumption throughout the study (9-19% increase over the control mean). These sporadic increases were consistent with the increased body weights of this group of animals and not considered treatment-related.

2. **Compound consumption:** The targeted values for test material intake were 10, 100, and 1000 mg/kg/day. Male mice from the low-, middle-, or high-dose groups received acceptable time-weighted average dosages of 10.3, 102, or 1030 mg/kg/day, respectively; female mice from the low-, middle-, or high-dose received acceptable time-weighted average dosages of 10.2, 103, or 1010 mg/kg/day, respectively.

D. OPHTHALMOSCOPIC EXAMINATION: Examinations performed on all animals pre-study and prior to termination (day 85) revealed no treatment-related findings.

E. BLOOD ANALYSES:

1. **Hematology:** There were no significant changes in any of the hematologic parameters for either male or female mice.

2. **Clinical chemistry:** The only clinical chemistry parameter affected by ingestion of XDE-742/BAS-770H was serum cholesterol in the high-dose males and females (summary Table 3). In males of the 1000 mg/kg/day dose group, the mean serum cholesterol was increased

22% compared to controls and exceeded the historical control range (170 > 112 – 158 mg/dL). In females, the serum cholesterol level was statistically identified at the $p<0.05$ level and was 30% greater than the control value. The 100 mg/dL mean cholesterol level in females was at the upper end of the historical control range (historical controls 76 – 100 mg/dL). Analysis of the individual data showed that cholesterol levels in 4/8 males and 5/10 females of the high dose group exceeded their historical control counterparts (compared to 2/8 and 2/10 for the concurrent control groups males and females, respectively). This increase in serum cholesterol was considered to be treatment related but non-adverse.

Table 3. Serum Cholesterol of Mice Given XDE-742/BAS-770H for 90 Days

	Dose level (mg/kg/day)			
Parameter	0	10	100	1000
Males				
Group Ave. Cholesterol (mg/dL)	**139 ± 26**	**138 ± 24**	**139 ± 22**	**170 ± 39**
Ranked Individual data[1]	96	106	99	126
	115	111	123	137
	121	122	131	138
	141	124	140	158
	154	128	144	176
	154	156	146	190
	163	161	158	195
	168	166	169	242
	-	166	-	-
	-	-	-	-
Historical Controls	112, 137, 158, 157			
Females				
Group Ave. Cholesterol (mg/dL)	**77 ± 22**	**78 ± 18**	**88 ± 15**	**100 ± 13***
Ranked Individual data	48	57	72	74
	52	64	74	92
	59	66	76	94
	62	68	78	98
	77	71	80	99
	78	74	90	101
	82	79	97	102
	85	85	98	105
	106	94	103	106
	116	118	116	127
Historical Controls	76, 100, 84, 98			

[1] Data was not available for all males. Data was obtained from Appendix Tables 14 and 16 on pages 131-132 and 135-136 of the study report.
* Statistically different from control mean by Dunnett's test, alpha = 0.05.
Historical control based upon four dietary studies from June 1998 to February 2003.

F. URINALYSIS: Urinalysis was not performed for this study.

G. SACRIFICE AND PATHOLOGY:

1. **Organ weight:** The absolute and relative liver weights were increased for males given 1000 mg/kg/day and were statistically significant (Table 4). The absolute liver weight was

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increased 18.3% compared to controls while the relative liver weight was increased 12.3% due to the greater body weight in this dose group. Females given this high-dose level also had slightly increased liver weights (absolute weight increased 7.7%; relative weight increased 5.0% above controls), but these were not statistically identified and their relationship to treatment is uncertain. The female mice in this study were all slightly smaller than the historical controls (body weight 29.6-32.3 g), and the absolute liver weight of the controls, low- and middle-dose groups were all at or below the historical control range (1.447-1.578 g) while the relative liver weight of all dose groups was above the historical control range (4.736-4.892 g/100 g). Histological examination of the liver did not reveal any lesions or changes that would indicate hepatic injury; therefore the slight increases in liver weights in the high dose group males were not considered treatment related.

Table 4. Liver Weights of Mice Given XDE-742/BAS-770H

	Dose level (mg/kg/day)			
Parameter	0	10	100	1000
	Males			
Body Weight (g)	36.1 ± 2.7	35.8 ± 2.6	36.7 ± 2.7	38.3 ± 4.0
Historical Controls	37.1 – 42.7			
Liver (g)	1.986 ± 0.35	1.902 ± 0.16	1.997 ± 0.17	2.349 ± 0.29* (↑18)
Historical Controls	1.93 – 2.097			
Relative Liver (g/100 g bw)	5.483 ± 0.74	5.322 ± 0.46	5.439 ± 0.28	6.155 ± 0.67* (↑12)
Historical Controls	4.756 – 5.627			
	Females			
Body Weight (g)	27.9 ± 2.0	27.9 ± 1.9	28.3 ± 2.4	28.7 ± 2.2
Historical Controls	29.6 – 32.3			
Liver (g)	1.400 ± 0.19	1.434 ± 0.18	1.446 ± 0.19	1.508 ± 0.14 (↑8)
Historical Controls	1.447 – 1.578			
Relative Liver (g/100 g bw)	5.007 ± 0.42	5.127 ± 0.40	5.106 ± 0.50	5.257 ± 0.40 (↑5)
Historical Controls	4.736 – 4.892			

Data comes from Text Table 3 and text on page 27 and Tables 22 and 23 on pages 61-64 of the study report.
*indicates statistical significance at $p<0.05$

At 1000 mg/kg bw/day, absolute and relative adrenal weights were increased (not statistically significant) in males by 12 and 18%, respectively, and were decreased (not statistically significant) in females by 9 and 13%, respectively, compared to controls. These changes are not considered to be related to treatment in the absence of any treatment-related histological changes in the adrenal gland of these animals.

2. **Gross pathology:** There were no treatment-related gross pathologic observations.

3. **Microscopic pathology:** There were no treatment-related changes in any of the organs examined from either males or females.

III. DISCUSSION AND CONCLUSIONS:

A. **INVESTIGATORS' CONCLUSIONS:** "The only effects attributed to treatment with XDE-742/BAS-770H were identified in the high-dose group (1000 mg/kg/day). There were no treatment-related effects on body weight, feed consumption, ophthalmologic, clinical observations or hematologic parameters. Females given 1000 mg/kg/day had statistically-identified increased serum cholesterol (29.9% greater than controls), while males given this dose level also had increased cholesterol (22.3%) that was not statistically identified. The increased cholesterol was considered to be treatment related. The only treatment-related changes in male organ weights were statistically-identified higher absolute and relative liver weights for the 1000 mg/kg/day group (18.3% and 12.3% higher than controls, respectively). The absolute and relative liver weights of females given 1000 mg/kg/day were 7.7% and 5.0% greater than controls, respectively, and were not statistically identified. There were no treatment-related gross or histopathologic effects."

B. **REVIEWER COMMENTS:** There were no treatment-related effects on body weight, feed consumption, ophthalmology, clinical observations or hematologic parameters. Females given 1000 mg/kg/day had statistically-identified increased serum cholesterol (29.9% greater than controls), which was at the high-end of the historical control range (5/10 females had cholesterol levels in excess of the historical control range compared to 2/10 in the concurrent control group). Males at 1000 mg/kg/day also had increased cholesterol (22.3%) that was not statistically identified, likely due to one high dose male that had higher cholesterol levels than all the others (242 compared to <200 mg/dL). Half (4/8) of the high-dose males had cholesterol levels outside the historical control range (compared to 2/8 in the concurrent controls). The only other finding was a statistically-identified increase in absolute and relative liver weights for the 1000 mg/kg/day group males (18.3% and 12.3% higher than controls, respectively). The absolute and relative liver weights of females given 1000 mg/kg/day were 7.7% and 5.0% greater than controls, respectively, and were not statistically identified. Taken together, the increased cholesterol levels in males and females and the increased liver weights in males could indicate hepatic disease, however, there was no corroborating evidence of gross or histopathological changes in the liver. Therefore, these effects were not considered adverse.

The LOAEL was not observed. The NOAEL is 1000 mg/kg/day.

The registrant, Dow Chemical Company, originally prepared this STUDY PROFILE TEMPLATE (STP) (MRID 46908550) in HED's DER format. The HED reviewers may have added minor adjustments/additions to the original STP. The conclusions of the study and assignment of its classification as determined by HED reviewers are in the Executive Summary above.

C. **STUDY DEFICIENCIES:**
Blood clotting was not assessed.

EPA Reviewer: Linda L. Taylor, Ph.D. **Signature:** Linda L. Taylor
Reregistration Branch I, Health Effects Division (7509P) **Date** 01-03-08
EPA Secondary Reviewer: Kimberly Harper **Signature:** Kimberly D Harper
RAB2, Health Effects Division (7509P) **Date:** 12/10/07

Template version 02/06

TXR#: 0054347

DATA EVALUATION RECORD

STUDY TYPE: Subchronic Oral Toxicity [feeding] - dog; OPPTS 870.3150; OECD 409

PC CODE: 108702 **DP BARCODE:** D332276

TEST MATERIAL (PURITY): XDE-742 (98% a. i.) [pyroxsulam]

CHEMICAL: N-(5,7-dimethoxy[1,2,4]triazolo[1,5-a]pyrimidin-2-yl)-2-methoxy-4-(trifluromethyl)pyridine-3-sulfonamide

SYNONYMS: X666742, XDE-742, LY-666742, BAS-770H

CITATION: Stebbins, K. E., and Baker, B. S. (2003). XDE-742/BAS-770H: 90-Day Dietary Toxicity Study in Beagle Dogs. Toxicology & Environmental Research and Cunsulting. The Dow Chemical Company, Midland, Michigan. Laboratory Project Study ID: 021111, October 28, 2002-January 29, 2003. MRID 46908352. Unpublished.

Merriman, T.N. (2002) XDE-742: Range-finding and 28-Day Dietary Toxicity Study in Dogs. Toxicology & Environmental Research and Cunsulting. The Dow Chemical Company, Midland, Michigan. Laboratory Project Study ID: 011062, 26 February 2002. MRID 46908401. Unpublished.

SPONSOR: Dow AgroSciences LLC, Indianapolis, Indiana

EXECUTIVE SUMMARY: In a 90-day oral toxicity study (MRID 46908352, 46908401), XDE-742/BAS-770H (pyroxsulam) [Lot # E0952-52-01, TSN103826; 98.0% a. i.] was administered to 4 Beagle dogs/sex/dose in the diet at dose levels of 0, 0.03, 0.3, or 3.0% (equivalent to 0, 10.9, 91.3, or 884.1 mg/kg bw/day in males and 0, 10.4, 98.6, or 1142.4 mg/kg bw/day in females; hereafter mg/kg bw/day is referred to as mg/kg/day). Parameters evaluated included daily observations, detailed clinical observations, ophthalmic examinations, body weights, feed consumption, clinical pathology, organ weights, and gross and histopathologic examinations.

All dogs survived until study termination. There were no treatment-related clinical signs of toxicity, and no compound-related effects were observed on survival, ophthalmology, hematology, or gross pathology.

Body-weight gains overall were reduced in both sexes (males 34%/females 31%) at the high-dose level, although there was only a slight reduction in body weight at study termination (males 7%/females 3%). There were no other adverse treatment-related findings. **Therefore, the NOAEL is 884 (males)/1142 (females) mg/kg/day, the highest dose tested.**

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

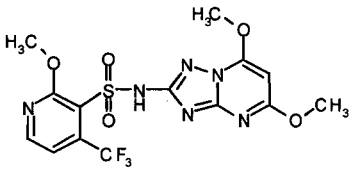
COMPLIANCE: Signed and dated GLP, Quality Assurance, and Data Confidentiality statements were provided.

I. MATERIALS AND METHODS:

A. MATERIALS:

1. Test material: XDE-742/BAS-770H

Description:	(solid) white powder
Lot/batch #:	E0952-52-01/TSN103826
Purity:	98.0% a.i.
Compound stability:	stable in lab diet (#5007) for at least 24 days at concentrations ranging from 0.001%-5%
CAS # if TGAI:	422556-08-9
Structure:	



2. Vehicle and/or positive control: control diet (LabDiet Certified Canine Diet #5007)

3. Test animals:

Species:	Dog
Strain:	Beagle
Age/weight at study initiation:	approximately 6 months old; males: 6.46-8.50 kg; females: 5.53-6.93 kg
Source:	Marshall Research Laboratories, North Rose, NY
Housing:	individual pens
Diet:	LabDiet® Certified Canine Diet #5007 *ad libitum*; PMI Nutrition International
Water:	Municipal *ad libitum*
Environmental conditions:	**Temperature:** 20.3-23.2°C **Humidity:** 42.1-63.3% **Air changes:** 12-15/hr **Photoperiod:** 12 hrs dark/12 hrs light
Acclimation period:	at least 25 days

B. STUDY DESIGN:

1. In life dates: Start: October 28, 2002; End: January 29, 2003.

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

TABLE 1: Study Design

Test group	Conc. in diet (%)/Nominal Dose to animal (mg/kg/day)	Actual Dose (mg/kg/day) (M/F)	# Male	# Female
Control	0/0	0/0	4	4
Low	0.03/10	10.9/10.4	4	4

Mid	0.3/100	91.3/98.6	4	4
High	3/1000	884.1/1142.4	4	4

3. **Dose selection rationale:** The dose levels were selected based on the results from a 28-day dog study [MRID 46908401] where oral administration *via* the diet at dose levels up to 30000 ppm (3% in diet) resulted in no adverse effects on survival, clinical signs of toxicity, and no toxicologically-meaningful differences in mean body weight, body-weight gain, food consumption, clinical pathology data, gross necropsy findings, or organ weights. It is noted, however, that males at the high-dose level displayed an overall (days 1-28) negative body-weight gain (-146 g) compared to the body-weight gains of the control (+292 g), low- (+184 g), and mid-dose (+593 g) groups. Similarly, high-dose females displayed a negative body-weight gain overall (-61 g) compared to the body-weight gains of the control (+150 g), low- (+239 g), and mid-dose (+235 g) groups. The 3% level was expected to be well tolerated, and the remaining dose levels were expected to provide dose-response data for any treatment-related effects at the high dose.

4. **Diet preparation and analysis:** High-dose diets were prepared every two weeks by mixing appropriate amounts of test substance with ground PMI Certified Canine Diet #5007. Mid-dose diets were prepared by diluting the high-dose diet with control lab diet, and the low-dose diets were prepared by diluting the mid-dose diet with control lab diet. The test material was determined (previously) to be stable for at least 24 days at concentrations ranging from 0.001% to 5%, which spanned the diet concentrations used in the study and, therefore, stability was not determined in this study. Homogeneity of the low- and high-dose diets and analysis of all dose levels plus control were determined for week 1, week 5, and week 13 of the study.

Results:

Homogeneity analysis: The % relative standard deviations for homogeneity were reported as 0.916% to 5.88%, confirming the homogeneity of the mixture.

Stability analysis: Determined previously (stable for at least 24 days).

Concentration analysis: % of target ranged from 93% to 105%.

5. **Statistics:** Means and standard deviations were calculated for all continuous data. All parameters examined statistically were first tested for equality of variance using Bartlett's test. If significant at alpha = 0.01, then the data were subjected to a transformation to obtain equality of the variances. The transformations examined are the common log, the inverse, and the square root, in that order. In-life body weight, urine specific gravity, hematology parameters (excluding RBC indices, differential WBC), coagulation, and clinical chemistry parameters were evaluated using a repeated measures (RM) analysis of variance (ANOVA) for time (the repeated factor), sex, and dose. A Bonferroni correction was applied to the alpha level to compensate for the multiple comparisons with the control group, which controlled the experiment-wise error rate. The corrected comparison-wise alpha level of 0.02 is reported so direct comparison can be made to the p-values generated. Terminal body weight, organ weight (absolute and relative, excluding ovaries, uterus, epididymides, and testes), and urine volume were evaluated using a two-way ANOVA with the factors of sex and dose. Differences between the groups were primarily

detected by the dose factor. For these parameters, the first examination was whether the sex-dose interaction was significant at alpha = 0.05; if it was, a oneway ANOVA was then done separately for each sex. Comparisons of individual dose groups to the control group were made with a Dunnett's test only when a statistically significant dose effect existed (alpha = 0.05). Results for ovaries, uterus, epididymides, and testes weight (absolute and relative) were analyzed using a one-way ANOVA. If significant dose effects were determined, then separate doses were compared to controls using a Dunnett's test. Feed consumption data were evaluated by Bartlett's test for equality of variances. Exploratory data analysis was performed by a parametric analysis of variance (ANOVA); when significant at alpha = 0.05, followed by Dunnett's test (alpha = 0.05). Descriptive statistics only (means and standard deviations) were reported for body weight gains, RBC indices, and WBC differential counts.

These analyses are considered appropriate.

C. METHODS:

1. Observations:

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

1b. Clinical examinations: Clinical examinations were conducted pre-exposure and weekly throughout the study and included cage-side, hands-on, and open-field observations. The examination begins at the head of the dog and gradually works towards the tail [body movements, behaviors, changes in posture, resistance to removal from cage, eye observations, degree of salivation, muscle tone, extensor-thrust response, reactivity to handling, open field observations (responsiveness to touch, gait evaluation)].

2. **Body weight:** Animals were weighed during the pre-exposure period and weekly during the exposure period. Body-weight gains were calculated.

3. **Food consumption and compound intake:** Food consumption for each dog was determined and mean daily diet consumption was calculated as g food/day. Compound intake (mg/kg bw/day) values were calculated as using actual feed concentrations, body weights, and feed consumption data.

4. **Ophthalmoscopic examination:** Eyes were examined pre-exposure and during the week prior to sacrifice using indirect ophthalmoscopy. Eyes were also examined during necropsy.

5. **Hematology and clinical chemistry:** Blood was collected *via* venipuncture of the jugular vein from all fasted dogs pre-exposure, during week 7, and on the day of sacrifice for hematology and clinical chemistry from all surviving animals. The CHECKED (X) parameters were examined.

a. Hematology:

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

* Recommended for 90-day oral non-rodent studies based on Guideline 870.3150

b. Clinical chemistry:

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

* Recommended for subchronic non-rodent studies based on Guideline 870.3150

6. **Urinalysis:** Urine was collected from fasted (16 hours) dogs (metabolism cages) pre-exposure, during week 7, and during the last week of the study. The CHECKED (X) parameters were examined.

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

* Recommended for subchronic non-rodent studies based on Guideline 870.3150

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

X	DIGESTIVE SYSTEM
X	Tongue
X	Salivary glands*
X	Esophagus*
X	Stomach*
X	Duodenum*
X	Jejunum*
X	Ileum*
X	Cecum*
X	Colon*
X	Rectum*
X	Liver*+
X	Gall bladder*+
X	Pancreas*
X	**RESPIRATORY**
X	Trachea*
X	Lung*
X	Nose*
X	Pharynx*
X	Larynx*

X	CARDIOVASC./HEMAT.
X	Aorta thoracic*
X	Heart*+
X	Bone marrow*
X	Lymph nodes*
X	Spleen*+
X	Thymus*
X	**UROGENITAL**
X	Kidneys*+
X	Urinary bladder*
X	Testes*+
X	Epididymides*+
X	Prostate*
X	Ovaries*+
X	Uterus*+
X	Mammary gland*

X	NEUROLOGIC
X	Brain*+
X	Peripheral nerve*
X	Spinal cord (3 levels)*
X	Pituitary*
X	Eyes (optic nerve)*
X	**GLANDULAR**
X	Adrenal gland*+
	Lacrimal gland
X	Parathyroid*+
X	Thyroid*+
X	**OTHER**
X	Bone (sternum and/or femur)
X	Skeletal muscle
X	Skin*
X	All gross lesions and masses*

* 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:** There were no treatment-related clinical signs in any of the dogs of either sex.

2. **Mortality:** All dogs survived to terminal sacrifice (90 days).

B. BODY WEIGHT AND WEIGHT GAIN: During the first week of dosing, all male dogs in the treated groups gained weight, but 3 of the 4 control male dogs lost weight (-0.063 kg to -0.403 kg). Males at the high-dose level displayed a slight decrease in body weight by study termination (7%), but body-weight gain overall was decreased 34% compared to control. Females at the high-dose level also displayed a slight decrease in body weight (3%), but body-weight gain overall was decreased 31% compared to control. Negative body-weight gains were observed at the high-dose level (both sexes) during the second week.

TABLE 2. Mean (±SD) body weight/body-weight gain (kg) [a] data				
Interval	Dose (%)			
	Control	0.03	0.3	3

TABLE 2. Mean (±SD) body weight/body-weight gain (kg) [a] data				
Interval	Dose (%)			
	Control	0.03	0.3	3
MALES				
Body weight				
Day 1	7.63±0.63	7.61±0.76	7.58±0.85	7.51±0.36
Day 8	7.54±0.59	8.05±0.74	7.86±0.90	7.75±0.35
Day 15	7.57±0.25	8.16±0.98	7.93±0.90	7.73±0.37
Day 57	8.50±0.94	8.95±0.91	8.88±1.04	7.97±0.48 (↓6)
Day 78	9.20±1.08	9.26±0.96	9.26±1.30	8.41±0.53 (↓9)
Day 92	9.06±1.04	9.45±0.97	9.42±1.42	8.46±0.50 (↓7)
Body-weight gain				
Days 1-8	-0.082±0.266	0.442±0.297	0.274±0.178	0.235±0.062
Days 8-15♪	0.030	0.107	0.076	-0.016
Days 1-29♪	0.504	0.709	0.600	0.341 (↓32)
Days 29-64♪	0.641	0.840	0.818	0.412 (↓36)
Days 64-92 ♪	0.287	0.292	0.419	0.193 (↓33)
Days 1-92	1.433±0.492	1.841±0.628	1.837±0.765	0.946±0.169 (↓34)
FEMALES				
Body weight				
Day 1	5.98±0.19	6.02±0.32	6.09±0.49	6.16±0.61
Day 8	6.15±0.30	6.13±0.40	6.28±0.68	6.34±0.71
Day 15	6.41±0.44	6.24±0.41	6.48±0.73	6.28±0.62
Day 57	6.87±0.71	7.03±0.38	7.18±0.83	6.55±1.16
Day 78	7.22±0.73	7.31±0.54	7.62±0.88	6.90±1.13
Day 92	7.19±0.81	7.31±0.64	7.59±1.03	7.00±1.23
Body-weight gain				
Days 1-8	0.174±0.158	0.111±0.110	0.191±0.257	0.177±0.141
Days 8-15♪	0.253	0.102	0.192	-0.057
Days 1-29♪	0.629	0.576	0.646	0.137 (↓78)
Days 29-64♪	0.304	0.583	0.718	0.393
Days 64-92 ♪	0.276	0.131	0.138	0.309
Days 1-92	1.209±0.636	1.289±0.451	1.502±0.601	0.839±0.686 (↓31)

[a] Data obtained from Tables 8 & 9, pages 59-64 in the study report; (% of control); ♪calculated by reviewer

C. FOOD CONSUMPTION AND COMPOUND INTAKE:

1. **Food consumption:** Food consumption was consistently lower than control at the high-dose level for males (10%-27% less than control), but a similar decrease was not observed in females.

2. **Compound consumption:** Actual test material intake was close to the intended dose.

TABLE 3: Compound Consumption				
Test group	Conc. in diet (%)	Intended Dose (mg/kg/day)	Male (actual)	Female (actual)
Control	0	0	0	0
Low	0.03	10	10.9±0.6	10.4±0.9
Mid	0.3	100	91.3±6.4	98.6±10.7
High	3	1000	884.1±73.2	1142.4±63.0

Data from Tables12 & 13, pages 67-68 of the report

3. **Food efficiency:** Food efficiency data were not provided. Males at the high-dose level

ingested 13% less food overall than the control males and gained 34% less body weight. High-dose females ingested 12% more food than the control females and gained 31% less body weight.

D. OPHTHALMOSCOPIC EXAMINATION: There were no treatment-related findings in either sex.

E. BLOOD ANALYSES:

1. **Hematology:** There were no treatment-related findings in any of the hematology parameters monitored in either sex.

2. **Clinical chemistry:** Females at the high-dose level displayed higher cholesterol and alkaline phosphatase values than the control at weeks 7 and 13 [Table 4.] With the exception of week 13 cholesterol values, these high-dose values exceeded the historical control also. NOTE: Females at the mid-dose level displayed both alkaline phosphatase and cholesterol values that were lower than the historical control range during the pre-test measurement, and with the exception of week 13 cholesterol values, were also lower during the study.

TABLE 4. Clinical Chemistry Data

Observation	Dose group (mg/kg/day)			
	Control	10	100	1000
Males				
Cholesterol (mg/dL)				
Pre-test [143-207]	167±35	140±25	163±14	152±13
Week 7 [158-179]	163±34	133±26	153±17	174±13
Week 13 [158-193]	177±32	147±21	152±15	173±15
Alkaline phosphatase (μ/L)				
Pre-test [97-214]	156±78	139±10	143±6	116±23
Week 7 [120-163]	145±77	115±13	127±17	148±40
Week 13 [92-117]	109±52	89±18	89±11	102±15
Females				
Cholesterol (mg/dL)				
Pre-test [130-186]	151±8	129±10	124±4	154±23
Week 7 [167-181]	151±15	149±17	160±14	200±25
Week 13 [179-216]	168±11	177±28	184±40	214±29
Alkaline phosphatase (μ/L)				
Pre-test [116-206]	153±24	125±12	109±20	117±27
Week 7 [105-125]	124±20	103±9	95±22	133±41
Week 13 [77-102]	95±27	70±12	69±8	117±24

[a] Data obtained from Tables 32-37, page 87-92 in the study report;
[Historical control]: range from 14 dietary studies (pre-exposure data); 5 dietary studies (7-week data); 4 dietary studies (13-week data)

F. URINALYSIS: There were no changes in any of the urinalysis parameters monitored for either sex.

G. SACRIFICE AND PATHOLOGY:

1. **Organ weight:** At the high-dose level, relative liver weights (males 113%/females 133% of control) were significantly increased compared to the control in both sexes (Table 5). High-dose

females displayed a significant increase in absolute liver weight (128% of control) also. There appeared to be a dose-related decrease in thymus weight (absolute and relative) in males, and the ovary and uterine weights were decreased in the high-dose females. With regard to the uterus weight, 1 of the 4 control females displayed a uterine weight less than 10 grams (1.26 grams) while 3 of the 4 high-dose females displayed a uterine weight of less than 2.2 grams (highest 9.62 grams).

TABLE 5. Organ Weight Data [a]				
Organ	Dose (mg/kg bw/day)			
	0	10	100	1000
MALES				
Terminal body weight	8768±1063	9238±1081	9061±1316	8210±510 (94)
Liver				
absolute	234±37	265±25	232±27	250±22 (107)
relative	2.70±0.62	2.88±0.22	2.58±0.27	3.04±0.14 (113)
Thymus				
absolute	12.946±5.336	12.878±3.216	10.512±1.443 (81)	8.775±1.579 (68)
relative	0.144±0.044	0.138±0.020	0.119±0.029 (83)	0.107±0.016 (74)
FEMALES				
Terminal body weight	6857±741	6938±702	7411±1036	6701±1194
Liver				
absolute	193±33	206±28	200±47	248±42 (128)
relative	2.80±0.22	2.97±0.22	2.68±0.41	3.71±0.44 (133)
Ovary				
absolute	1.517±0.868	1.459±1.173	1.648±0.599	0.959±0.676 (63)
relative	0.022±0.014	0.020±0.015	0.022±0.006	0.014±0.010 (64)
Uterus				
absolute	9.114±5.432	7.127±4.463	10.293±6.662	3.813±3.884 (42)
relative	0.130±0.075	0.099±0.055	0.133±0.077	0.057±0.058 (44)

[a] Data obtained from Tables 56 & 57, page117-120 in the study report

2. **Gross pathology:** There were no treatment-related gross pathologic observations reported in either sex at any dose level.

3. **Microscopic pathology:** The only histopathologic finding considered treatment-related was a very slight panlobular hepatocellular hypertrophy, which was observed in 3 of the 4 high-dose females. This finding is consistent with the increase in liver weight observed in the high-dose females. A similar finding was not observed in males.

III. DISCUSSION AND CONCLUSIONS:

A. **INVESTIGATORS' CONCLUSIONS:** The mean in-life body weights of the high-dose males and females combined over the duration of the study were statistically identified as lower than controls. At the end of the study, the mean body weight gains for the high-dose males and females were 34% and 31% lower than controls, respectively. High-dose females had treatment-related increases in cholesterol concentration and alkaline phosphatase activity. High-dose animals had treatment-related, statistically identified higher relative liver weights averaged across both sexes, relative to control. In addition, the mean absolute liver weight of high-dose females was greater than controls but not statistically identified. The higher liver weights corresponded to treatment-related very slight panlobular hepatocellular hypertrophy in 3 of 4 high-dose females. The no-observed-effect level (NOEL) for male and female dogs administered XDE-742.BAS-770H in the diet for 90 days was 0.3 % (approximately 91.3 mg/kg/day for males and 98.6 mg/kg/day for females).

B. **REVIEWER COMMENTS:** In general, the test material was well tolerated. Dogs of both sexes displayed reduced body-weight gains overall at the high-dose level (males 34%/females 31%), but body weights were only slightly lower at study termination (males 7%/females 3%). Females

at the high dose displayed increased cholesterol and alkaline phosphatase levels throughout the study. Increased liver weight (absolute and relative) was observed in females at the high dose, along with an increased incidence of slight panlobular hepatocellular hypertrophy. Males at the high dose displayed an increase in relative liver weight with no accompanying microscopic lesions. These findings do not appear to be adverse but rather may be adaptive in nature. This is supported by the findings in the chronic oral toxicity study in dogs in which similar liver effects were observed on longer-term exposure with no increase in severity of the findings. No histopathological lesions were observed following chronic exposure at dose levels up 2% (620 mg/kg/day in males and 589 mg/kg/day in females). The effects observed following the 90-day exposure period [reduced body-weight gain, increased cholesterol concentration (females), alkaline phosphatase activity (females), increased liver weight, and an increased incidence of panlobular hepatocellular hypertrophy (females)] are not considered adverse. **The NOAEL is 884 (males)/1142 (females) mg/kg/day, the highest dose tested.**

The registrant, the Dow Chemical Company, originally prepared this STUDY PROFILE TEMPLATE (STP) (MRID 46908551, 86908555) in HED's DER format. The HED reviewers may have added minor adjustments/additions to the original STP. The conclusions of the study and assignment of its classification as determined by HED reviewers are in the Executive Summary above.

C. **STUDY DEFICIENCIES:** Food efficiency data were not provided. However, this does not affect study interpretation.

however, the processes of the laboratory and the study involved are inspected in regular intervals.

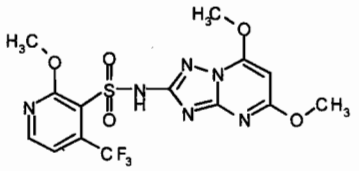
I. MATERIALS AND METHODS:

A. MATERIALS:

1. **Test material:** XDE-742/BAS 770H

Description:	solid, white-beige
Lot/batch #:	E0952-52-01
Purity:	98.0 % a.i.
Compound stability:	produced 7/25/02;stable until 7/30/04
CAS #:	422556-08-9

Structure:



2. **Vehicle and/or positive control:** Aqua bidest is listed in the Methods section, but no description, Lot/Batch #, or purity information are reported. A stability analysis of XDE-742/BAS 770H in doubly-distilled water and dimethylformamide was provided (page 49 of the study report; dated September 23, 2003).

3. **Test animals:**

Species:	Rat	
Strain:	Wistar CrlG1xBrlHan:WI	
Age/weight at study initiation:	60±1 day old	
Source:	Charles River, Sulzfeld, Germany	
Housing:	Individual stainless steel wire mesh cages	
Diet:	Ground Kliba maintenance diet mouse/rat "GLP" meal, *ad libitum*	
Water:	municipal, *ad libitum*	
Environmental conditions:	**Temperature:**	20-24°C
	Humidity:	30%-70%
	Air changes:	not provided
	Photoperiod:	12 hrs dark/12 hrs light
Acclimation period:	8 days	

B. STUDY DESIGN:

1. **In life dates:** Start: September 2, 2003; End: September 25, 2003.

2. **Animal assignment:** Animals were assigned (distributed according to body weight; computer randomization) to the test groups noted in Table 1.

TABLE 1: Study design			
Test group	**Dose (mg/kg bw/d)**	**# Male**	**# Female**
Control	0	3	3
Treated	1000	3	3

3. **Dose selection rationale:** This is a range-finding study, and the limit dose was selected.

4. **Preparation and treatment of animal skin:** The test material was administered as a paste in Aqua bidest (prepared twice a week) for about 2 weeks (5 days a week). Shortly before the first application (one day) and weekly thereafter, the fur of each test animal was clipped from the dorsal and dorsolateral areas of the trunk (at least 10% of the body surface). The applied quantity of the test substance was 2 grams/kg body weight, based on the most recent body weight measurement. The test substance/vehicle suspension was evenly applied to the skin, and the skin was covered for 6 hours after application using a semiocclusive dressing consisting of 4 layers of porous gauze dressing ("Verbandmull Ph.Eur", Lohmann GbbH & Co KG.) and an elastic dressing (Fixomull Stretch, Beierdorf AG). The dressings were removed after 6 hours and the application areas were cleaned with lukewarm water. At the end of the application period, all surviving rats were sacrificed.

   Rats in the control group were exposed to the vehicle using the same procedure as described for the treated rats.

5. **Statistics:** Means and standard deviations of each test group were calculated for food consumption, body weight, body-weight change, and food efficiency. A comparison of each group with the control was performed using Dunnett's test (2-sided).

C. METHODS:

1. **Observations:**

   1a. **Cageside observations:** Animals were observed daily for overt signs of toxicity or mortality (twice daily) Monday through Friday and once daily on weekends.

   1b. **Clinical examinations:** Clinical examinations, including a detailed examination of the skin, were performed just before each daily treatment.

   1c. **Neurological evaluations:** Not performed.

2. **Body weight:** Animals were weighed prior to initiation of the study (for randomization purposes), on day 0 (start of application), and at weekly intervals.

3. **Food consumption:** Food consumption (g food/animal/day) was determined weekly (individually) and food efficiency was calculated.

4. **Ophthalmoscopic examination:** Eyes were not examined.

5. **Hematology and clinical chemistry:** Blood was not collected for hematology or clinical chemistry assessment.

6. **Urinalysis:** Urine was not collected for analysis.

7. **Sacrifice and pathology:** At the end of the application period, all animals were sacrificed under CO_2 anesthesia without further examination.

II. RESULTS:

A. OBSERVATION(s):

1. **Clinical signs of toxicity:** There were no treatment-related signs of toxicity in either sex.

2. **Mortality:** All rats survived until study termination.

3. **Neurological evaluations:** not performed.

4. **Dermal Irritation:** There were no signs of local skin irritation.

B. BODY WEIGHT AND WEIGHT GAIN: There were no adverse effects on body weight or body-weight gains in either sex.

Table 2. Average body weights and body weight gains during 2 weeks of dermal treatment [a]

Dose (mg/kg/day)	Body weights (g±SD)			Body-weight gain	
	Day 0	Day 7	Day 14	Days 0-7	Days 0-14
Males					
0	238.1±3.0	251.0±0.9	267.9±6.3	13.0±2.1	29.8±5.6
1000	236.6±4.7	253.6±3.7	270.6±4.9	17.0±3.5	34.0±2.4
Females					
0	166.4±5.1	178.0±3.0	186.0±7.9	11.6±4.3	20.2±9.6
1000	165.6±1.0	179.9±3.2	191.3±9.9	14.3±2.3	25.7±9.0

[a] Data from pages 32-35 of the report; n=3

C. FOOD CONSUMPTION AND EFFICIENCY:

1. **Food consumption:** There were no adverse effects on food consumption.

2. **Food efficiency:** Food efficiency was comparable between the groups and for both sexes.

D. OPHTHALMOSCOPIC EXAMINATION: not performed.

E. BLOOD ANALYSES: not performed.

1. **Hematology:** N/A

2. **Clinical chemistry:** N/A

F. URINALYSIS: N/A

G. SACRIFICE AND PATHOLOGY: Examinations were not performed.

1. **Organ weight:** N/A

2. **Gross pathology:** N/A

3. **Microscopic pathology** – N/A

III. DISCUSSION AND CONCLUSIONS:

A. **INVESTIGATORS' CONCLUSIONS:** XDE-742 / BAS 770H was administered to groups of 3 male and 3 female Wistar rats by the dermal route (6 hours/day, 5 days/week, semi-occlusive dressing) for 2 weeks at dose levels of 0 (vehicle), and 1000 mg/kg/day in order to evaluate whether Wistar rats will tolerate a 4-week dermal application up to the limit dose. Regarding the performed clinical examinations, no substance-related effects were observed at 1000 mg/kg/day. Therefore, a 4-week dermal toxicity study with a high dose level of 1000 mg/kg/day can be justified.

B. **REVIEWER COMMENTS:** There were no adverse effects observed on any parameter monitored in either sex following two weeks of dermal exposure to XDE-742/BAS 770H at the limit dose. Skin irritation was not observed.

The registrant, the Dow Chemical Company, originally prepared this STUDY PROFILE TEMPLATE (STP) (MRID 46908552) in HED's DER format. The HED reviewers may have added minor adjustments/additions to the original STP. The conclusions of the study and assignment of its classification as determined by HED reviewers are in the Executive Summary above.

C. **STUDY DEFICIENCIES:** None that would affect study interpretation. This is a range-finding study.

EPA Reviewer: Linda Taylor, Ph.D. **Signature:** Alan C. Levy for
Reregistration Branch I, Health Effects Division (7509P) **Date:** 1-3-08
EPA Secondary Reviewer: Kimberly Harper **Signature:**
RAB2, Health Effects Division (7509C) **Date:** 12/10/07

Template version 02/06

TXR#: 0054347

DATA EVALUATION RECORD

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

PC CODE: 108702 **DP BARCODE:** D332276

TEST MATERIAL (PURITY): XDE-742 (98% a.i.); pyroxsulam

CHEMICAL: N-(5,7-dimethoxy[1,2,4]triazolo[1,5-a]pyrimidin-2-yl)-2-methoxy-4-(trifluromethyl)pyridine-3-sulfonamide

SYNONYMS: X666742, XDE-742, LY-666742, BAS-770H, XR-742

CITATION: E. W. Carney and B. Tornesi. (2005). XDE-742: Oral Gavage Developmental Toxicity Study In CRL:CD(SD) Rats. Toxicology & Environmental Research and Consulting, The Dow Chemical Company, Midland, Michigan, 48674. Study ID: 051053, (13 October 2005). MRID 46908355. Unpublished

Schneider, S. (2004). XDE-742 / BAS 770H – Maternal Toxicity Study in Wistar Rats (Range-Finding) Oral Administration (gavage). Experimental Toxicology and Ecology. BASF Aktiengesellschaft, Germany. Project No. 10R0298/03022, (March 23, 2004). MRID 46908402. Unpublished.

SPONSOR: Dow AgroSciences LLC, 9330 Zionsville Road, Indianapolis, Indiana 46268

EXECUTIVE SUMMARY: In a developmental toxicity study (MRID 46908355), pyroxsulam [XDE-742 (98% a.i.; E0952-52-01)] was administered to 26 time-mated Crl:CD (SD) female rats/dose *via* gavage at dose levels of 0, 100, 300, or 1000 mg/kg bw/day from days 6 through 20 of gestation.

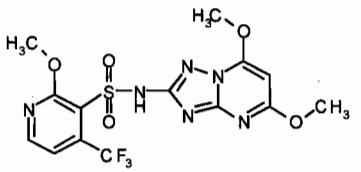
There were no treatment-related effects in survival, clinical signs, body weight, body-weight gain, food consumption, liver or kidney weights, or cesarean parameters, including pregnancy rates, resorption rates, litter size, numbers of corpora lutea or implantations, percent pre- and post-implantation losses, fetal sex ratio, fetal body weights, or gravid uterine weights. **The maternal NOAEL is 1000 mg/kg bw/day (limit dose), the highest dose tested.**

I. MATERIALS AND METHODS

A. MATERIALS:

1. Test material: XDE-742

Description:	white powder
Lot/batch #:	E0952-52-01
Purity:	98.0%. a.i.
Compound stability:	XDE-742 was found to be stable in METHOCEL* at concentrations ranging from 0.25-250 mg/ml for at least 21 days
CAS #of TGAI:	422556-08-9
Structure:	See below



2. Vehicle and/or positive control: 0.5% METHOCEL A4M Lot/Batch # (not provided)

3. Test animals:

Species:	Rat
Strain:	Crl:CD(SD)
Age/weight at study initiation:	10-11 weeks old; 200-250 grams
Source:	Charles River Laboratories Inc. (Portage, MI)
Housing:	Individually in stainless steel cages
Diet:	LabDiet® Certified Rodent Diet #5002 (PMI Nutrition International, St. Louis, Missouri) in meal form *ad libitum*
Water:	municipal *ad libitum*
Environmental conditions:	**Temperature:** 22±1°C **Humidity:** 40%-70% **Air changes:** 12-15/hr **Photoperiod:** 12 hrs dark/12 hrs light
Acclimation period:	one week

B. PROCEDURES AND STUDY DESIGN

1. In life dates: Start: March 20, 2005; End: April 13, 2005

2. Mating: Sexually mature (virgin) females were mated with sexually mature males of the same strain at the supplier's facility. Confirmation of mating was determined by the presence of a copulatory plug, and the day that a plug was detected was designated as day 0 of gestation [GD 0]. On GD21, all surviving animals were euthanized and necropsied.

3. Animal assignment: Animals were stratified by body weight and then randomly assigned to dose groups using a computer program designed to increase the probability of uniform group

* Trademark of The Dow Chemical Company

mean body weights, as indicated in Table 1.

TABLE 1. Animal assignment				
Dose (mg/kg bw/day)	0	100	300	1000
Number of Females♪	26	26	26	26

♪time-mated

4. **Dose selection rationale:** The dose levels were selected based on the results from a range-finding study [MRID 46908402] in which 10 time-mated Wistar rats were administered via gavage XDE-742/BAS 770H at dose levels up to the limit dose [1000 mg/kg/day] from gestation day 6 to gestation day 19. Salivation (transient) was observed in all dams at 1000 mg/kg/day immediately after dosing, but there were no other effects reported at this dose or the two other dose levels [100 and 300 mg/kg/day].

5. **Dosage preparation and analysis:** XDE-742 was administered as a suspension in an aqueous vehicle of 0.5% METHOCEL A4M such that a dose volume of 4 ml/kg body weight yielded the targeted dose. The frequency of the test material-vehicle mixture preparation was not reported; however, it appears that the doses were prepared on the day of dosing. All suspensions from the first mix were analyzed prior to the start of dosing to verify homogeneous distribution of the test material in the vehicle. Analysis of all dosing suspensions from the first mix were initiated prior to the start of dosing using high performance liquid chromatography (HPLC) with ultraviolet detection and external standards to determine concentrations.

Results:

Homogeneity analysis: XDE-742 was homogeneously distributed throughout the dosing suspensions as verified by analysis of multiple aliquots at different levels in the storage vessels. The relative standard deviation ranged from 0.37 to 0.74%.

Stability analysis: XDE-742 was found to be stable in METHOCEL at concentrations ranging from 0.25 – 250 mg/kg for at least 21 days. Concentrations after 21 days of storage range from 99.6 to 104% of the initial concentration. The study report did not specify under what conditions the dosing solutions were stored.

Concentration analysis: Analysis of all dosing suspensions from the first mix revealed mean concentrations of XDE-742 ranging from 98.4 to 101% of targeted concentrations.

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 *via* gavage on gestation days 6 through 20, in a volume of 4 mL/kg of body weight/day. There was no statement whether dosing was based on the body weight on the most recent body weight determination or on a particular day of gestation.

C. OBSERVATIONS:

1. **Maternal observations and evaluations:** Animals were observed approximately one hour post dose (hand-held examination) and were checked for mortality or clinical signs twice daily. Body weights were recorded on gestation days (GD) 0 (by supplier) and daily during GD 6-21. Food consumption data were recorded for all animals on GD 3-6, 6-9, 9-12, 12-15, 15-18, and 18-21. Dams (not fasted) were sacrificed on day 21 of gestation (euthanized by carbon dioxide inhalation) and a limited gross pathologic examination (necropsy) was performed. The sequence of the maternal necropsies was counterbalanced across groups to control for potential confounding influences of timing on fetal growth and skeletal ossification. Examinations at sacrifice consisted of an examination of the external tissues and all orifices. The thoracic and abdominal cavities were opened and the viscera were examined. The stomach, liver, and kidneys were dissected from the carcass and incised, and any obvious gross pathologic alterations were recorded. The weight of the liver, kidneys, and gravid uterus were recorded. Representative sections of liver, kidneys, and gross lesions were preserved. Microscopic examination of tissues was not performed. A detailed examination of the reproductive tract was performed and the number and position of implantations, viable fetuses, dead fetuses, and resorptions (early and late) were recorded. The number of ovarian corpora lutea were counted for each dam with one or more viable fetuses. The uteri of females without visible implantations were stained with a 10% aqueous solution of sodium sulfide and examined for evidence of early resorptions in order to verify pregnancy status. The maternal examinations were conducted without knowledge of the treatment group assignment.

2. **Fetal evaluations:** The sex of all fetuses was recorded and the body weight of all viable fetuses determined. All fetuses were given an external examination that included observations on body proportions, the head and face (including closure of the palate), abdomen, spine, extremities, genitalia, rectum and tail. All viable fetuses were euthanized by sublingual oral administration of sodium pentobarbital solution. At least one half of all the fetuses in each litter were chosen randomly via computer for visceral examination conducted by dissection under a low power stereomicroscope for evidence of visceral alterations. The visceral examination included observation of the thymus, trachea, esophagus, lungs, great vessels, heart (external and internal), liver, gastrointestinal tract, pancreas, spleen, kidney (sectioned), adrenal glands, ureters, bladder and reproductive organs. The heads of these fetuses were removed, placed in Bouin's fixative and serially sectioned to allow for inspection of the eyes, brain, nasal passages and tongue. The remaining fetuses not selected for visceral examination were then skinned, eviscerated, preserved in alcohol and double stained with Alcian Blue and Alizarin Red S for cartilage and bone. After staining, skeletons were macerated and cleared. A thorough evaluation of the fetal skeleton was conducted on the remaining fetuses not selected for visceral examination. However, a fetus may have been intentionally changed from one selected for visceral examination to one processed for skeletal examination (and vice versa) if it was deemed that such examination provided more meaningful data about a suspected abnormality. Fetal examinations were conducted without knowledge of the treatment group assignment.

D. DATA ANALYSIS:

1. **Statistical analyses:** Maternal body weights, maternal body weight gains, organ weights (absolute and relative), fetal body weights and feed consumption were evaluated by Bartlett's test (alpha = 0.01) for equality of variances. Based on the outcome of Bartlett's test, a parametric or non-parametric analysis of variance (ANOVA) was performed. If the ANOVA was significant at alpha = 0.05, analysis by Dunnett's test (alpha = 0.05) or the Wilcoxon Rank-Sum test (alpha = 0.05) with Bonferroni's correction was performed, respectively. Feed consumption values were excluded from analysis if the feed was spilled or scratched.

   Frequency of pre- and post-implantation loss (calculations shown below), and fetal alterations were analyzed using a censored Wilcoxon test with Bonferroni's correction. The number of corpora lutea, implantations, and litter size were evaluated using a non-parametric ANOVA (alpha = 0.05) followed by the Wilcoxon Rank-Sum test (alpha = 0.05) with Bonferroni's correction. Pregnancy rates were analyzed using the Fisher exact probability test (alpha = 0.05) with Bonferroni's correction. Fetal sex ratios were analyzed using a binomial distribution test. Females lacking visible implantations at the scheduled necropsy were excluded from the appropriate analyses. Statistical outliers were identified, using a sequential method (alpha = 0.02), and if excluded, were excluded for sound scientific reasons. Both Dunnett's test and Bonferroni's correction correct for multiple comparisons to the control group to keep the experiment-wise alpha at 0.05. Both were reported at the experiment-wise alpha level.

2. **Indices:** The following indices were calculated from cesarean section records of animals in the study:

   $$\text{Pre-implantation loss}^* = \frac{(\text{No. corpora lutea} - \text{implantations})}{\text{No. corpora lutea}} \times 100$$

   $$\text{Post-implantation loss}^* = \frac{(\text{No. implantations} - \text{viable fetuses})}{\text{No. implantations}} \times 100$$

   * Note: Percent pre- and post-implantation loss was determined for each litter, followed by calculation of the mean of these litter values.

3. **Historical control data:** Historical control data were not provided to allow comparison with concurrent controls.

II. RESULTS:

A. MATERNAL TOXICITY:

1. **Mortality and clinical observations:** All females survived to scheduled sacrifice, and there were no treatment-related findings.

2. **Body weight:** Body weight data are summarized in Table 2. There were no adverse effects on body weight or body-weight gain.

TABLE 2. Mean (±SD) maternal body weight/gain (g) [a]				
Interval	**Dose in mg/kg bw/day (# of Dams)**			
	Control (22)	**100 (24)**	**300 (20)**	**1000 (21)**
Pretreatment				
Body weight				
Day 0	225.8±9.9	221.3±11.7	222.8±9.2	224.8±8.3
Day 6	257.0±13.8	249.7±15.7	256.0±11.1	258.0±12.2
Body-weight gain				
Days 0-6	31.2±10.1	28.4±9.7	33.3±7.9	33.2±6.0
Treatment				
Body weight				
Day 9	273.0±15.6	266.5±16.1	275.6±11.7	277.0±14.3
Day15	315.1±18.7	307.0±21.6	320.6±14.0	320.7±18.8
Body-weight gain				
Days 6-9	15.9±3.9	16.8±3.1	19.5±3.8*	18.9±4.0*
Days 6-21	154.0±17.1	146.5±24.2	162.8±15.7	161.4±18.9
Posttreatment				
Body weight				
Day 21	411.0±24.7	396.2±34.2	418.8±20.9	419.5±27.3
Body-weight gain				
Days 0-21	185.2±23.0	174.9±29.3	196.0±20.4	194.6±22.0

Data from Tables 5 and 6 (pages 31-32) of the report
* statistically different ($p < 0.05$) from the control.

3. **Food consumption:** Food consumption was comparable among the groups.

4. **Gross pathology**: No treatment-related changes were observed. Liver and kidney weights were comparable among the groups.

5. **Cesarean section data:** There were no significant, treatment-related, effects on pregnancy rates, resorption rates, litter size, numbers of corpora lutea or implantations, percent preimplantation loss, percent postimplantation loss, fetal sex ratios, fetal body weights, or gravid uterine weights at any dose level.

TABLE 3 Cesarean section observations [a]				
Observation	**Dose (mg/kg bw/day)**			
	0	**100**	**300**	**1000**
# Animals assigned (mated)	26	26	26	26
# Animals pregnant	22	24	20	21
Pregnancy rate (%)	84.6	92.3	76.9	80.8
# Nonpregnant	4	2	6	5
Maternal wastage				
No. died	0	0	0	0
No. died pregnant	0	0	0	0
No. died nonpregnant	0	0	0	0
No. aborted	0	0	0	0
No. Premature delivery	0	0	0	0
Total No. corpora lutea♪	313	319	278	326
Corpora lutea/dam	14.2±1.7	13.3±1.9	13.9±1.7	15.5±2.9
Total No. implantations♪	292	286	265	280
(Implantations/dam)	13.3±1.8	11.9±2.9	13.3±1.6	13.3±1.9
Total No. litters	22	24	20	21
Total No. live fetuses♪	271	269	251	268

TABLE 3 Cesarean section observations [a]				
Observation	Dose (mg/kg bw/day)			
	0	100	300	1000
(Live fetuses/dam)	12.3±2.1	11.2±3.0	12.6±1.6	12.8±1.9
Total No. dead fetuses (Dead fetuses/dam)	0 0	0 0	0 0	0 0
Total No. resorptions♪	21	17	14	12
Early	21	17	13	12
Late	0	0	1	0
Resorptions/dam	1.0±1.5	0.7±1.4	0.7±0.7	0.6±0.6
Resorptions/dam w/ resorptions	1.5 (21/14)	2.1 (17/8)	1.3 (14/11)	1.1 (12/11)
Litters with total resorptions	0	0	0	0
Mean fetal weight (g)	5.81±0.28	5.92±0.32	5.86±0.29	5.77±0.36
Males	5.94±0.23	6.11±0.33	6.03±0.30	5.92±0.31
Females	5.69±0.33	5.71±0.30	5.73±0.28	5.59±0.41
Sex ratio (% male)	47	51	45	52
Preimplantation loss (%)	6.6±8.6	10.3±17.4	4.3±7.9	12.1±15.6
Postimplantation loss (%)	7.1±9.9	5.5±11.0	5.2±5.3	4.4±4.6
Gravid Uterine Weight (g)	97.80±13.95	91.43±22.71	101.86±13.83	101.85±15.52

[a] Data obtained from Table 10 (page 39) in the study report
♪ calculated using data from Appendix Table 7 (pages 184-187)

B. **DEVELOPMENTAL TOXICITY:** The incidence of external, visceral, and skeletal variations and malformations observed were comparable among the groups. There were no statistically-significant differences in the incidence of any fetal malformation or variation in any of the treated groups compared to the control. The alterations observed in the fetuses of the treated dams occurred at low frequencies and/or there was no dose-response [Tables 4a-4c]. The incidence of malformed fetuses did not demonstrate a treatment-related effect, as evidenced by the occurrence of two malformed fetuses in the control, one in the 100 mg/kg/day group, nine in the 300 mg/kg/day group, and three in the 1000 mg/kg/day group.

1. **External examination**: There were no fetal external variations or malformations identified in the control group (Table 4a). No toxicologically meaningful fetal external variations or malformations were identified in any of the treated groups compared to the control. At 100 mg/kg/day, one fetus had a domed head, in addition to visceral malformations (see Table 4b). At 300 mg/kg/day, one fetus had exencephaly, spina bifida, clubfoot, and flexure of the forelimb, as well as skeletal malformations (see Table 4c). One fetus at 1000 mg/kg/day had meningoencephalocele, as well as additional skeletal malformations (see Table 4c).

TABLE 4a. External examinations [a]				
Observations	Dose (mg/kg bw/day)			
	0	100	300	1000
No. Fetuses(litters) examined	271 (22)	269 (24)	251 (20)	268 (21)
No. Fetuses(litters) affected	0 (0)	1 (1)	1 (1)	1 (1)
Domed skull+	0 (0) [b]	1 (1)[1]	0 (0)	0 (0)
Exencephaly skull+	0 (0)	0 (0)	1 (1)[2]	0 (0)
Meningoencephalocele skull+	0 (0)	0 (0)	0 (0)	1 (1)[3]
Spina bifida vertebral column+	0 (0)	0 (0)	1 (1)[2]	0 (0)
Club foot hindlimb+	0 (0)	0 (0)	1 (1)[2]	0 (0)
Flexure forelimb+	0 (0)	0 (0)	1 (1)[2]	0 (0)

[a] Data obtained from Table 11, pages 40-41 in the study report.
[b] Fetal (litter) incidence; + considered a malformation
[1-8] malformations denoted with the same superscript were noted in a single fetus

2. **Visceral examination:** The incidence of visceral and craniofacial variations or malformations in the treated groups was comparable to the incidence in the control. One control fetus had situs inversus and another had a kidney hemorrhage. In the 100 mg/kg/day group there was one fetus with a misshapen heart, misshapen atrium (abnormal atrium lobulation) and a ventriclular septal defect; this fetus also had a domed skull noted above. One fetus of the 100 mg/kg/day group and 3 fetuses (same litter) in the 1000 mg/kg/day group displayed cyst testis. At 300 and 1000 mg/kg/day, there was one fetus in each group with a missing testis. Also, one fetus with a hypoplastic testis was observed in the high-dose group. One 300 mg/kg/day group fetus had a dark color tissue mass located within the brain.

TABLE 4b. Visceral examinations [a]

Observations	Dose (mg/kg bw/day)			
	0	100	300	1000
#Fetuses(litters) examined	141 (22)	140 (24)	130 (20)	141 (21)
#Fetuses(litters) affected	1 (1)	1 (1)	1 (1)	2 (2)
Hypoplastic testis+	0 (0) [b]	0 (0)	0 (0)	1 (1)
Missing testis+	0 (0)	0 (0)	1 (1)	1 (1)
Situs invertus+	1 (1)	0 (0)	0 (0)	0 (0)
Misshapen atrium	0 (0)	1 (1)[1]	0 (0)	0 (0)
Misshapen heart+	0 (0)	1 (1)[1]	0 (0)	0 (0)
Septal defect ventricle+	0 (0)	1 (1)[1]	0 (0)	0 (0)

[a] Data obtained from pages Table 11, pages 41-43 in the study report.
[b] Fetal (litter) incidence
[1-8] malformations denoted with the same superscript were noted in a single fetus

3. **Skeletal examination:** The incidence of fetal skeletal variations and malformations was comparable among the groups. One control fetus and 3 fetuses in the same litter at 300 mg/kg/day displayed an extra thoracic vertebra and centra, and an extra thoracic rib. An extra sternebra was also observed in the same 3 fetuses at 300 mg/kg/day.

Additional findings in the 300 mg/kg/day group included one fetus with numerous skeletal malformations, including missing interparietal, missing occipital, and fused occipital condyle (right occipital condyle fused to the atlas). Observations made on this fetus were consistent with the exencephaly malformation identified during external examination. Other skeletal findings in this fetus included the presence of fused cervical vertebrae and cleft cervical centra, cleft thoracic vertebrae and centra, fused thoracic vertebrae, fused thoracic ribs and a missing lumbar vertebra and centra. A second fetus in the 300 mg/kg/day group had cervical hemivertebrae; extra, fused and misshapen cervical ribs, fused thoracic vertebrae, thoracic hemivertebrae, fused thoracic ribs, misshapen thoracic ribs, and misshapen sternebrae. A third fetus in the same dose group had an extra cervical rib and sternoschisis. In the 1000 mg/kg/day group, one fetus had skeletal malformations (cleft cervical and thoracic centra and lumbar hemivertebra). External examinations of this same fetus had identified another malformation (meningoencephalocele).

TABLE 4c. Skeletal examinations [a]				
Observations [b]	Dose (mg/kg bw/day)			
	0	100	300	1000
#Fetuses(litters) examined	130 (22)	129 (24)	121 (20)	127 (21)
#Fetuses(litters) affected	1 (1)	0 (0)	7 (5)	1 (1)
Fused occipital condyles+	0 (0) [b]	0 (0)	1 (1)[2]	0 (0)
Missing interparietal+	0 (0)	0 (0)	1 (1)[2]	0 (0)
Missing occipital+	0 (0)	0 (0)	1 (1)[2]	0 (0)
Cleft thoracic vertebrae+	0 (0)	0 (0)	1 (1)[2]	0 (0)
Extra thoracic vertebrae+	1 (1)[4]	0 (0)	3 (1)[5,6,7]	0 (0)
Fused cervical vertebrae+	0 (0)	0 (0)	1 (1)[2]	0 (0)
Fused thoracic vertebrae+	0 (0)	0 (0)	2 (2)[2,8]	0 (0)
Hemivertebra certival vertebrae+	0 (0)	0 (0)	1 (1)[8]	0 (0)
Hemivertebra thoracic vertebrae+	0 (0)	0 (0)	1 (1)[8]	0 (0)
Hemivertebra lumbar vertebrae+	0 (0)	0 (0)	0 (0)	1 (1)
Missing lumbar vertebrae+	0 (0)	0 (0)	1 (1)[2]	0 (0)
Cleft cervical centra+	0 (0)	0 (0)	1 (1)[2]	1 (1)
Cleft thoracic centra+	0 (0)	0 (0)	1 (1)[2]	1 (1)
Extra thoracic centra+	1 (1)[4]	0 (0)	3 (1)[5,6,7]	0 (0)
Fused thoracic centra+	0 (0)	0 (0)	1 (1)[8]	0 (0)
Missing lumbar centra+	0 (0)	0 (0)	1 (1)[2]	0 (0)
Extra cervical rib+	0 (0)	0 (0)	2 (2)[8,9]	0 (0)
Extra thoracic rib+	1 (1)[4]	0 (0)	3 (1)[5,6,7]	0 (0)
Fused cervical rib+	0 (0)	0 (0)	1 (1)[8]	0 (0)
Fused thoracic rib+	0 (0)	0 (0)	2 (2)[2,8]	0 (0)
Misshapen cervical rib+	0 (0)	0 (0)	1 (1)[8]	0 (0)
Misshapen thoracic rib+	0 (0)	0 (0)	1 (1)[8]	0 (0)
Missing rib+	0 (0)	0 (0)	1 (1)	0 (0)
Extra sternebrae+	0 (0)	0 (0)	3 (1)[5,6,7]	0 (0)
Misshapen sternebrae+	0 (0)	0 (0)	1 (1)[8]	0 (0)
Sternoschisis sternebrae+	0 (0)	0 (0)	1 (1)[9]	0 (0)

[a] Data obtained from Table 11, pages 43-49 in the study report.

[b] Fetal (litter) incidence

[1-8] malformations denoted with the same superscript were noted in a single fetus

III. DISCUSSION AND CONCLUSIONS:

A. **INVESTIGATORS' CONCLUSIONS:** Administration of XDE-742 via oral gavage at dose levels up to 1000 mg/kg/day produced no treatment-related maternal toxicity and no indications of embryo/fetal toxicity or teratogenicity at any dose level. Therefore, under the conditions of this study, the no-observed-effect level (NOEL) for maternal and the embryo/fetal toxicity was 1000 mg/kg/day.

B. **REVIEWER COMMENTS:** Adverse effects were not observed at any dose level in either the maternal animals or in the offspring following oral exposure to the maternal animal during gestation days 6 through 20. The doses were adequate for assessing the developmental toxicity potential of pyroxsulam in the rat. The **maternal toxicity NOAEL is 1000 mg/kg/day** [limit dose], the highest dose tested. The **developmental toxicity NOAEL is 1000 mg/kg/day** also.

1. **Maternal toxicity:** Maternal toxicity was not observed at any dose level, including the limit dose.

2. **Developmental toxicity:** Developmental toxicity was not observed at any dose level, including the limit dose.

   a. **Deaths/resorptions:** There were no dead fetuses, and the incidence of resorptions was comparable among the groups.

   b. **Altered growth:** No adverse effects were observed.

   c. **Developmental variations:** The incidence of developmental variations was comparable among the groups.

   d. **Malformations:** The incidence of malformations was comparable among the groups.

C. **STUDY DEFICIENCIES:** None that would adversely affect study interpretation.

EPA Reviewer: Linda Taylor, Ph.D. **Signature:** Linda Taylor
Reregistration Branch I, Health Effects Division (7509P) **Date:** 01-03-08
EPA Secondary Reviewer: Kimberly Harper **Signature:** Kimberly D Harper
RAB2, Health Effects Division (7509P) **Date:** 12/07/07

Template version 02/06

TXR#: 0054347

ABBREVIATED DATA EVALUATION RECORD

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

PC CODE: 108702 **DP BARCODE:** D332276

TEST MATERIAL (PURITY): XDE-742 (98% a.i.) [pyroxsulam]

CHEMICAL: N-(5,7-dimethoxy[1,2,4]triazolo[1,5-a]pyrimidin-2-yl)-2-methoxy-4-(trifluromethyl)pyridine-3-sulfonamide

SYNONYMS: X666742, XDE-742, LY-666742, BAS-770H

CITATION: Sloter, E. D. (2005) Oral Dose Range-Finding Prenatal Developmental Toxicity Study of XDE-742 in Rabbits. WIL Research Laboratories, LLC. Laboratory Report Number WIL-406014; Dow Study Number 041136, December 21, 2005. MRID 46908415. Unpublished.

SPONSOR: The Dow Chemical Company, Midland, Michigan

EXECUTIVE SUMMARY: In a range-finding developmental toxicity study (MRID 46908415), pyroxsulam [XDE-742 (98% a.i.; Lot# E0952-52-01)] was administered orally to 6 New Zealand white rabbits/dose *via* gavage in 0.5% methylcellulose at dose levels of 0, 10, 100, 300, 600, or 1000 mg/kg bw/day from days 6 through 28 of gestation (mg/kg bw/day is alternately referred to as mg/kg/day).

All rabbits in the 1000 mg/kg/day group were euthanized on gestation day 16 or 17. Euthanization took place prior to the scheduled sacrifice because of severe body-weight losses and reduced food intake (>50%) in two of the six dams. Clinical signs observed in this group included small feces and decreased defecation.

At the remaining dose levels (10-600 mg/kg/day), there were no treatment-related effects on survival. Clinical signs (small feces and decreased defecation) were noted at 300 and 600 mg/kg/day. Slightly lower body weights were observed at 300 (3%-4%) and 600 (3%-5%) mg/kg/day during the dosing period, and body-weight gains were reduced, but there was wide variation (large standard deviations). Food consumption was reduced at 600 mg/kg/day throughout the dosing period (approximately 20% overall). No adverse effect was observed on

the number of corpora lutea, implantations, resorptions, viable fetuses, fetal body weight, or external fetal morphology up to a dose level of 600 mg/kg/day. Based on these findings, the dose levels selected (30, 100, and 300 mg/kg/day) for the definitive rabbit developmental toxicity study [MRID 46908354] are considered inadequate for assessing developmental toxicity in the rabbit.

The maternal LOAEL is 1000 mg/kg bw/day, based on severely decreased body weights and food consumption, leading to early termination of the treatment group. The maternal NOAEL is 600 mg/kg bw/day.

The developmental LOAEL was not identified. The developmental NOAEL is 600 mg/kg bw/day.

The developmental toxicity study (range-finding) in the rabbit is classified acceptable (non-guideline), and it does not satisfy the guideline requirement for a developmental toxicity study (OPPTS 870.3700; OECD 414) in the non-rodent. The purpose of the study was to determine the dose levels for the definitive study [MRID 46908354].

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

I. MATERIALS AND METHODS:

A. MATERIALS: same as definitive study [MRID 46908354]

B. PROCEDURES AND STUDY DESIGN

1. **In life dates:** Start: December 13, 2004; End: January 11, 2005.

2. **Mating:** Time-mated females were obtained from Covance Research Products, Inc. It appears that mating was one-one. It would appear that the day of mating was designated as gestation day 0.

3. **Animal Assignment:** Animals were assigned [computerized randomization procedure based on body-weight stratification in a block design] to dose groups as indicated in Table 1.

Table 1. Animals/group (mg/kg/day)					
control	10	100	300	600	1000
6	6	6	6	6	6

II. RESULTS:

A. MATERNAL TOXICITY:

1. **Mortality and clinical observations:** On gestation days 16 and 17, all females (all pregnant) the 1000 mg/kg/day dose level were euthanized due to excessive toxicity in one-third of the rabbits in this group [6-7 days without eating and > 400-gram loss in body weight]. Prior to sacrifice, clinical signs in this group included small feces and/or decreased defecation in four females from gestation days 7 through group termination (Table 2). These findings were reported to correspond to sustained decrements in food consumption and mean body weight losses in the group. One pregnant female at the 300 mg/kg/day dose level was found dead on gestation day 25. This female displayed decreased defecation (gestation days 19-25) and reductions in body weight and food consumption (gestation days 17-25). The cause of death was not determined. Small feces and/or decreased defecation were noted three females at 300 mg/kg/day and two females at 600 mg/kg/day, starting on gestation day 12 and continuing throughout the study.

Table 2. Clinical Signs (total occurrence/# animals)						
Sign	Dose in mg/kg/day (# of does)					
	Control	10	100	300	600	1000
Excreta						
Small feces	0/0	0/0	1/1	3/2	7/2	18/4
Decreased defecation	0/0	0/0	0/0	8/2	6/2	15/3

2. **Body weight:** Body weight and body-weight gain data are summarized in Table 3. Prior to dosing, the 1000 mg/kg/day group displayed the largest negative body-weight gain (-68 grams) compared to the control (-30 grams) and other dose groups (from –26 grams to +16 grams). An assessment of the individual body-weight data show that one of the 6 does at 600 mg/kg/day displayed minimal body-weight gain (33 grams overall) compared to the other does in that group (range of 104-693 grams) during the days 6-29 time period. Two of the 6 does at 1000 mg/kg/day had a negative body-weight gain (-454 and –498 grams) compared to the other 4 does (50, 51,

56, and 103 grams) during the days 6-16 time period. Individual body weight/gain data are shown in Table 4 for the 600 and 1000 mg/kg/day and control groups.

Table 3. Body Weight/Gain (grams)

	Dose in mg/kg/day					
	0	**10**	**100**	**300**	**600**	**1000**
Body weight						
Day 0	3722 ± 259	3730 ± 230	3699 ± 210	3711 ± 199	3720 ± 205	3691 ± 211
Day 6	3692 ± 268	3704 ± 204	3678 ± 219	3727 ± 132	3731 ± 163	3623 ± 215
Day 15	4022 ± 293	4003 ± 222	3981 ± 212	3911 ± 329	3895 ± 173	3545 ± 416
Day 16	4030 ± 308	4006 ± 222	4001 ± 216	3940 ± 344	3891 ± 185	3508 ± 467
Day 17	4047 ± 299	4030 ± 215	4031 ± 235	3962 ± 352	3904 ± 183	3833 ± 149 [3]
Day 21	4138 ± 285	4078 ± 200	4089 ± 233	3958 ± 398	3933 ± 217	-
Day 29	4219 ± 302	4166 ± 209	4191 ± 272	4084 ± 336	4032 ± 222	-
Pretreatment: BW gain						
Days 0-4	-52 ± 10	-31 ± 53	-53 ± 43	5 ± 99	-10 ± 75	-75 ± 57
Days 4-6	22 ± 22	5 ± 66	32 ± 38	11 ± 45	21 ± 35	8 ± 31
Days 0-6♪	-30	-26	-21	16	11	-68
Treatment: BW gain						
Days 6-7	139 ± 74	128 ± 44	121 ± 59	88 ± 102 (63)	73 ± 85 (53)	34 ± 51 (24)
Days 7-8	2 ± 49	30 ± 39	-11 ± 25	-14 ± 41	-24 ± 21	-45 ± 20
Days 8-9	38 ± 26	5 ± 23	26 ± 41	31 ± 39	8 ± 33 (21)	-14 ± 31
Days 9-10	35 ± 23	20 ± 17	40 ± 33	20 ± 16	6 ± 34 (17)	-11 ± 34*
Days 10-11	8 ± 22	14 ± 15	-1 ± 22	-9 ± 34	12 ± 14	-34 ± 83
Days 12-13	40 ± 18	48 ± 18	16 ± 32	30 ± 30	26 ± 14	5 ± 65 (13)
Days 13-14	23 ± 15	16 ± 28	60 ± 22	22 ± 23	28 ± 23	-27 ± 49*
Days 14-15	40 ± 21	34 ± 25	29 ± 20	17 ± 53	11 ± 17	15 ± 43
Days 15-16	8 ± 19	3 ± 36	20 ± 17	29 ± 31	-4 ± 22	-37 ± 68
Days 16-17	16 ± 29	24 ± 11	30 ± 21	22 ± 19	14 ± 13	14 ± 25
Days 6-16	339 ± 138	302 ± 108	323 ± 118	213 ± 239 (63)	159 ± 147 (47)	-115 ± 280**
Days 6-29	527 ± 221	462 ± 84	513 ± 228	357 ± 241 (68)	301 ± 239 (57)	-
Posttreatment: BW gain						
Days 28-29	14 ± 16	16 ± 33	29 ± 13	38 ± 39	25 ± 27	-

[a] Data obtained from Tables 4-5, pages 44-54 in the study report; n = 6 (except n=5 in the 300 mg/kd/day group) [unless]; ♪ calculated by reviewer; (% of control)

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

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

Table 4. Individual Body Weight/Gain (grams)

	Animal #					
600 mg/kg/day	**1**	**2**	**3**	**4**	**5**	**6**
Body weight						
Day 0	3758	3583	3928	3986	3583	3482
Day 6	3756	3599	3953	3889	3639	3551
Day 7	3811	3646	3926	4012	3855	3575
Day 8	3784	3601	3926	3964	3834	3574
Day 21	3798	3711	4083	3969	4272	3763
Day 28	3775	3832	4245	3992	4286	3909
Day 29	3789	3834	4265	3993	4332	3978
Pretreatment: BW gain						
Days 0-4	-35	48	23	-149	48	8
Days 4-6	33	-32	2	52	8	61
Days 0-6	-2	16	25	-97	56	69
Treatment: BW gain						
Days 6-7	55	47	-27	123	216	24

Table 4. Individual Body Weight/Gain (grams)						
	Animal #					
Days 6-29	33	235	312	104	693	427
Days 6-16	32	73	73	174	437	167
1000 mg/kg/day	**7**	**8**	**9**	**10**	**11**	**12**
Body weight						
Day 0	3451	3512	4033	3733	3625	3791
Day 6	3329	3475	3935	3690	3557	3753
Day 7	3298	3542	3958	3807	3582	3757
Day 8	3263	3467	3894	3776	3560	3714
Day 12	3099	3134	3915	3807	3581	3773
Day 15	2994	3057	3976	3795	3653	3794
Day 16	2831	3021	3986	3740	3660	3809
Day 17	-	-	3974	-	3677	3847
Pretreatment: BW gain						
Days 0-4	-131	13	-132	-45	-101	-56
Days 4-6	9	-50	34	2	33	18
Days 0-6	-122	-37	-98	-43	-68	-38
Treatment: BW gain						
Days 6-7	-31	67	23	117	25	4
Days 6-12	-230	-341	-20	117	24	20
Days 6-16	-498	-454	51	50	103	56
Control	**13**	**14**	**15**	**16**	**17**	**18**
Body weight						
Day 0	4158	3496	3500	3607	3894	3675
Day 6	4158	3445	3474	3597	3840	3637
Day 7	4229	3634	3621	3850	3956	3693
Day 8	4183	3589	3625	3938	3972	3689
Day 12	4299	3630	3680	4075	4131	3701
Day 15	4433	3714	3809	4132	4255	3789
Day 16	4465	3710	3824	4128	4282	3773
Day 17	4479	3784	3826	4123	4295	3773
Pretreatment: BW gain						
Days 0-4	-51	-52	-66	-37	-48	-57
Days 4-6	51	1	40	27	-6	19
Days 0-6	0	-51	26	10	-54	-38
Treatment: BW gain						
Days 6-7	71	189	147	253	116	56
Days 6-12	141	185	206	478	291	64
Days 6-16	307	265	350	531	442	136

[a] Data obtained from Tables 15-16, pages 78-79, 90-94, 96-98, and 112-119 in the study report;

3. **Food consumption:** Food consumption data were not provided for the pre-dosing period. At the 1000 mg/kg/day dose level, decreased food consumption (25%-59% of control) was observed throughout the dosing interval (days 6-16). Table 5 compares the individual food consumption data for the does at the 600 and 1000 mg/kg/day dose levels compared to the control.

Table 5. Individual Food Consumption Data (grams/animal data)						
	Animal #					
600 mg/kg/day	**1**	**2**	**3**	**4**	**5**	**6**
g/animal/day						
Days 6-16	137	104	133	142	212	138
Days 6-29	113	103	137	116	190	138
g/kg/day						
Days 6-16	36	29	33	35	54	38
Days 6-29	30	28	34	29	46	37
1000 mg/kg/day	**7**	**8**	**9**	**10**	**11**	**12**
g/animal/day						
Days 6-16	20 .	20	62	115	132	101
g/kg/day						
Days 6-16	6	6	16	30	37	27
Control	**13**	**14**	**15**	**16**	**17**	**18**
g/animal/day						
Days 6-16	172	200	210	254	214	134
Days 6-29	153	184	184	234	166	118
g/kg/day						
Days 6-16	40	55	57	64	52	36
Days 6-29	35	47	48	56	39	31

[a] Data obtained from Tables 18-19, pages 126-173 in the study report;

4. **Gross pathology:** No treatment-related findings were reported.

5. **Cesarean section data:** No adverse effects were observed [Table 6]. The numbers of corpora lutea, implantations, resorptions, or viable fetuses, pre- and post-implantation losses, and fetal body weights were comparable among the groups.

TABLE 6 Cesarean section observations [a]

Observation	Dose in mg/kg/day					
	0	10	100	300	600	1000
# pregnant (%)	6	6	6	6	6	6
Maternal wastage						
# died	0	0	0	1	0	6
# aborted	0	0	0	0	0	0
# premature delivery	0	0	0	0	0	0
Total # corpora lutea	65	57	57	51	54	-
Corpora lutea/doe	10.8 ± 1.3	9.5 ± 1.9	9.5 ± 1.2	10.2 ± 2.3	9.0 ± 1.4	-
Total # implantations	58	51	51	49	51	-
Implantations/doe	9.7 ± 1.4	8.5 ± 2.1	8.5 ± 1.5	9.8 ± 2.3	8.5 ± 1.8	-
Total # litters	6	6	6	5	6	-
Total # live fetuses	54	51	51	46	51	-
Live fetuses/doe	9.0 ± 0.9	8.5 ± 2.1	8.5 ± 1.5	9.2 ± 2.3	8.5 ± 1.8	-
Dead fetuses	0	0	0	0	0	-
Total # resorptions	4	0	0	3	0	-
Early	2	0	0	3	0	-
Late	2	0	0	0	0	-
Resorptions/doe	0.7 ± 1.2	0	0	0.6 ± 0.9	0	-
Early	0.3 ± 0.5	0	0	0.6 ± 0.9	0	-
Late	0.3 ± 0.8	0	0	0	0	-
Litters w/ total resorptions	0	0	0	0	0	-
Mean fetal weight	43.1 ± 4.0	45.7 ± 4.3	44.5 ± 1.5	43.1 ± 4.3	45.4 ± 5.6	-
Sex ratio (% males)	NP	NP	NP	NP	NP	-
Preimplantation loss (%)	10.9 ± 3.8	10.3 ± 13.8	10.4 ± 12.2	4.0 ± 5.8	6.1 ± 6.8	-
Postimplantation loss (%)	6.0 ± 10.3	0	0	6.2 ± 8.5	0	-

[a] Data obtained from Tables 10 and 22, pages 67, 186-191 in the study report; NP = not provided

III. DISCUSSION AND CONCLUSIONS:

A. **INVESTIGATOR'S CONCLUSIONS:** Based on the results, dose levels of 30, 100, and 300 mg/kg/day were selected for the definitive prenatal developmental toxicity study of XDE-742 administered orally by gavage to pregnant rabbits. A high-dose level of 300 mg/kg/day was selected based on evidence of slight maternal toxicity consisting of decreased fecal output, decreased mean body weight and food consumption at that dose level. The low-dose level of 30 mg/kg/day was selected as a 10-fold decrease from the high-dose level. The mod-dose of 100 mg/kg/day was chosen as a value within an approximate 3-fold range between the high and dose doses.

B. **REVIEWER COMMENTS:** The selection of the 300 mg/kg/day dose level as the highest dose to be tested in the definitive study is not adequately justified in light of the caesarian section data, which demonstrates no adverse effect on the number of corpora lutea, implantations, resorptions, viable fetuses, and fetal body weight at a dose level of 600 mg/kg/day. Additionally, body weights were comparable to the control throughout the study at 600 mg/kg/day, and 4 of the 6 does at 1000 mg/kg/day displayed less than a 10% body-weight deficit compared to the control during the gestation days 6-16 dosing period prior to sacrifice on gestation day 16/17. Although body-weight gains were significantly reduced at 1000 mg/kg/day, there was a large standard deviation due mainly to 2 of the 6 rabbits. Additionally, all six of the 1000 mg/kg/day rabbits displayed a negative body-weight gain (-38 grams to -122 grams) during the pre-dosing (GD 0-6) period compared to three of the six control rabbits (-38 grams to -51 grams).

NOTE: The cover page of the report lists the Study Initiation Date as December 14, 2004 and the Study Completion Date as December 21, 2005. On page 16 of the report under Key Study Dates, December 13, 2004 is listed as the first gestation day 0 (experimental starting date) and January 11, 2005 is listed as the Experimental completion/termination date. The Quality Assurance report is dated December 21, 2005, as is the study report (Study Director signature).

EPA Reviewer: Linda L. Taylor, Ph.D. **Signature:** Linda L. Taylor
Reregistration Branch I, Health Effects Division (7509P) **Date:** 11-03-08
EPA Secondary Reviewer: Kimberly Harper **Signature:** Kimberly D Harper
RAB2, Health Effects Division (7509P) **Date:** 12/12/07

Template version 02/06

TXR#: 0054347

DATA EVALUATION RECORD

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

PC CODE: 108702 **DP BARCODE:** D332276

TEST MATERIAL (PURITY): XDE-742 (98% a.i.) [pyroxsulam]

CHEMICAL: N-(5,7-dimethoxy[1,2,4]triazolo[1,5-a]pyrimidin-2-yl)-2-methoxy-4-(trifluromethyl)pyridine-3-sulfonamide

SYNONYMS: X666742, XDE-742, LY-666742, BAS-770H

CITATION: Sloter, E. D. (2005) Oral Prenatal Developmental Toxicity Study of XDE-742 in Rabbits. WIL Research Laboratories, LLC. Laboratory Report Number WIL-406015; Dow Study Number 041145, December 21, 2005. MRID 46908354. Unpublished.

SPONSOR: The Dow Chemical Company, Midland, Michigan

EXECUTIVE SUMMARY: In a developmental toxicity study (MRID 46908354), pyroxsulam [XDE-742 (98% a. i.; Lot# E0952-52-01)] was administered to 26 New Zealand White rabbits/dose *via* gavage in 0.5% methylcellulose at dose levels of 0, 30, 100, or 300 mg/kg bw/day from days 6 through 28 of gestation.

At the highest dose tested, no adverse effects were observed on maternal survival, clinical signs, body weight/gain, food consumption, gross pathology, number of corpora lutea, implantation sites, resorptions, live fetuses, sex ratio, or fetal body weight. There were no treatment-related effects on the incidence of external, visceral, or skeletal malformations or developmental variations.

When combined with the results of the dose range-finding study (MRID 46908415), the maternal LOAEL is 1000 mg/kg bw/day, based on severely decreased body weights and food consumption, leading to early termination of the treatment group. The maternal NOAEL is 600 mg/kg bw/day.

The developmental LOAEL was not identified. The developmental NOAEL is 600 mg/kg

bw/day.

Combined with the dose range-finding study, this developmental toxicity study in the rabbit is classified acceptable/guideline, and it does satisfy the guideline requirement for a developmental toxicity study (OPPTS 870.3700; OECD 414) in the rabbit.

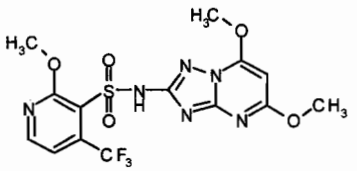
COMPLIANCE: Signed and dated GLP, Quality Assurance, and Data Confidentiality statements were provided.

I. MATERIALS AND METHODS:

A. MATERIALS:

1. **Test material:** XDE-742

Description:	TSN103826, fine, light tan powder
Lot/batch #:	Lot# E0952-52-01
Purity:	98.0±0.2 % a.i.
Compound stability:	Considered stable at room temperature
CAS #of TGAI:	422556-08-9
Structure:	See below



2. **Vehicle and/or positive control:** 0.5% methylcellulose; Lot # SG01012N03; premium grade

3. **Test animals:**

Species:	rabbit
Strain:	New Zealand White
Age/weight at study initiation:	5 months old at time of mating
Source:	Covance Research Products, Inc., Denver, PA
Housing:	individual stainless steel cages
Diet:	Certified Rabbit LabDiet® 5322 *ad libitum*
Water:	Reverse osmosis-purified drinking water *ad libitum* It should be noted that faulty watering devices limited water availability to 7 animals in the 100 mg/kg bw/day group during the second week of dose administration; these devices were replaced and all animals were also given supplemental water bottles.
Environmental conditions:	**Temperature:** 65.4-68.1°F **Humidity:** 53.6-62.1% **Air changes:** 10/hr **Photoperiod:** 12 hrs dark/12 hrs light
Acclimation period:	not specified; time-mated females arrived on gestation days 1-3; dosing began on GD 6

B. PROCEDURES AND STUDY DESIGN

1. **In life dates:** Start: March 29, 2005; End: June 14, 2005

2. **Mating:** Time-mated females were obtained from Covance Research Products, Inc. It appears that mating was one-one. It would appear that the day of mating was designated as gestation day 0.

3. **Animal Assignment:** Animals were assigned [computerized randomization procedure based on body-weight stratification in a block design] to dose groups as indicated in Table 1.

TABLE 1: Animal Assignment				
Dose (mg/kg bw/day)	**0**	**30**	**100**	**300**
Nunber of Females	**26**	**26**	**26**	**26**

4. **Dose selection rationale:** The dose levels were selected based on the results from a range-finding study [MRID 46908415] in which significant body-weight losses and decreased food consumption were reported at the limit dose (1000 mg/kg/day). All rabbits in the 1000 mg/kg/day group were euthanized on gestation day 16 or 17 prior to the scheduled sacrifice due to body-weight losses and reduced food intake (>50%). Clinical signs observed in this group included small feces and decreased defecation. At the remaining dose levels (10-600 mg/kg/day), there were no treatment-related effects in survival.

   The selection of the 300 mg/kg/day dose level as the highest dose to be tested in the definitive study is not adequately justified in light of the caesarian section data, which demonstrates no adverse effect on the number of corpora lutea, implantations, resorptions, viable fetuses, fetal body weight at a dose level of 600 mg/kg/day. Additionally, body weights were comparable to the control (slightly lower) throughout the study at 300 (2%-4%) and 600 (3%-5%) mg/kg/day, and 4 of the 6 does at 1000 mg/kg/day displayed less than a 10% body-weight deficit compared to the control during the gestation day 6-16 dosing period prior to sacrifice on gestation day 16/17. Although body-weight gains were significantly reduced at 1000 mg/kg/day, there was a large standard deviation due mainly to 2 of the 6 rabbits. Additionally, all six of the 1000 mg/kg/day rabbits displayed a negative body-weight gain (-38 grams to -122 grams) during the pre-dosing (GD 0-6) period compared to three of the six control rabbits (-38 grams to -51 grams). Food consumption was reduced at 600 mg/kg/day throughout the dosing period (approximately 20% overall), and two does at the 1000 mg/kg/day dose level stopped eating by gestation day 10 (abbreviated DER appended).

5. **Dosage preparation and analysis:** Test material-vehicle mixture was prepared approximately weekly by mixing appropriate amounts of test substance with 0.5% methylcellulose with storage at room temperature, protected from light. Prior to the start of the study, stability of the test substance in 0.5% methylcellulose was evaluated for a period of 21 days at ambient conditions by the sponsor. Concentration was evaluated during the second and last weeks of dose administration and homogeneity (top, middle, and bottom) of the test mixture was evaluated once, prior to dose administration.

Results:
Homogeneity analysis: 3.52% - 7.83% (relative standard deviation)

Stability analysis: N/A

Concentration analysis: 98.5%-115%

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

6. **Dosage administration:** All doses were administered once daily by gavage on gestation days 6

through 28, in a volume of 4 mL/kg of body weight/day. Dosing was based on the body weight of the most recent body-weight determination.

C. OBSERVATIONS:

1. **Maternal observations and evaluations:** The animals were checked for mortality or clinical signs twice daily (am and pm). Individual detailed clinical observations were recorded from the day of receipt through gestation day 29 (prior to dosing and during the treatment period). Additionally, the rabbits were observed for signs of toxicity approximately 1 hour post dose. Body weight data were recorded on gestation days 0 (by supplier) and 6-29 (daily). Individual food consumption data were recorded daily on gestation days 6-29. Dams were sacrificed on day 29 of gestation by i.v. injection of sodium pentobarbital via the marginal ear vein. Dams were sacrificed on day 29 of gestation. Examinations at sacrifice were conducted without knowledge of treatment group and consisted of: examination of the thoracic, abdominal, and pelvic cavities; examination of the uterus and ovaries; and recording the number of corpora lutea/ovary were recorded; number and location of all fetuses, early and late resorptions, and total number of implantation sites.

2. **Fetal evaluations:** All fetal examinations (external, visceral and skeletal) were performed blind to treatment group. Each viable fetus was examined externally and individually weighed. The detailed external examination included an examination of the eyes, palate and external orifices. Each viable fetus was subjected to a visceral examination, which included the heart and major blood vessels. The sex of each fetus was determined by internal examination. Kidneys were examined and graded for renal papillae development. Heads from all fetuses were examined by a mid-coronal slice. External, visceral, and skeletal findings were recorded as developmental variations or malformations, and the findings were summarized by (1) presenting the incidence of an observation both as the number of fetuses and the number of litters available for examination, and (2) considering the litter as the basic unit for comparison and calculating the number of affected fetuses in a litter on a proportional basis as follows:

$$\text{Summation per Group (\%)} = \frac{\sum \text{Viable Fetuses Affected/Litter (\%)}}{\text{No. Litters/Group}}$$

where:

$$\text{Viable Fetuses Affected/Litter (\%)} = \frac{\text{\# Viable Fetuses Affected/Litter}}{\text{\# Viable Fetuses/Litter}} \times 100$$

D. DATA ANALYSIS:

1. **Statistical analyses:** All statistical tests were performed using appropriate computing devices or programs. Where applicable, the litter was used as the experimental unit.

   Mean maternal body weights, body weight changes, and food consumption, gravid uterine weights, numbers of corpora lutea, implantation sites and viable fetuses, and fetal body weights (separately by sex and combined) were subjected to a parametric one-way analysis of variance (ANOVA) to determine intergroup differences. If the ANOVA revealed statistically significant intergroup variance, Dunnett's test was used to compare the test article-treated groups to the control group. Mean litter proportions (percent per litter) of prenatal data (viable and nonviable fetuses, early and late resorptions, total resorptions, pre- and

postimplantation loss and fetal sex distribution), total fetal malformations and developmental variations (external, visceral, skeletal and combined) and each particular external, visceral and skeletal malformation or variation were subjected to the Kruskal-Wallis nonparametric ANOVA test to determine intergroup differences. If the ANOVA revealed statistically significant intergroup variance, Dunn's test was used to compare the test article-treated groups to the control group.

2. **Indices:** The following indices were calculated from cesarean section records of animals in the study:

Group mean litter basis:

$$\text{postimplantation loss/litter} = \frac{\text{\# dead fetuses, resorptions (early/late)/group}}{\text{\# gravid females/group}}$$

Proportional litter basis:

$$\text{Summation per group (\%)} = \frac{\sum \text{postimplantation loss/litter (\%)}}{\text{\# litters/group}}$$

where

$$\text{postimplantation loss/litter (\%)} = \frac{\text{\# dead fetuses, resorptions (early/late)/litter}}{\text{\# implantation sites/litter}} \times 100$$

3. **Historical control data:** Historical control data were provided to allow comparison with concurrent controls. Data were provided from 14 studies conducted by WIL Research Laboratories, LLC using time-mated New Zealand White [Hra:(NZW)SPF] rabbits.

II. RESULTS:

A. MATERNAL TOXICITY:

1. **Mortality and clinical observations:** The following observations were reported: There was one death in each of the treatment groups but none in the control group. However, these were most likely due to dosing error rather than to test material exposure. There was one abortion, which occurred at the low-dose level; however, the historical control data (1 abortion out of 297 pregnancies) indicate that a single abortion is not an unusual event. One high-dose doe delivered her litter on gestation day 29 (scheduled day of sacrifice). Decreased defecation was reported in 6 control, 6 low-dose, 20 mid-dose, and 10 high dose does. The higher occurrence (during gestation days 14-21) of this clinical sign at the mid-dose level was considered related to reduced water availability on GD 13; the majority of the incidences of this clinical sign at the high-dose occurred on GD 24-29.

2. **Body weight:** Body weight data are summarized in Table 2. Body weights were comparable among the groups throughout the gestation period. The significant reduction in body-weight gain observed during the day 13-18 interval for does from the 100 mg/kg/day dose group was attributed to the temporary lack of water for this group. Does from the 300 mg/kg/day dose group exhibited reduced body weight gain when compared to controls during certain intervals of

the gestation period (e.g., days 7-8, 13-14, 14-15, 12-18), after dosing was completed (24% of control for days 28-29), and a slightly lower total body weight gain throughout the dosing period (82% of control for days 6-29). However, this slight change is not considered to be adverse given the transient nature of the change (for certain intervals, the mean body weight gain at 300 mg/kg bw/day was double control). Furthermore, mean body weights were not affected by treatment and it is not uncommon for rabbits to experience erratic body weight gains/losses during gestation.

TABLE 2 Mean (±SD) maternal body weight/ gain (g) [a] data

Interval	Dose in mg/kg bw/day (# of Dams)			
	Control (23)	30 (26)	100 (26)	300 (25)
Body weight				
Day 0	3574 ± 250	3583 ± 287	3582 ± 25	3580 ± 272
Day 6	3516 ± 247	3532 ± 283	3548 ± 246	3542 ± 230
Day 13	3836 ± 276	3795 ± 307	3762 ± 240	3802 ± 278
Day 14	3867 ± 279	3813 ± 297	3734 ± 256	3812 ± 283
Day 21	4015 ± 317	3982 ± 318	3906 ± 286	3964 ± 273
Day 29	4037 ± 344	4012 ± 278	3983 ± 286	3980 ± 277
Pretreatment: body-weight gain				
Days 0-6	-58 ± 89	-52 ± 77	-34 ± 71	-37 ± 83
Treatment: body-weight gain				
Days 6-7	149 ± 81	105 ± 77	101 ± 92	122 ± 86
Days 7-8	23 ± 38	24 ± 36	26 ± 51	5 ± 30 (22)
Days 12-13	15 ± 26	28 ± 44	-8 ± 64	32 ± 36
Days 13-14	30 ± 31	19 ± 60	-28 ± 80**	10 ± 47 (33)
Days 14-15	38 ± 30	41 ± 34	-5 ± 61	23 ± 44 (61)
Days 12-18	145 ± 69	148 ± 80	40 ± 159**	118 ± 79 (81)
Days 6-29	521 ± 231	491 ± 132	423 ± 195 (81)	428 ± 250 (82)
Posttreatment: body-weight gain				
Days 28-29	25 ± 48	21 ± 38	16 ± 39 (64)	6 ± 53 (24)
Corrected BW gain	-74.4 ± 234.8	-72.5 ± 166.6	-146.5 ± 203.3	-116.4 ± 230.9

[a] Data obtained from Tables 4-6, pages 50-60 in the study report; (% of control)
** Statistically different ($p < 0.01$) from the control.

3. **Food consumption:** Food consumption was comparable to the control at the low-dose level throughout the study [Table 3]. At the mid-dose level, there was a slight decrease in food consumption throughout the dosing period and a statistically-significant decrease in food consumption during the weeks 12-17 period during which water availability was limited. Decreased (79%-95% of control) food consumption was noted throughout most of the dosing period at the high-dose level compared to the control, although statistical significance was not attained. These decreases were not considered to be adverse due to the small degree of change.

TABLE 3 Mean (±SD) maternal food consumption (g) [a]

Interval	Dose in mg/kg bw/day (# of does)			
	Control (23)	30 (26)	100 (26)	300 (25)
Days 6-7	250 ± 51	238 ± 57	238 ± 58	237 ± 61
Days 12-13	194 ± 40	183 ± 54	130 ± 73** (67)	174 ± 44
Days 13-14	176 ± 33	164 ± 53	86 ± 74** (49)	149 ± 44 (85)
Days 14-15	195 ± 56	190 ± 63	93 ± 88** (48)	155 ± 55 (79)
Days 15-16	192 ± 41	191 ± 41	116 ± 79** (60)	157 ± 60 (82)
Days 16-17	191 ± 42	193 ± 53	135 ± 60** (71)	171 ± 47 (90)
Days 17-18	204 ± 54	196 ± 60	167 ± 74 (82)	178 ± 61 (87)
Days 6-29	168 ± 30	167 ± 19	152 ± 31 (90)	158 ± 28 (94)

[a] Data obtained from Tables 7, pages 61-65 in the study report; (% of control)

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

4. **Gross pathology:** Macroscopic findings were comparable among the groups.

5. **Cesarean section data:** Data are summarized in Table 4. There were no adverse effects observed on any of the parameters monitored. There were no dead fetuses. The numbers of corpora lutea, implantations, resorptions, or viable fetuses, pre- and post-implantation losses, and fetal body weights were comparable among the groups. The value for % males was lowest at the high-dose level but within the historical control range (41.5%-61.7%).

TABLE 4 Cesarean section observations [a]

Observation	Dose (mg/kg bw/day)			
	0	30	100	300
No. Animals assigned (mated)	26	26	26	26
No. Animals pregnant	23	26	26	25
Pregnancy rate (%)	88.5	100	100	96.2
No. Nonpregnant	3	0	0	1
Maternal wastage				
No. died	0	1	1	1
No. Died pregnant	0	1	1	1
No. Died nonpregnant	0	0	0	0
No. Aborted	0	1	0	0
No. Premature delivery	0	0	0	1
Total No. corpora lutea Corpora lutea/Dam	204 9.7 ± 2.2	224 9.3 ± 2.1	248 9.9 ± 2.2	235 9.8 ± 1.8
Total No. implantations (Implantations/Dam)	213 9.3 ± 2.0	215 9.0 ± 2.0	241 9.6 ± 2.3	216 9.0 ± 2.3
Total No. litters	23	24	25	24
Total No. live fetuses (Live fetuses/Dam)	244 8.9 ± 1.8	208 8.7 ± 2.1	232 9.3 ± 2.4	204 8.5 ± 2.4
Total No. dead fetuses (Dead fetuses/Dam)	0	0	0	0
Total No. resorptions	9	7	9	12
Early	3	6	6	5
Late	6	1	3	7
Resorptions/Dam	0.4 ± 0.7	0.3 ± 0.6	0.4 ± 0.7	0.5 ± 0.7
Early	0.1 ± 0.34	0.3 ± 0.61	0.2 ± 0.66	0.2 ± 0.41
Late	0.3 ± 0.54	0.0 ± 0.20	0.1 ± 0.33	0.3 ± 0.46
Litters with total resorptions	0	0	0	0
Mean fetal weight (g)	43.2 ± 5.3	42.4 ± 4.1	42.3 ± 5.9	42.1 ± 5.9
Males	44.1 ± 5.4	42.6 ± 4.1	43.1 ± 6.1	41.6 ± 5.8
Females	42.2 ± 5.6	42.0 ± 4.7	41.3 ± 6.4	42.3 ± 6.9
Sex ratio (% male)	53.5	51.0	53.2	45.2
Preimplantation loss (%)	4.4 ± 7.5	4.2 ± 9.4	3.0 ± 8.5	7.7 ± 16.0
Postimplantation loss (%)	3.8 ± 6.3	3.3 ± 6.8	4.1 ± 8.6	5.7 ± 7.5

[a] Data obtained from Tables 10-11, pages 72-75 in the study report.

B. DEVELOPMENTAL TOXICITY:

The total number (fetuses/litter) of malformations (external, soft tissue, and skeletal) was 2/2, 0/0, 12/4, and 6/5 in the control, 30, 100, and 300 mg/kg/day dose groups, respectively.

1. **External examination:** No external developmental variations were reported in any fetus (Table 5a). External malformations were observed in one fetus (carpal flexure, bilateral; no apparent skeletal origin) at 100 mg/kg/day and in one fetus (localized edema in the hindpaw) at 300 mg/kg/day. The incidence of carpal flexure is within the laboratory's historical control data range (malformation was noted in 5/2978 fetuses and in 5/292 litters) and is not considered treatment-related. No incidences of localized edema were noted in the historical control database, but this single incidence at 300 mg/kg/day is not considered to be toxicologically significant.

TABLE 5a. External examinations [a]

Observations [b]	Dose (mg/kg bw/day)			
	0	30	100	300
No. Fetuses(litters) examined	204 (23)	208 (24)	232 (25)	204 (24)
No. Fetuses(litters) affected	0	0	1	1
Carpal and/or tarsal flexure	0 (0)[c]	0 (0)	1 (1)	0 (0)
Localized fetal edema	0 (0)	0 (0)	0 (0)	1 (1)

[a] Data obtained from Table 12, page76 in the study report.

[b] Some observations may be grouped together.

[c] Fetal (litter) incidence

2. **Visceral examination:** There were no dose-related effects on the incidence of visceral malformations or developmental variations, although visceral malformations were observed only in the 100 and 300 mg/kg/day dose groups (Table 5b). Lobular dysgenesis of the liver (caudate lobe fused to the right lobe) was observed in 6 fetuses of one 100 mg/kg/day litter and in 1 fetus in one 300 mg/kg/day litter. This visceral malformation has not been observed in the laboratory's historical control, which consists of 14 studies. Lobular agenesis of the lung (right accessory lobe absent) was observed in 3 fetuses of one 100 mg/kg/day litter and in 1 fetus of one 300 mg/kg/day litter; the incidence at the 100 mg/kg/day dose level is outside the historical control [one fetus in one litter; in 4 of 14 studies; total # fetuses (2577); total # litters (292)]. One 100 mg/kg/day fetus had a malpositioned kidney, and this malformation was observed in one fetus in the laboratory's historical control data from 14 studies. It was noted that this 100 mg/kg/day fetus was a low-weight-for-term fetus with ta body weight of 24.1 grams compared to both the mean fetal body weight of the litter (36.3 grams) and group mean (42.3 grams). One 300 mg/kg/day fetus had an interventricular septal defect (an opening in the anterior portion of the septum), which was within the historical control incidence (1 fetus in each of two different studies).

   Soft tissue developmental variations observed in the study included a major blood vessel variation (left carotid artery arose from the brachiocephalic trunk; highest incidence in control), accessory spleen, extra papillary muscle with attached chordae tendineae and retrocaval ureters. Small gallbladders were observed only in the treated groups [0/0, 7/5, 7/4, and 6/6 (fetuses/litters) in the control, low, mid, and high dose groups, respectively], but there was no dose-response. This developmental variation was noted in 12 of the 14 studies in the historical control (for a total of 24 fetuses in 19 litters), with the highest incidence being 7 fetuses in 4 litters (most common incidence of 1-2 fetuses in 1-2 litters).

TABLE 5 b. Visceral examinations [a]				
Observations [b]	Dose (mg/kg bw/day)			
	0	30	100	300
No. Fetuses(litters) examined	204 (23)	208 (24)	232 (25)	204 (24)
No. Fetuses(litters) with malformations	0 (0)	0 (0)	10 (3)	3 (2)
Malformations				
Malpositioned kidney	0 (0) [c]	0 (0)	1 (1)	0 (0)
Liver, lobular dysgenesis	0 (0)	0 (0)	6 (1)	1 (1)
Interventicular septal defect	0 (0)	0 (0)	0 (0)	1 (1)
Lungs, lobular agenesis	0 (0)	0 (0)	3 (1)	1 (1)
Variations				
Accessory spleen	18 (9)	34 (16)	24 (11)	27 (12)
Extra papillary muscle w/ attached chordae tendineae	4 (4)	2 (2)	12 (6)	4 (3)
Major blood vessel variation	25 (9)	17 (7)	16 (11)	11 (6)
Small gallbladder	0 (0)	7 (5)	7 (4)	6 (6)
Retrocaval ureter	7 (5)	7 (5)	3 (2)	1 (1)

[a] Data obtained from Table 12, page 76 and Table 14, page 81 in the study report.
[b] Some observations may be grouped together.
[c] Fetal (litter) incidence

3. **Skeletal examination:** There were few skeletal malformations (# fetuses/# litters: 2/2, 0/0, 1/1, and 2/2 in the control, low, mid, and high dose groups, respectively; Table 5c). Vertebral anomaly with an associated rib anomaly was observed in one fetus at 100 and one fetus at 300 mg/kg/day. This malformation consisted of absent ribs, arches, and/or centra, malpositioned arches and centra and/or a 13th rudimentary rib in this study and was observed in 8 of the 14 historical control studies (total of 13 fetuses in 12 litters). The 100 mg/kg/day fetus also had a costal cartilage anomaly consisting of fused and malpositioned costal cartilage; this malformation occurred in 2 of the 14 historical control studies (1 fetus in each study). The rib anomaly observed in one fetus at 300 mg/kg/day was noted in 5 of the 14 historical control studies (total of 7 fetuses in 6 litters). Skeletal variations occurred at similar incidences in all dose groups.

TABLE 5c. Skeletal examinations [a]				
Observations [b]	Dose (mg/kg bw/day)			
	0	30	100	300
No. Fetuses(litters) examined	204 (23)	208 (24)	232 (25)	204 (24)
No. Fetuses(litters) with malformations	2 (2)	0 (0)	1 (1)	2 (2)
Interrupted ossification of rib(s)	1 (1) [c]	0 (0)	0 (0)	0 (0)
Sternebra(e) maligned (severe)	1 (1)	0 (0)	0 (0)	0 (0)
Rib anomaly	0 (0)	0 (0)	0 (0)	1 (1)
Vertebral anomaly w/ or w/out associated rib anomaly	0 (0)	0 (0)	1 (1)	1 (1)
Costal cartilage anomaly	0 (0)	0 (0)	1 (1)	0 (0)

[a] Data obtained from Table 12, page76 n the study report.
[b] Some observations may be grouped together.
[c] Fetal (litter) incidence

III. DISCUSSION AND CONCLUSIONS:

A. **INVESTIGATOR'S CONCLUSIONS:** No definitive adverse signs of maternal or developmental toxicity were observed in the study. A slight decrease in mean food consumption was observed at 300 mg/kg/day, but the decrease was transient, not statistically significant, and there were no corresponding effects on body weight or any other sign of maternal toxicity. The effect was, therefore, not considered adverse. The maternal NOAEL was considered to be 300 mg/kg/day, the highest dose tested based on the absence of adverse effects following oral exposure *via* gavage to the pregnant rabbit. In the absence of any treatment-related embryo/fetal effects or terata, the developmental toxicity NOAEL was considered to be 300 mg/kg/day (exposure *via* gavage to the pregnant rabbit).

B. **REVIEWER COMMENTS:** At the highest dose tested, there were no adverse effects observed on maternal survival, clinical signs, body weight/gain, food consumption, gross pathology, numbers of corpora lutea, implantation sites, resorptions, live fetuses, sex ratio, or fetal body weight. There were no treatment-related effects on the incidence of external, visceral, or skeletal malformations or developmental variations. In the absence of any treatment-related effect, both the maternal toxicity NOAEL and the developmental toxicity NOAEL are 300 mg/kg/day, the highest dose tested. However, it appears that the pregnant rabbits would have tolerated a higher dose level; *e.g.,* 600 mg/kg/day and possibly 1000 mg/kg/day, based on the range-finding results.

1. **Maternal toxicity:** No treatment-related, adverse effects were observed on any parameter monitored.

2. **Developmental toxicity:** No treatment-related, adverse effects were observed on the incidence of external, visceral, or skeletal malformations or developmental variations.

   a. **Deaths/resorptions:** There were no dead fetuses, and the incidence of resorptions (early and late) was comparable among the groups.

   b. **Altered growth:** No adverse effect was observed on growth.

   c. **Developmental variations:** Developmental variations were comparable among the groups.

   d. **Malformations:** There was no treatment-related effect on the incidence of external, visceral, or skeletal malformations.

C. **STUDY DEFICIENCIES:** In the dose selection assessment, there is no discussion regarding why a dose level greater than 300 mg/kg/day was not considered. The selection of the 300 mg/kg/day dose level as the highest dose to be tested is not adequately justified in light of the caesarian section data in the range-finding study [MRID 46908415], which demonstrate no adverse effect on the number of corpora lutea, implantations, resorptions, viable fetuses, fetal body weight at a dose level of 600 mg/kg/day. Additionally, body weights of the pregnant rabbits in the range-finding study were comparable to the control at 600 mg/kg/day and only 12%-13% lower than control at 1000 mg/kg/day. Of the three 1000 mg/kg/day does euthanized on gestation day 16, 2 displayed a body-weight deficit of 25%-30%, whereas the third displayed a 7% deficit.

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The three 1000 mg/kg/day does euthanized on gestation day 17 had a body-weight deficit of 2%-9%.

EPA Reviewer: Linda Taylor, Ph.D. **Signature:** Linda Taylor
Reregistration Branch I, Health Effects Division (7509P) **Date:** 01-03-08
EPA Secondary Reviewer: Alan C. Levy, Ph.D. **Signature:** Alan C. Levy
Registration Action Branch 2, Health Effects Division (7509P) **Date:** 1-3-2008

Template version 02/06

TXR#: 0054347

DATA EVALUATION RECORD

STUDY TYPE: Reproduction and Fertility Effects Study – rat OPPTS 870.3800; OECD 416.

PC CODE: 108702 **DP BARCODE:** 332276

TEST MATERIAL (PURITY): XDE-742 (98% a.i.); pyroxsulam

CHEMICAL: (N-(5,7-dimethoxy[1,2,4]triazolo[1,5-a]pyrimidin-2-yl)-2-methoxy-4-(trifluromethyl)pyridine-3-sulfonamide)

SYNONYMS: triazolopyrimidine herbicide; X666742, XDE-742, LY-666742, BAS-770H

CITATION: Carney, E. W., Zablotny, C. L., and K. E. Stebbins. (2005). XDE-742: Two Generation Dietary Reproductive Toxicity Study in CD Rats. Toxicology & Environmental Research and Consulting, The Dow Chemical Company, Midland, Michigan, 48674. Study ID: 041012, (September 19, 2005). MRID 46908404. Unpublished.

Schneider, S. (2004). XDE-742 / BAS 770H – One-Generation Reproduction Toxicity Study in Wistar Rats (Range-Finding) Oral Administration (Diet). Experimental Toxicology and Ecology. BASF Aktiengesellschaft, Germany. Project No. 15R0298/03023, (March 23, 2004). MRID 46908403. Unpublished.

SPONSOR: Dow AgroSciences LLC, 9330 Zionsville Road, Indianapolis, Indiana 46268 and BASF Aktiengesellschaft, Germany.

EXECUTIVE SUMMARY: In a 2-generation reproduction study (MRIDs 46908404 and 46908403), pyroxsulam (98% a.i., EO952-52-01, TSN103826) was administered to 27 CD (CrlCD(SD) IGC BR) rats/sex/dose in the diet at the nominal dose levels of 0, 100, 300, or 1000 mg/kg bw/day (mg/kg bw/day is alternately referred to as mg/kg/day). There was one breeding per generation.

There were no adverse effects on parental survival, clinical signs, body weight/gain, and food consumption in either sex in either generation. Alterations in organ weights were not consistent between generations and/or did not demonstrate a dose-response. There were no treatment-related alterations in gross or histopathology in either sex or generation. **The parental systemic NOAEL is 1000 mg/kg bw/day in both sexes (the highest dose tested), based on the lack of any significant**

effect on any parameter monitored.

There was no adverse effect on the survival, growth, organ weights (brain, spleen, thymus), or development (onset of puberty) of the offspring of either generation. **The offspring NOAEL is 1000 mg/kg bw/day, the highest dose tested.**

The reproductive NOAEL is 1000 mg/kg bw/day (both sexes), based on the lack of an adverse effect on any parameter of reproductive function of the parental animals, including estrous cyclicity and periodicity, sperm measures, mating, conception, fertility or gestation indices, post-implantation loss, time to mating, or gestation length in either generation.

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

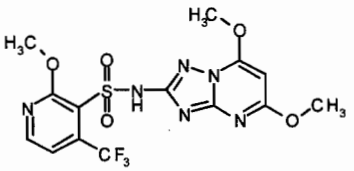
COMPLIANCE: Signed and dated GLP, Quality Assurance, and Data Confidentiality statements were provided.

I. MATERIALS AND METHODS:

A. MATERIALS:

1. Test material: XDE-742

Description:	White powder
Lot/batch #:	E0952-52-01, TSN103826
Purity:	98% a.i.
Compound stability:	Previously reported to be stable in rodent diet for up to 36 days at concentrations ranging from 0.005-5% (MRID 46908349)
CAS # of TGAI:	422556-08-9
Structure:	See below



2. Vehicle and/or positive control: no vehicle used (diet)

3. Test animals:

Species:	Rat
Strain:	CD (Crl: CD (SD) IGC BR)
Age at study initiation:	(P) 6 wks; (F_1) approximately 4 wks
Wt. at study initiation:	(P) Males: 236.5 ± 12.3-236.8 ± 12.4 g; Females: 176.7 ± 9.6-177.0 ± 9.7 g (F_1) Males: 94.0 ± 12.3-98.4 ± 12.6 g; Females: 84.7 ± 10.9-91.4 ± 9.7 g
Source:	Charles River Laboratories Inc. (Portage, Michigan)
Housing:	singly in stainless steel cages, except during breeding (one male : one female) and littering phases; during littering, dams (and their litters) were housed in plastic cages (ground corn cob nesting material) from ~day 19 of gestation until completion of lactation.
Diet:	LabDiet® Certified Rodent Diet #5002 (PMI Nutrition International, St. Louis, Missouri) in meal form, *ad libitum*
Water:	municipal water source, *ad libitum*
Environmental conditions:	**Temperature:** 22 ± 1°C **Humidity:** 40-70 % **Air changes:** 12-15/hr **Photoperiod:** 12 hrs dark/12hrs light
Acclimation period:	At least one week prior to study initiation

In life dates: Start: March 5, 2004; End: November 23, 2004

B. PROCEDURES AND STUDY DESIGN:

1. **Mating procedure:** Breeding for the P1 and P2 adults commenced after approximately ten weeks of treatment. Each female was placed with a single male from the same test group (1:1 mating) until mating occurred or two weeks elapsed. During each breeding period, daily vaginal lavage samples were evaluated for the presence of sperm as an indication of mating. The day on which sperm were detected or a vaginal copulatory plug was observed *in situ* was considered GD 0. The sperm- or plug-positive (presumed pregnant) females were then removed from the males

and returned to their home cages. In cases where a breeding male died, a substitute partner (from the same dose group) that had already completed mating was provided. If mating had not occurred after two weeks, the animals were separated without further opportunity for mating. If available, one rat/sex/litter was randomly selected for the P2 mating to produce the F2 generation. More than one weanling may have been selected from the litters, if necessary, to achieve 27 breeding pairs/dose level for the second generation. Cohabitation of P2 male and female littermates was avoided. A second breeding of the first or second generation adults was not conducted.

2. **Study schedule:** The P parental animals were given test diets for 10 weeks before they were mated (16 weeks of age), and the F_1 parental animals were not mated until 10 weeks after they were selected from the F_1 litters. Selection of parents for the F_1 generation was made when the pups were 21 days of age, and the mated animals in the study were approximately 13 weeks of age at mating.

3. **Animal assignment:** Animals were randomly (stratified by body weight and then randomly assigned to treatment groups using a computer program designed to increase the probability of uniform group mean body weights and standard deviations at study initiation) assigned to test groups as shown in Table 1.

TABLE 1. Animal assignment

Test group	Dose in diet [a] (mg/kg/day)	Animals/group			
		P1 Males	P1 Females	P2 Males	P2 Females
Control	0	27	27	27	27
Low (LDT)	100	27	27	27	27
Mid (MDT)	300	27	27	27	27
High (HDT)	1000	27	27	27	27

[a] Diets administered from approximately 10 weeks prior to mating, through breeding, gestation, and lactation for two generations

4. **Dose selection rationale:** The dose levels were selected based on the results from a one-generation (range-finding) reproduction study [MRID46908403] where dietary administration of up to 1000 mg/kg/day [for approximately 6 weeks prior to breeding, throughout breeding, gestation (3 weeks) and lactation (3 weeks)] did not result in any treatment-related effects in females. However, males at 1000 mg/kg/day displayed decreased terminal body weights (6% lower than controls). There were no treatment-related gross pathological findings or any adverse effects on reproductive function or neonatal growth and survival of the offspring. The highest dose (1000 mg/kg/day; the limit dose) was selected for the 2-generation reproduction study. In another range-finding study [MRID 46908402], presumed pregnant Wistar rats were administered XDE-742 *via* gavage at dose levels up to 1000 mg/kg/day during gestation days 6-19 to assess maternal toxicity. No adverse effects were observed. A separate DER has not been generated for either range-finding study.

5. **Dosage preparation and analysis:** Diets were prepared by serially diluting a concentrated test material-feed mixture (premix) with ground feed. Premixes were prepared periodically throughout the study based on stability data. Diets were prepared weekly for approximately ten weeks prior to breeding of the P1 adults. The concentrations of the test material in the diets were calculated from the most recent body weight and feed consumption data. Initial concentrations of test material in

the diet were calculated from pre-exposure body weights and feed consumption data. To avoid potential overdosing during the breeding period, animals co-housed were provided with the lower of the two concentrations (female diet) for the respective dose group. During gestation, females from each dose group were provided with the appropriate dietary concentration of XDE-742 given during breeding. Dietary concentrations supplied during lactation were adjusted using historical control feed consumption data for lactating females to account for the large and rapid increase in feed consumption (2-3x increase) typical for rats in late lactation. Until all litters were weaned, weanlings received a diet containing the same concentration of XDE-742 that was given to the P1 females during the third week of lactation. Dams awaiting necropsy received a diet containing the same concentration XDE-742 that was given during the breeding period until all litters have finished the lactation phase. Dietary concentrations for the P2 generation were calculated as described for the P1 animals.

Results: Homogeneity Analysis: Analyses confirmed that the test material was homogeneously distributed in the diets. Percent relative standard deviation (RSD) values, which were calculated from the variance between top and bottom concentrations within test diets, ranged from 2.96 to 5.01%. All values were within this range with the exception of our outlier value (14.3%).

Stability Analysis: XDE-742 was previously reported to be stable in rodent diets for up to 36 days at concentrations ranging from 0.005 to 5% (MRID 46908349). Test diets for the current study were prepared and used within these stability limits.

Concentration Analysis: Analyses of the premixes and test diets for concentration verification were conducted on four different occasions during the study, representing all dose levels, sexes, and major study phases (note that one of the analyses was done on a breeding diet which is common to both sexes). The average concentrations of XDE-742 in the diets fed to the animals over the entire study period were 102, 101, and 103% of the target for the male 100, 300, and 1000 mg/kg/day dose levels, and 100, 97.2, 104% of target for the female 100, 300, and 1000 mg/kg/day dose levels, 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.

C. OBSERVATIONS:

1. **Parental animals:** Observations and the schedule for those observations are summarized in the following tables (Tables 2a and 2b).

TABLE 2a. Key Study Parameters and Schedule

Study Events and Parameters	No. Animals	Timing (both generations unless indicated otherwise)
Cage-side examinations	All	At least twice daily
Clinical observations – males	All	Weekly throughout the study
Clinical observations – females	All	Weekly during pre-breeding period; GD 0, 7, 14, and 21; LD 0, 1, 4, 7, 14, and 21
Body weights – females	All	Weekly during pre-breeding period; GD 0, 7, 14, and 21; LD 1, 4, 7, 14, and 21
Body weights – males	All	Weekly throughout study
Feed consumption – females	All	Weekly during pre-breeding period; GD 0, 7, 14, and 21; LD 1, 4, 7, 11, 14, 17, 19, and 21
Feed consumption – males	All	Weekly during pre-breeding period through breeding period
Estrous cycle evaluation – females	All	Daily for 3 weeks prior to mating and during cohabitation; day of necropsy
Reproductive performance	All	N/A
Gross necropsy – adult females	All	After LD 21 or at least 24 days after evidence of mating or end of mating period for females not delivering a litter
Gross necropsy – adult males	All	After litters have been born
Organ weights	All	At necropsy
Sperm motility – males	All	At necropsy
Sperm count (testicular and epididymal)	All control and high-dose males*	Post-necropsy
Sperm morphology	All control and high-dose males*	Post-necropsy
Histopathology in P1 and P2 (including oocyte quantification P2 only)♪	All control and high dose animals*	Post-necropsy

GD = gestation day, LD = lactation day, N/A = Not Applicable. ♪report indicates histopathology performed on both generations (template P2 only should be for oocyte quantification); * Lower dose groups also evaluated as appropriate (see text). Table from template [MRID 46908601] submitted by Dow

TABLE 2b. Key Study Parameters and Schedule

Study Events and Parameters	No. Animals	Timing (both generations unless indicated otherwise)
Dam/Litter clinical observations	All	PND 0, 1, 4, 7, 14, 21
Dam/Litter cageside observations	All	Daily from PND 0 – 21
No. of live & dead pups	All	PND 0, 1, 4, 7, 14, and 21
Pup sex & body weight	All	PND 1, 4 (BC and AC), 7, 14, and 21
External alterations♪	All	PND 1, 4 (BC and AC), 7, 14, and 21
Culling	All	PND 4
Weaning	All	PND 21
Gross necropsy – weanlings	3/sex/litter	PND 22
Organ weights – weanlings	1/sex/litter	PND 22
Vaginal Opening (VO)	All F1 females selected to become P2 parents	PND 28 until achieved or PND 43
Preputial Separation (PPS)	All F1 males selected to become P2 parents	PND 35 until achieved or PND 53
Body Weight at Pubertal Onset	All F1 animals selected to become P2 parents	Day of VO or PPS acquisition

Table from template [MRID 46908601] submitted by Dow; ♪ inserted by reviewer; PND = postnatal day, BC = Before Culling, AC = After Culling.

2. **Litter observations:** According to the report, the following litter observations (X) were made.

F1/F2 Litter Observations

Observations	Time of Observation (lactation day)						
	Day 0	Day 1	Day 4[a]	Day 4[b]	Day 7	Day 14	Day 21
Number of live pups	X	X	X	X	X	X	X
Number of dead pups	X	X	X	X	X	X	X
Sex of each pup (M/F)		X	X	X	X	X	X
Pup weight		X	X	X	X	X	X
External alterations	X	X	X	X	X	X	X
Culling				X			
Weaning							X

[a]Before standardization (culling); [b]after standardization (culling)

On day 4 postpartum, F1 and F2 litters were standardized to a maximum of 8 pups/litter by randomly ordering the pups in each litter by sex. Pups to be culled were then randomly selected using a computer generated selection procedure, so that four males and four females (whenever possible) remained in each litter. Litters with fewer than eight pups were not culled. Excess pups were euthanized and discarded.

Dead pups were sexed and examined grossly if possible for external and internal abnormalities;

the report did not indicate whether an attempt was made to determine a possible cause of death for pups born or found dead.

All F1 weanlings selected for mating were observed daily for vaginal opening beginning on postnatal day 28 or preputial separation beginning on day 35. The age and body weights at the time of landmark acquisition were recorded. Because there was not a treatment-related effect on the F1 sex ratio, age at vaginal opening, or age at preputial separation, anogenital distance was not measured in the F2 pups.

3. **Postmortem observations:**

a. **Parental animals:** Adult males (fasted) were sacrificed after completion of their respective mating periods when it was determined that they were no longer needed for assessment of reproductive effects. Adult females (fasted) were terminated after weaning of their litters, or at least 24 days after the end of the mating period for females not producing a litter. Vaginal lavage smears were prepared from all surviving P1 and P2 females for later determination of estrous cycle stage. The animals were anesthetized by the inhalation of CO_2 and weighed. The tracheas were exposed and clamped, and the animals were euthanized by decapitation.

A complete necropsy was conducted on all animals. The necropsy included an examination of the external tissues and all orifices. The head was removed, the cranial cavity opened and the brain, pituitary and adjacent cervical tissues were examined. The eyes were examined *in situ* by application of a moistened microscope slide to each cornea. The skin was removed from the carcass, the thoracic and abdominal cavities were opened and the viscera examined. All visceral tissues were dissected from the carcass, re-examined, and selected tissues were incised. The nasal cavity was flushed via the nasopharyngeal duct and the lungs were distended to an approximately normal inspiratory volume with neutral, phosphate-buffered 10% formalin using a hand-held syringe and blunt needle.

The uteri of all females were stained with an aqueous solution of 10% sodium sulfide for approximately two minutes and were examined for the presence and number of implantation sites. After evaluation, uteri were gently rinsed with saline and preserved in neutral phosphate-buffered 10% formalin.

During routine working hours, any animals found dead or euthanized prior to the scheduled necropsy were necropsied on that day. However, animals euthanized or found dead outside working hours were refrigerated until the next scheduled workday, at which time they were necropsied. Similar necropsy procedures were followed for these animals except that terminal body and organ weights were not recorded and the testes, epididymides and ovaries were preserved in neutral, phosphate-buffered 10% formalin.

The following tissues were weighed (X) and/or examined microscopically (Z):

XZ	Ovaries	XZ	Testes
XZ	Uterus with oviducts and cervix	XZ	Epididymides
XZ	Vaginal/cervix (weighed with uterus)	XZ	Prostate
Z	Gross Lesions	XZ	Seminal vesicles with coagulating glands

X	Brain	XZ	Pituitary (after fixation)
XZ	Liver	XZ	Kidneys
XZ	Adrenal glands	X	Spleen
Z	Mammary gland (females only)	X	Thyroid with parathyroids (after fixation)

Table from template [MRID 46908601] submitted by Dow

b. Offspring: The F_1 offspring not selected as parental animals and all F_2 offspring were sacrificed at 22 days of age. Pups were anesthetized with CO_2, weighed and euthanized by decapitation. These animals (3 pups/sex/litter) were subjected to postmortem macroscopic examination as described above for adults (weanlings not fasted overnight). One pup/sex/litter was randomly selected for the collection of brain, spleen, uterus and thymus weights. Representative samples of grossly abnormal tissues were collected from all weanlings at the scheduled necropsy. In addition, the brain, spleen, uterus and thymus, were saved for the weanlings selected for organ weight measurements in the event that an effect on organ weight was observed and/or future evaluation was desirable. Pups found dead as well as the brain, spleen, uterus, thymus and gross lesions were preserved in neutral, phosphate-buffered 10% formalin.

D. DATA ANALYSIS:

1. **Statistical analyses:** Parental body weights, gestation and lactation body weight gains, litter mean body weights, feed consumption, sperm count, follicle count, percent total and progressively motile sperm, mean estrous cycle length and organ weights (absolute and relative) were evaluated by Bartlett's test for equality of variances. Based upon the outcome of Bartlett's test, either a parametric or nonparametric analysis of variance (ANOVA) was performed. If the ANOVA was significant, a Dunnett's test or the Wilcoxon Rank-Sum test with Bonferroni's correction were performed. Feed consumption values were excluded from analysis if the feed was spilled or scratched.

   Gestation length, age at vaginal opening, age at preputial separation, average time to mating, and litter size were analyzed using a nonparametric ANOVA. If the ANOVA was significant, the Wilcoxon Rank-Sum test with Bonferroni's correction was performed. Sperm morphology was arcsine transformed and analyzed using a parametric ANOVA. Slides containing less than 200 sperm were excluded from analysis. If the ANOVA was significant, the Dunnett's test was performed. Statistical outliers were identified by the sequential method and were routinely excluded from feed consumption only. Other outliers, if excluded, were excluded from analysis for documented, scientifically sound reasons. The mating, conception, fertility and gestation indices were analyzed by the Fisher exact probability test with Bonferroni's correction. Evaluation of the neonatal sex ratio on postnatal day 1 was performed by the binomial distribution test. Gender was determined for pups born dead on postnatal day 0 and these data were included in sex ratio calculations. Survival indices, post-implantation loss, and other incidence data among neonates were analyzed using the litter as the experimental unit by the censored Wilcoxon test as modified with Bonferroni's correction. Non-pregnant females, females with resorptions only, or females found to be pregnant after staining of their uteri were excluded from gestation and lactation body weights and body weight gains, feed consumption and organ weights. Both the Dunnett's test and Bonferroni's correction corrected for multiple

comparisons to the control to keep the experiment-wise error rate at 0.05. Both were reported at the experiment-wise alpha level.

Because numerous measurements were statistically compared in the same group of animals, the overall false positive rate (Type I errors) was greater than the nominal alpha levels. Therefore, along with statistical analyses, the final interpretation of the data considered other factors such as dose-response relationships and whether the results were consistent with other biological and pathological findings or historical control values.

The procedures used are standard analyses and are considered appropriate.

2. **Indices:**

Reproductive and offspring viability indices: The following reproductive and offspring viability indices were provided in the study report:

- Female mating index = (No. females with evidence of mating/No. paired) x 100
- Male mating index = (No. males with evidence of mating/No. paired) x 100
- Female conception index = (No. females with evidence of pregnancy/No. mated) x 100
- Male conception index = (No. males siring a litter/No. mated) x 100
- Female fertility index = (No. females with evidence of pregnancy/No. paired) x 100
- Male fertility index = (No. males siring a litter/No. paired) x 100
- Gestation index = (No. females delivering a viable litter/No. females delivering a litter) x 100
- Gestation survival index = percentage of delivered pups alive at birth
- Post-implantation loss = (No. implants – No. viable offspring)/(No. implants) x 100
- Day 1 or 4 pup survival index = (No. viable pups on day 1 or 4/No. born live) x 100
- Day 7, 14, or 21 pup survival index = (No. viable pups on day 7, 14 or 21/No. viable pups after culling) x 100

3. **Historical control data:** Historical control data were not provided.

II. RESULTS:

A. PARENTAL ANIMALS

1. **Mortality and clinical signs:** There was no evidence of a treatment-related effect on survival in either sex in either generation. Clinical signs were comparable among the groups in both sexes and both generations.

2. **Body weight and food consumption:** Body weight and body-weight gains were comparable among the male groups in both generations and among the P2 females. P1 female body weights were comparable among the groups. Body-weight gains were slightly lower (83%-89% of

control) during the first week of dosing in the treated P1 female groups compared to the control, but there was no dose-response.

Food consumption was comparable among the male groups in both generations. Food consumption was slightly lower (92%-93% of control) during the first week of dosing in the treated P1 female groups compared to the control, but there was no dose-response. Similarly, P2 females displayed a slightly lower food consumption during the last week of the premating period, but there was no dose-response.

Reported body weight, body-weight gain, and selected food consumption results are summarized in Tables 3a and 3b.

TABLE 3a. Mean (±SD) Body weight/gain and food consumption - pre-mating [a]

Observations/study day	Dose group (mg/kg/day)			
	0	100	300	1000
[P1] Generation males - Pre-mating				
Mean body weight (g)				
day –2	236.5 ± 12.3	236.5 ± 12.6	236.5 ± 12.6	236.8 ± 12.4
day 6	286.7 ± 15.0	286.3 ± 18.0	287.5 ± 15.6	287.3 ± 15.4
day 69	510.6 ± 44.3	505.1 ± 43.3	514.1 ± 55.9	511.4 ± 41.4
Mean body-weight gain (g) ♪				
days –2-69	274.1	268.6	277.6	274.6
Mean food consumption (g/day)				
days 1-8	25.1 ± 1.2	25.1 ± 1.7	25.6 ± 1.9	25.8 ± 1.3
days 64-71	26.8 ± 2.2	25.9 ± 2.1	27.3 ± 3.1	28.3 ± 2.3
[P1] Generation females - pre-mating				
Mean body weight (g)				
day –2	176.8 ± 9.8	176.7 ± 9.6	176.9 ± 9.7	177.0 ± 9.7
day 6	200.5 ± 13.4	196.3 ± 12.4	198.1 ±11.0	197.4±13.7
day 13	220.0 ± 18.1	212.8 ± 15.5	215.5 ± 14.1	215.4± 16.6
day 69	288.9 ± 27.2	288.0 ± 25.8	289.6 ± 21.5	283.8 ± 21.0
Mean body-weight gain (g)♪				
days –2-6	23.7	19.6 (83)	21.2 (89)	20.4 (86)
days 6-13	19.5	16.5 (85)	17.4 (89)	18.0 (92)
days –2-69	112.1	111.3	112.7	106.8 (95)
Mean food consumption (g/day)				
days 1-8	19.4 ± 1.9	17.8 ± 1.3* (92)	18.0 ± 1.4* (93)	18.1 ± 1.4* (93)
days 64-71	19.2 ± 2.1	18.8 ± 1.4	18.7 ± 1.2	19.4 ± 1.7

[a] Data obtained from Tables 14, 15, 22 & 23, pages 63, 65, 73 & 75 in the study report. ♪calculated by reviewer; (% of control)
* $p<0.05$.

TABLE 3b. Mean (±SD) Body weight/gain and food consumption - pre-mating [a]

Observations/study day	Dose group (mg/kg/day)			
	0	100	300	1000
[P2] Generation males – Pre-mating				
Mean body weight (g)				
day 1	95.9 ± 12.9	98.4 ± 12.6	95.3 ± 11.6	94.0 ± 12.3
day 72	536.3 ± 43.2	544.0 ± 51.1	547.3 ± 46.2	533.4 ± 48.4
Mean body-weight gain (g)				
days 1-72	440.4	445.6	452.0	439.4
Mean food consumption (g/day)				
days 1-4	16.2 ± 1.9	16.9± 1.8	16.7 ±1.1	15.8 ± 1.8
days 4-11	20.9 ± 2.5	21.8 ± 2.2	21.1 ± 2.1	20.5 ± 2.3
days 67-74	29.1±1.9	30.2 ± 2.7	30.5 ± 2.6	30.2 ± 2.8
[P2] Generation females - pre-mating				

TABLE 3b. Mean (±SD) Body weight/gain and food consumption - pre-mating [a]

Observations/study day	Dose group (mg/kg/day)			
	0	100	300	1000
Mean body weight (g)				
day 1	88.2 ± 10.8	91.4 ± 9.7	84.7 ±10.9	84.9 ±10.6
day 72	287.3 ± 26.4	289.1 ± 31.2	285.0 ± 26.0	282.4 ± 33.3
Mean body-weight gain (g)♪				
days 1-72	199.1	197.7	200.3	197.5 (99)
days 1-9	48.6	48.8	48.0	50.0
Mean food consumption (g/day)				
days 1-4	14.2 ±1.0	14.4 ± 1.5	13.9 ± 1.4	14.0 ± 1.5
days 4-11	17.0 ± 1.4	17.4 ± 1.9	16.6 ± 1.3	16.9 ± 1.7
days 67-74	19.9 ± 1.8	18.9 ± 1.9 (95)	18.5 ±1.1* (93)	18.7 ±1.5* (94)

[a] Data obtained from Tables 18, 19, 22, 23, 28, 29, page 68, 70, 73, 75, 80, 82 in the study report. ♪calculated by reviewer; (% of control); * $p<0.05$.

Selected group mean body weights and food consumption values for pregnant or nursing dams were summarized in the report as follows (Tables 4 and 5).

There were no treatment-related effects on body weight or food consumption during gestation. Overall body weight gains during the lactation period were increased (not statistically significant) in P1 and P2 females from the 1000 mg/kg bw/day dose group (123-163% of control). There was no comparable increase in food consumption during the lactation period. The increased body weight is not considered to be an adverse effect.

TABLE 4. Body Weight Gains and Food Consumption- Gestation[a]				
Body weights and Food consumption/study day	**Dose Level (mg/kg/day)**			
	0	**100**	**300**	**1000**
P1 Generation Females – Gestation				
Mean body weight gain (g) Gestation day 0-7	39.5±7.9	36.8 ± 9.7	32.4±11.2	37.6± 7.7
Mean body weight gain (g) Gestation day 7-14	34.4± 7.9	31.2 ± 6.6	30.4 ± 9.5	32.0 ± 6.7
Mean body weight gain (g) Gestation day 14-21	86.7 ± 13.5	87.9 ±10.7	83.3 ±16.0	86.8 ±13.0
Mean body weight gain (g) Gestation day 0-21	160.5 ± 20.7	155.9 ± 13.6	146.1 ± 21.4*	156.4 ± 16.2
Mean food consumption (g/day) Gestation day 0-7	24.4 ± 2.2	23.8 ± 2.2	22.7 ± 2.4*	24.4 ± 2.2
Mean food consumption (g/day) Gestation day 7-14	26.3 ± 2.5	25.8 ± 2.0	24.9 ± 2.0	26.4 ± 2.5
Mean food consumption (g/day) Gestation day 14-21	25.5 ± 2.7	24.3 ± 2.0	23.8 ± 1.8*	25.5 ± 1.7
P2 Generation Females – Gestation				
Mean body weight gain (g) Gestation day 0-7	35.7 ± 7.7	34.5 ± 5.2	35.5 ± 7.3	36.7 ± 5.7
Mean body weight gain (g) Gestation day 7-14	31.4 ± 6.6	31.9 ± 7.9	30.3 ± 8.4	33.7 ± 8.3
Mean body weight gain (g) Gestation day 14-21	81.1 ±20.8	75.5 ±16.0	76.8 ±23.7	82.1 ±15.2
Mean body weight gain (g) Gestation day 0-21	148.2 ± 23.6	141.9 ± 17.7	142.6 ± 30.0	152.5 ± 18.1
Mean food consumption (g/day) Gestation day 0-7	23.2 ± 1.9	22.3 ± 2.0	22.6 ± 2.2	23.3 ± 1.9
Mean food consumption (g/day) Gestation day 7-14	26.0 ± 2.2	25.1 ± 2.6	24.6 ± 2.6	25.7 ± 2.0
Mean food consumption (g/day) Gestation day 14-21	25.5 ± 2.8	23.9 ± 2.8	24.6 ± 2.4	25.3 ± 2.7

[a]Data extracted from pages (66, 71, 77 and 84) of the study report.
* alpha = 0.05.

TABLE 5. Body Weight Gains - Lactation[a]				
Body weights/study day	**Dose Level (mg/kg/day)**			
	0	**100**	**300**	**1000**
P1 Generation Females – Lactation				
Mean body weight gain (g) Lactation day 1-4	13.2 ± 11.9	14.7 ± 9.0	15.3 ± 8.4	14.3 ± 11.8
Mean body weight gain (g) Lactation day 4-7	8.3 ± 11.7	4.7 ± 10.5	8.2 ± 8.3	11.4 ± 11.3
Mean body weight gain (g) Lactation day 7-14	22.6 ± 10.7	21.6 ± 11.1	13.4 ± 12.8*	24.2 ± 11.6
Mean body weight gain (g) Lactation day 14-21	-18.2 ± 14.2	-21.5 ± 10.0	-17.6 ± 3.7	-18.0 ± 11.3
Mean body weight gain (g) Lactation day 1-21	25.9 ± 15.3	19.4 ± 11.1	19.2 ± 14.3	31.9 ± 11.2
Mean food consumption (g/day) Lactation day 1-4	31.8 ± 4.8	31.7 ± 4.2	31.9 ± 5.2	32.9 ± 5.6
Mean food consumption (g/day) Lactation day 4-7	40.6 ± 2.5	40.2 ± 4.3	42.4 ± 5.9	42.6 ± 5.6
Mean food consumption (g/day) Lactation day 7-11	53.1 ± 4.1	51.6 ± 4.8	50.5 ± 5.4	55.5 ± 4.1
Mean food consumption (g/day) Lactation day 11-14	61.9 ± 4.0	60.6 ± 5.8	58.5 ± 5.8	62.8 ± 5.7
Mean food consumption (g/day)	65.5 ± 5.3	63.8 ± 5.1	64.1 ± 5.9	67.5 ± 5.2

TABLE 5. Body Weight Gains - Lactation[a]				
Body weights/study day	**Dose Level (mg/kg/day)**			
	0	**100**	**300**	**1000**
Lactation day 14-17				
Mean food consumption (g/day) Lactation day 17-19	67.6 ± 6.7	65.0 ± 6.4	64.9 ± 8.5	67.2 ± 4.8
Mean food consumption (g/day) Lactation day 19-21	80.8 ± 7.7	78.5 ± 9.0	71.3 ± 8.5*	76.1 ± 9.4
P2 Generation Females – Lactation				
Mean body weight gain (g) Lactation day 1-4	15.0 ± 12.1	17.6 ± 8.0	18.9 ± 7.5	21.1 ± 10.5
Mean body weight gain (g) Lactation day 4-7	9.9 ± 7.8	8.6 ± 10.2	8.8 ± 9.9	10.0 ± 11.0
Mean body weight gain (g) Lactation day 7-14	4.4 ± 10.6	13.4 ± 13.4*	9.0 ± 9.9	17.3 ± 12.0*
Mean body weight gain (g) Lactation day 14-21	-13.6 ± 11.6	-22.5 ± 12.3	-17.8 ± 16.6	-22.8 ± 13.1
Mean body weight gain (g) Lactation day 1-21	15.7 ± 13.2	17.2 ± 15.4	18.9 ± 16.9	25.6 ± 14.2
Mean food consumption (g/day) Lactation day 1-4	31.6 ± 5.6	32.7 ± 6.2	34.8 ± 5.8	35.1 ± 5.6
Mean food consumption (g/day) Lactation day 4-7	40.1 ± 6.0	42.6 ± 6.1	43.4 ± 4.9	43.7 ± 6.0
Mean food consumption (g/day) Lactation day 7-11	52.0 ± 5.0	53.1 ± 4.9	53.6 ± 4.5	56.5 ± 4.1*
Mean food consumption (g/day) Lactation day 11-14	58.0 ± 5.9	60.4 ± 5.8	60.4 ± 8.2	63.0 ± 5.7
Mean food consumption (g/day) Lactation day 14-17	62.0 ± 7.1	63.6 ± 6.1	65.5 ± 5.6	67.3 ± 7.3*
Mean food consumption (g/day) Lactation day 17-19	60.5 ± 10.4	66.2 ± 5.6*	66.0 ± 6.8	67.5 ± 7.9*
Mean food consumption (g/day) Lactation day 19-21	76.9 ± 7.9	77.5 ± 8.2	76.6 ± 11.1	75.3 ± 9.9

[a]Data extracted from pages (67, 72, 79, and 86) of the study report. (% of control)
* alpha = 0.05.

3. **Test substance intake:** Based on food consumption and body weight, the doses expressed as mean daily mg test substance/kg body weight during the 10-week pre-mating period are presented in Table 6. The values for the P1 generation are considered to be representative of the test substance intake for the entire study.

TABLE 6. Mean test substance intake during premating (mg/kg body weight/day)						
	Male			**Female**		
	100	**300**	**1000**	**100**	**300**	**1000**
P_1	106.05	321.29	1078.18	103.83	311.21	1042.83
P_2	112.36	340.70	1138.07	104.35	315.93	1048.83

Data from Text Table 2, page 33 of the study report

4. **Reproductive function:**

   a. **Estrous cycle length and periodicity:** There was no evidence of an effect on estrous cyclicity at any dose level in either the P1 or P2 females. The number of small, growing, and total ovarian follicles were comparable between control and high-dose F2 females (Table 7).

TABLE 7. Mean (±SD) Estrous Cycle Data				
Observation	**Dose group (mg/kg/day)**			
	0	**100**	**300**	**1000**
[P1] Females				
Mean days per cycle	4.1±0.3	4.1±0.4	4.1±0.3	4.2±0.4
% of days in♪				
estrus	26.1±2.8	25.2±2.6	26.8±3.0	25.9±3.7
diestrus	57.3±6.3	57.9±7.3	59.3±6.9	59.6±5.9
[P2] Females				
Mean days per cycle	4.3±0.4	4.1±0.2	4.2±0.3 (26)	4.1±0.4
% of days in♪				
estrus	24.4±3.1	25.2±2.2	25.5±4.8	24.7±3.3
diestrus	61.0±6.3	60.1±6.2	61.2±7.6	59.3±6.4

[a] Data obtained from Tables 55 & 56, page 145-146 in the study report; n= 27 unless ( ); ♪calculated by reviewer using data from Appendix Tables 39 (pages 499-502) & 42 (pages 511-514)

b. <u>Sperm measures</u>: Results from the evaluation of sperm parameters revealed no differences among the groups in either generation.

TABLE 8. Sperm Motility and Progressive Motility				
Observation	**Dose group (mg/kg/day)**			
	0	**100**	**300**	**1000**
P1 Males				
% motile	92.2±19.4	98.1±1.3	97.4±2.7	90.2±26.0
% progressively motile	83.5±18.7	87.1±4.6	87.5±5.5	81.9±24.0
n=	26	27	26	27
P2 Males				
% motile	96.4±2.6	96.9±1.7	95.0±4.2	95.8±3.1
% progressively motile	87.8±4.2	84.6±7.5	83.0±7.8	85.7±6.0
n=	26	25	27	26

[a] Data obtained from Appendix Tables 30 & 31, page 475-482 in the study report

Table 9. Sperm Counts/Proportion of Abnormal Sperm		
Parameter	**0 mg/kg/day**	**1000 mg/kg/day**
P1 males		
Epididymal sperm counts		
total sperm (10^6)	294.3±82.9	308.1±105.3
concentration/gram epididymis (10^6)	942.3±272.7	974.5±265.8
Testicular sperm counts		
Total sperm (10^6)	251.8±69.3	257.9±77.5
concentration/gram testes (10^6)	149.4±30.3	144.5±38.4
Proportion of abnormal sperm (total)	0.020±0.016	0.056±0.195♪
P2 males		
Epididymal sperm counts		
total sperm (10^6)	344.9±102.5	353.9±119.0
concentration/gram epididymis (10^6)	1121.5±257.0	1088.6±338.5
Testicular sperm counts		
Total sperm (10^6)	299.6±73.9	327.2±76.6
concentration/gram testes (10^6)	165.5±37.0	181.2±31.6
Proportion of abnormal sperm (total)	0.019±0.009	0.021±0.011

Data from Tables 49-54, pages 139-144 of report; n=25-27; ♪2 outliers (0.000 & 0.990) included in mean

5. <u>Reproductive performance</u>: Results for the parental animals are summarized in Tables 10 and 11. There did not appear to be an effect of treatment on reproductive performance in either generation.

TABLE 10. Reproductive Performance P1 Generation[a]

Reproductive Performance	Dose Group (mg/kg/day)			
	0	100	300	1000
Mean precoital interval (days)	2.9 ± 1.4	2.1 ± 1.3	3.0 ± 1.9	3.3 ± 2.8
MALES				
Mating Index % (number)[b]	92.6 (25/27)	100 (27/27)	96.2 (25/26)	100 (27/27)
Conception Index % (number)[c]	96.0 (24/25)	92.6 (25/27)	100 (25/25)	92.6 (25/27)
Fertility Index % (number)[d]	88.9 (24/27)	92.6 (25/27)	96.2 (25/26)	92.6 (25/27)
Fertility not determined	0	0	1♪	0
Intercurrent deaths	0	0	1	0
FEMALES				
Mating Index % (number)[e]	92.6 (25/27)	100 (27/27)	96.3 (26/27)	100 (27/27)
Conception Index % (number)[f]	96.0 (24/25)	92.6 (25/27)	100 (26/26)	92.6 (25/27)
Fertility Index % (number)[g]	88.9 (24/27)	92.6 (25/27)	96.3 (26/27)	92.6 (25/27)
Fertility not determined	0	0	0	0
Intercurrent deaths	0	0	0	0
Gestation Index %[h]	100	100	100	100
Median gestation interval (days)	21.6 ± 0.5	21.6 ± 0.5	21.7 ± 0.5	21.7 ± 0.5
Number of live litters	24	25	26	25

[a] Data extracted from pages 147-148 of the report; Table 13 from template (MRID 46908601) submitted by Dow
♪died prior to mating;
[b] (# Males that mated /total # males co-housed with females) X 100%.
[c] (# Males that sired a litter/# males mated) X 100%.
[d] (# Males that sired a litter/# males co-housed with female) X 100%.
[e] (# Females with evidence of mating/# females co-housed with males) X 100%.
[f] (# Females with evidence of pregnancy/# females mated) X 100%.
[g] (# Females with evidence of pregnancy/# females co-housed with males) X 100%.
[h] (# Females delivering a litter/# females pregnant) X 100%

TABLE 11. Reproductive Performance P2 Generation[a]

Reproductive Performance	Dose Group (mg/kg/day)			
	0	100	300	1000
Mean precoital interval (days)	2.8 ± 2.0	3.9 ± 2.8	3.0 ± 2.0	3.1 ± 2.0
MALES				
Mating Index % (number)[b]	96.3 (26/27)	96.0 (24/25)	96.2 (25/26)	96.3 (26/27)
Conception Index % (number)[c]	88.5 (23/26)	95.8 (23/24)	88.0 (22/25)	88.5 (23/26)
Fertility Index % (number)[d]	85.2 (23/27)	92.0 (23/25)	84.6 (22/26)	85.2 (23/27)
Fertility not determined	0	2/27	1/27	0
Intercurrent deaths	0	2	1	0
FEMALES				
Mating Index % (number)[e]	96.3 (26/27)	96.3 (26/27)	96.2 (25/26)	96.3 (26/27)
Conception Index % (number)[f]	88.5 (23/26)	96.2 (25/26)	88.0 (22/25)	88.5 (23/26)
Fertility Index % (number)[g]	85.2 (23/27)	92.6 (25/27)	84.6 (22/26)	85.2 (23/27)
Fertility not determined	0	0	1/27	0
Intercurrent deaths	0	0	1	0
Gestation Index %[h]	100	100	100	100
Median gestation interval (days)	21.5 ± 0.5	21.8 ± 0.4	21.5 ± 0.5	21.5 ± 0.5
Number of live litters	23	25	22	23

[a] Data extracted from page 149 of the study report; Table 14 from template (MRID 46908601) submitted by Dow
[b] (# Males that mated /total # males co-housed with females) X 100%.
[c] (# Males that sired a litter/# males mated) X 100%.
[d] (# Males that sired a litter/# males co-housed with female) X 100%.
[e] (# Females with evidence of mating/# females co-housed with males) X 100%.
[f] (# Females with evidence of pregnancy/# females mated) X 100%.

[g] (# Females with evidence of pregnancy/# females co-housed with males) X 100%.
[h] (# Females delivering a litter/# females pregnant) X 100%

6. Parental postmortem results:

a. Organ weights: In general, changes in organ weights reflected the slight differences in body weight and/or were not consistent between generations. For example, there was an apparent dose-related (slight) decrease in prostate weight in the P1 males (94% of control at 1000 mg/kg/day) and an apparent dose-related (slight) increase in P2 males (109% of control at 1000 mg/kg/day). P2 males dosed at 1000 mg/kg/day displayed a significantly higher (112% of control) relative thyroid weight, which was within the historical control range (0.0039-0.0048 g/100 g from 2 studies conducted since 2002). The report noted a significantly lower absolute spleen weight in the P1 females dosed at 1000 mg/kg/day (89% of concurrent control; 94% of the low end of the historical control range of 0.558 – 0.602 g from 4 studies conducted since 2002). Selected absolute and relative (to body weight) organ weight values are presented in the following tables (Tables 12a and 12b).

Organ	TABLE 12a. Organ Weight (P1 and P2 Males) Dose Group (mg/kg/day) 0	100	300	1000
MALES (absolute; grams)				
Brain				
P1	2.124±0.118	2.144±0.129	2.127±0.086	2.159±0.095
P2	2.225±0.109	2.188±0.125	2.213±0.079	2.230±0.114
Prostate				
P1	1.283±0.187	1.239±0.245	1.224±0.277	1.207±0.236
P2	1.191±0.226	1.252±0.241	1.257±0.259	1.294±0.189
Seminal vesicles				
P1	1.823±0.252	1.811±0.214	1.802±0.244	1.772±0.307
P2	1.688±0.327	1.764±0.338	1.851±0.304	1.789±0.365
Testes				
P1	3.382±0.594	3.616±0.312	3.574±0.251	3.490±0.608
P2	3.815±0.373	3.856±0.299	3.831±0.274	3.921±0.394
Epididymides				
P1	1.374±0.196	1.411±0.085	1.412±0.100	1.370±0.203
P2	1.419±0.161	1.451±0.107	1.487±0.138	1.510±0.150
Thyroid				
P1	0.0236±0.0038	0.0238±0.0041	0.0239±0.0041	0.0246±0.0038
P2	0.0248±0.0042	0.0269±0.0041	0.0257±0.0039	0.0274±0.0047
Terminal body weight				
P1	558.8±49.8	549.2±64.6	553.0±66.6	544.6±51.2
P2	608.7±52.0	623.1±72.2	613.4±55.2	597.7±66.2
MALES (relative to body weight; grams/100 grams)				
Brain				
P1	0.382±0.033	0.394±0.037	0.390±0.047	0.400±0.041
P2	0.368±0.033	0.355±0.043	0.363±0.034	0.377±0.037
Prostate				
P1	0.234±0.041	0.228±0.050	0.227±0.067	0.225±0.055
P2	0.196±0.038	0.203±0.041	0.206±0.044	0.219±0.037
Testes				
P1	0.610±0.121	0.665±0.078	0.653±0.069	0.645±0.123
P2	0.630±0.071	0.625±0.070	0.628±0.055	0.662±0.084
Epididymides				
P1	0.248±0.041	0.260±0.029	0.258±0.027	0.253±0.042
P2	0.236±0.030	0.236±0.029	0.243±0.024	0.255±0.031
Thyroid				

Organ	TABLE 12a. Organ Weight (P1 and P2 Males)			
	Dose Group (mg/kg/day)			
	0	100	300	1000
P1	0.0043±0.0007	0.0044±0.0008	0.0044±0.0008	0.0045±0.0007
P2	0.0041±0.0006	0.0043±0.0006	0.0042±0.0006	0.0046±0.0007*

[a] Data extracted from Tables 38 & 40 (pages 91-92, 96-97) of the study report; * $p<0.05$

Organ	TABLE 12b. Organ Weight (P1 and P2 Females)			
	Dose Group (mg/kg/day)			
	0	100	300	1000
FEMALES (absolute; grams)				
Brain				
P1	2.011±0.106	1.971±0.134	1.977±0.109	1.959±0.093
P2	2.042±0.099	2.032±0.113	2.019±0.095	2.004±0.121
Spleen				
P1	0.595±0.110	0.562±0.064	0.574±0.090	0.527±0.053*
P2	0.599±0.091	0.583±0.069	0.591±0.090	0.570±0.073
Terminal body weight				
P1	308.3±21.5	310.4±24.0	302.4±19.9	300.1±18.8
P2	308.7±23.7	310.1±26.7	307.6±27.7	307.8±28.1
FEMALES (relative to body weight; grams/100 grams)				
Brain				
P1	0.655±0.049	0.638±0.062	0.656±0.050	0.655±0.042
P2	0.664±0.051	0.658±0.053	0.660±0.044	0.655±0.061
Spleen				
P1	0.193±0.027	0.182±0.020	0.191±0.033	0.176±0.017
P2	0.195±0.028	0.189±0.023	0.193±0.029	0.186±0.018

[a] Data extracted from Tables 39 & 41 (pages 93-95, 98-99) of the study report; * $p<0.05$

b. Pathology:

1. **Macroscopic examination:** No treatment-related alterations were observed at necropsy in either sex of either generation. The gross pathologic findings were comparable among the groups.

2. **Microscopic examination:** No treatment-related microscopic lesions were observed in either sex of either generation. The histologic findings were comparable among the groups. The numbers of small and growing ovarian follicles from P2 females were comparable between the control and high-dose groups.

Table 13. Results of ovarian follicle counting (mean ± SD)[a]			
Dose group (mg/kg bw/day)	Small follicles	Growing follicles	Total follicles
0	81 ± 23	39 ± 7	120 ± 27
1000	94 ± 25 (116)	44 ± 8 (113)	138 ± 29 (115)

[a] Data extracted from Table 46 (page 136) of the study report; (% of control)

B. OFFSPRING:

1. **Viability and clinical signs:** The following findings were reported:

Mean litter size litter and viability (survival) results of pups during lactation are summarized from the report in Table 14. The report stated that there were no effects of treatment at any dose level on gestation indices, post-implantation loss, pup survival, or pup sex ratio in either generation. The only parameter reported as significantly altered was F2 pup survival, which was significantly increased on PND 21 at the 300 mg/kg/day dose level. This reviewer notes that the number born dead (F1 only) at the 1000 mg/kg/day dose level (11 pups; 3.3%; 5 litters; 20%) is twice the number of pups in any of the other groups (5 pups; 1.4%-1.5%; 4, 2, 2 litters; 17%, 8%, 8% in control, low dose, mid dose, respectively). The number of litters affected in the control (4 litters; 17%) is similar to the number at the high dose (5 litters; 20%). There was one complete litter loss (12 pups) in the F2 generation at 300 mg/kg bw/day between days 0 and 4. The dam that lost the litter had no remarkable clinical or necropsy observations.

TABLE 14. Litter parameters for F_1 and F_2 generations [a]

Observation	Dose group (mg/kg/day)			
	0	100	300	1000
F_1 Generation				
Mean implantation sites♪	15.8±1.5 [378]	15.5±1.5 [386]	14.5±3.1 [378]	15.1±2.2 [377]
Number born live (%)	339/344 (98.5)	354/359 (98.6)	352/357 (98.6)	325/336 (96.7)
Number born dead (%)	5 (1.5)	5 (1.4)	5 (1.4)	11 (3.3)
Sex ratio day 1 (% males)	47	53	50	49
# Deaths days 0-4 (%)	3/339 (0.9)	4/354 (0.8)	6/352 (1.7)	3/325 (0.9)
# Deaths days 4-21 (%)	1/192 (0.5)	1/200 (0.5)	2/202 (1.0)	2/200 (1.0)
Mean litter size Day 0	14.1±2.0	14.2±1.8	13.5±3.1	13.0±2.4
Day 1	14.0±1.9	14.0±1.9	13.5±3.1	12.9±2.5
Day 4 [b]	13.9±1.8	13.9±1.9	13.3±3.1	12.8±2.4
Day 4 [c]	8.0±0.0	8.0±0.0	7.8±0.9	8.0±0.0
Day 7	8.0±0.2	8.0±0.0	7.7±0.9	8.0±0.2
Day 14	8.0±0.2	8.0±0.2	7.7±0.9	8.0±0.2
Day 21	8.0±0.2	8.0±0.2	7.7±0.9	7.9±0.3
Birth index♫	91.0	93.0	94.4	89.1
Live birth index	98.5	98.6	98.6	96.7
Viability index	98.2	98.0	98.3	98.5
Lactation index	99.5	99.5	99.0	99.0
F_2 Generation				
Mean implantation sites♪	13.96±3.8 [321]	14.2±2.5 [354]	14.1±2.4 [310]	14.3±1.9 [330]
Number born live (%)	290/292 (99.3)	322/328 (98.2)	286/286 (100)	310/311 (99.7)
Number born dead (%)	2 (0.7)	6 (1.8)	0	1 (0.3)
Sex ratio day 1 (% males)	49	49	53	52
# Deaths days 0-4 (%)	9/290 (3.1)	12/322 (3.7)	18/286 (6.3)	13/310 (4.2)
# Deaths days 4-21 (%)	5/175 (2.9)	6/194 (3.1)	0/166 (0)*	4/184 (2.2)
Mean litter size Day 0	12.6±3.6	12.9±2.9	13.0±3.0	13.8±3.3
Day 1	12.3±3.7	12.8±2.9	12.8±3.0	13.3±2.0
Day 4 [b]	12.2±3.6	12.4±3.2	12.2±4.0	12.9±2.1
Day 4 [c]	7.6±1.3	7.8±0.8	7.5±1.7	8.0±0.0
Day 7	7.5±1.3	7.6±1.4	7.5±1.7	7.9±0.5
Day 14	7.4±1.3	7.6±1.6	7.5±1.7	7.8±0.5
Day 21	7.4±1.3	7.5±1.6	7.5±1.7	7.8±0.5
Birth index♫	91.0	92.7	92.3	94.5
Live birth index	99.3	98.2	100	99.7
Viability index	96.9	96.3	93.7	95.8
Lactation index	97.1	96.9	100	97.8

[a] Data obtained from Tables 57-60, pages 147-152 in the study report. ♪not provided in study report or template (determined by reviewer using individual data from Appendix tables 60 (pages 618-649, 677-703, 733-759, 798-834) and 61 (877-911, 941-968,

997-1024, 1061-1096); [total]; ♫ calculated as # implantations minus # born x 100; [b] Before standardization (culling); [c] After standardization (culling)

2. **Body weight:** Offspring body weights were comparable among the groups (both sexes and generations). Selected mean pup body weight data are presented in Table 15. Litter weight data were not provided.

TABLE 15. Mean (±SD) Pup Body Weights (g) [a]

Lactation Day	Dose group (mg/kg/day)							
	0	100	300	1000	0	100	300	1000
	F_1 Pups – male				F_2 Pups – male			
1	7.1±0.8	7.1±0.7	7.0±0.7	7.2±0.6	7.0±0.8	7.1±0.7	6.7±0.6	6.8±0.7
4 b	10.1±1.2	10.0±1.2	10.0±1.1	10.2±1.1	10.3±1.4	9.8±1.5	9.8±1.2	9.6±1.4
4 c	10.0±1.3	10.0±1.2	10.0±1.2	10.2±1.1	10.3±1.4	9.8±1.5	9.7±1.1	9.7±1.4
7	16.8±2.0	16.7±1.6	16.5±1.6	16.5±1.7	16.4±1.8	16.1±1.8	15.8±1.8	15.6±2.1
14	34.8±3.0	35.2±3.1	34.9±2.9	34.8±2.4	34.5±2.6	34.1±2.3	34.3±3.1	33.8±3.0
21	56.6±5.3	56.7±5.9	55.9±4.9	55.7±4.6	55.4±3.9	55.5±4.3	54.8±5.4	54.1±6.1
	F_1 Pups – female				F_2 Pups – female			
1	6.8±0.8	6.7±0.7	6.7±0.7	6.7±0.5	6.6±0.7	6.7±0.6	6.4±0.5	6.4±0.7
4 b	9.7±1.4	9.6±1.2	9.6±1.3	9.7±1.1	9.8±1.3	9.3±1.4	9.4±1.0	9.3±1.2
4 c	9.7±1.4	9.6±1.2	9.6±1.2	9.7±1.1	9.7±1.4	9.3±1.4	9.4±1.0	9.4±1.3
7	16.0±2.1	16.1±1.7	15.7±1.6	15.7±1.8	15.7±2.0	15.0±2.5	15.3±1.5	15.2±1.8
14	33.5±3.3	34.1±3.2	33.5±2.8	33.4±2.6	33.3±2.2	32.7±2.3	33.2±2.5	33.7±2.3
21	54.5±5.5	55.1±5.7	53.2±4.6	53.4±5.0	53.2±3.9	53.0±3.7	53.0±4.6	53.6±3.9

[a] Data obtained from Tables 61-62, pages 153-154 in the study report; table from template (MRID 46908601) submitted by Dow
[b] Before standardization (culling); [c] After standardization (culling); n = 21-26

3. **Sexual maturation:** Sexual maturation was not adversely affected in either sex of P2 animals.

Table 15. Sexual Maturation	Dose Group (mg/kg/day)			
	0	100	300	1000
MALES				
days to preputial separation	44.9±2.2	44.7±2.3	44.3±2.1	45.3±2.8
mean body weight (grams)	246.9±25.1	244.2±25.4	248.8±23.2	251.9±29.5
FEMALES				
days to vaginal opening	31.9±1.3	32.3±1.4	32.0±1.5	32.6±1.6
mean body weight (grams)	113.5±11.7	115.3±13.8	113.2±10.2	115.3±13.0

[a] Data extracted from Tables 63-64 (pages 155-156) of the study report; n=27

4. **Offspring postmortem results:**

   a. **Organ weights:** Brain, spleen, and thymus weights were provided for the F1 and F2 weanlings of both sexes, as well as uterus weight for the female F1 and F2 weanlings. The report noted a statistically-identified, lower relative brain weight of the F1 female weanlings at 100 mg/kg/day. However, there was no dose-response in the 300 and 1000 mg/kg/day female F1 weanlings, and the finding is not considered related to treatment. There were no other differences noted.

b. Pathology:

1. **Macroscopic examination:** There were no treatment-related gross pathologic observations in the F1 or F2 weanlings reported.

2) **Microscopic examination:** Since there were no treatment-related gross pathologic observations in the F1 or F2 weanlings, histological examinations were not performed.

III. DISCUSSION AND CONCLUSIONS:

A. INVESTIGATORS' CONCLUSIONS: XDE-742 did not reveal evidence of systemic toxicity or demonstrate adverse effects on any parameter of reproductive function of the parental animals. Additionally, there was no adverse effect on survival, growth, or development of the offspring. Based upon the absence of any significant effects, the no-observed-effect-level (NOEL) for parental and reproductive toxicity was considered to be 1000 mg/kg/day, the highest dose tested.

B. REVIEWER COMMENTS: XDE-742 was well tolerated by both sexes throughout the premating and mating periods at dose levels that included the limit dose (1000 mg/kg/day, highest dose tested). No adverse effects were observed in either generation on the reproductive function of either sex or on the survival, growth, and development of the offspring. The NOAEL for reproductive toxicity is 1000 mg/kg/day, based on the lack of any significant adverse effect on any parameter monitored. Similarly, the NOAEL for parental and offspring toxicity is 1000 mg/kg/day, the highest dose tested.

The registrant, the Dow Chemical Company, originally prepared this STUDY PROFILE TEMPLATE (STP) (MRID 46908601) in HED's DER format. The HED reviewers may have added minor adjustments/additions to the original STP. The conclusions of the study and assignment of its classification as determined by HED reviewers are in the Executive Summary above.

C. STUDY DEFICIENCIES: There were no deficiencies that would adversely impact study interpretation. However, it is noted that the Study Design section of the report indicated that the histopathological examination of the testes included a qualitative assessment of stages of spermatogenesis. The report stated that the presence and integrity of the 14 stages of spermatogenesis were qualitatively evaluated; microscopic evaluation included a qualitative assessment of the relationships between spermatogonia, spermatocytes, spermatids, and spermatozoa seen in cross sections of the seminiferous tubules; progression of these cellular associations defined the cycle of spermatogenesis. Additionally, sections of both testes were examined for the presence of degenerative changes (e.g., vacuolation of the germinal epithelium, a preponderance of Sertoli cells, sperm stasis, inflammatory changes, mineralization, and fibrosis). These data were not provided.

EPA Reviewer: Linda L. Taylor, Ph.D. **Signature:** Linda L. Taylor
Reregistration Branch I, Health Effects Division (7509P) **Date:** 01-03-08
EPA Secondary Reviewer: Kimberly Harper **Signature:** Kimberly D Harper
RAB2, Health Effects Division (7509P) **Date:** 12/12/07

TXR#: 0054347

DATA EVALUATION RECORD

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

PC CODE: 108702 **DP BARCODE:** D332276

TEST MATERIAL (PURITY): XDE-742 (98% a.i.) [pyroxsulam]

CHEMICAL: N-(5,7-dimethoxy[1,2,4]triazolo[1,5-a]pyrimidin-2-yl)-2-methoxy-4-(trifluromethyl)pyridine-3-sulfonamide

SYNONYMS: X666742, XDE-742, LY-666742, BAS-770H

CITATION: Stebbins, K. E., and Dryzga, M. D. (2004). XDE-742: One-Year Dietary Toxicity Study in Beagle Dogs. Toxicology & Environmental Research and and Development. The Dow Chemical Company, Midland, Michigan. Laboratory Report No: 031012, May 20-21, 2003 – May 19-20, 2004. MRID 46908405. Unpublished.

SPONSOR: Dow AgroSciences LLC, Indianapolis, Indiana.

EXECUTIVE SUMMARY: In a chronic toxicity study (MRID 46908405), XDE-742 (pyroxsulam) [Lot # E0952-52-01, TSN103826; 98.0% a.i.] was administered to 4 Beagle dogs/sex/dose in the diet at dose levels of 0, 0.05, 0.3, or 2.0 % [equivalent to 0, 13.2, 93.0, or 619.6 mg/kg bw/day (males)/0, 17.1, 88.7, or 589.1 mg/kg bw/day (females)] for 12 months (mg/kg bw/day is hereafter referred to as mg/kg/day).

There were no adverse treatment-related effects on mortality, clinical signs, body weight, food consumption, hematology, clinical chemistry, organ weights, or gross and histologic pathology in either sex. **The NOAEL is 619.6 (males)/589.1 (females) mg/kg/day, the highest dose tested.**

This chronic study in the dog is acceptable (guideline), and it satisfies the guideline requirement for a chronic oral study [OPPTS 870.4100, OECD 452] in the dog.

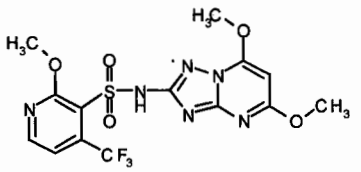
COMPLIANCE: Signed and dated GLP, Quality Assurance, and Data Confidentiality statements were provided.

I. MATERIALS AND METHODS

A. MATERIALS:

1. Test material: XDE-742

Description:	white powder
Lot/batch #:	E0952-52-01/TSN103826
Purity:	98.0% a.i.
Compound stability:	stable in lab diet (#5007) for at least 24 days at concentrations ranging from 0.001%-5%
CAS # of TGAI:	422556-08-9
Structure:	



2. Vehicle and/or positive control: control diet (LabDiet Certified Canine Diet #5007)

3. Test animals:

Species:	Dog	
Strain:	Beagle	
Age/weight at study initiation:	Approximately 6 months old; males: 6.73-9.19 kg; females: 6.44-8.34 kg	
Source:	Marshall Research Laboratory, North Rose, NY	
Housing:	individual pens	
Diet:	LabDiet® Certified Canine Diet #5007 *ad libitum*; PMI Nutrition International	
Water:	municipal *ad libitum*	
Environmental conditions:	**Temperature:**	20.3-23.1°C
	Humidity:	41.6-63.8%
	Air changes:	12-15/hr
	Photoperiod:	12 hrs dark/12 hrs light
Acclimation period:	4 weeks	

B. STUDY DESIGN:

1. In life dates: Start: May 20-21, 2003; End: May 19-20, 2004

2. Animal assignment: Animals were stratified by pre-exposure body weight and then randomly assigned to the test groups (Table 1) using a computer program.

TABLE 1: STUDY DESIGN

Test group	Conc. in diet (%)	Target dose to animal (mg/kg bw/day)	Actual Dose (mg/kg/day)		Main study 12 months	
			Male	Female	Male	Female
Control	0	0	0	0	4	4
Low (LDT)	0.05	17	13.2	17.1	4	4
Mid (MDT)	0.3	100	93.0	88.7	4	4
High (HDT)	2.0	667	619.6	589.1	4	4

3. **Dose selection rationale:** The dose levels were selected based on the results from a subchronic feeding study [MRID 46908352; separate DER] in which 4 dogs/sex/group were administered the test material in the diet at dose levels up to 3% (males 884/females 1142 mg/kg/day) for 90 days. Body-weight gains overall were reduced in both sexes (males 34%/females 31%) at the high-dose level, although there was only a slight reduction in body weight at study termination (males 7%/females 3%). The only other treatment-related findings (high-dose only) were increases in cholesterol concentration (female), alkaline phosphatase activity (female), increased liver weight (males relative; females absolute and relative), and a slight increase in the incidence of panlobular hepatocellular hypertrophy (female). The effects observed following 90 days of exposure at the high-dose level (3% concentration in diet) are not considered adverse [NOAEL is 884 (males)/1142 (females) mg/kg/day]. The highest dose selected for the one-year study (2%) was expected to produce decreased body-weight gains, and the other two dose levels were expected to provide dose-response data for any treatment-related effects observed at the 2% dose level.

4. **Diet preparation and analysis:** High-dose diets were prepared every two weeks by mixing appropriate amounts of test substance with ground PMI Certified Canine Diet #5007. Mid-dose diets were prepared by diluting the high-dose diet with control lab diet, and the low-dose diets were prepared by diluting the mid-dose diet with control lab diet. Homogeneity of the low- and high-dose diets was determined prior to study initiation and at 4, 8, and 12 months. Analysis of the test material in the diet was performed at the same time points (all dose levels).

Results:

Homogeneity analysis: The % relative standard deviations for homogeneity were reported as 2.96% to 10.6%, confirming the homogeneity of the mixture, with the exception of one analysis of male 2% batch where the standard deviation was 14.5%. This diet was re-mixed (9.82%).

Stability analysis: Determined previously (stable for at least 24 days).

Concentration analysis: LC-MS analysis indicated 85% to 103% of target concentrations, with one exception (female 667 mg/kg/day group; 77.5% fed for one week); the mean concentrations in the diet ranged from 91.5%-97.8% of the target concentration, which indicated an acceptable agreement between actual and targeted levels.

5. **Statistics:** Means and standard deviations were calculated for all continuous data. All parameters examined statistically were first tested for equality of variance using Bartlett's test. If significant at alpha = 0.01, then the data were subjected to a transformation to obtain equality of the variances. The transformations examined are the common log, the inverse, and the square root, in that order. In-life body weight, urine specific gravity, hematology parameters (excluding RBC indices, differential WBC), coagulation, and clinical chemistry parameters were evaluated using a repeated measures (RM) analysis of variance (ANOVA) for time (the repeated factor), sex, and dose. A Bonferroni correction was applied to the alpha level to compensate for the multiple comparisons with the control group, which controlled the experiment-wise error rate. The

corrected comparison-wise alpha level of 0.02 is reported so direct comparison can be made to the p-values generated. Terminal body weight, organ weight (absolute and relative, excluding ovaries, uterus, epididymides, and testes), and urine volume were evaluated using a two-way ANOVA with the factors of sex and dose. Differences between the groups were primarily detected by the dose factor. For these parameters, the first examination was whether the sex-dose interaction was significant at alpha = 0.05; if it was, a oneway ANOVA was then done separately for each sex. Comparisons of individual dose groups to the control group were made with a Dunnett's test only when a statistically significant dose effect existed (alpha = 0.05). Results for ovaries, uterus, epididymides, and testes weight (absolute and relative) were analyzed using a one-way ANOVA. If significant dose effects were determined, then separate doses were compared to controls using a Dunnett's test. Feed consumption data were evaluated by Bartlett's test for equality of variances. Exploratory data analysis was performed by a parametric analysis of variance (ANOVA); when significant at alpha = 0.05, followed by Dunnett's test (alpha = 0.05). Descriptive statistics only (means and standard deviations) were reported for body weight gains, RBC indices, and WBC differential counts.

These analyses are considered appropriate.

C. METHODS:

1. **Observations:** Animals were inspected once daily for signs of toxicity and twice daily for moribundity/mortality. Clinical examinations were conducted pre-exposure and weekly throughout the study and included cage-side, hands-on, and open-field observations. The examination begins at the head of the dog and gradually works towards the tail [body movements, behaviors, changes in posture, resistance to removal from cage, eye observations, degree of salivation, muscle tone, extensor-thrust response, reactivity to handling, open field observations (responsiveness to touch, gait evaluation)].

2. **Body weight:** Animals were weighed during the period prior to study initiation, weekly during the first 13 weeks, and at least once per month thereafter.

3. **Food consumption and compound intake:** Food consumption for each animal was determined and mean daily diet consumption was calculated as g food/kg body weight/day. Food efficiency was not determined, and compound intake (mg/kg bw/day) values were calculated as time-weighted averages from the consumption and body weight gain data.

4. **Ophthalmoscopic examination:** Eyes were examined pre-exposure and during the week prior to sacrifice using indirect ophthalmoscopy. Eyes were also examined during necropsy.

5. **Hematology and clinical chemistry:** Blood was collected *via* venipuncture of the jugular vein from all fasted dogs pre-exposure, at the months 3- and 6-month time points, and during the week prior to sacrifice for hematology and clinical chemistry from all surviving animals. The CHECKED (X) parameters were examined.

a. Hematology:

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

* Recommended for chronic non-rodent studies based on Guideline 870.4100b

b. Clinical chemistry:

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

* Recommended for chronic non-rodent studies based on Guideline 870.4100b

6. **Urinalysis:** Urine was collected from fasted (16 hours) dogs (metabolism cages) pre-exposure, at 3- and 6-month time points, and during the last week of the study. The CHECKED (X) parameters were examined.

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

* Recommended for chronic non-rodent studies based on Guideline 870.4100b.

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

X	DIGESTIVE SYSTEM
X	Tongue
X	Salivary glands*
X	Esophagus*
X	Stomach*
X	Duodenum*
X	Jejunum*
X	Ileum*
X	Cecum*
X	Colon*
X	Rectum*
X	Liver*+
X	Gall bladder*+
X	Pancreas*
X	**RESPIRATORY**
X	Trachea*
X	Lung*
X	Nose*
X	Pharynx*
X	Larynx*

X	CARDIOVASC./HEMAT.
X	Aorta thoracic*
X	Heart*+
X	Bone marrow*
X	Lymph nodes*
X	Spleen*+
X	Thymus*
X	**UROGENITAL**
X	Kidneys*+
X	Urinary bladder*
X	Testes*+
X	Epididymides*+
X	Prostate*
X	Ovaries*+
X	Uterus*+
X	Mammary gland*

X	NEUROLOGIC
X	Brain*+
X	Peripheral nerve*
X	Spinal cord (3 levels)*
X	Pituitary*
X	Eyes (optic nerve)*
X	**GLANDULAR**
X	Adrenal gland*+
	Lacrimal gland
X	Parathyroid*+
X	Thyroid*+
X	**OTHER**
X	Bone (sternum and/or femur)
X	Skeletal muscle
X	Skin*
X	All gross lesions and masses*

* Recommended for chronic oral non-rodent studies based on Guideline 870.4100b
\+ Organ weight required for non-rodent studies.

II. RESULTS:

A. OBSERVATIONS:

1. **Clinical signs of toxicity:** There were no treatment-related clinical signs in any of the dogs of either sex.

2. **Mortality:** All dog of both sexes survived until study termination.

3. **Neurological evaluations:** A neurological examination was not performed.

B. BODY WEIGHT AND WEIGHT GAIN: No significant changes in body weight or body-weight gain were reported for either sex.

TABLE 2: Mean body weights (BW) and body weight gains (BWG)[a]				
kilograms±SD	**Control**	**0.05%**	**0.3%**	**2.0%**
MALES				
Initial BW day 1	7.68±0.77	7.86±0.52	7.59±0.67	8.07±0.87
Final BW day 365	10.38±0.84	10.42±1.00	9.97±0.96	10.75±1.67
BWG Wk 1 (% Control)	0.276±0.162	0.294±0.133	0.219±0.230 (79)	0.103±0.146 (37)
BWG Wk 1-13 (% Control)	1.80±0.30	1.87±0.24	1.64±0.21 (92)	1.48±0.42 (82)
BWG Wk 13-26 (% Control) ♪1	0.566	0.570	0.546	0.687
BWG Wk 26-52 (% Control) ♪2	0.335	0.124 (37)	0.192 (57)	0.516

Overall BWG Wk -1-52	2.70±0.40	2.56±0.56	2.38±0.34 (95)	2.68±1.13
FEMALES				
Initial BW	7.25±0.47	7.25±0.20	7.60±0.51	7.14±0.51
Final BW	8.89±0.84	8.77±0.57	9.30±1.66	8.75±1.16
BWG Wk 1 (% Control)	-0.133±0.245	0.118±0.114	0.027±0.388	0.246±0.129
BWG Wk 1-13 (% Control)	0.865±0.265	0.685±0.656 (79)	1.249±0.957	1.145±0.438
BWG Wk 13-26 (% Control) ♪1	0.401	0.717	-0.169	0.170 (42)
BWG Wk 26-52 (% Control) ♪2	0.366	0.121	0.621	0.299
Overall BWG Wk -1-52	1.631±0.403	1.522±0.755	1.702±1.218	1.614±1.196

% Control = Percent from control; ♪calculated by reviewer; 1(days 92-176); 2 (days 176-365)
[a] Data obtained from Tables 9 and 10, pages 71-86 in the study report.

C. FOOD CONSUMPTION AND COMPOUND INTAKE:

1. **Food consumption:** Food consumption was comparable among the groups for both sexes.

2. **Compound consumption:** The mean test material intake for the 12-month study is shown in Table 3.

Table 3. Compound Consumption

Targeted dose (%)	0	0.05	0.3	2.0
Targeted dose (mg/kg/day)	0	17	100	667
Actual dose (mg/kg/day)				
male	0	13.2±1.9	93.0±12.1	619.6±84.4
female		17.1±1.8	88.7±12.5	589.1±85.4

Data obtained from Table 2, page 28 of the study report

3. **Food efficiency:** Food efficiency data were not provided.

D. OPHTHALMOSCOPIC EXAMINATION: There were no treatment-related alterations in either sex.

E. BLOOD ANALYSES:

1. **Hematology:** Females at the high-dose level displayed lower mean red blood cell counts, hematocrit, and hemoglobin concentration at the 12-month time point compared to the concurrent and the historical control values (Table 4). For each of these parameters, the high-dose females displayed a decreased value relative to the previous 6-month value, whereas, with the exception of the 6-month control RBC value, all parameters increased over the previous time point value for each of the other groups. Increased platelet counts were observed throughout the study in males at the mid-and high-dose levels and in the high-dose females compared to the concurrent and historical controls; however, pre-exposure values were also higher than the concurrent control, and the males did not display a dose response. There were no microscopic changes in bone marrow or peripheral blood smears that correlated with these findings.

TABLE 4. Hematology Data				
Observation	**Dose (%)**			
	Control	**0.05**	**0.3**	**2.0**
Females				
Mean RBC count ($x10^6$/ µL)				
Pre-test [5.77-6.67]	6.62±0.57	5.77±0.18	6.09±0.76	5.92±0.29
3 month [6.61-7.86]	6.85±0.51	6.29±0.60	6.54±0.59	6.58±0.56
6 month [6.34-7.34]	6.81±0.27	6.68±0.77	6.75±0.83	6.68±0.63
12 month [6.76-7.14]	7.07±0.19	6.90±0.53	6.78±0.67	6.44±0.72
Mean hemoglobin concentration (g/dL)				
Pre-test [13.0-15.8]	14.9±1.4	13.7±0.5	14.4±1.2	13.5±1.0
3 month [14.4-17.1]	15.4±1.0	14.7±1.4	15.2±1.4	14.6±1.6
6 month [14.9-16.5]	15.5±0.7	15.8±1.8	15.7±1.8	15.2±1.6
12 month [15.6-16.4]	16.5±0.5	16.6±1.4	16.3±1.5	14.9±1.6
Mean hematocrit (%)				
Pre-test [39.7-46.7]	43.8±3.8	39.6±1.4	42.0±3.9	39.7±2.7
3 month [41.7-51.8]	43.8±3.0	41.3±3.9	43.1±4.3	41.2±3.7
6 month [43.6-49.6]	44.6±1.6	45.1±5.2	45.2±5.9	43.6±4.1
12 month [44.2-48.4]	48.2±1.9	47.8±4.1	47.1±5.0	43.1±4.5
Mean platelet count ($x10^3$/ µL)				
Pre-test [257-409]	358±68	313±11	332±27	380±163
3 month [277-365]	296±49	284±21	303±75	405±116
6 month [298-328]	299±41	302±20	315±65	411±75
12 month [309-369]	316±28	324±60	346±66	386±126
Males				
Mean platelet count ($x10^3$/ µL)				
Pre-test [250-436]	287±24	298±50	383±64	364±54
3 month [211-328]	246±69	301±73	336±43	336±37
6 month [220-326]	225±55	256±33	357±62	322±30
12 month [223-305]	250±30	284±21	351±39	337±41

[a] Data obtained from Tables 23-30, pages 113-128 in the study report; [Historical control]: range from 13 dietary studies (pre-exposure data); 6 dietary studies (3 month data); 3 dietary studies (6 month data); and 3 dietary studies (12 month data), performed since 1999

2. **Clinical chemistry:** At the high-dose level, males displayed increased cholesterol concentrations throughout the study compared to the concurrent and historical controls, but females showed increased levels only at the 12-month time point. Alkaline phosphatase activity was increased throughout the study in the high-dose males compared to the concurrent and historical control values. High-dose females displayed increased alkaline phosphatase activity pre-dose (127% of control) and at the 12-month interval (128% of control), with the latter value being greater than the historical control. However, the magnitude of the increase over the concurrent control was the same for both time points. Similar increases in these two parameters were observed in the 90-day oral toxicity study in dogs, although the increases were observed only in the female at a diet concentration level of 3%. There were no histopathologic correlates to the observed increases in cholesterol concentration or alkaline phosphatase activity.

TABLE 4. Clinical Chemistry Data				
Observation	**Dose group (%)**			
	Control	**0.05**	**0.3**	**2.0**
Males				
Cholesterol (mg/dL)				
Pre-test [143-188]	153±15	142±19	147±12	158±13
3 month [160-193]	162±8	167±24	163±11	202±26 (125)

TABLE 4. Clinical Chemistry Data				
Observation	Dose group (%)			
	Control	0.05	0.3	2.0
6 month [158-195]	178±13	175±18	159±8	198±23 (111)
12 month [168-173]	183±17	178±27	169±16	225±17 (123)
Alkaline phosphatase (µ/L)				
Pre-test [103-426]	152±28	135±18	127±15	142±15
3 month [57-117]	72±18	85±29	56±18	117±70 (163)
6 month [54-74]	58±11	62±16	42±15	75±25 (129)
12 month [45-73]	40±7	68±49	33±17	98±60 (245)
Females				
Cholesterol (mg/dL)				
Pre-test [136-185]	153±17	145±8	153±23	132±34
3 month [168-200]	204±64	166±19	208±34	177±38
6 month [173-208]	211±42	188±59	223±74	199±51
12 month [176-214]	225±100	183±15	221±25	265±68 (118)
Alkaline phosphatase (µ/L)				
Pre-test [101-206]	128±26	135±42	142±26	162±67 (127)
3 month [65-102]	95±41	97±14	82±18	79±13
6 month [60-70]	66±37	88±30	79±26	70±12
12 month [48-71]	73±49	95±41	82±34	101±44 (128)

[a] Data obtained from Tables 39-46, page 145-160 in the study report; [Historical control]; (% of control); Historical control: range from 13 dietary studies (pre-exposure data); 6 dietary studies (3 month data); 3 dietary studies (6- and 12-month data), performed since 1999

F. **URINALYSIS:** There were no treatment-related alterations in any of the urinalysis parameters monitored for either sex.

G. **SACRIFICE AND PATHOLOGY:**

1. **Organ weight:** Liver weights were significantly increased in both sexes at the high-dose level compared to the concurrent and historical controls.

TABLE 5. Liver Weight Data [a]				
Organ	Dose (%)			
	0	0.5	0.3	2.0
MALES				
Terminal body weight				
Liver				
Absolute [254-283]	260.2±38.1	248.0±15.9	258.8±22.0	322.9±44.5
Relative [2.16-2.50]	2.56±0.21	2.43±0.09	2.66±0.18	3.13±0.40
FEMALES				
Terminal body weight				
Liver				
Absolute [217-237]	225.0±31.7	230.7±13.4	233.3±18.1	271.4±25.5
Relative [2.45-2.91]	2.61±0.50	2.82±0.41	2.59±0.41	3.22±0.53

[a] Data obtained from Tables 71-71, pages201-208 in the study report. bolded considered treatment-related

2. **Gross pathology:** Gross pathological findings were comparable among the groups for both sexes.

3. **Microscopic pathology:** No treatment-related histopathologic lesions were observed in either sex at concentration levels in the diet up to 2%.

III. DISCUSSION AND CONCLUSIONS:

A. **INVESTIGATORS' CONCLUSIONS:** There were no treatment-related effects on daily observations, detailed clinical observations, ophthalmologic examinations, body weights, feed consumption, urinalysis, or gross or histopathologic examinations. Females from the high-dose group had treatment-related minimal decrements in red blood cell count, hematocrit, and hemoglobin concentration at the 12-month sampling interval. High-dose males and females had treatment-related increases in cholesterol concentration and alkaline phosphatase activity. High-dose dogs had treatment-related, statistically-identified higher absolute and relative liver weights analyzed across both sexes, relative to controls. The relative liver weights for high-dose males and females were 22.4% and 23.2% higher than controls, respectively. However, there were no histopathologic correlates to the treatment-related hematologic, clinical chemistry, and liver weight effects, and therefore, these changes are considered to be of minimal toxicological significance. The no-observed-effect level (NOEL) for male and female dogs administered XDE-742 in the diet for one year was 0.3% (approximately 93.0 mg/kg/day for males and 88.7 mg/kg/day for females.

B. **REVIEWER COMMENTS:** The test material was well tolerated by both sexes. Body weights/body-weight gains and food consumption were comparable throughout the study for both sexes. Females at the high-dose level displayed lower mean red blood cell counts, hematocrit, and hemoglobin concentration at the 12-month time point compared to the concurrent and the historical control values. High-dose males displayed increased cholesterol concentrations throughout the study compared to both the concurrent and historical controls, but females showed increased levels only at the 12-month time point. Alkaline phosphatase activity was increased throughout the study in the high-dose males compared to both the concurrent and historical control values, but the increased alkaline phosphatase activity observed in the high-dose females was observed pre-dose also. There were no histopathologic correlates to the observed decreases in hematology parameters or the increases in cholesterol concentration or alkaline phosphatase activity. Similar increases in the latter two parameters were observed in the 90-day oral toxicity study in dogs, although the increases were observed only in the female at a diet concentration level of 3%. Increased liver weights were observed at the high-dose in both sexes, but there were no histopathologic correlates to the increased organ weight. Liver weights were also observed in the 90-day dog study in both sexes, and the high-dose females displayed slight panlobular hepatocellular hypertrophy. None of the findings in either study are considered adverse. **The NOAEL is 619.6 (males)/589.1 (females) mg/kg/day, the highest dose tested.**

The registrant, the Dow Chemical Company, originally prepared this STUDY PROFILE TEMPLATE (STP) (MRID 46908602) in HED's DER format. The HED reviewers may have added minor adjustments/additions to the original STP. The conclusions of the study and assignment of its classification as determined by HED reviewers are in the Executive Summary above.

C. **STUDY DEFICIENCIES:** None that would adversely affect study interpretation.

EPA Reviewer: Kimberly Harper **Signature:** Kimberly D Harper
RAB2, Health Effects Division (7509P) **Date:** 12/14/07
EPA Secondary Reviewer: Alan Levy **Signature:** Alan C. Levy
RAB2, Health Effects Division (7509P) **Date:** 1-3-2008

Template version 02/06

TXR#: 0054347

DATA EVALUATION RECORD

STUDY TYPE: Carcinogenicity feeding study - mouse; OPPTS 870.4200a [§83-2a]; OECD 451.

PC CODE: 108702 **DP BARCODE: 332276**

TEST MATERIAL (PURITY): Pyroxsulam (98.0%) (N-(5,7-dimethoxy[1,2,4]triazolo[1,5-a]pyrimidin-2-yl)-2-methoxy-4-(trifluoromethyl)pyridine-3-sulfonamide)

SYNONYMS: X666742, XR-742, XDE-742

CITATION: Johnson, K.A., D.V.M., Ph.D. ; M. D. Dryzga, B.S. ; B. L. Yano, D.V.M., Ph.D. (2005). XDE-742: 18-Month Dietary Oncogenicity Study in CD-1 Mice. Toxicology & Environmental Research and Consulting, The Dow Chemical Company, Midland, Michigan. Study ID: 031015, 15 December 2005. MRID 46908406. Unpublished

SPONSOR: Dow AgroSciences LLC, 9330 Zionsville Road, Indianapolis, Indiana 46268

EXECUTIVE SUMMARY:

In a carcinogenicity study (MRID 46908406) pyroxsulam (98.0% a.i., E0952-52-01/TSN103826) was administered to 50 CD-1 mice/sex/dose in their diet at nominal dose levels of 0, 10, 100, or 1000 mg/kg bw/day for 18 months (mg/kg bw/day alternately referred to as mg/kg/day). Animals were evaluated by daily cage side observation and periodic handheld detailed clinical examination. Body weight and food consumption were measured weekly for the first 13 weeks and monthly thereafter. Ophthalmic examinations were conducted pre-exposure and prior to necropsy. All mice had a complete necropsy examination with white blood cell (WBC) and differential WBC counts and weights of selected organs at the scheduled necropsy. Tissues were examined histopathologically from all control and high-dose group mice, as well as all mice that died or were euthanized in moribund condition. The kidneys, liver, lungs, ovaries, and all relevant gross lesions from the low- and intermediate-dose groups at the terminal necropsy were also examined histopathologically.

There were no effects of XDE-742 consumption with regards to survival, clinical examinations, body weights and body weight gains, or food consumption. There were no effects related to treatment for either ophthalmic examinations or total or differential WBC counts.

Treatment-related effects occurred in the liver of male mice given 1000 mg/kg bw/day, with the mean absolute and relative liver weights increased by 26.4% and 31.6%, respectively, increased incidence of liver masses at necropsy and histopathologically increased incidence of foci of altered cells (hepatocytes).

The **LOAEL is 1000 mg/kg bw/day, based on increased absolute and relative liver weights and increased incidence of clear cell foci of alteration (hepatocytes) in males.** The **NOAEL is 100 mg/kg bw/day. There were no treatment-related, adverse effects in females.**

At the doses tested, there was an increase in tumor incidence in male mice in the low- and high-dose groups with regards to hepatocellular adenomas and/or carcinomas when compared to controls. There was no increase tumor response in the mid-dose males compared to controls. Male mice in the high dose group were more likely to have one or more adenomas (4/50 vs 14/50), carcinomas (1/50 vs 4/50), and adenomas and/or carcinomas (6/50 vs 15/50) than controls. The increased incidence of tumors in the high dose group did not achieve statistical significance. The tumors were determined to be unrelated to treatment due to the highly variable background levels of liver tumors in the CD-1 mouse, especially the males. A study published in 2005 by Charles River stated the historical control ranges for adenomas and carcinomas in male CD-1 mice from 52 separate studies were 3-28% and 2-16%, respectively. This indicates that liver tumors in male mice are fairly common and highly variable, suggesting that liver tumors in mice might be better held to a higher statistical standard of $p<0.01$ instead of $p<0.05$. The liver adenomas in the low- and high-dose groups in this study slightly exceeded the historical control range published by Charles River. There was a tendency of affected mice to have both foci of altered cells and multiple tumors (adenomas and/or carcinomas). CARC determined that the tumor incidence was unrelated to treatment because of the highly variable nature of liver tumors in mice. Other points taken into consideration were 1) the lack of a clear dose response, 2) SAR – none of the other chemicals in this pesticide class are linked to liver tumors, 3) pyroxsulam is not mutagenic, 4) the mouse metabolism study indicated a dose-response in internal exposure, but there was no clear dose-response in tumors, 5) there was no increase in basophilic foci, which is more commonly linked to tumor formation than clear cell foci, and 6) there was no tumor response in female mice.

This carcinogenicity study in mice is acceptable/guideline and satisfies the guideline requirement for a carcinogenicity study [OPPTS 870.4200; OECD 451] in rats.

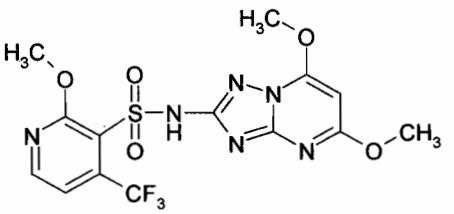
COMPLIANCE: Signed and dated GLP, Quality Assurance and Data Confidentiality statements were provided.

United States Environmental Protection Agency, *Health Effects Test Guidelines*, OPPTS 870.4200 (Carcinogenicity) EPA712-C-98-211, August 1998 with the exception, that the frequency and number of animals having detailed clinical observations were modified. Ten mice/sex/dose level were evaluated monthly for the first 12 months and then quarterly at 15 and 18 months. This modification was reviewed and accepted by the USEPA for a similar combined chronic toxicity/oncogenicity study using rats conducted in this laboratory (memorandum from Dr. W. F. Sette, Toxicology Branch, Health Effects Division, to J. I. Miller, Herbicide Branch, Registration Division, 19 July 2001).

I. MATERIALS AND METHODS:

A. MATERIALS:

1. Test material:

1.	**Test Material:**	XDE-742
	Description:	Solid (powder), white
	Lot/Batch #:	E0952-52-01; TSN103826
	Purity:	98.0% XDE-742
	Compound Stability:	A previous 28-day toxicity study with Fischer 344 rats (MRID 46908351) showed XDE-742 stable for at least 36 days in the feed at concentrations ranging from 0.005% to 5%. This range spanned the diet concentrations used in this study; therefore, additional stability data were not obtained.
	CAS #:	422556-08-9
		

2. Vehicle and/or positive control: LabDiet® Certified Rodent Diet #5002 (PMI Nutrition International)

3. Test animals:

3.	**Test animals:**	
	Species:	Mice
	Strain:	CD-1 [Crl:CD1(ICR)]
	Age/weight at study initiation:	Approximately 6 weeks Males: 24.2 – 33.2 g Females: 17.9 – 26.4 g
	Source:	Charles River Laboratories Inc. (Portage, Michigan)
	Housing:	Animals were housed one per cage in stainless steel cages after assignment to the study. Cages had wire-mesh floors that were suspended above absorbent paper and contained a feed container and a

		pressure activated nipple-type watering system.	
	Feed and Water:	Animals were provided LabDiet® Certified Rodent Diet #5002 (PMI Nutrition International, St. Louis, Missouri) in meal form. Feed and municipal water were provided *ad libitum*. Drinking water obtained from the municipal water source was periodically analyzed for chemical parameters and biological contaminants by the municipal water department.	
	Environmental conditions:	**Temperature:**	22 ± 1°C (one exception when the temperature was 36.4°C)
		Humidity:	40-70% (one exception when the relative humidity was 22.1%)
		Air changes:	12-15 times/hour
		Photoperiod:	12-hour light/dark
	Acclimation period:	13 days prior to the start of the study.	

B. STUDY DESIGN:

1. **In life dates:** Start: April 14-15, 2003 – October 12-14, 2004 (males) and October 15, 18, and 19, 2004 (females)

2. **Animal assignment/dose levels:** Animals were stratified by pre-exposure body weight and then randomly assigned to treatment groups using a computer program. Animals placed on study were uniquely identified via subcutaneously implanted transponders (BioMedic Data Systems, Seaford, Delaware), which were correlated to unique alphanumeric identification numbers.

TABLE 1: Study design main study 18 months

Test group	Nominal Dose (mg/kg/day)	Number of Animals/Sex	Actual Dose (mg/kg/day)	
			Male	Female
Control	0	50	0	0
Low (LDT)	10	50	10	10
Mid (MDT)	100	50	100	101
High (HDT)	1000	50	932	1012

3. **Dose selection:** The high dose was chosen based on results of a 90-day dietary mouse study (MRID 46908351). The high dose also represents the maximum or limit dose specified in USEPA OPPTS 870.4200 guidelines (1998). The mid- and low-dose levels were expected to provide dose-response data for any treatment-related effects observed in the high-dose group.

4. **Diet preparation and analysis:** Diets were prepared by serially diluting a concentrated test material-feed mixture (premix) with ground feed. Premixes were mixed periodically

throughout the study based on stability data. Initial concentrations of test material in the diet were calculated from historical body weights and food consumption data. Subsequently, the concentrations of the test material in the feed were adjusted weekly for the first 13 weeks of the study and at 4-week intervals thereafter, based upon the most recent body weight and food consumption data.

The homogeneity of the low-dose female and the high-dose male diets was determined pre-exposure, and during months 4, 8, 12, and 16. The method used for analyzing the test material in feed was a solvent extraction method followed by analysis using liquid chromatography-mass spectrometry (LC-MS) and solvent standards incorporating an internal standard.

Analyses of all dose levels, plus control and premix, were conducted pre-exposure and at approximately 4, 8, 12, and 16 months.

Results:

Homogeneity analysis: The homogeneity of XDE-742 in rodent feed was determined on five separate mixing batches (mixed pre-exposure and at 4, 8, 12, and 16 months) for the 10 mg/kg/day female and 1000 mg/kg/day male test diets, the lowest and highest concentrations used in the study. The diets were homogeneously mixed, with relative standard deviations for all diets sampled between 1.27% and 10.8%.

Stability analysis: Stability data was completed in the 28-day feeding study in rats (MRID 46908548). XDE-742 was shown to be stable for at least 36 days in the feed at concentrations ranging from 0.005 – 5%, which encompasses the concentrations used in this study. Therefore, additional stability data were not generated for this study.

Concentration analysis: The concentrations of XDE-742 were determined for the control, premix, and test diets from all treatment levels on five separate mixing batches (mixed pre-exposure and at 4, 8, 12, and 16 months). Mean analyzed concentrations for the premix and each dose level ranged from 91.3% (premix) to 105% (low-dose males) of the targeted concentration, which were considered acceptable. Analytical results of the individual samples varied between 80.2-114% of the target concentration of XDE-742. The 80.2% of target concentration value was outside the laboratory's acceptable range of ± 15%. However, this value was determined for the premix while the analyzed concentrations of the diets prepared from this premix and fed to the animals were 103-114% of target and within the laboratory's acceptable range.

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

5. **Statistics:** Means and standard deviations were calculated for all continuous data. Body weights, feed consumption, organ weights, and total WBC counts were evaluated by Bartlett's test for equality of variances (alpha = 0.01). Based on the outcome of Bartlett's test, exploratory data analyses were performed by a parametric or nonparametric analysis of variance (ANOVA). If the ANOVA was significant at alpha = 0.05, it was followed,

respectively, by Dunnett's test or the Wilcoxon Rank-Sum test with a Bonferroni correction for multiple comparisons to the control. The experiment-wise alpha level of alpha = 0.05 was reported for Dunnett's test and Wilcoxon Rank-Sum test. Detailed clinical observation (DCO) incidence scores were statistically analyzed by a z-test of proportions comparing each treated group to the control group at alpha = 0.05. Descriptive statistics only (means and standard deviations) were reported for body weight gains, feed efficiency, and differential WBC counts. Statistical outliers were identified by a sequential test (alpha = 0.02), but routinely excluded only from feed consumption statistics. Outliers may have been excluded from other analyses only for documented, scientifically sound reasons.

Gross pathologic observations were tabulated and considered in the interpretation of final histopathologic data, but were not evaluated statistically. The cumulative incidence of histopathologic observations for all animals scheduled for the terminal sacrifice was used in the statistical analysis. For tissues where all animals in all dose groups were scheduled to be examined, the incidences of specific histopathologic observations were first tested for deviation from linearity (alpha = 0.01) using ordinal spacing of the doses. If linearity was not rejected, the data was then tested for a linear trend using the Cochran-Armitage Trend test. If the trend was statistically significant at alpha = 0.02, or if significant deviation from linearity was found, incidences for each dose group were compared to that of the control group using a pairwise Chi-square test with Yates' continuity correction (alpha = 0.05, two-sided). For tissues that were evaluated from all control and high-dose animals, but only from selected animals in the low- and intermediate-dose groups, statistical analysis consisted of the pairwise comparisons of control and high dose using the pairwise Chi-square test with Yates' continuity correction (alpha = 0.05, two-sided). Rare tumors, those with a background incidence of less than or equal to 1%, were considered significant in the Chi-square test with Yates' continuity correction at alpha = 0.10, two-sided.

Differences in mortality patterns were tested by the Gehan-Wilcoxon procedure for all animals scheduled for terminal sacrifice.

C. METHODS:

1. Observations:

1a. Cageside observations: A cage-side examination was conducted at least once a day, preferably at the same time each day (usually in the morning). The animals were not hand-held for these observations unless deemed necessary. Significant abnormalities that could be observed included, but were not limited to: decreased/increased activity, repetitive behavior, vocalization, incoordination/limping, injury, neuromuscular function (convulsion, fasciculation, tremor, twitches), altered respiration, blue/pale skin and mucous membranes, severe eye injury (rupture), alterations in fecal consistency, and fecal/urinary quantity. Moribund animals not expected to survive until the next observation period were humanely euthanized that day. Any animals found dead were necropsied as soon as was practical. In addition, all animals were observed for morbidity, mortality, and the availability of feed and water at least twice daily

1b. Clinical examinations: Detailed clinical observations (DCO) were conducted on the first ten surviving animals/sex/dose level at approximately the same time each examination day. Observations were conducted according to an established format at baseline and monthly for 12 months, and then at 15 and 18 months. Examinations included cage-side, hand-held, and open-field observations that were recorded by category or using explicitly defined scales (ranked).

Clinical examinations (consisting of the categorical portion of the DCO) were conducted on all animals once a month from months 9-18. This examination included a careful, hand-held evaluation of the skin, fur, mucous membranes, respiration, nervous system function (including tremors and convulsions), and animal behavior. Animals were observed for general behavior and appearance, respiration, nervous system function (including tremors and convulsions) and any other signs of clinical toxicity. In addition, all animals were examined for unusual swelling or palpable masses concurrent with this hand-held clinical or detailed clinical observations. The time of onset, location, dimensions, appearance, and progression of each palpable mass were recorded.

2. **Body weight:** All mice were weighed during the pre-exposure period, weekly during the first 13 weeks of the study and at approximately monthly intervals thereafter. Body weight gains were calculated throughout the study.

3. **Food consumption and compound intake:** Food consumption was determined pre-exposure, weekly during the first 13 weeks of the study and at approximate monthly intervals thereafter for all animals by weighing food containers at the start and end of a measurement cycle. Consumption was calculated using the following equation:

$$\text{Food consumption (g/day)} = \frac{\text{(initial weight of feed container – final weight of feed container)}}{\text{(\# of days in measurement cycle) (\# of animals per cage)}}$$

Food Efficiency: Food efficiencies were calculated using mean body weight gains and mean feed consumption data from the first 13 weeks of the study using the following equation:

$$\text{Food efficiency} = \frac{\text{(g feed consumed/day)}}{\text{(g body weight gain/day)}}$$

Compound Intake: The actual test material intake (TMI) was calculated upon completion of the study using test material concentrations in the feed, actual body weights (BW) and measured feed consumptions using the following equation:

$$TMI = \frac{\left(\text{feed consumption}\left(\frac{\text{g}}{\text{day}}\right)\right) * (1000\ \text{mg/g}) * \frac{\text{(\% of test material in feed)}}{100}}{\frac{\left(\frac{\text{Current BW [g]} + \text{Previous BW [g]}}{2}\right)}{1000\ \text{g/kg}}}$$

4. **Ophthalmoscopic examination:** The eyes of all animals were examined by a veterinarian pre-exposure and prior to the scheduled necropsy using indirect ophthalmoscopy. One drop

of 0.5% tropicamide ophthalmic solution was instilled in each eye to produce mydriasis prior to the indirect ophthalmic examination. Eyes were also examined by a prosector during necropsy using a moistened glass slide pressed to the cornea.

5. **Hematology and clinical chemistry:**

a. **Hematology:** Blood smears were made from all surviving animals via sample collection from the pedal vein (12 months) or orbital sinus (18 months). A white blood cell (WBC) count and differential WBC count were determined from all animals at the terminal sacrifice (18 months) using an Advia 120 Hematology Analyzer (Bayer Corporation, Tarrytown, New York). Blood from moribund animals, anesthetized with CO_2, was obtained from the orbital sinus or tail vein. Blood smears were not obtained from animals that died spontaneously. A differential WBC count, as derived from the blood smears, was not determined from the 12-month samples or animals that were moribund due to the absence of effects in the mice surviving to 18 months.

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

X = parameter examined
* Minimum required for carcinogenicity studies (Control and HDT unless effects are observed) based on Guideline 870.4200 & OECD 451

b. **Clinical chemistry:** Clinical chemistry is not required for carcinogenicity studies based on Guideline 870.4200 & OECD 451 and was not examined as part of this study.

6. **Urinalysis:** Urinalysis is not required for carcinogenicity studies based on Guideline 870.4200 & OECD 451 and was not examined as part of this study.

7. **Sacrifice and pathology:** A complete necropsy was conducted on all animals. Non-fasted mice were anesthetized by the inhalation of CO_2, weighed, and blood samples obtained from the orbital sinus. Their tracheas were exposed and clamped, and the animals were euthanized by decapitation. A gross pathological examination was conducted and the checked (X) tissues were collected for histological examination. The (XX) organs, in addition, were weighed. Tissues were examined histopathologically from all controls and high dose animals and all animals that died or were sacrificed in moribund condition. The liver, lungs, kidneys, ovaries, and relevant gross lesions were examined histopathologically from mice in the low and middle dose groups from the scheduled necropsy.

	DIGESTIVE SYSTEM		CARDIOVASC./HEMAT.		NEUROLOGIC
X	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* (Mediastinal/Mesenteric)	X	Pituitary*
X	Duodenum*	X	Tissues (Mediastinal/Mesenteric)	X	Eyes (retina, optic nerve)*
X	Jejunum*	XX	Spleen*+		**GLANDULAR**
X	Ileum*	X	Thymus	XX	Adrenal gland*+
X	Cecum*			X	Lacrimal gland
X	Colon*		**UROGENITAL**	X	Mammary gland*
X	Rectum*	XX	Kidneys*+	X	Parathyroids*
XX	Liver*+	X	Urinary bladder*	X	Thyroids*
X	Gall bladder*	XX	Testes*+		**OTHER**
X	Pancreas*	XX	Epididymides*+	X	Bone
	RESPIRATORY	X	Prostate*	X	Skeletal muscle
X	Trachea*	X	Seminal vesicle*/ Coagulating glands	X	Skin*
X	Lung*++	XX	Ovaries*+	X	All gross lesions and masses*
X	Nose*	XX	Uterus*+	X	Oral tissues
X	Pharynx*	X	Cervix	X	Auditory sebaceous glands
X	Larynx*	X	Oviducts		
		X	Vagina		

X = parameter examined, XX = parameter weighed
* Required for carcinogenicity studies based on Guideline 870.4200.
+Organ weight required in carcinogenicity studies.
++Organ weight required if inhalation route.

II. RESULTS:

A. OBSERVATIONS:

1. **Clinical signs of toxicity:** There were no treatment-related effects on the detailed clinical observations due to ingestion of XDE-742 at any dose level.

There were no cage-side, clinical, palpable mass or detailed clinical observations ascribed to ingestion of XDE-742. The most common observation was dermatitis, which was first observed for males on day 57 and for females on day 120 (results shown in Table 1). Dermatitis occurred in all dose groups, including controls, and the incidence gradually, but irregularly, progressed from 1 or 2 mice per dose level to ≤ 20% near the end of the study. The dermatitis was commonly noted initially at the margin of the ears (pinnas). In some mice the inflammation remained localized to the ear while in others it progressed to adjacent sites. According to the study report, in some mice the dermatitis healed normally, while in others the distal portion of the pinna gradually was lost prior to the ulcerated area being covered by

scar tissue (these were termed "missing ears – sloughed" on the clinical examination and "inflammation, healed" at necropsy). Progressive dermatitis initially involving the pinna with progression to adjacent sites has been reported, particularly in CD-1 mice, and has been commonly found in the performing laboratory in oncogenicity studies with this strain. Late in the study, more mice given XDE-742 were noted to have dermatitis than the controls, but this was considered to be a non-treatment related variability due to: 1) the lack of a dose response in females; 2) the higher incidence was for all anatomic sites combined whereas there were smaller differences at the various anatomic sites; 3) the final incidence after necropsy and histopathologic examination did not have a dose-responsive pattern; and 4) dermatitis is a common spontaneous occurrence in CD-1 mice.

Table 1. Incidence of Dermatitis in Male and Female Mice *

Days on Study	Dose (mg/kg/day)			
	0	10	100	1000
Males				
57	0	0	0	2
113	1	3	1	2
169	0	3	2	1
197	0	3	2	1
253	0	2	0	2
309	5	4	6	3
365	4	3	6	4
421	3	4	6	6
505	1	5	6	10
547	7	4	4	8
Females				
120	-	1	-	-
169	0	0	0	0
225	0	1	0	0
253	0	1	0	0
309	0	1	4	4
365	0	5	3	3
421	0	6	3	2
477	0	4	4	5
533	2	2	4	8

- data not reported

*Data were selected from Tables 7 (males) and 8 (females) on pages 66 and 76 of the study report, respectively. Intervals were selected based upon the time of first occurrence and at approximate 50 day intervals thereafter.

2. **Mortality:** The mortality rates at the end of the study were 22, 20, 20, and 24% for males and 22, 28, 20, and 20% for females in the control, 10, 100, and 1000 mg/kg/day groups, respectively. There were no treatment-related statistically identified differences in the overall moribundity/ mortality in male or female mice given XDE-742 when compared to the control animals.

B. **BODY WEIGHT:** There were no treatment-related effects in male or female body weights or body weight gains at any dose level.

TABLE 2: Mean body weights (BW) and body weight gains (BWG)[a]				
g±SD	0	10	100	1000
MALES initial BW	28.8 ± 1.8	28.0 ± 2.0	28.6 ± 1.8	28.3 ± 1.8
Day 8	31.0 ± 2.0	30.4 ± 2.0	30.9 ± 1.9	30.7 ± 2.1
Day 92	40.6 ± 3.3	40.5 ± 3.9	41.6 ± 5.0	39.0 ± 3.1
Day 204	44.0 ± 4.4	45.2 ± 5.7	46.4 ± 6.1	43.4 ± 4.7
Day 316	46.0 ± 5.2	46.9 ± 6.6	48.0 ± 6.2	44.6 ± 5.5
Final BW Day 547	45.6 ± 5.4	46.0 ± 7.2	47.3 ± 5.1	43.9 ± 5.0
BWG Day 1 -8	2.2 ± 0.7	2.4 ± 1.0	2.3 ± 0.8	2.4 ± 0.7
BWG Day 1-92	11.8 ± 2.2	12.5 ± 3.3	13.0 ± 4.2	10.7 ± 2.2
BWG Day 92-204	3.4	4.7	4.8	4.4
BWG Day 204-316	2.0	1.7	1.6	1.2
BWG Day 316-547	-0.4	-0.9	-0.7	-0.7
Overall BWG Days 1-547	16.8 ± 4.7	17.9 ± 6.4	18.6 ± 4.8	15.6 ± 4.6 (↓7)
FEMALES initial BW	22.1 ± 1.3	22.0 ± 1.4	22.1 ± 1.4	21.4 ± 1.6
Day 8	24.0 ± 1.5	23.6 ± 1.4	23.4 ± 1.6	23.3 ± 1.6
Day 92	30.3 ± 2.9	30.5 ± 2.7	30.4 ± 3.4	29.4 ± 2.6
Day 204	34.0 ± 3.6	34.4 ± 2.8	34.5 ± 4.4	33.4 ± 3.2
Day 316	35.8 ± 4.1	35.8 ± 3.7	36.0 ± 4.9	35.1 ± 3.9
Final BW Day 547	37.8 ± 4.6	38.2 ± 4.6	39.1 ± 5.7	37.7 ± 5.1
BWG Day 1-8	1.9 ± 0.8	1.7 ± 0.8	1.4 ± 0.8	1.9 ± 0.6
BWG Day 1-92	8.1± 2.7	8.5 ± 2.2	8.5 ± 2.6	8.0 ± 1.9
BWG Day 92-204	3.7	3.9	4.1	4.0
BWG Day 204-316	1.8	1.4	1.5	1.7
BWG Day 316-547	2.0	2.4	3.1	2.6
Overall BWG Days 1-547	15.6 ± 4.7	16.3 ± 4.0	17.0 ± 5.4	16.3 ± 4.5

( ) = Percent of control
[a] Data obtained from pages 84-93 in the study report.

C. FOOD CONSUMPTION AND COMPOUND INTAKE:

1. **Food consumption:** There were no effects on feed consumption that were attributed to XDE-742. Mean feed consumption of males given XDE-742 was often statistically identified as higher than controls throughout the study, particularly the high-dose level. However, these differences were considered spurious as the changes were minor and there were no body weight effects. Mean feed consumption of female mice was comparable to controls with only a few statistically identified differences, which varied as to the dose level affected and whether the differences were increased or decreased.

2. **Compound consumption:** The mean XDE-742 consumptions over the course of the study were 0, 10, 100, or 932 mg/kg/day (0, 86, 860, or 7982 ppm) for males and 0, 10, 101, or 1012 mg/kg/day (0, 62, 593, or 5891 ppm) for females in the control, low-, intermediate- and high-dose groups, respectively.

3. **Food efficiency:** There were no treatment related effects on food efficiency.

D. OPHTHALMOSCOPIC EXAMINATION: The eyes of all mice were within normal limits at the pre-exposure examination. Prior to study termination (day 542), ophthalmic examinations indicated low incidences (0-5) of cloudy/opaque cornea, irregular corneal surface, cloudy lens, and pale fundus. These observations were found in all groups without a clear dose response and were not considered treatment related.

E. BLOOD ANALYSES:

1. **Hematology:** Neither the mean total WBC counts nor the differential WBC counts were affected by ingestion of XDE-742.

2. **Clinical chemistry:** Not applicable

F. URINALYSIS: Not applicable

G. SACRIFICE AND PATHOLOGY:

1. **Organ weight:** Effects attributed to ingestion of XDE-742 were limited to the 1000 mg/kg/day dose level and included increased absolute and relative mean liver weights of males and decreased absolute and relative mean kidney weights of both sexes given this high-dose level (summarized in Table 3), all of which were statistically significant except the relative kidney weight of males. These organ weights were also outside the laboratory historical control range of recent 18-month studies using CD-1 mice. The absolute and relative liver weights of males given 1000 mg/kg/day were increased 26.4% and 31.6% above controls. The liver weights of males given 100 mg/kg/day and females given 1000 mg/kg/day were almost identical to, or slightly less than, their respective controls.

   The absolute and relative kidney weights of male and female mice given 1000 mg/kg/day were decreased 6.2% - 11.8% from controls, but were not considered adverse due to lack of corroborating changes in histopathology.

   Absolute and relative mean ovary weights of females given 100 or 1000 mg/kg/day XDE-742 and the absolute uterine weight of females in the 100 mg/kg/day dose group were significantly increased compared to control. There is no clear dose response in absolute or relative ovary weights. The absolute and relative ovary weights of the control, low, and high dose groups all fall below the historical control range, while the mid-dose group exceeded or was near the upper end of the historical control range. The absolute uterine weights did show a dose response; however, all the dose groups fell within the historical control range.

TABLE 3. Organ Weight Effects in CD-1 Mice Given XDE-742[1]

	Dose Level (mg/kg/day)				
	Historical Controls[2]	0	10	100	1000
Parameter	**Males**				
Final Body Weight (g)	39.6–48.2	45.6	46.0	47.1	44.1
Absolute Liver (g)	2.161–2.491	2.411	2.412	2.490	3.048$ (↓26.4)
Relative Liver (g/100g bw)	5.215–5.536	5.284	5.277	5.298	6.955$ (↓31.6)
Absolute Kidneys (g)	0.842–0.884	0.875	0.842	0.854	0.791* (↓10)
Relative Kidneys (g/100g bw)	1.857–2.128	1.932	1.845	1.824	1.812 (↓6)
	Females				
Final Body Weight (g)	35.5–38.4	37.4	37.9	38.4	36.7
Absolute Liver (g)	-	2.145	2.118	2.136	2.034
Relative Liver (g/100g bw)	-	5.748	5.574	5.529	5.555
Absolute Kidneys (g)	0.548–0.663	0.569	0.538	0.553	0.502* (↓12)
Relative Kidneys (g/100g bw)	1.440–1.834	1.539	1.429	1.445	1.378* (↓10)
Absolute Ovaries (g)	0.074–0.161	0.034	0.052	0.185$ (↑444)	0.068$ (↑100)
Relative Ovaries (g/100g bw)	0.197–0.438	0.091	0.141	0.369$ (↑305)	0.186$ (↑104)
Absolute Uterus (g)	0.919-1.837	1.042	1.502	1.459$ (↑40)	1.832 (↑76)

- data not available

* Statistically different from control mean by Dunnett's test, alpha = 0.05.

$ Statistically different from control mean by Wilcoxon's test, alpha = 0.05.

[1] Data obtained from Text Table 6 on page 32 and Table 24 on page 108 of the study report.

[2] Range of control values from four 18-month dietary oncogenicity studies necropsied between 12/2001 and 5/2004 in this laboratory.

2. **Gross pathology:** The only observation suggestive of a response to treatment was an increased incidence of "Mass-Nodule" of the liver in males given 1000 mg/kg/day (Table 4). Twelve males given 1000 mg/kg/day were noted to have one or more masses at necropsy vs. six controls. Additionally, male mice given 1000 mg/kg/day tended to have

more than one hepatic mass/nodule with seven having multiple gross hepatic masses vs. two controls with multiple masses. Gross masses were not all primary hepatocellular neoplasms (*i.e.,* liver involvement by hemangioma, hemangiosarcoma and lymphosarcoma may also have been diagnosed as "Mass-Nodule" at necropsy); thus final interpretation was dependent upon histopathologic examination.

All groups of females given XDE-742 had an increased incidence of ovarian cysts than controls but the incidence was similar across the dose range from 10 to 1000 mg/kg/day and was not considered treatment related. Historical control data was not provided for ovarian cysts.

Table 4. Summary of Gross Pathologic Observations in CD-1 Mice Given XDE-742[1]

	Dose Level (mg/kg/day)							
	Males				Females			
Organ/Observation	0	10	100	1000	0	10	100	1000
Liver (number examined)	50	50	50	50	50	50	50	50
Mass/Nodule; any lobe, any descriptor, any size, - one	4	6	5	5	1	3	0	2
- two	1	2	1	3	0	0	1	0
- three	1	0	1	4	0	0	0	0
- four	0	1	0	0	0	0	0	0
Total Mice with Mass/ Nodule; any lobe, any descriptor, any size, any number	6	9	7	12	1	3	1	2
Ovaries (number examined)	-	-	-	-	50	50	50	50
No visible Lesions	-	-	-	-	31	23	19	17
Cyst, unilateral	-	-	-	-	8	10	13	13
Cyst, bilateral	-	-	-	-	10	17	15	16

[1] Data obtained from Text Table 7 on page 33 of the study report and Table 25 on page 116 of the study report.

3. **Microscopic pathology:**

a. Non-Neoplastic: Foci of altered cells were categorized by the cytoplasmic staining of the majority of the cells in the focus. Apparent treatment-related increases in the numbers of clear (vacuolated) cell foci (statistically significant) and lesser increases in the numbers of mixed or eosinophilic cell foci occurred in males given 1000 mg/kg/day. Foci of altered cells are relatively uncommon in control CD-1 mice, with the historical control incidence data presented in Table 6 below. The incidence of foci of altered cells in the liver of male mice given 10 or 100 mg/kg/day and females from all dose levels was low and similar to controls.

All groups of females given XDE-742 had incidences of uterine hyperplasia. Although there were 11 instances of moderate hyperplasia in the 1000 mg/kg/day dose group compared to 5 in the control, this was not statistically identified. There is no clear trend of increasing severity of uterine hyperplasia; it is considered neither treatment-related nor adverse. Historical control data was not provided.

Table 5. Non-Neoplastic Findings in CD-1 Mice Given XDE-742

	Dose Level (mg/kg/day)							
	Males				Females			
	0	10	100	1000	0	10	100	1000
Liver (number examined)	50	50	50	50	50	50	50	50
Focus of Altered Cells, hepatocyte, - basophilic, one or more	1	2	1	2	0	2	0	1
- clear, one or more	0	0	0	7*	0	1	1	0
- eosinophilic, one or more	0	0	1	3	1	0	1	1
- mixed, one	2	1	0	5	1	0	0	0
Number of Mice with Focus of Altered Cells, hepatocyte, any descriptor, any number, (total)	2	3	2	12*	2	3	2	2
Number of Mice with a Focus of Altered Cells, any descriptor, and a primary hepatocyte tumor (Adenoma and/or Carcinoma)[a]	1	0	1	7	0	0	0	0
Hyperplasia	0	0	0	0	1	0	0	0
Hypertrophy, centrilobular/midzonal (very slight-slight)	23	19	19	28	3	2	4	4
Necrosis, hepatocyte focal (very slight)	2	0	4	4	3	2	4	1
Vacuolization, hepatocyte centrilobular/midzonal	4	1	2	6	0	0	0	1
Uterus, Hyperplasia, cystic endometrial	-	-	-	-	41	38	43	43
Very slight	-	-	-	-	24	16	18	20
Slight	-	-	-	-	12	18	21	11
Moderate	-	-	-	-	5	3	4	11
Severe	-	-	-	-	0	1	0	1

* Statistically significant difference by Yates Chi-Square, alpha = 0.05, two-sided.

[a] Not statistically analyzed.

Data obtained from Text Table 8 on page 35 and Table 26 on page 172 of the study report.

Table 6. Historical Control Values: Foci of Cellular Alteration in the Liver of Male CD-1 Mice from 18-Month Dietary Carcinogenicity Studies

Organ/Observation	Study			
	A	B	C	D
Liver (number examined)	50	50	50	50
Focus of Cellular Alteration, basophilic, hepatocyte, one	1[a]	0	1	0
Focus of Cellular Alteration, clear, hepatocyte, one	0	0	0	0
Focus of Cellular Alteration, eosinophilic, hepatocyte, one	0	0	0	3[b]
Focus of Cellular Alteration, mixed, hepatocyte, one	1[a]	0	0	0

Study A necropsied 12/2001; Study B necropsied 05/2003; Study C necropsied 12/2003; Study D necropsied 04-05/2004.
[a] Graded as focal, very slight rather than counted.
[b] Diagnosed as combined category of 1-5 foci.
Data obtained from Text Table 9 on page 35 of the study report.

b. Neoplastic: Hepatocellular tumors, both adenomas and carcinomas, were increased in males given 1000 mg/kg/day, although the differences were not statistically identified as there was no clear dose-response relationship (the trend test p value was 0.0716 for total mice with adenomas and 0.0669 for total mice with adenoma and/or carcinoma). The incidence of mice with hepatic adenomas and/or carcinomas in the control males, as well as those given 100 mg/kg/day, was similar to historical controls (Table 8).

Although the incidence of mice with hepatocellular adenomas was not statistically identified, the incidence in males receiving 1000 mg/kg/day exceeded historical controls provided in the study report and many of the affected high-dose mice had multiple hepatocellular tumors. Seven of the fourteen high-dose male mice with adenomas had multiple adenomas (Table 7). Additionally, three high-dose male mice had hepatocellular carcinomas in addition to one or more adenomas (#03A1534, one adenoma; #03A1525, two adenomas; and #03A1542, three adenomas). While multiple hepatic tumors were particularly common in males given 1000 mg/kg/day, they were also noted in all other dose levels including controls. One control male had six hepatic adenomas (along with basophilic and mixed cell foci), five males given 10 mg/kg/day had two adenomas and one from this dose group had five, while two from the 100 mg/kg/day dose group had multiple hepatic tumors (three adenomas in #03A1487 and two adenomas and two carcinomas in #03A1486). Multiplicity of hepatocellular tumors is relatively uncommon in historical control male CD-1 mice in the test laboratory (Table 8) with two adenomas found in a single control male mouse in two studies and one mouse that had one adenoma and one carcinoma in another study.

Despite the increased incidence and multiplicity of hepatocellular tumors in males given 1000 mg/kg/day, these liver tumors apparently arose late in the study and did not result in early mortality. Fourteen of the 15 males from the high-dose group with hepatocellular tumors survived until the scheduled necropsy. The only animal given 1000 mg/kg/day with a

hepatocellular tumor that was removed early from study was euthanized in moribund condition late in the study (day 533). This mouse (#03A1534) had one hepatocellular adenoma and one carcinoma but the cause of death was renal amyloidosis. Two deaths in males (weeks 73 and 77) of the 100 mg/kg/day dose group were attributed to hepatocellular carcinoma.

The incidence of hepatocellular tumors in mice given 100 mg/kg/day was similar to controls while the incidence of hepatocellular adenomas was increased in males given 10 mg/kg/day. This increase was considered spurious biological variation due to the lack of a dose-response relationship in the males given 100 mg/kg/day. In contrast to the other hepatic effects noted in the males given 1000 mg/kg/day, both the mean liver weights and the incidence of foci of altered cells were similar to controls in both the low- and intermediate-dose levels. There were no liver effects in females given up to 1000 mg/kg/day that were attributed to treatment. The mean liver weights of females from all dose levels were almost identical to controls and the incidence of both foci of altered cells and hepatocellular adenomas was low and similar to controls.

The CARC concluded, however, that the tumors in the low- and high-dose groups were not treatment-related due to the highly variable background levels of liver tumors in the CD-1 mouse, especially the males. A study published in 2005 by Charles River stated the historical control ranges for adenomas and carcinomas in male CD-1 mice from 52 separate studies were 3-28% and 2-16%, respectively. This indicates that liver tumors in male mice are fairly common and highly variable, suggesting that liver tumors in mice be held to a higher statistical standard of $p<0.01$, instead of $p<0.05$. The liver adenomas in the low- and high-dose groups in this study are at the high-end of the historical control range published by Charles River, but do not significantly exceed it. The historical control data also showed that liver tumors are more common in male mice than in female mice. Other points taken into consideration were 1) the lack of a clear dose response, 2) SAR – none of the other chemicals in this pesticide class are linked to liver tumors, 3) pyroxsulam is not mutagenic, 4) the mouse metabolism study indicated a dose-response in internal exposure, but there was no clear dose-response in tumors, 5) there was no increase in basophilic foci, which is more commonly linked to tumor formation than clear cell foci, and 6) there was no tumor response in female mice.

Table 7. Neoplastic Histopathologic Observations in Livers of CD-1 Mice Given XDE-742

	Dose Level (mg/kg/day)							
	Males				Females			
Organ/Observation	0	10	100	1000	0	10	100	1000
Liver (number examined)	50	50	50	50	50	50	50	50
Adenoma, hepatocyte, benign, primary - one	3	7	7	7	3	1	0	1
- two	1	5	1	1	0	0	0	0
- three	0	0	1	5	0	0	0	0
- four	0	0	0	1	0	0	0	0
- five	0	1	0	0	0	0	0	0
- six	1	0	0	0	0	0	0	0

Total mice with Adenoma, hepatocyte, total (one or more)	5	13	9	14	3	1	0	1
Carcinoma, hepatocyte, malignant without metastasis, - one	1	0	1	4	0	0	0	0
- two	0	0	1	0	0	0	0	0
Total mice with Adenoma (any number) and/or Carcinoma (any number)	6	13	10	15	3	1	0	1

Data obtained from Text Table 10 on page 37 of the study report.

Table 8. Historical Control Values: Primary Hepatocellular Neoplasms in Male CD-1 Mice from 18-Month Dietary Oncogenicity Studies

Organ/Observation	Study			
	A	B	C	D
Liver (number examined)	50	50	50	50
Adenoma, hepatocyte, benign, primary - one	8	1	5	7
Adenoma, hepatocyte, benign, primary - two	0	1	0	1
Carcinoma, hepatocyte, malignant without metastasis - one	2	1	0	1
Carcinoma, hepatocyte, malignant with metastasis - one	1	0	0	0
Total Mice with Adenoma and/or Carcinoma	10	3	5	9

Study A necropsied 12/2001; Study B necropsied 05/2003; Study C necropsied 12/2003; Study D necropsied 04-05/2004.
Data obtained from Text Table 11 on page 38 of the study report.

III. DISCUSSION AND CONCLUSIONS:

A. <u>INVESTIGATORS' CONCLUSIONS</u>: CD-1 mice given diets providing up to 1000 mg XDE-742/kg/day for 18 months tolerated the dosing well with no adverse in-life effects attributed to treatment. Parameters without adverse treatment-related effects included clinical observations, survival, body weight, feed consumption, and ophthalmologic examinations. Total and differential WBC counts of mice from the scheduled termination were also not affected by XDE-742.

Males given 1000 mg/kg/day had a higher incidence and number of hepatic "Mass-Nodules" observed at necropsy and the mean absolute and relative liver weights of this dose group were increased and statistically identified. Histopathologically, males given 1000 mg/kg/day had a greater incidence of foci of altered hepatocytes and increased incidence and multiplicity of hepatocyte adenomas and/or carcinomas that were attributed to ingestion of XDE-742. However, the increased tumor incidence was moderate and the difference from controls was not statistically identified. The hepatic adenomas and carcinomas did not appear to occur

early in the study as all but one (Day 533) were found in mice surviving to the scheduled termination. The incidence of hepatocellular tumors in males given 100 mg/kg/day was similar to concurrent and historical controls, but those given 10 mg/kg/day were greater than the controls. The incidence of hepatocellular adenomas in males given 10 mg/kg/day was therefore not dose related and was not statistically identified, not accompanied by liver weight increases nor increased incidence of foci of altered cells. Thus, the increase in males given 10 mg/kg/day was interpreted to be unrelated to treatment (*i.e.*, biological variation). The only other treatment-related effect noted at 1000 mg/kg/day was slightly decreased absolute and relative mean kidney weights, present in both males and females. However, this was not accompanied by treatment-related histopathologic effects and was regarded as toxicologically not adverse.

Thus, under conditions of this study, ingestion of 1000 mg XDE-742/kg/day caused increased liver weights, increased number of foci of altered hepatocytes and increased incidence and numbers of hepatocyte adenomas and carcinomas only in male mice. The no-observed-effect level was 100 mg/kg/day for both males and females. The only treatment-related effect in females given 1000 mg/kg/day was slightly decreased kidney weight and this level was considered a no-observed-adverse-effect level for females.

B. REVIEWER COMMENTS: There were no effects of XDE-742 consumption with regards to mortality, clinical examinations, body weights and body weight gains, or food consumption. There were no effects related to treatment for either ophthalmic examinations or total and differential WBC counts.

Treatment-related effects occurred in the liver of male mice given 1000 mg/kg/day, with the mean absolute and relative liver weights increased by 26.4% and 31.6%, respectively, and histopathologically increased incidence of foci of altered cells (hepatocytes, males only).

The tumors in the low- and high-dose groups were not considered treatment-related due to the highly variable background levels of liver tumors in the CD-1 mouse, especially the males. A study published in 2005 by Charles River stated the historical control ranges for adenomas and carcinomas in male CD-1 mice from 52 separate studies were 3-28% and 2-16%, respectively. This indicates that liver tumors in male mice are fairly common and highly variable, suggesting that liver tumors in mice be held to a higher statistical standard of $p<0.01$, instead of $p<0.05$. The liver adenomas in the low- and high-dose groups in this study are at the high-end of the historical control range published by Charles River, but do not significantly exceed it. The historical control data also showed that liver tumors are more common in male mice than in female mice. Other points taken into consideration were 1) the lack of a clear dose response, 2) SAR – none of the other chemicals in this pesticide class are linked to liver tumors, 3) pyroxsulam is not mutagenic, 4) the mouse metabolism study indicated a dose-response in internal exposure, but there was no clear dose-response in tumors, 5) there was no increase in basophilic foci, which is more commonly linked to tumor formation than clear cell foci, and 6) there was no tumor response in female mice.

The **LOAEL is 1000 mg/kg bw/day, based on increased absolute and relative liver weights and increased incidence of clear cell foci of alteration (hepatocytes) in males.** The **NOAEL is 100 mg/kg bw/day. There were no treatment-related, adverse effects in females.**

The registrant, Dow Chemical Company, originally prepared this STUDY PROFILE TEMPLATE (STP) (MRID 46908603) in HED's DER format. The HED reviewers may have added minor adjustments/additions to the original STP. The conclusions of the study and assignment of its classification as determined by HED reviewers are in the Executive Summary above.

C. STUDY DEFICIENCIES:

None

EPA Reviewer: Kimberly Harper **Signature:** Kimberly D Harper
RAB2, Health Effects Division (7509P) **Date:** 12/12/07
EPA Secondary Reviewer: Alan Levy **Signature:** Alan C. Levy
RAB2, Health Effects Division (7509P) **Date:** 1-3-2008

Template version 02/06

TXR#: 0054347

DATA EVALUATION RECORD

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

PC CODE: 108702 **DP BARCODE:** D332276

TEST MATERIAL (PURITY): (N-(5,7-dimethoxy[1,2,4]triazolo[1,5-a]pyrimidin-2-yl)-2-methoxy-4-(trifluoromethyl)pyridine-3-sulfonamide), XDE-742 (98.0%)

SYNONYMS: BAS-770H, XR-742, X666742

CITATION: Stebbins, K. E., and K. J. Brooks. XDE-742: two-year chronic toxicity/oncogenicity and chronic neurotoxicity study in Fischer 344 rats. Toxicology & Environmental Research and Consulting, The Dow Chemical Company, Midland, Michigan. Study ID: 031014, 02 November 2005. MRID 46908407. Unpublished

SPONSOR: Dow AgroSciences LLC, 9330 Zionsville Road, Indianapolis, Indiana 46268

EXECUTIVE SUMMARY: This study (MRID 46908407) was conducted to evaluate the potential chronic toxicity and oncogenicity of XDE-742 (N-(5,7-dimethoxy[1,2,4]triazolo[1,5-a]pyrimidin-2-yl)-2-methoxy-4-(trifluoromethyl)pyridine-3-sulfonamide) to rats. Groups of 65 male and 65 female Fischer 344 rats were fed diets formulated to provide 0, 10, 100, or 1000 mg/kg/day. Ten rats/sex/dose were necropsied after one year (chronic toxicity group), five rats/sex/dose were necropsied after one year (chronic neurotoxicity group), and the remaining 50 rats/sex/dose were fed the respective diets for up to two years and necropsied (oncogenicity group). The chronic neurotoxicity study has been reported separately (MRID 46908411).

There were no treatment related adverse effects on mortality, clinical signs, ophthalmology, hematology, clinical chemistry, histopathology.

There were no body weight/body weight gain effects in males at any dose. Females given 1000 mg/kg/day had treatment-related statistically identified lower mean body weights at most time-points when compared to controls. At 12 and 24 months, body weight gains for females given 1000 mg/kg/day were 7-8% lower than controls. The decrement in body weight gain was interpreted to be a non-adverse effect, because the lower weights did not worsen during the second year of the study, and the body weights at most time-points throughout the study were within historical control ranges. Feed consumption for females administered 1000 mg/kg/day was statistically identified as lower than controls between test days 8 through 84. This decrement in feed consumption was interpreted to be treatment-related, and corresponded to the

lower body weights. For the remainder of the study, the feed consumption of females given 1000 mg/kg/day was comparable to controls at most time-points. There were no treatment-related effects on body weights or feed consumption of females given 10 or 100 mg/kg/day.

Treatment-related changes in organ weights consisted of higher mean absolute (4.1%) and relative (8.8%) liver weights in males given 1000 mg/kg/day at 12 months only, and higher mean absolute (6%) and relative (11%) liver weights in females given 1000 mg/kg/day at 24 months. The higher relative liver weights were statistically identified as different from controls. The liver weight changes were interpreted to be non-adverse, based on the lack of any corresponding clinical pathologic or histopathologic liver effects.

No treatment-related increase in neoplasms was observed in either male or female rats at any dose level, indicating that XDE-742 did not have an oncogenic potential under the conditions of this study

A LOAEL was not observed in this study. The no-observed-adverse-effect level (NOAEL) was 1000 mg/kg/day for both sexes.

This chronic/carcinogenicity study in the rats is acceptable and satisfies the guideline requirement for a chronic/carcinogenicity study (OPPTS 870.4300); OECD 453 in rats.

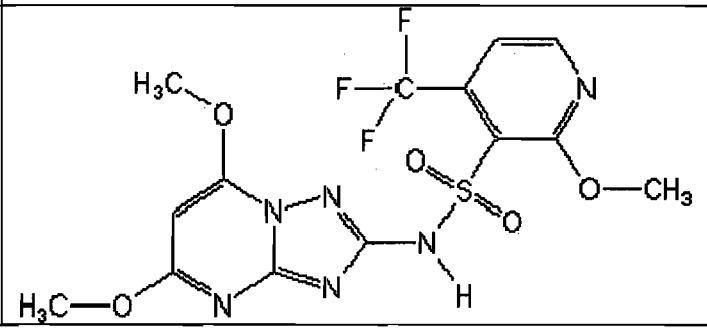
COMPLIANCE: Signed and dated GLP, Quality Assurance and Data Confidentiality statements were provided.

United States Environmental Protection Agency, Health Effects Test Guidelines, OPPTS 870.4300 (Combined Chronic Toxicity/Carcinogenicity) EPA712-C-98-212, August 1998, with the exception that ten oncogenicity animals were evaluated monthly for the first 12 months and quarterly from 12-24 months (rather than weekly detailed clinical observations on all animals for two years.) This modification was reviewed and accepted by the USEPA for a similar combined chronic toxicity/oncogenicity study (memorandum from Dr. W. F. Sette, Toxicology Branch, Health Effects Division, to J. I. Miller, Herbicide Branch, Registration Division. 19 July 2001). Organisation for Economic Co-Operation and Development. *OECD Guideline for the Testing of Chemicals*, Guideline 453 (Combined Chronic Toxicity/Carcinogenicity Studies), 12 May 1981, with the exception that ten (rather than twenty) rats/sex/dose group were used for clinical pathology evaluations.

I. MATERIALS AND METHODS:

A. MATERIALS:

1.	**Test Material:**	XDE-742
	Description:	Powder, white
	Lot/Batch #:	E0952-52-01; TSN103826
	Purity:	98.0% XDE-742
	Compound Stability:	A previous 28-day toxicity study with Fischer 344 rats demonstrated that XDE-742 was stable for at least 36 days in the feed at

		concentrations ranging from 0.005% to 5%. This range spanned the diet concentrations used in this study; therefore additional stability data was not obtained.
	CAS #:	422556-08-9
	Structure:	

2. **Vehicle and/or positive control:** LabDiet® Certified Rodent Diet #5002 (PMI Nutrition International, St. Louis, Missouri).

3.	**Test animals:**		
	Species:	Rats	
	Strain:	Fischer 344	
	Age/weight at study initiation:	Approximately 6 weeks of age Body weights (g) on study day 1: males 125.8-127.4, females 106.1-106.5	
	Source:	Charles River Laboratories Inc. (Raleigh, North Carolina)	
	Housing:	Animals were housed two per cage in stainless steel cages for the entire study. The male animals were transferred to larger cages after one year to meet individual space requirements. Cages had wire-mesh floors and were suspended above catch pans. Cages contained feed crocks and pressure activated, nipple-type watering systems.	
	Feed and Water:	Animals were provided LabDiet® Certified Rodent Diet #5002 (PMI Nutrition International, St. Louis, Missouri) in meal form. Feed and municipal water were provided *ad libitum*. Analyses of the feed were performed by PMI Nutrition International to confirm the diet provided adequate nutrition and to quantify the levels of selected contaminants. Drinking water obtained from the municipal water source was periodically analyzed for chemical parameters and biological contaminants by the municipal water department. In addition, specific analyses for chemical contaminants were conducted at periodic intervals by an independent testing facility. There were no contaminants found in either the feed or water that would adversely impact the results of this study.	
	Environmental conditions:	Temperature: Humidity: Air changes: Photoperiod:	22 ± 1°C (test day 182/181 for males/females), the temperature was 28.9°C 40-70% (test day 182/181 for males and females), the relative humidity was 35.5% 12-15 times/hour 12-hour light/dark

	Acclimation period:	Approximately two weeks prior to the start of the study.

B. STUDY DESIGN:

1. **In life dates:** Test material administration began April 10, 2003 (males) and April 11, 2003 (females). The chronic toxicity animals were necropsied on April 12 and 13, 2004 (test day 369) for males and females, respectively, and the neurotoxicity animals were necropsied during April 13-16 (test days 370-373 for males and test days 369-372 for females). All surviving male and female oncogenicity rats were necropsied during April 11-15, 2005 (April 11, 13, and 14 or test days 733, 735 and 736 for males and April 12, 14, and 15 or test days 733, 735, and 736 for females).

2. **Animal assignment/dose levels:** Animals were stratified by pre-exposure body weight and then randomly assigned to treatment groups using a computer program. Animals placed on study were uniquely identified via subcutaneously implanted transponders (BioMedic Data Systems, Seaford, Delaware), which were correlated to unique alphanumeric identification numbers (Table 1).

Test Group	Nominal Dose (mg/kg/day)	Actual Ave. Dose M/F (mg/kg/day)	Onco. Study 24 Months		Chronic Study 12 Months		Neuro Study 12 Months	
			Male	Female	Male	Female	Male	Female
Control	0	0/0	50	50	10	10	5	5
Low (LDT)	10	10.1/10.2	50	50	10	10	5	5
Mid (MDT)	100	101.0/101.6	50	50	10	10	5	5
High (HDT)	1000	1012/1018	50	50	10	10	5	5

3. **Dose selection:** The high-dose (limit test) was chosen based on results of the 90-day dietary rat study. The mid- and low-dose levels were expected to provide dose-response data for any treatment-related effects observed in the high-dose group. The highest dose tested was the limit dose for this type of study (1000 mg/kg/day).

4. **Diet preparation and analysis:** Diets were prepared by serially diluting a concentrated test material-feed mixture (premix) with ground feed. Premixes were mixed periodically throughout the study based on stability data. Initial concentrations of test material in the diet were calculated from historical body weights and feed consumption data. Subsequently, the concentrations of the test material in the feed were adjusted weekly for the first 13 weeks of the study and at 4-week intervals thereafter, based upon the most recent body weight and feed consumption data.

   The homogeneity of the low-dose female and the high-dose male diets were determined prior to the start of dosing and at approximately 4, 8, 12, 18, and 23 months. For analyzing the test material in feed, a solvent extraction method was followed by analysis using liquid chromatography-mass spectrometry (LC-MS) and solvent standards incorporating an internal standard.

Analyses of all dose levels, plus control and premix, were determined pre-exposure and at approximately 4, 8, 12, 18, and 23 months.

Results:

Homogeneity analysis: The homogeneity of XDE-742 in rodent feed was determined on six separate mixing batches (mixed prior to study start and at approximately 4, 8, 12, 18, and 24 months) for the 10 mg/kg/day female and 1000 mg/kg/day male test diets, the lowest and highest concentrations used in the study. The diets were homogeneously mixed, with relative standard deviations for all diets sampled between 1.40% and 4.98%.

Stability analysis: Stability of XDE-742 was determined for at least 36 days in the feed at concentrations ranging from 0.005 to 5%.

Concentration analysis: The concentrations of XDE-742 were determined for the control, premix, and test diets from all treatment levels on six separate mixes (mixed prior to study start and at approximately 4, 8, 12, 18 and 23 months) and were found to be acceptable. Mean concentrations for each dose level for the six time-points ranged from 97.4-105% of the targeted concentrations. Analytical results varied from 92.9% to 109% of the target concentration of XDE-742 for each individual sample. XDE-742 was not found in control feed at any time.

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

5.. **Statistics:** Means and standard deviations were calculated for all continuous data. Body weights, food consumption, organ weights, urine volume, urine specific gravity, clinical chemistry, coagulation, and appropriate hematologic data were evaluated by Bartlett's test for equality of variances (alpha = 0.01). Based on the outcome of Bartlett's test, exploratory data analyses were performed by a parametric or non-parametric analysis of variance (ANOVA). If the ANOVA was significant at alpha = 0.05, it was followed, respectively, by Dunnett's test or the Wilcoxon Rank-Sum test with a Bonferroni correction for multiple comparisons to the control. Since the multiple comparisons to control are not orthogonal, a correction needs to be made to control the type one error rate. The Bonferroni correction can be used whether the tests are independent or not. The experiment-wise alpha level of 0.05 was reported for Dunnett's test and Wilcoxon Rank-Sum test. DCO incidence scores were statistically analyzed by a z-test of proportions comparing each treated group to the control group at alpha = 0.05. Descriptive statistics only (means and standard deviations) were reported for body weight gains, RBC indices, and differential WBC counts. Statistical outliers were identified by a sequential test (alpha = 0.02), but routinely excluded only from food consumption statistics. Outliers may have been excluded from other analyses only for documented, scientifically sound reasons.

For tissues where all animals in all dose groups were scheduled to be examined, the incidences of specific histopathologic observations were first tested for deviation from linearity (alpha = 0.01) using ordinal spacing of the doses. If linearity was not rejected, the data was then tested for a linear trend using the Cochran-Armitage Trend test. If the trend was statistically significant at alpha = 0.02 or if significant deviation from linearity was found, incidences for each dose group

were compared to that of the control group using a pairwise Chi-square test with Yates' continuity correction (alpha = 0.05, two-sided). For tissues evaluated from all control-dose and high-dose rats but only from selected rats in the intermediate-dose groups, statistical analysis consisted of the pairwise comparisons of control and high-dose groups using the pairwise Chi-square test with Yates' continuity correction (alpha = 0.05, two-sided). Rare tumors, those with a background incidence of less than or equal to 1%, were considered significant in the Chi-square test with Yates' continuity correction at alpha = 0.10, two-sided.

Differences in mortality patterns were tested by the Gehan-Wilcoxon procedure for all animals scheduled for terminal sacrifice. There was no significant effect noted (alpha = 0.05) and, therefore, mortality adjusted analyses were not conducted.

C. METHODS:

1. Observations:

1a. Cageside observations: A cage-side (general) clinical examination was conducted at least once a day, preferably at the same time each day (usually in the morning). This examination was performed with the animals in their cages and was designed to: 1) detect significant clinical abnormalities that were clearly visible upon a limited examination, and 2) to monitor the general health of the animals. Significant clinical abnormalities observed could have included, but were not limited to: activity, repetitive behavior, vocalization, incoordination/lameness, injury, neuromuscular function (convulsion, fasciculation, tremor and twitches), altered respiration, blue/pale skin and mucous membranes, severe eye injury (rupture), fecal consistency, and fecal/urinary quantity. Moribund animals not expected to survive until the next observation period were humanely euthanized that day. Any animal found dead was necropsied as soon as was practical. At least twice daily, usually at the beginning and end of each day, animals were also observed for morbidity and mortality and the availability of feed/water.

1b. Clinical examinations: A complete detailed clinical observation (DCO) was conducted pre-exposure and monthly for 12 months, then at 15, 18, 21, and 24 months on the first ten surviving animals from the oncogenicity group. Baseline (day 1) clinical observations were conducted on all animals not receiving DCOs. Palpable tumor observations (categorical observations) were conducted monthly from months 12-24 on all animals. Observations were conducted according to an established format and at approximately the same time each examination day. Examinations included cage-side, hand-held and open-field observations recorded categorically or using explicitly defined scales (scored). Categorical observations (detailed and palpable tumor), clinical, and cage-side non-scheduled observations in which only positive findings were documented, were summarized collectively as clinical observations.

1c. Neurological evaluations: Neurological parameters were examined on a subset of animals in this study at the midway point of the exposure period. The methods and results are presented independently (see MRID 46908411).

2. **Body weight:** The rats were weighed during the pre-exposure period, weekly during the first 13 weeks of the study and then at approximately monthly intervals during the remainder of the study. Body weight gains were calculated throughout the study.

3. **Food consumption and compound intake:** Food consumption data were collected pre-exposure, weekly during the first 13 weeks of the study and then at approximate monthly intervals thereafter for all animals. Food containers were weighed at the start and end of a measurement cycle and consumption was calculated using the following equation:

$$\text{Food consumption (g/day)} = \frac{\text{(initial weight of feed container – final weight of feed container)}}{\text{(\# of days in measurement cycle) (\# of animals per cage)}}$$

Test material intake (TMI) was calculated for 0-12 months and 12-24 months using test material concentrations in the feed, actual body weights (BW) and measured feed consumption.

$$TMI = \frac{(\text{feed consumption}\left(\frac{\text{g}}{\text{day}}\right)) * (1000\ \text{mg/g}) * \frac{(\%\ \text{of test material in feed})}{100}}{\frac{\left(\frac{\text{Current BW [g]} + \text{Previous BW [g]}}{2}\right)}{1000\ \text{g/kg}}}$$

4. **Ophthalmoscopic examination:** The eyes of all animals were examined by a veterinarian pre-exposure and prior to the scheduled necropsy using indirect ophthalmoscopy. One drop of 0.5% tropicamide ophthalmic solution was instilled in each eye to produce mydriasis prior to the indirect ophthalmic examinations. A prosector also examined eyes during necropsy using a moistened glass slide pressed to the cornea.

5. **Hematology and clinical chemistry:** Blood samples were collected from the orbital sinus of fasted animals anesthetized with isoflurane or CO_2. Samples were taken from the ten rats/sex/dose of the chronic toxicity group at three and six months (after the FOB testing) and at 12 months. At 18 and 24 months, blood samples were taken from the first ten surviving rats/sex/dose group. The checked (X) parameters were examined.

a. **Hematology:** Blood samples were mixed with ethylenediamine-tetraacetic acid (EDTA). Blood smears were prepared, stained with Wright's stain and archived for potential future evaluation, if warranted. Hematologic parameters were assayed using a Technicon H•1E Hematology Analyzer (Bayer Corporation, Tarrytown, New York).
At 24 months, blood was collected and blood smears were prepared on all surviving rats from the oncogenicity group at the scheduled necropsy. Total white blood cell counts and differential white blood cell counts were determined for all surviving rats. No blood samples were obtained from animals that died prior to the end of the dosing period. Blood was collected and blood smears prepared from all moribund rats. Smears were stained, cover-slipped, and archived.

Blood samples were collected in sodium citrate tubes, centrifuged, and plasma collected and assayed using an ACL9000 (Instrumentation Laboratory, Lexington, Massachusetts).

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*		
	(Thromboplastin time)		
	(Clotting time)		
X	(Prothrombin time)		

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

b. **Clinical chemistry:** Serum was separated from cells as soon as possible following blood collection. Serum parameters were measured using a Hitachi 914 Clinical Chemistry Analyzer (Boehringer-Mannheim, Indianapolis, Indiana).

	ELECTROLYTES		OTHER
X	Calcium	X	Albumin*
X	Chloride	X	Blood creatinine*
	Magnesium	X	Blood urea nitrogen*
X	Phosphorus	X	Total Cholesterol*
X	Potassium*		Globulins
X	Sodium*	X	Glucose*
	ENZYMES (more than 2 hepatic enzymes)*	X	Total bilirubin
X	Alkaline phosphatase (ALK)*	X	Total serum protein (TP)*
	Cholinesterase (ChE)		Triglycerides
	Creatine phosphokinase		Serum protein electrophores
	Lactic acid dehydrogenase (LDH)		
X	Serum alanine amino-transferase (ALT/ SGPT)*		
X	Serum aspartate amino-transferase (AST/ SGOT)*		
	Gamma glutamyl transferase (GGT)*		
	Glutamate dehydrogenase		

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

6. **Urinalysis:** Urine was collected from all surviving, non-fasted rats from the chronic toxicity group at 3, 6, and 12 months (after the FOB testing) and from the first ten surviving rats/sex/dose group from the oncogenicity group at 18 and 24 months. Animals were housed in metabolism cages and urine collected overnight (approximately 16 hours).

X	Appearance*	X	Glucose*
X	Volume*	X	Ketones
X	Specific gravity / osmolality*		Bilirubin
X	pH*	X	Blood*

X	Sediment (microscopic)		Nitrate
X	Protein*	X	Urobilinogen

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

7. **Sacrifice and pathology**: Fasted rats were anesthetized by the inhalation of CO_2 and weighed; blood samples were obtained from the orbital sinus. The animals were euthanized by decapitation.

A complete necropsy was conducted on all animals by a veterinary pathologist assisted by a team of trained individuals. All animals that died and those sacrified on schedule were subjected for histological evaluation [note: tongue and auditory sebaceous glands were grossly examined and preserved]. A gross pathological examination was conducted and the checked (X) tissues were collected for histological examination. 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* (Mediastinal/Mesenteric)	X	Pituitary*
X	Duodenum*	X	Tissues (Mediastinal/Mesenteric)	X	Eyes (retina, optic nerve)*
X	Jejunum*	XX	Spleen*+		GLANDULAR
X	Ileum*	X	Thymus	XX	Adrenal gland*+
X	Cecum*			X	Lacrimal gland
X	Colon*		UROGENITAL	X	Mammary gland*
X	Rectum*	XX	Kidneys*+	X	Parathyroids*
XX	Liver*+	X	Urinary bladder*	X	Thyroids*
	Gall bladder*	XX	Testes*+	X	Coagulating Glands
X	Pancreas*	XX	Epididymides*+		OTHER
	RESPIRATORY	X	Prostate*	X	Bone
X	Trachea*	X	Seminal vesicle*	X	Skeletal muscle
X	Lung*++	XX	Ovaries*+	X	Skin*
X	Nose*	XX	Uterus*+	X	All gross lesions and masses*
X	Pharynx*	X	Cervix	X	Oral Tissues
X	Larynx*	X	Vagina		Auditory Sebaceous Glands
		X	Oviducts		

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

II. RESULTS:

A. OBSERVATIONS:

1. **Clinical signs of toxicity:** The only treatment-related clinical observation was an increased incidence of perineal urine soiling in males and females given 100 or 1000 mg/kg/day. The increased urine soiling was first noted during months 4 (males) and 5 (females) at the 1000 mg/kg/day dose level, and during months 16 (males) and 15 (females) at the 100 mg/kg/day dose level. This finding was consistent with treatment-related perineal soiling noted in animals given 1000 mg/kg/day from the previously conducted XDE-742 90-day dietary toxicity study in Fischer 344 rats. The urine soiling was interpreted to be a non-adverse effect, based on the lack of any corresponding histopathologic urinary tract effects and the absence of alterations in urinalysis parameters.

   Masses were infrequently found on the categorical portion of DCO examinations through the first 12 months of study. As the study progressed, the incidence of palpable masses increased; however, there was no evidence of a dose-response relationship in the incidence of palpable masses. The ultimate disposition of palpable masses was addressed following the

gross and histopathologic examination of these lesions.

2. **Mortality:** After 24 months, there were no statistically- identified differences in mortality for either males or females.
3. **Neurological evaluations:** The results of the neurological evaluation are presented in MRID 46908411.

B. BODY WEIGHT: Females given 1000 mg/kg/day had a treatment-related decrease in mean body weights for most of the study (Table 2). The decreased body weights were statistically identified on test day 29 through test day 680. At 12 months (test day 365), the mean body weight for females given 1000 mg/kg/day was 4.4% lower than controls. At 24 months (test day 729), the mean body weight for females given 1000 mg/kg/day was 4.5% lower than controls. This decrement in body weight was interpreted to be a non-adverse effect, because the lower weights did not worsen during the second year of the study, and the body weights at most of the time-points throughout the study were within historical control ranges of dietary or oral gavage toxicity studies performed recently at this laboratory. There were no treatment-related effects on body weights of females given 10 or 100 mg/kg/day.

Differences in body weights were also reflected in lower body weight gains for females given 1000 mg/kg/day. At 12 months (day 365), the mean body weight gain for females given 1000 mg/kg/day was 8% lower than controls. The decrease in body weight gain continued until study termination, at which time females given 1000 mg/kg/day were 7% lower than controls. The decrement in body weight gain was interpreted to be a non-adverse effect, which did not result in any decline in the clinical condition of the animals, and did not impact the assessment of oncogenicity potential. There were no treatment-related effects on body weight gains of females given 10 or 100 mg/kg/day, nor of males from any dose group.

Table 1. Body Weights of Fischer 344 Rats Given XDE-742 – Selected Intervals

	Dose Level (mg/kg/day)				
Test Day/Week	0	Historical[1]	10	100	1000
	Males (g)				
1/0	126.2 ± 6.3	NR	127.4 ± 6.1	126.4 ± 6.6	125.8 ± 7.0
29/4	245.5 ± 11.7	NR	251.2 ± 10.9	251.3 ± 10.5	247.5 ± 14.2
92/13	338.0 ± 15.3	NR	340.0 ± 15.2	338.9 ± 15.9	334.2 ± 20.9
204/29	401.1 ± 18.3	NR	400.3 ± 17.2	397.8 ± 19.2	391.9 ± 23.9
259/37	419.8 ± 19.5	NR	419.6 ± 17.8	417.1 ± 20.4	413.6 ± 25.2
365/52	449.1 ± 21.5	NR	447.9 ± 20.2	446.4 ± 23.9	444.3 ± 27.1
540/77	465.6 ± 24.1	NR	453.3 ± 21.2	457.8 ± 27.4	452.5 ± 23.8
729/104	418.2 ± 37.2	NR	429.4 ± 25.1	417.5 ± 36.5	428.8 ± 35.3
BWG W1-13	211.7 ± 13.4	NR	212.6 ± 13.1	212.6 ± 14.4	208.3 ± 17.8
BWG W1-52	322.8 ± 19.5	NR	320.6 ± 18.6	320.4 ± 22.7	318.5 ± 24.5
BWG W1-77	338.5 ± 21.5	NR	325.8 ± 20.5	330.8 ± 26.9	325.9 ± 22.4
BWG W1-104	292.0 ± 37.6	NR	300.7 ± 26.2	291.0 ± 37.0	302.2 ± 36.2
	Females (g)				
1/0	106.4 ± 5.1	103.9 - 118.5	106.5 ± 5.1	106.2 ± 5.4	106.1 ± 5.0
29/4	152.2 ± 7.0	144.2 – 161.8	152.1 ± 6.8	150.9 ± 7.1	148.4 ± 6.9*
92/13	188.3 ± 9.9	175.6 – 190.3	188.6 ± 8.4	187.8 ± 8.5	180.2 ± 9.2* (↓4)
204/29	211.4 ± 10.8	200.2 – 209.7	209.4 ± 10.5	208.2 ± 10.0	200.6 ± 9.8* (↓5)
260/37	219.7 ± 11.2	208.9 – 221.5	218.3 ± 11.3	216.6 ± 10.9	209.7 ± 10.7* (↓5)
365/52	235.0 ± 11.7	223.3 – 236.2	234.9 ± 14.9	233.9 ± 14.6	224.7 ± 12.5* (↓4)
540/77	275.1 ± 19.6	261.1 – 276.6	272.3 ± 23.3	271.9 ± 20.5	257.1 ± 19.1* (↓7)
729/104	295.9 ± 26.3	282.7 – 299.5	294.9 ± 26.5	293.3 ± 29.4	282.7 ± 25.8 (↓4)
BWG W1-13	81.9 ± 7.5	NR	82.1 ± 7.2	81.6 ± 6.2	74.1 ± 7.1 (↓10)
BWG W1-52	128.6 ± 10.3	NR	128.5 ± 13.0	127.5 ± 12.3	118.6 ± 10.4 (↓8)
BWG W1-77	168.5 ± 18.8	NR	165.9 ± 21.6	165.6 ± 20.7	151.4 ± 17.6 (↓10)
BWG W1-104	189.8 ± 25.7	NR	188.2 ± 24.4	186.9 ± 29.1	177.0 ± 24.9 (↓7)

Data obtained from Tables 17 and 18 on pages 110-116 and 117-123 of the study report.
NR – Not reported, ( ) - % difference compared to control
* Statistically Different from Control Mean by Dunnett's Test, alpha = 0.05.
[1]Historical controls group mean range from four studies conducted between 2002 and 2005, using data from the closest test days to those represented in the current study.

C. FOOD CONSUMPTION AND COMPOUND INTAKE:

1. **Food consumption:** Feed consumption for females administered 1000 mg/kg/day was statistically identified as lower than controls at all time-points between test days 8 through 84. This early decrement in feed consumption in females given 1000 mg/kg/day was interpreted to be treatment-related, and was associated with lower body weight and body weight gain. However, for the remainder of the study, the feed consumption of females given 1000 mg/kg/day was comparable to controls at most time-points. There were no treatment-related effects on feed consumption for females given 10 or 100 mg/kg/day or males at any dose level.

2. **Compound consumption (time-weighted average):** Over the course of the study, male rats from the low-, middle-, and high-dose groups received acceptable time-weighted average doses of 10.1, 101.0, and 1012 mg/kg/day, respectively; female rats from the low-, middle-,

and high-dose groups received acceptable time-weighted average doses of 10.2, 101.6, and 1018 mg/kg/day, respectively.

3. **Food efficiency:** Not Reported

D. **OPHTHALMOSCOPIC EXAMINATION:** Pre-exposure examination of all rats placed on study indicated they were all within normal limits. Variable numbers of male and female rats were observed with ocular hemorrhage, pale fundus, cloudy and/or vascularized cornea, opaque lens, periocular soiling, phthisis bulbi and/or missing eye at the 12- and 24-month intervals. Periocular soiling was considered to be a non-specific clinical sign that was unrelated to treatment. Eyes with pale fundus, opaque lens, or cloudy/vascularized cornea were considered to be spontaneous, age-related changes. The ocular hemorrhage, missing eyes, and phthisis bulbi were secondary to blood collection via the orbital sinus. All ophthalmic observations were interpreted to be unrelated to treatment due to their low incidence and lack of a dose-response relationship.

E. **BLOOD ANALYSES:**

1. **Hematology:** There were no treatment-related alterations in prothrombin times or hematologic parameters at any dose level. Males and females given 1000 mg/kg/day had minimal decreases in mean red blood cell counts and hematocrits at most of the sampling intervals, relative to controls (Table 2). The lower red blood cell counts and hematocrits for this dose level were only statistically identified at 6 months. These alterations were interpreted to be unrelated to treatment because the lower values did not significantly progress during the study, there was not a clear dose-response for many of the sampling intervals, and the majority of the values were within the historical control ranges of oral toxicity studies performed recently at the laboratory.

Table 2. Red Blood Cell and Hematocrit Parameters

Red Blood Cell Count (10^6/µl)	Males Dose (mg/kg/day)				
	0	Historical Controls	10	100	1000
3 months	8.60	8.27 – 9.19 [a]	8.60	8.64	8.46
6 months	8.83	8.60 – 9.28 [b]	8.60*	8.62	8.51*
12 months	9.06	8.70 – 8.87 [c]	8.87	8.88	8.75
18 months	8.52	8.11 – 8.66 [c]	8.73	8.56	8.33
24 months	6.87	6.65 - 7.80 [c]	8.48	7.00	7.63
Red Blood Cell Count (10^6/µl)	Females Dose (mg/kg/day)				
	0	Historical Controls	10	100	1000
3 months	7.93	7.42 – 8.27 [a]	7.81	7.73	7.64
6 months	7.86	7.41 – 8.14 [b]	7.87	7.85	7.52*
12 months	8.50	7.68 – 8.11 [c]	8.48	8.38	8.26
18 months	7.99	7.80 – 8.24 [c]	7.75	8.19	7.95
24 months	8.22	7.18 – 7.91 [c]	7.92	8.16	7.38
Hematocrit (%)	Males Dose (mg/kg/day)				
	0	Historical Controls	10	100	1000
3 months	40.2	41.6 – 46.3 [a]	40.1	40.4	39.9
6 months	43.0	42.7 – 44.2 [b]	41.9	42.3	41.9
12 months	46.9	43.0 – 44.5 [c]	45.9	46.2	45.7
18 months	42.5	40.2 – 43.1 [c]	43.5	42.8	41.9
24 months	41.1	37.7 – 42.0 [c]	48.1	40.2	43.1
Hematocrit (%)	Females Dose (mg/kg/day)				
	0	Historical Controls	10	100	1000
3 months	38.7	39.7 – 43.7 [a]	38.9	38.4	38.0
6 months	40.7	39.7 – 41.4 [b]	41.5	41.2	39.6*
12 months	45.9	41.2 – 43.4 [c]	46.0	45.7	44.9
18 months	42.0	40.8 – 42.4 [c]	41.3	42.9	41.5
24 months	47.1	39.9 – 42.5 [c]	45.8	46.1	42.0

Data collected from Text Table 4 on page 38 of the study report.
* Statistically different from control mean by Dunnett's Test, alpha = 0.05.
[a] Range from nine male and ten female studies conducted between 2001 and 2005.
[b] Range from three studies conducted between 2000 and 2005.
[c] Range from three studies conducted between 2002 and 2005.

2. **Clinical Chemistry:** Males given 1000 mg/kg/day had lower mean ALT activities and higher cholesterol concentrations at all sampling intervals (with the exception of lower cholesterol at 24 months). These values were statistically identified at 3, 6, and 12 months, and were interpreted to be treatment-related (Table 3). Toxicologically significant alterations in ALT are usually manifested by an increase, rather than a decrease, in this parameter. Although the mean cholesterol concentrations of males given 1000 mg/kg/day were significantly higher than controls during the first 12 months of the study, the values were within or only slightly outside the historical control ranges from recently conducted oral toxicity studies of this laboratory. There were no other treatment-related alterations in the clinical chemistry or electrolyte parameters of males or females at any dose level.

TABLE 3. Clinical Chemistry Parameters

ALT (u/l)	Males Dose (mg/kg/day)				
	0	Historical Controls	10	100	1000
3 months	54	52 – 73 [a]	61	60	44[$]
6 months	101	85 – 94 [b]	93	96	65*
12 months	113	80 – 107 [c]	95	96	70*
18 months	67	71 – 83 [c]	85	62	57
24 months	122	55 – 104 [c]	55	68	54
Cholesterol (mg/dl)	Males Dose (mg/kg/day)				
	0	Historical Controls	10	100	1000
3 months	47	51 – 68 [a]	49	52	62*
6 months	56	69 – 80 [b]	59	64	74*
12 months	76	85 – 91 [c]	83	83	93*
18 months	113	108 – 127 [c]	111	122	128
24 months	201	149 – 164 [c]	131*	176	150*

Data obtained from Text Table 5 on page 39 of the study report.

* Statistically different from control mean by Dunnett's Test, alpha = 0.05.

$ Statistically different from control mean by Wilcoxon's Test, Alpha = 0.05.

[a] Range from nine studies conducted between 2001 and 2005.

[b] Range from three studies conducted between 2000 and 2005.

[c] Range from three studies conducted between 2002 and 2005.

F. URINALYSIS: There were no treatment-related alterations in urinalysis parameters at any dose level.

G. SACRIFICE AND PATHOLOGY:

1. **Organ weight:** The only treatment-related changes in organ weights at 12 months were higher mean absolute (4.1%) and relative (8.8%) liver weights in males given 1000 mg/kg/day (Table 4). The higher relative liver weight was statistically identified as different from controls ($p<0.05$). The increased absolute and relative liver weights were interpreted to be a non-adverse effect, based on the lack of any corresponding adverse clinical pathologic or histopathologic effects.

   The only treatment-related changes in organ weights at 24 months were higher mean absolute (6.1%) and relative (10.9%) liver weights in females given 1000 mg/kg/day (Table 4). The higher relative liver weight was statistically identified as different from controls. The increased absolute and relative liver weights were interpreted to be a non-adverse effect, based on the lack of any corresponding clinical pathologic or histopathologic effects.

Table 4. Selected organ weights for rats exposed to pyroxsulam for 12 and 24 months in their diet.

	Dose (mg/kg/day) – 12 months				
	0	Historical[1]	10	100	1000
Parameter	Males				
Final Body Weight (g)	421.4	NR	426.2	413.9	403.2
Absolute Liver (g)	10.987	10.388 - 10.968	11.119	10.676	11.436
Relative Liver (g/100g bw)	2.606	2.519 - 2.545	2.615	2.581	2.836*
Parameter	Females				
Final Body Weight (g)	219.5	218.0 - 218.5	219.5	216.7	206.4
Absolute Liver (g)	6.755	NR	5.567	5.448	5.602
Relative Liver (g/100g bw)	3.119	NR	2.538	2.513	2.715
	Dose (mg/kg/day) – 24 months				
	0	Historical[1]	10	100	1000
Parameter	Males				
Final Body Weight (g)	390.2	NR	401.5	393.8	397.4
Absolute Liver (g)	12.737	NR	12.404	12.422	12.809
Relative Liver (g/100g bw)	3.288	NR	3.102	3.159	3.244
	Females				
Final Body Weight (g)	273.5	274.0 - 278.9	273.9	274.8	260.3
Absolute Liver (g)	7.504	7.547 - 7.650	7.536	7.675	7.960
Relative Liver (g/100g bw)	2.761	2.730 - 2.811	2.758	2.803	3.062$

Data obtained from Tables 98-99 on pages 206-209 and Tables 103-104 on pages 235-238 of the study report.
*Statistically Different from Control Mean by Dunnett's Test, Alpha = 0.05.
$Statistically Different from Control Mean by Wilcoxon's Test, Alpha = 0.05.
[1]Historical controls group mean range from three studies conducted between 2002 and 2005.

2. **Gross pathology:** There were no treatment related findings at 12 months.

The only treatment-related gross pathologic observation was an increased incidence of perineal soiling in males given 100 or 1000 mg/kg/day, and in females given 1000 mg/kg/day. This finding was considered non-adverse due to the absence of alterations in urinalysis parameters.

3. **Microscopic pathology:**

a. **Non-neoplastic:** The only treatment-related histopathologic alteration noted at 12 months was a decreased incidence of basophilic foci of altered hepatocytes in females given 1000 mg/kg/day. The number of females with 1 to 5 basophilic foci of altered hepatocytes was 8, 7, 5, and 1 for the control, 10, 100 and 1000 mg/kg/day dose groups, respectively.

The only treatment-related histopathologic alteration in animals from the 24-month sacrifice was a decrease in the incidence of basophilic foci of altered hepatocytes, noted in males and females given 1000 mg/kg/day (Table 5). For males given 1000 mg/kg/day, the decrement was noted in the number of animals with 6 – 10 basophilic foci in the three standard sections of liver examined microscopically. In females given 1000 mg/kg/day, the decrement was noted in the number of animals with 11 – 20, and 21 or more basophilic foci. There was a concommitant increase in the number of females given 1000 mg/kg/day with 1 – 5 basophilic foci, which was reflective of the overall trend of fewer basophilic foci per animal in this dose group. Experimental models suggest that some foci may be precursors of hepatocellular

neoplasms. The lower incidence of basophilic foci was interpreted to be non-adverse, because toxicologically significant changes in basophilic foci are usually associated with an increased incidence.

Table 5. Histopathologic Liver Effects – 24 months

Sex	Males				Females			
Dose (mg/kg/day)	0	10	100	1000	0	10	100	1000
Number of Rats	50	50	50	50	50	50	50	50
Liver: Focus of basophilic hepatocytes; 1-5	25	15	18	17	2	1	4	21*
Liver: Focus of basophilic hepatocytes; 6-10	13	19	16	1*	7	8	9	14
Liver: Focus of basophilic hepatocytes; 11-20	4	3	0	0	18	23	17	4*
Liver: Focus of basophilic hepatocytes; 21 or more	0	0	0	0	19	14	14	0*

Data were obtained from Text Table 9 on page 45 of the study report.
*Statiscally identified by Yate's Chi-square test, alpha = 0.05, two-sided.

b. Neoplastic: There were no statistically-identified changes in the number of neoplasms for males or females at any dose level. This indicates that XDE-742 showed no carcinogenic potential under the conditions of this study. A few neoplasms were increased in incidence but not statistically identified (Table 6). Males given 1000 mg/kg/day had an increased incidence of hepatocellular adenoma, and females given 1000 mg/kg/day had an increased incidence of parafollicular cell adenomas of the thyroid gland. Neither of these neoplasms were treatment-related, because their incidence was within historical control ranges of dietary or oral gavage toxicity studies performed recently at this laboratory. Males given 100 or 1000 mg/kg/day had increased incidences of large granular lymphocyte (Fischer rat) leukemia. The increased incidence of leukemia was interpreted to be unrelated to treatment because of the lack of statistical significance, and the comparable or lower incidence of leukemia in females at all dose levels, relative to controls. Although the incidence of leukemia in males given 100 or 1000 mg/kg/day was outside the historical control range of dietary or oral gavage toxicity studies performed recently at this laboratory, it was within historical control ranges of 32 to 74% in male Fischer 344 rats from studies conducted by the National Toxicology Program (NTP). Data from the NTP indicate a highly variable incidence of Fischer rat leukemia in untreated animals used in two-year carcinogenicity studies.

Table 6. Selected Neoplastic Observations – 24 Months

Dose (mg/kg/day)	0	Historical[1]	10	100	1000
Number of rats	50	50 - 55	50	50	50
	MALES				
Hematopoietic/Lymphoid System: Leukemia, large granular lymphocyte, malignant, primary	20	12 - 19	20	28	29
Liver: Adenoma, hepatocyte, benign, primary	1	0 - 6	3	3	4
	FEMALES				
Hematopoietic/Lymphoid System: Leukemia, large granular lymphocyte, malignant, primary	12	8 - 12	6	8	11
Thyroid Gland: Adenoma, parafollicular cell, benign, primary	2	2 - 9	2	2	7

[1]Historical controls group mean range from four studies conducted between 2002 and 2005.

III. DISCUSSION AND CONCLUSIONS:

A. INVESTIGATORS' CONCLUSIONS: Groups of 65 male and 65 female Fischer 344 rats were fed diets formulated to provide 0 (controls), 10, 100, or 1000 mg XDE-742/kg/day for up to two years. The in-life phase of the study proceeded with no significant disease or toxicity problems. As expected, geriatric diseases typical of Fischer 344 rats were noted late in the study. Very few rats were removed from study prior to 12 months, after which moribund animals or spontaneous deaths gradually increased, but there were no statistically significant differences in mortality. Females given 1000 mg/kg/day had treatment-related statistically identified lower mean body weights at most time-points when compared to controls. At 12 and 24 months, body weight gains for females given 1000 mg/kg/day were 7.8% and 6.7% lower than controls, respectively. The decrement in body weight gain was interpreted to be a non-adverse effect, because the lower weights did not worsen during the second year of the study, and the body weights at most time-points throughout the study were within historical control ranges. Feed consumption for females administered 1000 mg/kg/day was statistically identified as lower than controls between test days 8 through 84. This decrement in feed consumption was interpreted to be treatment-related, and corresponded to the lower body weights. For the remainder of the study, the feed consumption of females given 1000 mg/kg/day was comparable to controls at most time-points. There were no treatment-related effects on body weights or feed consumption of females given 10 or 100 mg/kg/day, nor of males from any dose group.

The only treatment-related clinical observation was an increased incidence of perineal urine soiling in males and females given 100 or 1000 mg/kg/day. The increased urine soiling was first noted during months 4 and 5 in animals given 1000 mg/kg/day, and during months 15 and 16 in animals given 100 mg/kg/day. This observation was interpreted to be a non-adverse effect, based on the lack of any corresponding histopathologic urinary tract effects and the absence of alterations in urinalysis parameters.

Males given 1000 mg/kg/day generally had lower mean ALT activities and higher cholesterol concentrations at all sampling intervals. These values were statistically identified at the 3, 6, and 12-month sampling intervals, and were interpreted to be treatment-related, yet non-adverse effects based on the lack of any corresponding histopathologic alterations. Treatment-related changes in organ weights consisted of higher mean absolute (4.1%) and relative (8.8%) liver weights in males given 1000 mg/kg/day at 12 months only, and higher mean absolute (6.1%) and relative (10.9%) liver weights in females given 1000 mg/kg/day at 24 months. The higher relative liver weights were statistically identified as different from controls. The liver weight changes were interpreted to be non-adverse, based on the lack of any corresponding clinical pathologic or histopathologic liver effects.

The only treatment-related histopathologic alteration was a decrease in the incidence of basophilic foci of altered hepatocytes in females given 1000 mg/kg/day (12 and 24 months), and in males given 1000 mg/kg/day (24 months). This alteration was interpreted to be a non-adverse effect, because toxicologically significant changes in basophilic foci are usually associated with an increased incidence.

No treatment-related increase in neoplasms was observed in either male or female rats at any dose level, indicating that XDE-742 did not have an oncogenic potential under the conditions of this study. Based on the increased incidence of perineal soiling in males and females given 100 or 1000 mg/kg/day, the no-observed-effect level (NOEL) was 10 mg/kg/day. Since all treatment-related effects in males and females were interpreted to be non-adverse, the no-observed-adverse-effect level (NOAEL) was 1000 mg/kg/day for both sexes.

B. **REVIEWER COMMENTS**: There were no treatment related adverse effects on mortality, clinical signs, ophthalmology, hematology, clinical chemistry, histopathology.

Females given 1000 mg/kg/day had treatment-related statistically identified lower mean body weights (5-7%) at most time-points when compared to controls. Body weight gains were decreased throughout the study by 8-10% compared to controls. The decrement in body weight gain was interpreted to be a non-adverse effect, because the lower weights did not worsen during the second year of the study, and the body weights at most time-points throughout the study were within historical control ranges. Feed consumption for females administered 1000 mg/kg/day was statistically identified as lower than controls between test days 8 through 84. This decrement in feed consumption corresponded to the lower body weights in the first 13 weeks of the study. Body weights and body weight gains remained minimally decreased throughout the study after food consumption values returned to control levels.

Treatment-related changes in organ weights consisted of higher mean absolute (4.1%) and relative (8.8%) liver weights in males given 1000 mg/kg/day at 12 months only, and higher mean absolute (6.1%) and relative (10.9%) liver weights in females given 1000 mg/kg/day at 24 months. The higher relative liver weights were statistically identified as different from controls. The liver weight changes were interpreted to be non-adverse, based on the lack of any corresponding clinical pathologic or histopathologic liver effects.

The incidence of large granular lymphocyte leukemia was outside of the historical control range from this lab for all groups of males in this study, and there was no statistical identification of any group exceeding the control incidence level. Spontaneous leukemia incidence rates from NTP indicate a variable range of leukemia incidence (22-68% for males Fischer-344 rats). The female incidence rate from this study is within historical control range for the lab. Therefore, it is likely that the leukemia incidence rate in this study is not treatment-related.

The LOAEL for this study was not observed. The NOAEL is 1000 mg/kg/day.

The incidence of large granular lymphocyte leukemia was outside of the historical control range from this lab for all groups of males in this study, and there was no statistical identification of any group exceeding the control incidence level. Spontaneous leukemia incidence rates from NTP indicate a variable range of leukemia incidence (32-74% for males Fischer-344 rats). The female incidence rate from this study is within historical control range for the lab. Therefore, it is likely that the leukemia incidence rate in this study is not treatment-related.

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C. STUDY DEFICIENCIES:

None

EPA Reviewer: Kimberly Harper **Signature:** Kimberly D Harper
RAB2, Health Effects Division (7509P) **Date:** 12/12/07
EPA Secondary Reviewer: Alan C. Levy, PhD **Signature:** Alan C. Levy
RAB2, Health Effects Division (7509P) **Date:** 1-3-2008

Template version 02/06

TXR#: 0054347

DATA EVALUATION RECORD

STUDY TYPE: ***In vitro* Bacterial Gene Mutation** *(Salmonella typhimurium, E. coli)/* mammalian activation gene mutation assay; OPPTS 870.5100[1] [§84-2]; OECD 471 (formerly OECD 471 & 472).

PC CODE: 108702 **DP BARCODE:** D332276

TEST MATERIAL (PURITY): XDE-742 / BAS 770 H (98.0%)

SYNONYMS: XR-742; XDE-742

CITATION: Engelhardt, G. and E. Leibold. *Salmonella typhimurium/Escherichia coli* Reverse Mutation Assay Standard Plate Test and Preincubation Method with XDE-742/BAS 770 H. Experimental Toxicology and Ecology, BASF Aktiengesellschaft, 67056 Ludwigshafen, Germany. Laboratory report # 40M0298/034051. (04 December 2003) MRID 46908414. Unpublished.

SPONSOR: BASF Aktiengesellschaft, 67056 Ludwigshafen, Germany.

EXECUTIVE SUMMARY: The objective of this study (MRID 46908414) was to evaluate the test article XDE-742 / BAS 770 H (98.0% a.i., EO952-52-01, TSN103826), for its ability to induce reverse mutations either in the presence or absence of mammalian microsomal enzymes at 1) the histidine locus in the genome of several strains (TA98, TA100, TA1535 and TA1537) of *Salmonella typhimurium* and at 2) the tryptophan locus of *Escherichia coli* strain WP2*uvr*A using both the plate incorporation and pre-incubation methodologies.

Initial and confirmatory plate incorporation mutagenicity assays were conducted with five concentrations of XDE-742 / BAS 770 H in the presence and absence of S9 mix, along with concurrent vehicle and positive controls using three plates per concentration. Concentrations tested in the initial assay with all tester strains were 0, 20, 100, 500, 2500, and 5000 µg per plate in both the presence and absence of S9 mix. The confirmatory plate incorporation assay was performed using concentrations of 0, 500, 750, 1000, 1500 and 2000 µg per plate based on the results of the initial assay where cytotoxicity was observed from about 750-1500 µg per plate depending upon the tester strain.

[1]870.5100 - Reverse mutation *E. coli* WP2 and WP2uvrA; *S. typhimurium* TA 97, TA98, TA100, TA1535, TA1537
870.5140 - Gene mutation *Aspergillus nidulans*
870.5250 - Gene mutation *Neurospora crassa*

Based on the negative findings observed using the plate incorporation methodology, a third assay was performed using a pre-incubation method both in the presence and absence of S9, with exposures from 62.5-2000 µg per plate. The results of the *S. typhimurium/E. coli* reverse mutation assay using both plate incorporation and pre-incubation methodologies indicate that under the conditions of this study, the test article, XDE-742 / BAS 770 H, did not cause a positive increase in the mean number of revertants per plate with any tester strain either in the presence or absence of microsomal enzymes prepared from Aroclor™ 1254-induced rat liver (S9).The positive controls induced the appropriate responses in the corresponding strains.

Under the conditions of this study, pyroxsulam did not induce an increase in revertant colonies compared to controls either in the presence or absence of metabolic activation up to concentrations that produced cytotoxicity and/or precipitation of the test compound; therefore XDE-742/BAS-770H is not a mutagenic agent in the bacterial reverse mutation test.

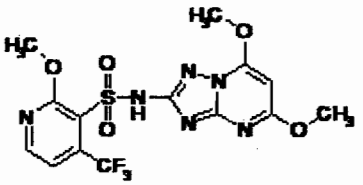
This study is classified as acceptable. This study satisfies the requirement for Test Guideline OPPTS 870.5100[2]; OECD 471 for *in vitro* mutagenicity (bacterial reverse gene mutation) data.

COMPLIANCE: Signed and dated GLP, Quality Assurance, and Data Confidentiality statements were provided. GLP deviation: Analytical confirmation of the concentrations of dosing solutions was not peformed.

2 870.5100 – Reverse mutation *E. coli* WP2 and WP2uvrA; *S. typhimurium* TA 97, TA98, TA100, TA1535, TA1537
870.5140 – Gene mutation *Aspergilius nidulans*
870.5250 – Gene mutation *Neurospora crassa*

I. MATERIALS AND METHODS:

A. MATERIALS:

1.	**Test Material:**	XDE-742 / BAS 770 H
	Description:	White-beige powder
	Lot/Batch #:	E0953-52-01
	Purity:	XDE-742 (98.0% a.i.)
	CAS #:	422556-08-9
	Chemical Structure	
	Solvent Used:	Dimethylformamide (DMF)

2.	**Control Materials:**	
	Negative:	DMF
	Solvent (final conc'n):	DMF at 100 µl per plate
	Positive:	Non-activation (-S9): N-methyl-N'-nitro-N-nitrosoguanidine : 5 µg/plate TA100, TA1535 4-nitro-o-phenylendiamine : 10 µg/plate TA98 9-Aminoacridine : 100 µg/plate TA1537 4-nitroquinoline-N-oxide: 5 µg/plate E.coli WP2 *uvr*A
		Activation (+S9) 2-Aminoanthracene (2-anthramine): 2.5 µg/plate TA98, TA100, TA1535, TA1537 2-Aminoanthracene: 60 µg/plate E.coli WP2 *uvr*A

3.	**Activation:** S9 derived from							
	X	Induced	X	Aroclor 1254	X	Rat	X	Liver
		Non-induced		Phenobarbitol		Mouse		Lung
				None		Hamster		Other
				Other		Other		

The concentrations of co-factors in the S9 mix were: NaH_2PO_4/Na_2HPO_4, pH 7.4 (15 mM), 0.25M Glucose-6-phosphate (5 mM), 0.10M NADP (4 mM), 0.825M KCl (33 mM), $MgCl_2$ (8 mM), and S9 Homogenate (10%).

4.	**Test organisms:** *S. typhimurium & E.coli* strains *[mark those that apply with x]*								
		TA97	X	TA98	X	TA100		TA102		TA104
	X	TA1535	X	TA1537		TA1538	X	E.coli WP2*uvr*A		
Properly maintained?							X	Yes		No
Checked for appropriate genetic markers (rfa mutation, R factor)?							X	Yes		No

5. **Test compound concentrations used:**
Non-activated and Activated conditions:

Initial Plate Incorporation Test: All tester strains at 0, 20, 100, 500, 2500, and 5000 µg per plate.

Confirmatory Plate Incorporation Assay: 0, 500, 750, 1000, 1500 and 2000 µg/plate.

Pre-incubation Assay:
TA1535 at 250, 500, 750, 1000 and 1500 µg/plate;
TA100 at 62.5, 125, 250, 500 and 750 µg/plate;
TA1537 and TA98 at 125, 250, 500 and 750 and 1000 µg/plate
WP2*uvr*A at 500, 750, 1000, 1500 and 2000 µg/plate.

All test article, vehicle controls, and positive controls were plated in triplicate.

B. TEST PERFORMANCE:

1. **Type of *Salmonella* assay:**
   - X standard plate test
   - X pre-incubation (20 minutes)
   - __ "Prival" modification *(i.e. azo-reduction method)*
   - __ spot test
   - __ other

2. **Protocol:** Tester strains were exposed to the test article via both the plate incorporation and the pre-incubation modification of the bacterial mutagenicity test.

Plate incorporation method: Test tubes containing 2.0 mL of overlay agar, consisting of 100 mL agar (0.6% agar + 0.6% NaCl) and 10 mL amino acid solution were kept in a water bath at 45°C, with the following components added:

0.1 mL test solution or vehicle
0.1 mL fresh bacterial culture (approximately 10^8 cells/mL)
0.5 mL S9 mix (in tests with metabolic activation) or

0.5 mL phosphate buffer (in tests without metabolic activation.

After mixing, the samples were poured onto minimal agar plates and incubated at 37°C for 48-72 hours in the dark before being counted. Minimal agar plates varied based on the bacterial strain.

Pre-incubation Method: In a test tube, 0.1 mL test solution or vehicle, 0.1 mL bacterial suspension and either 0.5 mL of S9 or phosphate buffer were mixed and incubated at 37°C for approximately 20 minutes using a shaker. Afterwards, 2 mL of soft agar was added, and after mixing, the samples were quickly poured onto agar plates. The plates were then incubated at 37°C for 48-72 hours in the dark before being counted.

Test article, vehicle controls, and positive controls were plated in triplicate. Condition of the bacterial background lawn was evaluated both macroscopically and microscopically (using a dissecting microscope) for indications of cytotoxicity and test article precipitate. Evidence of cytotoxicity was scored relative to the vehicle control plate and was recorded along with the revertant counts for all plates at that concentration. Revertant colonies were counted either by automated colony counter or by hand.

S9 fraction preparation: Sprague-Dawley rats were injected with 500 mg Aroclor 1254, as a solution in corn oil at a concentration of 20 g/100 mL, 5 days before sacrifice. The animals were housed under standard laboratory conditions for the 5 days, with pelleted feed and tap water available *ad libitum*. After 5 days, the rats were sacrificed, and the livers were prepared using sterile solvents and glassware. The livers were weighed and washed in an equivalent volume of 150 mM KCl solution, then cut into small pieces and homogenized in three volumes of KCl solution. After centrifugation of the homogenate at 9000 x g for 10 minutes at 4°C, 5 mL portions of the supernatant (S9 fraction) were stored at -70°C to -80°C.

The S9 mix was prepared fresh prior to each experiment by thawing the requisite amount of S9 fraction at room temperature and mixing 1 volume of S9 fraction with 9 volumes of S9 supplement (cofactors). The cofactors used were: $MgCl_2$ (8mM), KCl (33mM), glucose-6-phosphatase (5mM), NADP (4mM), and phosphate buffer (pH 7.4, 15 mM).

3. **Statistical analysis:** The mean and standard deviation were calculated for each replicate.

4. **Evaluation criteria:** Before assay data were evaluated, criteria for a valid assay had to be met. The following criteria were used to determine a valid assay:

Salmonella typhimurium tester strain cultures exhibited sensitivity to crystal violet to demonstrate the presence of the *rfa* wall mutation. Cultures of tester strains TA98 and TA100 exhibited resistance to ampicillin to demonstrate the presence of the pKM101 plasmid. Demonstrating the requirement for histidine (*Salmonella typhimurium*) or tryptophan (*Escherichia coli*), tester strain cultures exhibited a characteristic number of spontaneous revertants per plate within the normal range of the historical controls when plated along with the vehicle under selective conditions. Acceptable ranges for mean vehicle controls were as follows:

TA98	17	-	50
TA100	80	-	160
TA1535	10	-	25
TA1537	5	-	20
WP2*uvr*A	19	-	60

Demonstrating that appropriate numbers of bacteria were plated, density of tester strain cultures were greater than or equal to 1.0×10^8 bacteria per mL. Demonstrating that tester strains were capable of identifying a mutagen, the mean value of a positive control for a respective tester strain exhibited a significant increase over the mean value of the vehicle control for that strain. An acceptable positive control in the presence of S9 mix for a specific strain was evaluated as having demonstrated both integrity of the S9 mix and ability of the tester strain to detect a mutagen.

A minimum of three non-toxic concentrations was required to evaluate assay data. Cytotoxicity was detectable as a decrease in the number of revertant colonies per plate and/or by a thinning or disappearance of the bacterial background lawn. Thinning of the bacterial background lawn not accompanied by a reduction in the number of revertants per plate was not evaluated as an indication of cytotoxicity. Once criteria for a valid assay had been met, responses observed in the assay were evaluated.

For a test article to be considered positive, it had to produce at least a 2-fold concentration-related and reproducible increase in the mean revertants per plate over the mean revertants per plate of the appropriate vehicle control in at least one tester strain.

II. REPORTED RESULTS:

A. <u>Initial Plate Incorporation assay</u>: Six concentrations of XDE-742 in DMF at 0, 20, 100, 500, 2500 and 5000 µg per plate, were tested with TA98, TA100, TA1535, TA1537 and WP2*uvr*A in both the presence and absence of S9 mix using three replicates per concentration. Precipitate was observed at 2500 µg per plate and above. Cytotoxicity in terms of reduced background lawns and concentration-related decreases in revertant counts was observed with tester strains TA98 (Table 4), TA100 (Table 2), TA1535 (Table 1), TA1537 (Table 3) at 2500 µg per plate and above, in both the absence and presence of S9 mix, and at 5000 µg per plate for WP2*uvr*A (Table 5) under the same conditions. No significant concentration-related increases in revertant counts were observed in any tester strain either in the absence or presence of S9 metabolic activation.

B. <u>Mutagenicity assay</u>: Five concentrations of XDE-742 in DMF at 0, 500, 750, 1000, 1500 and 2000 µg per plate were tested with TA98, TA100, TA1535, TA1537 and WP2*uvr*A in both the presence and absence of S9 mix using three replicates per concentration. No precipitate was observed at any concentration. Cytotoxicity in terms of reduced background lawns and concentration-related decreases in revertant counts was observed with tester strains TA98 (Table 4) and TA1537 (Table 3) at 1500 µg per plate and above, in both the absence and presence of S9 mix. Similarly, cytotoxicity was observed for TA1535 (Table 1) at 1500 µg per plate in the absence and 2000 µg per plate in the presence of S9, and for TA100 (Table 2) at 750 µg per plate in the absence and 1500 µg per plate in the presence of S9.

WP2*uvr*A (Table 5) exhibited no cytotoxicity under any combination of concentrations or metabolic activation conditions. At 5000 mg/plate, there was a reduction in background growth; however, this dose was not considered cytotoxic because there was no significant reduction in the number of revertants per plate. No significant concentration-related increases in revertant counts were observed in any tester strain either in the absence or presence of S9 metabolic activation.

Table 1. Results from the initial and confirmatory standard plate test exposing TA1535 to XDE-742 with and without S9 activation.

	-S9				+S9 (1:9)				
Dose (µg/plate)	**REV**	**M**	**SD**	**FAC**	**REV**	**M**	**SD**	**FAC**	**Titre**
DMF	15, 18, 16	16	2	1.0	14, 20, 17	17	3	1.0	34, 26, 32
*	16, 16, 18	17	1	1.0	16, 18, 18	17	1	1.0	22, 24, 20
XDE-742									
20	14, 17, 13	15	2	0.9	16, 23, 18	19	4	1.1	-
100	17, 13, 16	15	2	0.9	12, 13, 19	15	4	0.9	-
500	18, 17, 14	16	2	1.0	12, 22 16	17	5	1.0	-
500*	14, 16, 16	15	1	0.9	15, 19, 16	17	2	1.0	-
750*	16, 18, 16	17	1	1.0	12, 17, 15	15	3	0.8	-
1000*	16, 14, 14	15	1	0.9	18, 12, 14	15	3	0.8	-
1500*	11, 7, 7	8	2	0.5	15, 16, 16	16	1	0.9	25, 16, 21
2000*	1, 1, 2 B	1	1	0.1	10, 7, 8	8	2	0.5	8, 7, 7
2500	2, 1, 1 B/P	1	1	0.1	3, 3, 6 B/P	4	2	0.2	1, 1, 0
5000	0, 0, 0 B/P	-	-	-	0, 0, 0 B/P	-	-	-	0, 0, 0
MNNG									
5.0	1136, 1044, 965	1048	86	64.2	-	-	-	-	-
5.0*	864, 931, 927	907	38	54.4					
2-AA 2.5									
2.5	-	-	-	-	116, 95, 89	100	14	5.9	-
2.5*	-	-	-	-	147, 112, 124	128	18	7.4	-

Data are from Appendix Table 1 and Table 6 on pages 33 and 38 of the study report.
(REV = revertants/plate, M = mean, SD = standard deviation, FAC = factor)
* data from the confirmatory standard plate test.
B: reduced background growth, P: precipitation, - data not appropriate/available
MNNG = N-methyl-N'-nitro-N-nitrosoguanidine, 2-AA = 2-aminoanthracene

Table 2. Results from the initial and confirmatory standard plate tests exposing TA100 to XDE-742 with and without S9 activation.

	-S9				+S9 (1:9)				
Dose (µg/plate)	**REV**	**M**	**SD**	**FAC**	**REV**	**M**	**SD**	**FAC**	**Titre**
DMF	107, 113, 102	107	6	1.0	107, 100, 111	106	6	1.0	22, 26, 20
*	106, 106, 120	111	8	1.0	101, 112, 113	109	7	1.0	26, 31, 27
XDE-742									
20	114, 105, 100	106	7	1.0	104, 100, 116	107	8	1.0	-
100	111, 104, 115	110	6	1.0	121, 115, 110	115	6	1.1	-
500	108, 114, 105	109	5	1.0	102, 90, 102	98	7	0.9	-
500*	102, 93, 88	94	7	0.9	100, 71, 101	91	17	0.8	-
750*	85, 25, 19	43	36	0.4	102, 65, 70	79	20	0.7	-
1000*	27, 17, 36	27	10	0.2	73, 64, 53	63	10	0.6	-
1500*	6, 6, 9	7	2	0.1	41, 22, 32	32	10	0.3	11, 14, 9
2000*	7, 11, 10 B	9	2	0.1	19, 16, 6	14	7	0.1	7, 3, 0
2500	11, 14, 21 B/P	15	5	0.1	14, 17, 17 B/P	16	2	0.2	0, 0, 0
5000	0, 0, 0 B/P	-	-	-	0, 0, 0 B/P	-	-	-	0, 0, 0
MNNG									
5.0	1237, 1156, 1007	1133	117	10.6	-	-	-	-	-
5.0*	1191, 1236, 925	1117	168	10.1					
2-AA 2.5									
2.5	-	-	-	-	993, 941, 903	946	45	8.9	-
2.5*	-	-	-	-	1046, 931, 982	986	58	9.1	-

Data are from Appendix Table 2 and Table 7 on pages 34 and 39 of the study report.
(REV = revertants/plate, M = mean, SD = standard deviation, FAC = factor)
* data from the confirmatory standard plate test.
B: reduced background growth, P: precipitation, - data not appropriate/available
MNNG = N-methyl-N'-nitro-N-nitrosoguanidine, 2-AA = 2-aminoanthracene

Table 3. Results from the initial and confirmatory standard plate tests exposing TA1537 to XDE-742 with and without S9 activation.

	-S9				+S9 (1:9)				
Dose (µg/plate)	**REV**	**M**	**SD**	**FAC**	**REV**	**M**	**SD**	**FAC**	**Titre**
DMF	8, 8, 12	9	2	1.0	8, 8, 13	10	3	1.0	36, 41, 35
*	5, 8, 8	7	2	1.0	10, 10, 5	8	3	1.0	27, 20, 19
XDE-742									
20	7, 11, 13	10	3	1.1	9, 12, 19	13	5	1.4	-
100	6, 14, 9	10	4	1.0	9, 9, 10	9	1	1.0	-
500	7, 9, 4	7	3	0.7	3, 7, 8	6	3	0.6	-
500*	9, 3, 2	5	4	0.7	4, 7, 8	6	2	0.8	-
750*	3, 6, 2	4	2	0.5	6, 2, 5	4	2	0.5	-
1000*	5, 5, 2	4	2	0.6	7, 4, 5	5	2	0.6	-
1500*	1, 1, 1	1	0	0.1	3, 3, 3	3	0	0.4	11, 14, 17
2000*	0, 0, 0 B	-	-	-	3, 1, 2	2	1	0.2	7, 4, 1
2500	1, 1, 2 B/P	1	1	0.1	2, 3, 2 B/P	2	1	0.2	0, 0, 0
5000	0, 0, 0 B/P	-	-	-	0, 0, 0	-	-	-	0, 0, 0
AAC									
100	471, 382, 426	426	45	45.7	-	-	-	-	-
100*	513, 464, 400	459	57	65.6	-	-	-	-	-
2-AA 2.5									
2.5	-	-	-	-	124, 141, 113	126	14	13.0	-
2.5*	-	-	-	-	114, 119, 105	113	7	13.5	-

Data are from Appendix Table 3 and Table 8 on pages 35 and 40 of the study report.
(REV = revertants/plate, M = mean, SD = standard deviation, FAC = factor)
* data from the confirmatory standard plate test.
B: reduced background growth, P: precipitation, - data not appropriate/available
AAC = 9-aminoacridine, 2-AA = 2-aminoanthracene

Table 4. Results from the initial and confirmatory standard plate tests exposing TA98 to XDE-742 with and without S9 activation.

	-S9				+S9 (1:9)				
Dose (µg/plate)	**REV**	**M**	**SD**	**FAC**	**REV**	**M**	**SD**	**FAC**	**Titre**
DMF	27, 29, 36	31	5	1.0	27, 40, 44	37	9	1.0	22, 31, 26
*	21, 25, 27	24	3	1.0	33, 30, 40	34	5	1.0	25, 37, 33
XDE-742									
20	29, 32, 26	29	3	0.9	38, 31, 30	33	4	0.9	-
100	27, 32, 36	32	5	1.0	49, 37, 33	40	8	1.1	-
500	27, 25, 22	25	3	0.8	36, 26, 24	29	6	0.8	-
500*	20, 23, 17	20	3	0.8	26, 38, 32	32	6	0.9	-
750*	18, 17, 17	17	1	0.7	36, 23, 32	30	7	0.9	-
1000*	21, 10, 14	15	6	0.6	20, 28, 23	24	4	0.7	-
1500*	2, 9, 5	5	4	0.2	17, 20, 15	17	3	0.5	18, 16, 12
2000*	4, 7, 5 B	5	2	0.2	14, 11, 11	12	2	0.3	7, 8, 8
2500	4, 8, 4 B/P	5	2	0.2	14, 11, 17 B/P	14	3	0.4	1, 1, 1
5000	0, 0, 0 B/P	-	-	-	0, 0, 0 B/P	-	-	-	0, 0, 0
NOPD									
10	760, 765, 725	750	22	24.5	-	-	-	-	-
10*	617, 709, 740	689	64	28.3	-	-	-	-	-
2-AA 2.5									
2.5	-	-	-	-	811, 786, 790	796	13	21.5	-
2.5*	-	-	-	-	513, 522, 600	545	48	15.9	-

Data are from Appendix Table 4 and Table 9 on pages 36 and 41 of the study report.
(REV = revertants/plate, M = mean, SD = standard deviation, FAC = factor)
* data from the confirmatory standard plate test.
B: reduced background growth, P: precipitation, - data not appropriate/available
NOPD = 4-nitro-o-phenylendiamine, 2-AA = 2-aminoanthracene

Table 5. Results from the initial and confirmatory standard plate tests exposing WP2 *uvrA* to XDE-742 with and without S9 activation.

	-S9				+S9 (1:9)				
Dose (µg/plate)	**REV**	**M**	**SD**	**FAC**	**REV**	**M**	**SD**	**FAC**	**Titre**
DMF	29, 25, 27	27	2	1.0	31, 39, 44	38	7	1.0	37, 29, 44
*	31, 38, 37	35	4	1.0	41, 35, 42	39	4	1.0	36, 30, 30
XDE-742									
20	27, 29, 20	25	5	0.9	41, 35, 36	37	3	1.0	-
100	25, 23, 28	25	3	0.9	36, 31, 40	36	5	0.9	-
500	24, 30, 25	26	3	1.0	41, 45, 36	41	5	1.1	-
500*	33, 29, 33	32	2	0.9	32, 31, 36	33	3	0.8	-
750*	29, 36, 34	33	4	0.9	31, 34, 35	33	2	0.8	-
1000*	33, 43, 30	35	7	1.0	31, 38, 30	33	4	0.8	-
1500*	30, 33, 22	28	6	0.8	38, 31, 30	33	4	0.8	22, 28, 31
2000*	37, 27, 22	29	8	0.8	30, 27, 31	29	2	0.7	22, 17, 24
2500	26, 24, 20 P	23	3	0.9	38, 40, 35 P	38	3	1.0	7, 4, 5
5000	14, 21, 12 B/P	16	5	0.6	29, 20, 27 B/P	25	5	0.7	5, 4, 2
4-NQO									
5.0	674, 619, 671	655	31	24.2	-	-	-	-	-
5.0*	640, 631, 570	614	38	17.4	-	-	-	-	-
2-AA 2.5									
60	-	-	-	-	235, 198, 247	227	26	6.0	-
60*	-	-	-	-	287, 219, 254	253	34	6.4	-

Data are from Appendix Table 5 and Table 10 on pages 37 and 42 of the study report.
(REV = revertants/plate, M = mean, SD = standard deviation, FAC = factor)
* data from the confirmatory standard plate test.
B: reduced background growth, P: precipitation, - data not appropriate/available
4-NQO = 4-nitroquinoline-N-oxide, 2-AA = 2-aminoanthracene

C. **Pre-incubation Assay:** Five concentrations of XDE-742 in DMF were evaluated in both the presence and absence of S9 as follows: TA1535 (Table 6) at 250, 500, 750, 1000 and 1500; TA100 (Table 7) at 62.5, 125, 250, 500 and 750; TA1537 (Table 8) and TA98 (Table 9) at 125, 250, 500 and 750 and 1000 and WP2*uvr*A (Table 10) at 500, 750, 1000, 1500 and 2000 µg/plate. Cytotoxicity in terms of reduced decreased revertant counts was observed at the highest concentration tested in each case. All data were acceptable, (vehicle and positive controls for each strain and activation condition were within acceptable criteria), and no positive increases in the mean number of revertants per plate were observed with any of the tester strains in either the presence or absence of S9 mix.

Table 6. Pre-incubation test exposing TA1535 to XDE-742with and without metabolic activation.

	-S9				+S9 (1:9)				
Dose (µg/plate)	**REV**	**M**	**SD**	**FAC**	**REV**	**M**	**SD**	**FAC**	**Titre**
DMF	17, 22, 14	18	4	1.0	17, 17, 15	16	1	1.0	25, 27, 32
XDE-742 250	17, 15, 12	15	3	0.8	18, 12, 19	16	4	1.0	-
500	14, 19, 14	16	3	0.9	15, 12, 11	13	2	0.8	-
750	12, 12, 14	13	1	0.7	17, 13, 12	14	3	0.9	-
1000	15, 11, 14	13	2	0.8	15, 15, 15	15	0	0.9	22, 28, 24
1500	12, 8, 5	8	4	0.5	12, 14, 11	12	2	0.8	12, 17, 11
MNNG 5.0	705, 775, 652	711	62	40.2	-	-	-	-	-
2-AA 2.5	-	-	-	-	127, 141, 108	125	17	7.7	-

Data are from Table 11 on page 44 of the study report.
(REV = revertants/plate, M = mean, SD = standard deviation, FAC = factor)
B: reduced background growth, P: precipitation, - data not appropriate/available
MNNG = N-methyl-N'-nitro-N-nitrosoguanidine, 2-AA = 2-aminoanthracene

Table 7. Pre-incubation test exposing TA100 to XDE-742with and without metabolic activation.

	-S9				+S9 (1:9)				
Dose (µg/plate)	**REV**	**M**	**SD**	**FAC**	**REV**	**M**	**SD**	**FAC**	**Titre**
DMF	107, 117, 105	110	6	1.0	108, 114, 121	114	7	1.0	34, 28, 22
XDE-742 62.5	102, 104, 99	102	3	0.9	108, 106, 127	114	12	1.0	-
125	100, 104, 106	103	3	0.9	130, 100, 96	109	19	1.0	-
250	107, 96, 93	99	7	0.9	105, 105, 96	102	5	0.9	-
500	103, 96, 97	99	4	0.9	107, 103, 95	102	6	0.9	16, 27, 25
750	93, 88, 70	84	12	0.8	96, 82, 91	90	7	0.8	14, 8, 10
MNNG 5.0	735, 635, 706	692	51	6.3	-	-	-	-	-
2-AA 2.5	-	-	-	-	1125, 1068, 1037	1077	45	9.4	-

Data are from Table 12 on page 45 of the study report.
(REV = revertants/plate, M = mean, SD = standard deviation, FAC = factor)
B: reduced background growth, P: precipitation, - data not appropriate/available
MNNG = N-methyl-N'-nitro-N-nitrosoguanidine, 2-AA = 2-aminoanthracene

Table 8. Pre-incubation test exposing TA1537 to XDE-742with and without metabolic activation.

	-S9				+S9 (1:9)				
Dose (µg/plate)	**REV**	**M**	**SD**	**FAC**	**REV**	**M**	**SD**	**FAC**	**Titre**
DMF	11, 8, 9	9	2	1.0	14, 8, 8	10	3	1.0	27, 27, 22
XDE-742 125	8, 8, 10	9	1	0.9	6, 9, 11	9	3	0.9	-
250	14, 12, 11	12	2	1.3	10, 8, 6	8	2	0.8	-
500	8, 6, 7	7	1	0.8	7, 9, 5	7	2	0.7	-
750	10, 8, 6	8	2	0.9	8, 8, 8,	8	0	0.8	6, 7, 8
1000	5, 5, 1	4	2	0.4	5, 6, 3	5	2	0.5	8, 8, 2
AAC 100	408, 398, 406	404	5	43.3	-	-	-	-	-
2-AA 2.5	-	-	-	-	127, 99, 105	110	15	11.0	-

Data are from Table 13 on page 46 of the study report.
(REV = revertants/plate, M = mean, SD = standard deviation, FAC = factor)
B: reduced background growth, P: precipitation, - data not appropriate/available
AAC = 9-aminoacridine, 2-AA = 2-aminoanthracene

Table 9. Pre-incubation test exposing TA98 to XDE-742with and without metabolic activation.

	-S9				+S9 (1:9)				
Dose (µg/plate)	**REV**	**M**	**SD**	**FAC**	**REV**	**M**	**SD**	**FAC**	**Titre**
DMF	31, 32, 29	31	2	1.0	34, 29, 36	33	4	1.0	35, 25, 41
XDE-742 125	25, 33, 21	26	6	0.9	32, 27, 25	28	4	0.8	-
250	36, 28, 24	29	6	1.0	33, 41, 36	37	4	1.1	-
500	21, 25, 25	24	2	0.8	28, 41, 41	37	8	1.1	-
750	19, 26, 14	20	6	0.6	29, 20, 24	24	5	0.7	12, 11, 8
1000	18, 12, 14	15	3	0.5	18, 24, 17	20	4	0.6	8, 17, 12
NOPD 100	967, 825, 867	886	73	28.9	-	-	-	-	-
2-AA 2.5	-	-	-	-	701, 803, 765	756	52	22.9	-

Data are from Table 14 on page 47 of the study report.
(REV = revertants/plate, M = mean, SD = standard deviation, FAC = factor)
B: reduced background growth, P: precipitation, - data not appropriate/available
NOPD = 4-nitro-o-phenylendiamine, 2-AA = 2-aminoanthracene

Table 10. Pre-incubation Test using WP2 *uvrA* with and without metabolic activation.

	-S9				+S9 (1:9)				
Dose (µg/plate)	**REV**	**M**	**SD**	**FAC**	**REV**	**M**	**SD**	**FAC**	**Titre**
DMF	32, 28, 36	32	4	1.0	33, 36, 41	37	4	1.0	28, 35, 52
XDE-742 500	27, 24, 25	25	2	0.8	28, 34, 44	35	8	1.0	-
750	21, 33, 36	30	8	0.9	41, 32, 20	31	11	0.8	-
1000	24, 28, 25	26	2	0.8	28, 26, 25	26	2	0.7	-
1500	24, 18, 24	22	3	0.7	30, 24, 17	24	7	0.6	28, 32, 42
2000	14, 14, 13	14	1	0.4	22, 18, 10	17	6	0.5	18, 25, 21
4-NQO 5.0	687, 632, 552	624	68	19.5	-	-	-	-	-
2-AA 60	-	-	-	-	251, 201, 196	216	30	5.9	-

Data are from Appendix Table 15 on page 48 of the study report.
(REV = revertants/plate, M = mean, SD = standard deviation, FAC = factor)
B: reduced background growth, P: precipitation, - data not appropriate/available
4-NQO = 4-nitroquinoline-N-oxide, 2-AA = 2-aminoanthracene

III. DISCUSSION AND CONCLUSIONS:

A. INVESTIGATOR'S CONCLUSIONS: Under the experimental conditions, it is concluded that XDE-742/BAS-770H is not a mutagenic agent in a bacterial reverse mutation test.

B. REVIEWER COMMENTS: In the three independent tests in this study, pyroxsulam did not cause an increase in revertant colonies in *Salmonella typhimurium* or *Escherichia coli* using the standard plate and/or the pre-incubation methods, either in the presence or absence of metabolic activation, up to concentrations that produced cytotoxicity and/or precipitation of the test compound. Positive control chemicals induced an increase in the number of revertant colonies compared to controls. **It is concluded that pyroxsulam is not a mutagenic agent in the bacterial reverse mutation test.**

The registrant, the Dow Chemical Company, originally prepared this STUDY PROFILE TEMPLATE (STP) (MRID 46908611) in HED's DER format. The HED reviewers may have added minor adjustments/additions to the original STP. The conclusions of the study and assignment of its classification as determined by HED reviewers are in the Executive Summary above. It should be noted that the STP provided information that was not explicitly included in the study report.

C. STUDY DEFICIENCIES: None

EPA Reviewer: Kimberly Harper **Signature:** Kimberly D Harper
RAB2, Health Effects Division (7509P) **Date:** 12/12/07
EPA Secondary Reviewer: Alan C. Levy, PhD **Signature:** Alan C. Levy
RAB2, Health Effects Division (7509P) **Date:** 1-3-2008

Template version 02/06

TXR#: 0054347

DATA EVALUATION RECORD

STUDY TYPE: *In Vitro* Mammalian Cells in Culture Gene Mutation assay in Chinese Hamster Ovary Cells; OPPTS 870.5300 [§84-2]; OECD 476.

PC CODE: 108702 **DP BARCODE:** D332276

TEST MATERIAL (PURITY): XDE-742 (98.0% a.i.)

SYNONYMS: (N-(5,7-dimethoxy[1,2,4]triazolo[1,5-a]pyrimidin-2-yl)-2-methoxy-4-(trifluoromethyl)pyridine-3-sulfonamide), X666742, XR-742

CITATION: S. D. Seidel, Ph.D., M. R. Schisler, B.S., J. M. Grundy, B.S. Evaluation of XDE-742 in the Chinese hamster ovary cell/hypoxanthine-guanine-phosphoribosyl transferase (CHO/HGPRT) forward mutation assay. Toxicology & Environmental Research and Consulting, The Dow Chemical Company, Midland, Michigan, 48674. Study ID: 041003, (2004). MRID 46908408. Unpublished to

SPONSOR: Dow AgroSciences LLC, 9330 Zionsville Road, Indianapolis, Indiana 46268

EXECUTIVE SUMMARY: The genotoxic potential of XDE-742 (98.0% a.i., Lot # EO952-52-01, TSN103826) was evaluated in the *in vitro* Chinese hamster ovary cell/hypoxanthine-guanine-phosphoribosyl transferase (CHO/HGPRT) forward gene mutation assay (MRID 46908408). In the preliminary cytotoxicity assay, cells were treated for 4 hours with either 0, 1.56, 3.125, 6.25, 12.5, 25, 50, 100, or 200 µg/mL XDE-742 dissolved in dimethyl sulfoxide (DMSO), both in the presence and absence of metabolic activation. In the gene mutation assay, cells were exposed 0, 12.5, 25, 50, 100, or 200 µg/mL, both in the presence and absence of metabolic activation. The highest concentration tested was based on the limit of solubility of the test material in the solvent (DMSO). The S9 fraction was prepared from the livers of Arochlor treated male Sprague-Dawley rats. The hypoxanthine-guanine phosphoribosyl transferase (HGPRT) locus was examined using 6-thioguanine (6-TG). The adequacy of the experimental conditions for detection of induced mutation was confirmed by employing positive control chemicals, ethyl methanesulfonate for assays without S9 and 20-methylcholanthrene for assays with S9.

XDE-742 was tested up to the limit of solubility (200 µg/mL in DMSO). There was no appreciable cytotoxicity at any dose tested. The number of TG^r mutants per 10^6 clonable cells in the treated groups was not significantly different than the controls. The mutant frequency of the control was consistent with recent historical control data for the performing laboratory. The positive control chemicals elicited a proper mutagenic response in the CHO cells at the HGPRT

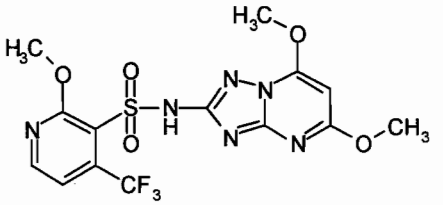
locus. **There was no evidence of induced mutant colonies over background.**

This study is acceptable and satisfies the guideline requirement for Mammalian cells in culture gene mutation assay in CHO-K_1-BH_4 cells; USEPA OPPTS 870.5300; OECD Guideline 476; EC, B.17.

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

I. MATERIALS AND METHODS

A. MATERIALS:

1.	**Test Material:**	XDE-742
	Description:	Powder, white
	Lot/Batch #:	EO952-52-01; TSN103826
	Purity:	98% XDE-742
	CAS #:	N/A
	Chemical Structure:	
	Solvent Used:	Dimethyl sulfoxide (DMSO, Sigma, St. Louis, Missouri)

2.	**Control Materials:**	
	Solvent control (final conc'n):	Dimethyl sulfoxide (DMSO) 1%
	Positive control:	Nonactivation: Ethyl methanesulfonate (EMS, CAS No. 62-50-0), 621 µg/ml
		Activation: 20-methylcholanthrene (20-MCA, CAS No. 56-49-5), 4 µg/ml

3.	**Activation**: S9 derived from							
	X	induced	X	Aroclor 1254	X	Rat	X	Liver
		non-induced		Phenobarbitol		Mouse		Lung
				None		Hamster		

The S-9 liver homogenate was purchased from Molecular Toxicology, Inc., Boone, North Carolina, and stored at approximately -100°C or below. Thawed S-9 was reconstituted at a final concentration of 10% (v/v) in a "mix". The S9 mix consisted of the following co-factors: 10 mM $MgCl_2 \cdot 6H_2O$ (Sigma), 5 mM glucose-6-phosphate (Sigma), 4 mM nicotinamide adenine dinucleotide phosphate (Sigma), 10 mM $CaCl_2$ (Fisher), 30 mM KCl (Sigma), and 50 mM sodium phosphate (pH 8.0, Sigma and Fisher). The reconstituted mix was added to the culture medium to obtain the desired final concentration of S9 in the culture, *i.e.*, 2% v/v. Hence, the final concentration of the co-factors in the culture medium is 1/5 of the concentrations stated above.

4.	**Test cells:** mammalian cells in culture				
			mouse lymphoma L5178Y cells		V79 cells (Chinese hamster lung fibroblasts)
		X	Chinese hamster ovary (CHO) cells		list any others

Media: The cells were routinely maintained in Ham's F-12 nutrient mix (GIBCO, Grand Island, New York) supplemented with 5% (v/v) heat-inactivated (56°C, 30 minutes), dialyzed fetal bovine serum (GIBCO), antibiotics and antimycotics (penicillin G, 100 units/ml; streptomycin sulfate, 0.1 mg/ml; fungizone, 25 µg/ml; GIBCO) and an additional 2 mM L-glutamine (GIBCO). The selection medium used for the detection of HGPRT$^-$ mutants was Ham's F-12 nutrient mix without hypoxanthine, supplemented with 10 µM 6-thioguanine (GIBCO) and 5% serum and the above-mentioned antibiotics.				
Properly maintained?	X	Yes		No
Periodically checked for Mycoplasma contamination?	X	Yes		No
Periodically checked for karyotype stability?	X	Yes		No
Periodically "cleansed" against high spontaneous background?	X	Yes		No

5. **Locus examined:** Hypoxanthine-guanine-phosphoribosyl transferase (HGPRT)
Selection Agent:

	Bromodeoxyuridine (BrdU)		8-Azaguanine (8-AG)		Ouabain
	Fluorodeoxyuridine (FdU)	X	6-Thioguanine (6-TG)		
	Trifluorothymidine (TFT)				

6. **Test compound concentrations used:**

Nonactivated conditions: Preliminary cytotoxicity assay A1: 0, 1.56, 3.125, 6.25, 12.5, 25, 50, 100, or 200 µg/mL
Mutagenicity assay B1: 0, 12.5, 25, 50, 100, or 200 µg/mL

Activated conditions: Preliminary cytotoxicity assay A1: 0, 1.56, 3.125, 6.25, 12.5, 25, 50, 100, or 200 µg/mL
Mutagenicity assay B1: 0, 12.5, 25, 50, 100, or 200 µg/mL

B. TEST PERFORMANCE:

1. **Toxicity Assay:** The cytotoxicity of the test material was assessed by determining the ability of the treated cells to form colonies. This assay was conducted for selecting concentrations of the test material to be used in the gene mutation assay. Cells were seeded into T-25 flasks (1.0×10^6 cells/flask) approximately 24 hours prior to treatment. Treatment was for approximately 4 hours with various concentrations of the test material with one replicate per dose in the presence and absence of S9 factor. After termination of treatment, the cells were trypsinized and replated at a density of 200 cells/dish into 60 mm dishes (three/dose) and the dishes incubated for 6-10 days to allow colony formation. The colonies were then fixed/stained with methanol/crystal violet. The number of colonies/dish were counted and the mean colonies/treatment were expressed relative to the solvent control value. The highest concentration tested in the toxicity assay was limited to the solubility of the test material in the solvent used to dissolve it.

Gene Mutation Assay: Each dose level was set up in duplicate from the time of treatment until the completion of the assay. The number of cells treated at each dose level was adjusted to yield at least 1×10^6 surviving cells. The cultures were trypsinized at the end of the treatment and replated at a density of 1×10^6 cells/100 mm dish (at least two dishes/replicate)

for phenotypic expression. In addition, 200 cells/60 mm dish (three dishes/replicate) were also plated to determine the toxicity and incubated for approximately 6-8 days to permit colony formation. During the phenotypic expression period (7-9 days), cells in the larger petri dishes were subcultured every 2-3 days and plated (at least two dishes/replicate) at a density of about 1 x 10^6 cells/100 mm petri dish. At each subculture, cells from various dishes within each replicate were pooled prior to replating. At the end of the expression period, the cultures were trypsinized and plated at a density of 2 x 10^5 cells/100 mm dish (a total of 10 dishes/treatment) in the selection media (Ham's F-12 without hypoxanthine and with 6-thioguanine) for the determination of HGPRT$^-$ mutants and 200 cells/60 mm dish (three dishes/treatment) in Ham's F-12 medium without hypoxanthine for determination of cloning efficiency. Treatments resulting in less than approximately 10% relative cell survival (based upon the concurrent toxicity assay results) were not used for determining either the cloning efficiency or mutation frequencies. The dishes were incubated for about 6-10 days and the colonies were fixed/stained with methanol/crystal violet. The mutation frequency (expressed as mutants per 10^6 clonable cells) for each replicate were calculated by the following formula:

$$MF = K \times (m / c)$$

Where:
$K = P_c \times 10^6 / P_m$
P_c = the number of cells plated for the survival plates
P_m = the number of cells plated for the mutation plates
c = the mean colonies per plate for survival plates for each replicate
m = the mean colonies per plate for mutation plates for each replicate

2. **Statistical methods:** The frequency of mutants per 10^6 clonable cells was statistically evaluated using a weighted analysis of variance; weights were derived from the inverse of the mutation frequency variance. The actual plate counts are assumed to follow a Poisson distribution therefore the mean plate count was used as an estimate of variance.

   If the analysis of variance was significant at alpha = 0.05, a Dunnett's t-test was conducted, comparing each treated group and the positive control to the negative control (alpha = 0.05, one-sided). An additional comparison of the positive control to the negative control (alpha = 0.05) was conducted using a linear contrast statement. Linear dose-related trend tests were performed if any of the pairwise comparisons of test material with the negative control yielded significant differences.

3. **Evaluation criteria:** For an assay to be acceptable, the mutant frequency in positive controls should have been significantly higher than the solvent controls. An additional criterion, was that the mutant frequency in the solvent controls should have been within reasonable limits of the laboratory historical control values and literature values. The test chemical was considered positive if it induced a statistically significant, dose related, reproducible increase in mutant frequency. The final interpretation of the data took into consideration such factors as the mutant frequency and cloning efficiencies in the solvent controls.

II. REPORTED RESULTS: The pH and osmolality of treatment medium containing approximately 200 µg/ml of the test material (limit of solubility in culture medium) and medium containing 1% DMSO were determined using a Denver Basic pH meter (Denver Instrument Co., Arvada, Colorado) and an Osmette A freezing point osmometer (Precision Systems, Inc., Natick, Massachusetts). Alterations in the pH and osmolality of the culture medium have been shown to induce false positive responses in *in vitro* genotoxicity assays. There was no appreciable change in either the pH or osmolality at this concentration as compared to the culture medium with solvent alone (culture medium with the test material, pH = 7.56, osmolality = 430 mOsm/kgH_2O; culture medium with 1% DMSO, pH = 7.70, osmolality = 427 mOsm/kgH_2O).

The analytically observed concentrations of the test material in the stock solutions ranged from 92.5 to 96.6 % of target.

A. PRELIMINARY CYTOTOXICITY ASSAY: In a preliminary toxicity assay, the test material was assayed at concentrations of 0, 1.56, 3.125, 6.25, 12.5, 25, 50, 100, and 200 µg/ml in the absence and presence of an externally supplied metabolic activation system (S9). The highest concentration tested was based upon the limitations imposed by the solubility of the test material in the solvent used to dissolve it. The treated cultures without and with S9 activation showed little to no toxicity with the relative cell survival (RCS) values ranging from 84.7 to 118.6 % in the absence of S9 activation and 86.1 to 160.9 % in the presence of S9 activation. Based upon the results of this assay, concentration levels of 0, 12.5, 25, 50, 100 and 200 µg/ml of the test material were selected for the gene mutation assay in the absence of S9 and presence of S9.

B. MUTAGENICITY ASSAY: In the mutagenicity assay, in the absence and presence of S9, little to no toxicity was observed as measured by relative cell survival (RCS) values (Table 1). In the absence of S9 activation RCS values ranged from 86.1 to 125.2 % and in the presence S9 the RCS values ranged from 72.5 to 100.8 %. The single low value (RCS value of 7.6%) observed for one of the replicates of the 200 µg/ml (+S9) was interpreted to be due to a technical error in seeding the correct number of cells.

The mutant frequencies observed in cultures treated with the test material in the absence and presence of S9 were not significantly different from the concurrent solvent control values (Table 1). All mutant frequencies were within a reasonable range of historical background values.

TABLE 1: Results of the Mutagenicity Assay using XDE-742.

Concentration		Mutagenicity Assay			
		-S9		+S9	
µg/ml		%RCS	MF	%RCS	MF
Neg. Control	0	100.2	10.2	108.6	10.7
Neg. Control	0	99.8	8.0	91.4	13.9
XDE-742	12.5	86.1	9.1	87.6	4.3
	12.5	90.0	4.4	100.8	6.9
	25	115.1	8.3	91.9	6.8
	25	100.8	6.6	87.1	6.2
	50	108.6	4.7	83.4	9.8
	50	93.5	3.8	72.5	4.2
	100	103.7	4.8	94.2	10.4
	100	105.5	6.5	94.7	16.9
	200	125.2	8.7	98.0	18.1
	200	87.3	8.2	7.6[a]	9.6
Historical Control[f] Average		NA	7.4	NA	8.2
Min-Max Range			1.2 -18.9		0 - 26.7
Pos. Control		26.2	823.6*	71.4	346.9*
Pos. Control		33.1	878.9*	71.4	367.0*

Data can be found in Tables 1-3 and 5 on pages 20-22 and 24 in the study report (MRID 46908408).
[f]The historical control data is a compilation of 46 (-S9) and 48 (+S9) experiments over a five year period between 1999-2004.
RCS = Relative Cell Survival
MF = TG^r Mutants per 10^6 clonable cells
*The frequency of TG^r mutants is significantly higher than the concurrent negative control value ($\alpha = 0.05$).
[a] Fewer cells plated due to a possible technical error.

The positive control chemicals induced significant increases in mutation frequencies and this data confirmed the adequacy of the experimental conditions for detecting induced mutations.

A confirmatory mutagenicity assay was not considered necessary since the initial test yielded clearly negative results.

Based upon the frequency of TG^r mutants recovered in cultures treated with the test material, it was concluded that XDE-742 was not mutagenic in the CHO/HGPRT gene mutation assay.

III. DISCUSSION AND CONCLUSIONS:

A. <u>INVESTIGATORS' CONCLUSIONS</u>: Based upon the frequency of TG^r mutants in cultures treated with the test material, it was concluded that XDE-742 was not mutagenic in the CHO/HGPRT gene mutation assay.

B. <u>REVIEWER COMMENTS</u>: XDE-742 was tested up to the limit of solubility (200 µg/mL in DMSO). There was no appreciable cytotoxicity at any dose tested. The number of TG^r mutants per 10^6 clonable cells in the treated groups was not significantly different than the controls. The mutant frequency of the control was consistent with recent historical control data for the performing laboratory. The positive control chemicals elicited a proper

mutagenic response in the CHO cells at the HGPRT locus. **There was no evidence of induced mutant colonies over background.**

The registrant, the Dow Chemical Company, originally prepared this STUDY PROFILE TEMPLATE (STP) (MRID 46908605) in HED's DER format. The HED reviewers may have added minor adjustments/additions to the original STP. The conclusions of the study and assignment of its classification as determined by HED reviewers are in the Executive Summary above.

C. STUDY DEFICIENCIES:

None

EPA Reviewer: Kimberly Harper **Signature:** Kimberly D Harper
RAB2, Health Effects Division (7509P) **Date:** 12/12/07
EPA Secondary Reviewer: Alan C. Levy, PhD **Signature:** Alan C. Levy
RAB2, Health Effects Division (7509P) **Date:** 1-3-2008

Template version 02/06

TXR#: 0054347

DATA EVALUATION RECORD

STUDY TYPE: ***In vitro* Mammalian Cytogenetics** – Chromosomal Aberration Assay Utilizing Rat Lymphocytes OPPTS 870.5375 [§84-2]; OECD 473

PC CODE: 108702 **DP BARCODE:** D332276

TEST MATERIAL (PURITY): XDE-742 (98.0% a.i.)

SYNONYMS: (N-(5,7-dimethoxy[1,2,4]triazolo[1,5-a]pyrimidin-2-yl)-2-methoxy-4-(trifluoromethyl)pyridine-3-sulfonamide), X666742, XR-742

CITATION: G. D. Charles, Ph.D., DABT, M. R. Schisler, B. S. (2004) Evaluation of XDE-742 in an *in vitro* chromosomal aberration assay utilizing rat lymphocytes. Toxicology & Environmental Research and Consulting, The Dow Chemical Company, Midland, Michigan, 48674. Laboratory report number 041005, August 23, 2004. MRID 46908409. Unpublished

SPONSOR: Dow AgroSciences LLC, 9330 Zionsville Road, Indianapolis, Indiana 46268

EXECUTIVE SUMMARY: XDE-742 (98.0% a.i., EO952-52-01, TSN103826) was evaluated in an *in vitro* chromosomal aberration assay (MRID 46908409) utilizing CD ISG rat lymphocytes. Approximately 48 hours after the initiation of whole blood cultures, cells were treated either in the absence or presence of S9 activation with concentrations ranging from 0 (solvent control) to 200 µg XDE-742 per ml of culture medium. The duration of treatment was 4 or 24 hours without S9 and 4 hours with S9. The highest concentration tested was based on the limit of solubility of the test material in the solvent used to dissolve it. Based upon the mitotic indices, cultures treated with targeted concentrations of 0 (solvent control), 50, 100, and 200 µg/ml in the absence (4 and 24 hour treatments) and presence (4 hour treatment) of S9 activation were selected for determining the incidence of chromosomal aberrations.

There were no significant increases in the frequencies of cells with aberrations in either the absence or presence of S9 activation. Cultures treated with the positive control chemicals (*i.e.*, mitomycin C without S9 and cyclophosphamide with S9) had significantly higher incidences of abnormal cells in the assay. Based upon these results, XDE-742 was considered to be non-genotoxic in this *in vitro* chromosomal aberration assay utilizing rat lymphocytes.

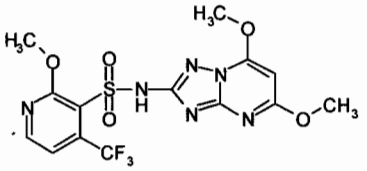
There was no evidence of increased chromosomal aberrations induced over background.

This study is acceptable and satisfies the guideline requirement for an *in vitro* mammalian cytogenetics assay (rat lymphocyte chromosomal aberration test); USEPA OPPTS 870.5375; OECD Guideline 473; EC, B.10; JMAFF, Mutagenicity Guidelines.

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

I. MATERIALS AND METHODS:

A. MATERIALS:

1.	**Test Material:**	XDE-742
	Description:	Powder, White
	Lot/Batch #:	EO952-52-01, TSN103826
	Purity:	98% XDE-742
	CAS #:	422556-08-9
	Chemical Structure:	
	Solvent Used:	Dimethyl sulfoxide (DMSO, Sigma, St. Louis, Missouri)

2.	**Control Materials:**	
	Solvent Control	Dimethyl sulfoxide (DMSO) 1%
	Positive Control:	Nonactivation: Mitomycin C (MMC, Sigma, CAS No. 50-07-7), 0.5 µg/ml (4 hour) and 0.05 µg/ml (24 hour) Activation: Cyclophosphamide monohydrate (CP, Sigma, CAS No. 6055-19-2), 4 µg/ml 0.75 µg/ml MMC (24 hour, nonactivation) and 6 µg/ml CP were also used and evaluated for cytotoxicity but were not used for assessment of chromosomal abberations.

3.	**Activation**: S9 derived from							
	X	Induced	X	Aroclor 1254	X	Rat	X	Liver
		Not-induced		Phenobarbitol		Mouse		Lung
				None		Hamster		Other (name)
				Other (name)		Other (name)		

The S9 homogenate was purchased from Molecular Toxicology, Inc., Boone, North Carolina, and stored at –100 °C or below. Thawed S9 was reconstituted at a final concentration of 10% (v/v) in a "mix". The S9 mix consisted of 10 mM $MgCl_2 \cdot 6H_2O$ (Sigma), 5 mM glucose-6-phosphate (Sigma), 4mM nicotinamide adenine dinucleotide phosphate (Sigma), 10mM $CaCl_2$ (Fisher, Fair Lawn, New Jersey), 30 mM KCl (Sigma), and 50 mM sodium phosphate (pH 8.0, Sigma and Fisher). The reconstituted mix was added to the culture medium to obtain the desired final concentration of S9 in the culture, *i.e.*, 2% v/v. Hence, the final concentration of the co-factors in the culture medium was 1/5 of the concentrations stated above.

4.	**Test cells:** Peripheral lymphocytes from male CD ISG (Outbred Crl:CD (SD)IGSBR rats

Media: RPMI 1640 medium (with 25 mM HEPES, GIBCO, Grand Island, New York) supplemented with 10% heat-inactivated dialyzed fetal bovine serum (GIBCO), antibiotics and antimycotics (Fungizone 0.25 µg/ml; penicillin G, 100 U/ml; and streptomycin sulfate, 0.1 mg/ml; GIBCO), 30 µg/ml PHA (HA16, Murex Diagnostics Ltd., Dartford, England), and an additional 2 mM L-glutamine (GIBCO).					
Properly maintained?		X	Yes		No
Periodically checked for *Mycoplasma* contamination?			Yes	X	No(N/A)
Periodically checked for karyotype stability?			Yes	X	No (N/A)
5.	**Test compound concentrations used:**				
	Nonactivated conditions:	Assay A1 – 4 hours: 0, 3.125, 6.25, 12.5, 25, 50, 100, and 200 µg/ml Assay A1 – 24 hours: 0, 1.56, 3.125, 6.25, 12.5, 25, 50, 100, and 200 µg/ml			
	Activated conditions:	Assay A1 – 4 hours: 0, 3.125, 6.25, 12.5, 25, 50, 100, and 200 µg/ml			

B. TEST PERFORMANCE:

1. **Preliminary cytotoxicity assay:** Not performed

2. **Cytogenetic assay:**

a.	**Cell exposure time:**	Test Material	Solvent Control	Positive Control
	Non-activated:	4 h & 24 h	4 h & 24 h	4 h & 24 h
	Activated:	4 h	4 h	4 h

b.	**Spindle inhibition**	
	Inhibition used/concentration:	Colcemid 1 µg/culture
	Administration time:	Approximately 3 hours prior to cell harvest

c.	**Cell harvest time after termination of treatment:**	Test Material	Solvent Control	Positive Control
	Non-activated:	20 & 0 h	20 & 0 h	20 & 0 h
	Activated:	20 h	20 h	20 h

d. **Details of slide preparation:** The cells were swollen by hypotonic treatment (0.075 M KCl), fixed with methanol:acetic acid (3:1), dropped on microscope slides, and stained in Giemsa.

e. **Metaphase analysis**

No. of cells examined per dose: 200 cells/treatment, positive controls 100 cells				
Scored for structural?	X	Yes		No
Scored for numerical?	X	Yes polyploid		No
Coded prior to analysis?	X	Yes		No

f. **Evaluation criteria:** For a test to be acceptable, the chromosomal aberration frequency in the positive control cultures should be significantly higher than the solvent controls. The

aberration frequency in the solvent control should be within reasonable limits of the laboratory historical values. A test chemical is considered positive in this assay if it induces a significant dose-related and reproducible increase in the frequency of cells with aberrations.

g. **Statistical analysis:** The properties of cells with aberrations (excluding gaps) were compared by the following statistical methods. At each dose level, data from the replicates was pooled. A two-way contingency table was constructed to analyze the frequencies of aberrant cells. An overall Chi-square statistic, based on the table, was partitioned into components of interest. Specifically, statistics were generated to test the global hypotheses: 1) no difference in the average number of cells with aberrations among the dose groups, and 2) no linear trend of increasing number of cells with aberrations with increasing dose. An ordinal metric (0, 1, 2, etc.) was used for the doses in the statistical evaluation. If either statistic was found to be significant at alpha = 0.05, versus a one-sided increasing alternative, pairwise tests (*i.e.* control vs. treatment) were performed at each dose level and evaluated at alpha = 0.05, again versus a one-sided alternative.

Polyploid cells were analyzed by the Fisher Exact probability test. The number of polyploid cells were pooled across replicates for the analysis and evaluated at alpha = 0.05. The data was analyzed separately based on the presence or absence of S-9 and based on the exposure time.

II. REPORTED RESULTS:

A. **ANALYTICAL ANALYSIS:** The pH of treatment medium containing approximately 203 µg XDE-742/ml of the test material (solubility limitations in the solvent) and medium containing 1% solvent (DMSO) was determined using a Denver Basic pH meter (Denver Instrument Co., Arvada, Colorado) and an Osmette A freezing point osmometer (Precision Systems, Inc., Natick, Massachusetts), respectively. There was no appreciable change in either the pH or osmolality at this concentration as compared to the culture medium with solvent alone (culture medium with the test material, pH = 7.29, osmolality = 428 mOsm/kg H_2O; culture medium with 1% DMSO, pH = 7.20, osmolality = 423 mOsm/kg H_2O).

The analytically detected concentrations of the test material in the stock solutions (Assay A1) varied from 93.6 to 102% of the target.

B. **CYTOGENETIC:** In the initial assay, with and without metabolic activation, the cultures did not display any toxicity as measured by mitotic indices. The relative mitotic indices for the treated cultures ranged from 106 – 121% without S9 and 91 – 111% with S9 compared to the controls. In the assay exposing the cultures for 24 hours in the absence of S9, the relative mitotic index of the treated groups ranged from 90-117%, indicating no appreciable toxicity. Therefore, cultures treated with 50, 100, and 200 µg/mL were chosen for the determination of chromosomal aberration frequencies and incidence of polyploidy. Among the cultures treated with the positive control chemicals for 4 hours, 0.5 µg/ml of MMC and 4 µg/ml of CP were selected for evaluation of aberrations in the absence and presence of S9, respectively. Cultures treated with 0.05 µg/ml MMC were selected for evaluation to serve as the positive control for the 24 hour assay in the absence of S9 (Table 1).

There were no significant increases in the incidence of polyploidy cells in any of the XDE-742 treated cultures or the positive controls as compared to the solvent control values Table 1).

TABLE 1. Mitotic Indices and Polyploidy Incidence in Rat Lymphocyte Cultures Exposed to XDE-742.

	Mean % mitotic index [a]			Incidence of polyploidy (%)		
Conc.	Assay A1			Assay A1		
µg/ml	-S9 (4 hr)	+S9 (4 hr)	-S9 (24 hr)	-S9 (4 hr)	+S9 (4 hr)	-S9 (24hr)
Negative control	14.0 (100)	16.8 (100)	13.8 (100)	0.5	1.0	1.0
1.56	ND	ND	13.2 (96)	ND	ND	ND
3.125	14.8 (106)	15.4(92)	15.1 (109)	ND	ND	ND
6.25	17.0 (121)	15.2 (91)	16.2 (117)	ND	ND	ND
12.5	16.0 (114)	17.3 (103)	13.2 (96)	ND	ND	ND
25	15.8 (113)	15.8 (94)	13.3 (96)	ND	ND	ND
50	16.8 (120)	16.6 (99)	12.4 (90)	0.5	2.0	0.5
100	16.1 (115)	18.5 (110)	12.8 (93)	1.5	1.5	1.0
200	15.8 (113)	18.7 (111)	14.0 (101)	0.0	0.5	1.0
Positive control [b]	11.5 (82)	13.7 (82)	8.7 (63)	0.0	0.5	0.0
Positive control [c]	ND	12.2 (73)	6.9 (50)			

Data are from Tables 2a-c and 3 on pages 23-26 of the study report (MRID 46908409).
[a] values in parenthesis are % relative mitotic index
[b] Positive control = 0.5 µg/ml MMC (-S9, 4 hr); 4 µg/ml CP (+S9, 4 hr); 0.05 µg/ml MMS (-S9, 24 hr)
[c] Positive control = 6 µg/ml CP (+S9, 4 hr); 0.075 µg/ml MMS (-S9, 24 hr)
ND = not done

In the 4 hr non-activation assay, the frequency of cells with aberrations in the solvent control was 2.0 % and the corresponding values at treatment levels of 50, 100, and 200 µg/ml were 1.5, 1.5, and 2.0%, respectively. In the activation assay, cultures treated with the test material at concentrations of 50, 100, and 200 µg/ml had aberrant cell frequencies of 2.5, 1.5, and 2.5, respectively as compared to the solvent control value of 1.0%. Statistical analyses of these data did not identify significant differences between the solvent control and any of the treated cultures without or with S9 activation (Table 2). The frequencies of aberrant cells observed in the test material treated cultures were within the laboratory historical background range.

In the non-activation assay, where cultures were treated continuously for 24 hr with the test material, the frequencies of aberrant cells in the solvent control was 2.5% and the corresponding values at concentration levels of 50, 100, and 200 µg/ml were 0.5, 2.5, and 3.0%, respectively (Table 2). There were no statistically significant differences between the test material treated cultures and the solvent control values and all values were within the laboratory historical background range.

Significant increases in the frequency of cells with aberrations were observed in cultures treated with the positive control chemicals. Aberrant cell frequencies in MMC (- S9, 4 hr treatment), CP (+ S9) and MMC (- S9, 24 hr treatment) cultures were 30, 32, and 27, respectively.

TABLE 2. Results of the Cytogenetic Assay using XDE-742.

	Total aberrations (excluding gaps) [a]			No. of cells with aberrations (excluding gaps) [a]		
Conc.	Assay A1 (n = 200 cells)			Assay A1 (n = 200 cells)		
µg/ml	-S9 (4 hr)	+S9 (4 hr)	-S9 (24 hr)	-S9 (4 hr)	+S9 (4 hr)	-S9 (24 hr)
Negative control	2.0	1.0	3.0	2.0	1.0	2.5
50	1.5	2.5	0.5	1.5	2.5	0.5
100	1.5	1.5	2.5	1.5	1.5	2.5
200	2.0	2.5	3.5	2.0	2.5	3.0
Positive control [b]	29.0	35.0	39.0	30.0*	32.0*	27.0*

Data are from Tables 4a-c and 5 on pages 27-30 of the study report (MRID 46908409).
[c]The historical control data is compiled from 129 (-S9) and 118 (+S9) experiments over an 11 year period between 1994-2004. The range of means for % cells with aberrations (excluding gaps) without metabolic activation was 0.6-1.8 with a min-max range of 0-6.0. The range of means for % cells with aberrations (excluding gaps) with metabolic activation was 0.8-2.1 with a min-max range of 0-6.5. No data for total aberrations were provided.
[a] Values are percentages
[b] Positive control = 0.5 µg/ml MMC (-S9, 4 hr); 4 µg/ml CP (+S9, 4 hr); 0.05 µg/ml MMC (-S9, 24 hr)
*Significantly different from negative controls, $\alpha = 0.05$

III. DISCUSSION AND CONCLUSIONS:

A. INVESTIGATOR'S CONCLUSIONS: It was concluded that under the experimental conditions used, XDE-742 was non-genotoxic in this *in vitro* chromosomal aberration study. A second assay with treatment of cultures in the presence of S9 was not considered necessary in this assay since the initial test yielded clearly negative results.

B. REVIEWER COMMENTS: There was no evidence of significant cytotoxicity at the doses tested; the highest dose was based on the limit of XDE-742 solubility in DMSO. Treatment with XDE-742 did not induce an increase in the number of polyploidy cells at any dose level, either in the presence or absence of metabolic activation. There were no significant increases in the frequencies of cells with aberrations in either the absence or presence of S9 activation. The test results were consistent with recent historical control data provided by the testing laboratory. Cultures treated with the positive control chemicals (*i.e.*, mitomycin C without S9 and cyclophosphamide with S9) had significantly higher incidences of abnormal cells in the assay. Based upon these results, XDE-742 was considered to be non-genotoxic in this *in vitro* chromosomal aberration assay utilizing rat lymphocytes.

Under the conditions of this study, there was no evidence of increased chromosomal aberrations induced over background up to the limit of solubility for the test compound.

The registrant, the Dow Chemical Company, originally prepared this STUDY PROFILE TEMPLATE (STP) (MRID 46908606) in HED's DER format. The HED reviewers may have added minor adjustments/additions to the original STP. The conclusions of the study and assignment of its classification as determined by HED reviewers are in the Executive Summary above.

C. STUDY DEFICIENCIES: None

EPA Reviewer: Kimberly Harper **Signature:** Kimberly D Harper
RAB2, Health Effects Division (7509P) **Date:** 12/12/07
EPA Secondary Reviewer: Alan C. Levy, PhD **Signature:** Alan C. Levy
RAB2, Health Effects Division (7509P) **Date:** 1-3-2008
Template version 02/06

TXR#: 0054347

DATA EVALUATION RECORD

STUDY TYPE: *In Vivo* Mammalian Cytogenetics - Erythrocyte Micronucleus Assay in Mice; OPPTS 870.5395 [§84-2]; OECD 474.

PC CODE: 108702 **DP BARCODE:** D332276

TEST MATERIAL (PURITY): XDE-742 (98.0% a.i.)

SYNONYMS: (N-(5,7-dimethoxy[1,2,4]triazolo[1,5-a]pyrimidin-2-yl)-2-methoxy-4-(trifluromethyl)pyridine-3-sulfonamide), X666742, XR-742, XDE-742

CITATION: P. J. Spencer, Ph.D., D.A.B.T. and J. Grundy, B.S. Evaluation of XDE-742 in the mouse bone marrow micronucleus test. Toxicology & Environmental Research & Consulting, The Dow Chemical Company, Midland, Michigan. Study ID: 041004, 1 October 2004. MRID 46908410. Unpublished

SPONSOR: Dow AgroSciences LLC, 9330 Zionsville Road, Indianapolis, Indiana 46268

EXECUTIVE SUMMARY: The *in vivo* genotoxic potential of XDE-742 (98.% a.i., EO952-52-01, TSN103826) was evaluated by examining the incidence of micronucleated polychromatic erythrocytes (MN-PCE) in the bone marrow (MRID 46908410). The test material was administered to 6 male CD-1 mice per dose by oral gavage on two consecutive days at dose levels of 0, 500, 1000, or 2000 (limit dose) mg/kg/day in 0.5% METHOCEL® solution. The 2000 mg/kg dose group had a satellite group of an additional 3 mice in case of mortality in the main study group. Treated mice were sacrificed at 24 hours after the second treatment for the collection of femoral bone marrow and evaluation of PCE (2000/animal) for MN. The proportion of PCE among erythrocytes was determined based upon 200 erythrocytes per animal and the results expressed as percentages. Mice treated with a single dose of 120 mg/kg bw cyclophosphamide monohydrate and sacrificed 24 hours later served as positive controls.

The dose levels were based upon the results of a range-finding test where doses up to 2000 mg/kg/day no treatment-related deaths, toxicity or changes in body temperatures were observed in male or female mice. The concentrations of the test material in the dosing solutions were verified by analytical methods. All animals were observed for clinical signs prior to dosing and at 2, 5, and 24 hours following each dosing. Body weights were collected prior to dosing and at sacrifice.
All animals survived to the end of the observation period.

There were no clinical signs of treatment related toxicity. There were no statistically significant differences in the frequencies of MN-PCE or percent PCE in groups treated with the test material as compared to the negative controls. There was a significant increase in the frequency of MN-PCE in the positive control chemical group as compared to the negative control group. There was no significant difference in the relative proportion of PCE among erythrocytes in the positive control group when compared to the negative control group.

Under the experimental conditions used, XDE-742 did not cause an increase in the number of micronucleated polychromatic erythrocytes in mouse bone marrow.

This study is classified as acceptable. This study satisfies the requirement for Test Guideline OPPTS 870.5395; OECD 474 for *in vivo* cytogenetic mutagenicity data.

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

I. MATERIALS AND METHODS:

A. MATERIALS:

1. Test material:

XDE-742

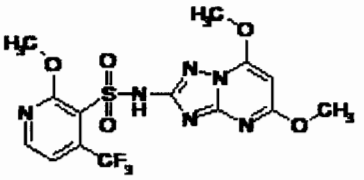
Description: White powder

Lot/Batch #: E0952-52-01 TSN103826

Purity: 98.0 % a.i.

CAS # of TGAI: 422556-08-9

Structure:



Solvent Used: 0.5% METHOCEL®

2. Control materials:

Vehicle: 0.5% METHOCEL — Final volume: 10 ml/kg bw — Route: oral gavage

Positive control : Cyclophosphamide monohydrate (CP), C.A.S. Number: 6055-19-2, Source : Sigma, St. Louis, Missouri — Final dose(s): 120 mg/kg bw — Route: oral gavage

3. Test animals:

Species: Mice

Strain: CD-1

Age/weight at study initiation: 8-9 weeks/31.9 – 33.5 g

Source: Charles River Laboratories (Portage, Michigan)

No. animals used per dose	6	Males	0	Females
Properly Maintained?	Y	Yes	-	No

4. Test compound administration:

	Dose levels	Final volume	Route
Preliminary	0, 500, 1000 or 2000 mg/kg/day	10 mL/kg	Oral gavage
Main study	0, 500, 1000 or 2000 mg/kg/day	10 mL/kg	Oral gavage

The dosing solutions were prepared on the day before each dose administration. A frozen stock solution of cyclophosphamide monohydrate (CP) dissolved in distilled water (thawed and brought to room temperature prior to use) served as the positive control. The vehicle used to mix the test material (0.5% METHOCEL®) served as the negative control. The concentrations of the test material in the dosing solutions were verified using high performance liquid chromatography with ultra violet detection (HPLC/UV).

B. TEST PERFORMANCE:

1. Treatment and sampling times:

a. Test compound:

Dosing:	☐	once	☒	twice (24 hrs apart)			☐	Other	
Sampling (after last dose):	☐	6 hr	☐	12 hr	☒	24 hr	☐	48 hr	☐ 72 hr

b. Negative and/or vehicle control:

Dosing:	☐	once	☒	twice (24 hrs apart)			☐	Other	
Sampling (after last dose):	☐	6 hr	☐	12 hr	☒	24 hr	☐	48 hr	☐ 72 hr

c. Positive control:

Dosing:	☒	once	☐	twice (24 hrs apart)			☐	Other	
Sampling (after last dose):	☐	6 hr	☐	12 hr	☒	24 hr	☐	48 hr	☐ 72 hr
Other:	☐		☐		☐		☐		

2. Tissues and cells examined:

Bone marrow: femoral

No. of polychromatic erythrocytes (PCE) examined per animal: 2000

No. of normochromatic erythrocytes (NCE; more mature RBCs) examined per animal: Up to 200

3. Details of slide preparation: The cell suspension was prepared by aspirating the bone marrow of the femur with a plastic syringe containing approximately 0.5 ml of fetal bovine serum (GIBCO, Grand Island, New York). The contents of the syringe were then transferred to a centrifuge tube containing serum and resuspended. The cells were centrifuged at approximately 1000 rpm for 5 minutes. The supernatant was discarded; the pellet was resuspended in a small amount of serum before transfer to slides. The Wedge smears were prepared on microscope slides using small portions of the cell suspension. The slides were allowed to air dry, fixed in cold methanol and stained with Wright-Giemsa using an automatic slide stainer, (Ames Hema-Tek, Miles Scientific, Naperville, Illinois).

All slides were coded, scored, and decoded upon completion to control for bias. Two thousand PCE were examined from each animal and the number of micronucleated polychromatic erythrocytes (MN-PCE) was recorded. Micronuclei were identified as darkly stained bodies with smooth contours and varying shapes such as round, almond, or ring. The ratio of PCE to NCE in the bone marrow was determined in the micronucleus test by examining 200 erythrocytes. The ratio was expressed as PCE X 100/PCE+NCE.

4. Evaluation criteria:

A test was considered valid if all of the following conditions were met:

- The range of MN-PCE values in the negative controls were within reasonable limits of the recent (past five years) laboratory background range.
- There was a significant increase in the incidence of MN-PCE in the positive control treatment as compared to the concurrent negative controls.
- The mean for % PCE value in one or more of the test material treated groups was $\geq$ 20% of the control value indicating no undue effect on erythropoiesis (toxicity).

A test material was considered positive in this assay if the following was met:

- Statistically significant increase in MN-PCE frequency at one or more dose levels accompanied by a dose response.

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A test material was considered negative in this assay if all of the following criteria were met:

- No statistically significant dose related increase in MN-PCE as compared to the negative control.

A test result not meeting the criteria for either the positive or the negative response was considered to be equivocal.

5. **Statistical methods:** The raw data on the counts of MN-PCE for each animal were first transformed by adding one to each count and then taking the natural log of the adjusted number. The transformed MN-PCE data and the data on percent PCE were analyzed separately by a one-way analysis of variance. Pairwise comparisons of treated vs. control groups were done, if the dose effect was significant, by Dunnett's t-test, one-sided (upper) for MN-PCE and two-sided for the percent PCE. Linear dose-related trend tests were performed only if any of the pairwise comparisons yielded significant differences. The alpha level at which all tests were conducted was 0.05.

The final interpretation of biological significance of the responses was based on both statistical outcome and scientific judgment.

II. REPORTED RESULTS:

A. PRELIMINARY TOXICITY ASSAY: Targeted dose levels of 500 (1/sex/dose), 1000 (4/sex/dose), and 2000 (4/sex/dose) mg/kg/day were used in the preliminary range-finding portion of the assay. There were no appreciable changes in the body weights of mice. There were no remarkable observations of toxicity or mortality among the treated animals during the in-life portion of the range-finding test. There were no remarkable changes in the body temperature of the mice that could be attributed to treatment with the test material.

B. MICRONUCLEUS ASSAY: The analytically determined concentrations of the test material in the dosing solutions used for the micronucleus test ranged from 95.0% to 98.6% of the targeted values. The test material was confirmed to be homogeneous in the vehicle (relative standard deviations of 1.62-3.35%).

Neither pyroxsulam nor the positive control had an effect on survival, clinical signs, or body weight. There were no indications of toxicity upon daily observation during the in-life portion of the micronucleus test.

A summary of the data on the frequencies of MN-PCE and percent PCE observed in various treatment groups of male mice is presented in Table 1. There were no significant differences in MN-PCE frequencies between the groups treated with the test material and the negative controls. The individual group means were within the historical control range for negative controls from recent studies at the lab (0.3-2.2 and 55.8-68.7, %MN-PCE and %PCE, respectively). The adequacy of the experimental conditions for the detection of induced micronuclei was ascertained from the observation of a significant increase in the frequencies of micronucleated polychromatic erythrocytes in the positive control group, which were also

within the range of historical control means (35.9-79.3).

The percent PCE values observed in the test material-treated animals or the positive control animals were not significantly different from the negative control values. However, the %PCE value for the positive control did fall within the historical control range of positive controls for the lab (42.8-55.0)

TABLE 1. Summary Micronucleated Polychromatic Erythrocytes (MN-PCE) Frequencies and % Polychromatic Erythrocytes (% PCE) – Males

			% MN-PCE		%PCE	
Dose (mg/kd/day)	Time of Sacrifice (hr)	n[a]	Mean	SD	Mean	SD
XDE-742 0	24	6	1.0	0.8	61.8	7.8
500	24	6	0.8	0.5	63.3	10.9
1000	24	6	0.4	0.4	59.3	5.5
2000	24	6	1.6	1.5	67.0	5.5
CP 120	24	6	63.4*	12.7	52.8	12.2

Data from Table 18 on page 40 of the study report (MRID 46908607).
[a] the number of animals per dose group where 2000 PCE were examined/animal for MN incidence and expressed as MN/1000 PCE (% MN-PCE)
*significantly different from control (alpha≤0.05)
CP = cyclophosphamide monohydrate

III. DISCUSSION AND CONCLUSIONS:

A. **INVESTIGATORS' CONCLUSIONS:** No treatment-related toxicity was observed in male mice administered two consecutive daily doses of XDE-742 up to the limit dose of 2000 mg/kg/day. Based on the results of the study, it was concluded that the test material, XDE-742, did not induce a significant increase in the frequencies of micronucleated bone marrow polychromatic erythrocytes when given as a single oral dose to male CD-1 mice on two consecutive days. Hence, XDE-742 is considered negative in this test system under the experimental conditions.

B. **REVIEWER COMMENTS:** The range finding study did not indicate any gender differences in survival, clinical signs, body weights, or body temperature. Therefore, only male mice were tested in the main study. Two oral doses of up to 2000 mg/kg/day spaced 24 hours apart did not have any effect on survival, body weight, or clinical signs in male CD-1 mice. The mean %MN-PCE and mean %PCE in the treated groups were not significantly different than the means of the controls and were within the historical control range for negative control groups from the performing lab, indicating that XDE-742 did not induce an increased incidence of micronucleated bone marrow polychromatic erythrocytes. The positive control chemical did produce a significantly higher mean %MN-PCE than the negative control group and was within the historical control range for positive control studies. The %PCE, though not statistically different from the negative controls in this study, was within the historical control range for positive control studies using cyclophosphamide monohydrate.

Therefore, XDE-742 did not induce increased micronucleated bone marrow polychromatic erythrocytes, and is therefore considered negative, under the conditions

of this study.

The registrant, the Dow Chemical Company, originally prepared this STUDY PROFILE TEMPLATE (STP) (MRID 46908607) in HED's DER format. The HED reviewers may have added minor adjustments/additions to the original STP. The conclusions of the study and assignment of its classification as determined by HED reviewers are in the Executive Summary above.

C. **STUDY DEFICIENCIES:** None

EPA Reviewer: Kimberly Harper **Signature:** Kimberly D Harper
RAB2, Health Effects Division (7509P) **Date:** 12/10/07
EPA Secondary Reviewer: Alan C. Levy, PhD **Signature:** Alan C. Levy
RAB2, Health Effects Division (7509P) **Date:** 1-3-2008

Template version 02/06

TXR#: 0054347

DATA EVALUATION RECORD

STUDY TYPE: Other Genotoxicity: Unscheduled DNA Synthesis in Primary Rat Hepatocytes/Mammalian Cell Cultures; OPPTS 870.5550 (*in vitro*) [§84-2]; OECD 486 (*in vivo*)

PC CODE: 108702 **DP BARCODE:** D332276

TEST MATERIAL (PURITY): XDE-742 (98%)

SYNONYMS: X666742, XR-742, BAS-770H, N-(5,7-dimethoxy[1,2,4]triazolo[1,5-a]pyrimidin-2-yl)-2-methoxy-4-(trifluoromethyl)-3-pyridinesulfonamide

CITATION: Beevers, C. (2006) XDE-742: Measurement of unscheduled DNA synthesis in mouse liver using an *in vivo/in vitro* procedure. Covance Laboratories Ltd. Harrogate UK. Covance Study Number 295/169, 04 November 2006. MRID 47022001. Unpublished

SPONSOR: The Dow Chemical Company, Midland, MI 48640 USA, for Dow AgroSciences LLC, 9330 Zionsville Road, Indianapolis, Indiana 46268

EXECUTIVE SUMMARY:

In an unscheduled DNA synthesis assay (MRID 47022001), male Crl:CD-1 (ICR) mice were administered vehicle control [0.5% (w/v) methylcellulose], treatment compound XDE-742 (98%, Lot # TSN103826) at doses of 1000 or 2000 mg/kg bw, or positive control compounds [Fast Garnet GBC (FG-GBC) 20 mg/mL or Dimethylnitrosamine (DMO) 1 mg/mL] hepatocytes were isolated approximately 12-14 hours or 2-4 hours after dosing.

Treatment with XDE-742 up to 2000 mg/kg yielded net nuclear grain (NNG) values similar to concurrent vehicle controls data and the lab's historical control ranges.

The positive controls induced did the appropriate response. **There was no evidence that unscheduled DNA synthesis, as determined by radioactive tracer procedures (NNG counts), was induced at concentrations up to 2000 mg/kg in Crl:CD-1 mouse hepatocytes.**

This study is classified as acceptable/guideline and satisfies does the guideline requirement for Test Guideline OPPTS 870.5550; OECD 482/486 for other genotoxic mutagenicity data.

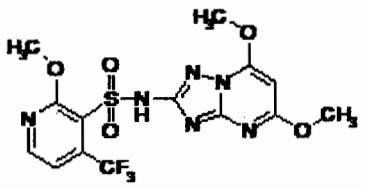
COMPLIANCE: Signed and dated GLP, Quality Assurance, and Data Confidentiality statements were provided.

I. MATERIALS AND METHODS:

A. MATERIALS:

1. **Test Material:**

Description:	XDE-742, off-white powder
Lot/Batch #:	TSN103826
Purity:	98% a.i.
Compound Stability:	Assigned expiry date 15 December 2009. XDE-742 was reported to be stable in 0.5% methylcellulose for 21 days. Data not provided.
CAS #:	422556-08-9
Structure:	



Solvent Used: 0.5% (w/v) aqueous methylcellulose (0.5% MC)

2. **Control materials:**

Negative control:	Methylcellulose	**Concentration:**	0.5%
Solvent:	Water	**Concentration:**	N/A
Positive control /solvent:	Fast Garnet GBC (FG-GBC) / Corn oil (12-14 hr sacrifice)	**Concentration:**	20 mg/mL
Positive control /solvent:	Dimethylnitrosamine (DMN) / Water (2-4 hr sacrifice)	**Concentration:**	1 mg/mL

3. **Test compound concentrations used:** 100 and 200 mg/mL (1000 and 2000 mg/kg)

4. **Media:** N/A

5. **Test cells:** Primary mouse hepatocytes, isolated from mice dosed with solvent, XDE-742 or positive control. Crl:CD-1 male mice were obtained from Charles River (UK) Ltd.

6. **Cell preparation:**

   a. **Perfusion technique**:
   Individual animals were anesthetized with halothane and maintained under deep anesthesia to prevent any likelihood of recovery. The hepatic portal vein was cannulated. The superior *vena cava* was cut. The hepatic portal cannula was then connected to a supply of 150 mL, calcium-free Buffer 1 (150 mM NaCl, 3.76 mM $NaHCO_3$, 4.84 mM Na_2HPO_4, 4.97 mM KCl, 1.24 mM KH_2PO_4, 0.62 mM $MgSO_4$, 0.62 mM $MgCl_2$, 10 µg/mL phenol red indicator) at a flow rate of approximately 20 mL/min. The

vena cava cannula was allowed to drain to waste and the liver washed free of blood. The liver was then perfused with approximately 400 mL Buffer 2 (142 mM NaCl, 24 mM $NaHCO_3$, 4.37 mM KCl, 1.24 mM KH_2PO_4, 0.62 mM $MgSO_4$, 0.62 mM $MgCl_2$, 10 µg/mL phenol red indicator), for approximately 5 minutes. Both buffers were gassed with 5% CO_2 in air (v/v).

When the reservoir of Buffer 2 reached approximately 300 mL, calcium and collagenase (approximately 80 mg of collagenase dissolved in 2 mL of 769 mM $CaCl_2$ and 10 mL of Buffer 2) were added. When the reticular pattern of the liver had begun to break up and the liver became 'spongy', the perfusion was stopped.

b. Hepatocyte harvest/culture preparation:
The gall bladder was removed and the liver cut free into a suitable container with pre-warmed Buffer 2. Hepatocytes were carefully teased out. The resulting hepatocyte suspension was gently washed through 150 µm nylon mesh with Williams E medium-Complete (WE-C) to a volume of approximately 50 mL. The suspension was centrifuged at approximately 40 x 'g' for 2-3 minutes. The resultant pellet was resuspended in WE-C. The centrifugation and resuspension procedure was repeated as necessary and the pellet resuspended finally in approximately 15 mL WE-C. A sample (0.5 mL) of this suspension was taken, diluted with an equal volume of 0.4% (w/v) trypan blue in phosphate buffered saline (PBS) and the proportion of viable cells (those with unstained nuclei) determined using an hemocytometer. The culture was diluted where possible to provide approximately 1.5 x 10^5 viable cells/mL.

B. TEST PERFORMANCE:

1. Cytotoxicity assay: N/A

2. UDS assay:

a. Treatment: Animals were weighed before dosing and the volume of vehicle, test article preparation or positive control to be administered was calculated based on a dose volume of 10 mL/kg. Animals were not fasted prior to dosing. All treatments were given via oral gavage to maximize exposure of the test article to the target organ.
Animals were dosed as follows:

Treatment	Dose (mg/kg bw)	Dose volume (mL/kg)	Number of animals dosed	
			12-14 hour experiment	2-4 hour experiment
0.5% MC	0	10	6	6
XDE-742	1000	10	6	6
XDE-742	2000	10	6	6
DMN	10	10	-	6
FG-GBC	200	10	6	-

Hepatocytes were prepared from three of the six animals in each dose group. Animals not required were humanely killed.

b. Preparation of Autoradiographs/Grain Development: For each hepatocyte suspension, 3 mL was added to each well of a 6-well multiplate containing 25 mm round plastic coverslips and incubated at 37°C in a 5% CO_2 in air (v/v) atmosphere for at least 90 minutes to allow cells to attach.

Medium was removed from the cells and the monolayers washed with 2 mL Williams E medium-Incomplete (WE-I) which was then replaced with 2 mL WE-I containing 10 µCi/mL [^{3}H] thymidine. After approximately 4 hours incubation at 37°C in a 5% CO_2 in air (v/v) atmosphere, the medium was removed and the cells washed with three changes of WE-I containing 0.25 mM thymidine. Cultures were then incubated overnight with 3 mL of the same medium.

To prepare for autoradiography, coverslips were washed with 2 mL phosphate buffered saline (PBS) and the cells fixed with three changes of 2 mL glacial acetic acid:ethanol (1:3 v/v). The coverslips were then washed four times with purified water, allowed to dry and mounted onto previously labelled microscope slides, cells side up, with DPX.

Three of the six slides from each animal were coated in Ilford K2 liquid emulsion (three were kept in reserve and were autoradiographed if required). Each slide was dipped individually into the emulsion, ensuring that no air-bubbles were generated. After gelling over ice, the slides were incubated in a light-tight box at room temperature for approximately 90 minutes to allow the emulsion to dry. The slides were then packed in light-tight boxes containing desiccant, sealed with tape and left refrigerated for 14 days. At the end of this time, the emulsion had developed and was fixed. The cell nuclei and cytoplasm were stained with Meyer's haemalum/eosin Y. Slides were dehydrated in ethanol, cleared in xylene and mounted with coverslips for microscopic examination.

c. Grain counting: Grain counting was performed using a microscope with a video camera connected to an image analysis system (Perceptive Instruments) and a computer programmed for automatic data capture. Where possible, nuclear and cytoplasmic grain counts were obtained from 100 cells per animal, 50 from each of two slides. Each slide was examined to ensure that the culture was viable. Each slide was scanned and cells scored such that there was no risk that the same cell was counted more than once. Nuclear and cytoplasmic grain counts were recorded, and the net grains/nucleus (net nuclear grains, NNG) and % cells in repair were determined. The following criteria were used for cell analysis:

- only cells with normal morphology were scored
- isolated nuclei with no surrounding cytoplasm were not scored
- cells without nuclear and/or cytoplasmic graining were not scored
- cells with unusual staining artefacts were not scored
- heavily labelled cells in S-phase were not scored
- all other normal cells, 100 per animal were scored
- all slides were analysed blind (coded).

e. **Evaluation criteria:** The test article was considered as positive in this assay if, at any dose and at either time point:

- the chemical yielded group mean NNG values greater than 0 NNG and 20% or more of cells responding (NNG values ≥ 5)
- a marked increase above solvent control levels was seen in both NNG and the percentage of cells in repair.

Cytoplasmic and nuclear grain count values as well as the concurrent negative control data were considered in relation to the overall NNG values of cultures from treated animals.

f. **Statistical analysis:** The following were calculated for each slide, animal and dose point:

- the population average NNG and standard deviation (SD)
- the percent of cells responding or in repair (≥ 5 NNG)
- the population average cytoplasmic and nuclear grain count.

No other statistical analysis was performed

II. REPORTED RESULTS:

A. PRELIMINARY CYTOTOXICITY ASSAY: N/A

B. UDS ASSAY:

There were no clinical signs reported for any vehicle or test-article treated animal. Bright yellow bedding was found in the cages of animals treated with the FG-GBC positive control; however, only two of the six mice were noted to have bright yellow urine.

In the 12-14 hr experiment, the mean NNG counts for both the 1000 and 2000 mg/kg bw treatment groups (0.3 and -0.3) were similar to concurrent (-0.3) and histrorical control values (-1.51). The percent of cells in repair for the XDE-742 treatment groups (1.7-2.7%) were similar to controls (1.3%) . The positive control chemical, FG-GBC, produced a sufficiently positive response to demonstrate the sensitivity of the system. (See Table 1)

In the 2-4 hr experiment, the mean NNG counts for both the 1000 and 2000 mg/kg bw treatment groups (1.0 and 0.4) were similar to concurrent (0.4) and histrorical control values (-1.51). The percent of cells in repair for the XDE-742 treatment groups (2.7-4.0%) were similar to controls (2.0%). The positive control chemical, DMN, produced a sufficiently positive response to demonstrate the sensitivity of the system. (See Table 2)

Table 1: XDE-742: group mean net nuclear grain count values, 12-14 hour

Dose (mg/kg bw)	Compound	Net nuclear grain count (NNG)		Percent of cells in repair (NNG ≥5)		Net nuclear grain count of cells in repair	
		mean	SD	mean	SD	mean	SD
0	0.5% MC	-0.3	0.6	1.3	1.2	6.8	0.6
HCD - Vehicle	-	-1.51 [a]	1.09 [b]	0.74 [a]	0.89 [b]	ND	ND
1000	XDE-742	0.3	0.5	1.7	1.5	5.9	0.8
2000	XDE-742	-0.3	0.4	2.7	1.5	6.3	0.5
200	FG-GBC	9.1	3.0	63.3	14.2	12.1	2.9
HCD - Positive control	FG-GBC	16.32 [c]	6.84 [b]	90.42 [c]	7.73 [b]	ND	ND

HCD Historical control data
a Mean of 20 data sets
b Standard error of mean
c Mean of 10 data sets
ND Not determined
Data obtained from page29 of the study report.

Table 2: XDE-742: group mean net nuclear grain count values, 2-4 hour

Dose (mg/kg bw)	Compound	Net nuclear grain count (NNG)		Percent of cells in repair (NNG ≥5)		Net nuclear grain count of cells in repair	
		mean	SD	mean	SD	mean	SD
0	0.5% MC	0.4	1.1	2.0	2.0	6.3	1.1
HCD - Vehicle	-	-1.51 [a]	1.09 [b]	0.74 [a]	0.89 [b]	ND	ND
1000	XDE-742	1.0	0.6	4.0	5.2	6.6	0.8
2000	XDE-742	0.4	0.3	2.7	0.6	5.2	0.0
10	DMN	35.8	8.9	94.0	9.5	37.2	6.7
HCD - Positive control	DMN	23.28 [c]	14.72 [b]	86.85 [c]	15.07 [b]	ND	ND

HCD Historical control data
a Mean of 20 data sets
b Standard error of mean
c Mean of 10 data sets
ND Not determined
Data obtained from page 29 of the study report.

III. DISCUSSION AND CONCLUSIONS:

A. INVESTIGATORS' CONCLUSIONS: In both UDS experiments, treatment with XDE-742 at doses up to 2000 mg/kg bw yielded NNG values consistent with the concurrent vehicle control data and the laboratory's historical control ranges (–0.3 to 1.0). No more than 4.0% of cells were seen in repair at any dose of XDE-742. These data confirm that NNG and/or % of cells in repair fell well below the thresholds for a positive response.

The data obtained in this study indicate that oral gavage treatment of male mice with 1000 or 2000 mg/kg bw XDE-742 did not result in increased UDS in hepatocytes isolated approximately 12-14 or 2-4 hours after dosing. It was concluded that XDE-742 had no genotoxic activity detectable in this test system under the experimental conditions employed.

B. REVIEWER COMMENTS: In both UDS experiments (12-14 hrs or 2-4 hrs), treatment with XDE-742 at doses up to 2000 mg/kg bw yielded NNG values (–0.3 to 1.0) consistent with the concurrent vehicle control data (-0.3 to 0.4) and the laboratory's historical control ranges (99% confidence limits = -4.39 to 1.29; observed range -3.8 to 0.2). No more than 4.0% of cells were seen in repair at any dose of XDE-742.

The positive control chemicals (FG-GBC and DMN) produced sufficient results to demonstrate the sensitivity of the system and test design.

There was no evidence that unscheduled DNA synthesis, as determined by radioactive tracer procedures (NNG counts), was induced at concentrations up to 2000 mg/kg in Crl:CD-1 mouse hepatocytes.

The registrant, the Dow Chemical Company, originally prepared this STUDY PROFILE TEMPLATE (STP) (MRID 47022002) in HED's DER format. The HED reviewers may have added minor adjustments/additions to the original STP. The conclusions of the study and assignment of its classification as determined by HED reviewers are in the Executive Summary above.

C. STUDY DEFICIENCIES:

There are no deficiencies that would affect the interpretation of the study.

EPA Reviewer: Linda L. Taylor, Ph.D. **Signature:** Linda Taylor
Reregistration Branch I, Health Effects Division (7509P) **Date:** 01-03-08
EPA Secondary Reviewer: Kimberly Harper **Signature:** Kimberly D Harper
RAB2, Health Effects Division (7509P) **Date:** 12/12/07

Template version 02/06

TXR#: 0054347

DATA EVALUATION RECORD

STUDY TYPE: Chronic Neurotoxicity (feeding) - rat OPPTS 870.6200b (OECD 424)

PC CODE: 108702 **DP BARCODE:** D332276

TEST MATERIAL (PURITY): XDE-742 (98.0% a.i.) [pyroxsulam]

CHEMICAL: N-(5,7-dimethoxy[1,2,4]triazolo[1,5-a]pyrimidin-2-yl)-2-methoxy-4-(trifluro methyl)pyridine-3-sulfonamide

SYNONYMS: X666742, XDE-742, LY-666742, BAS-770H

CITATION: Maurissen, J. P., Andrus, A. K., Yano, B. L., *et al.* (2005) XDE-742: Chronic Neurotoxicity Study in Fischer 344 Rats. Toxicology & Environmental Research and Consulting. The Dow Chemical Company, Midland, Michigan. Laboratory Project Study ID: 031014, April 3, 2003 – April 16, 2004. MRID 46908411. Unpublished

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EXECUTIVE SUMMARY:

In a chronic neurotoxicity study (MRID 46908411), XDE-742 (pyroxsulam; 98.0% a.i., Lot # E0952-52-01; TSN103826) was administered to 10 Fischer 344 rats/sex/group at dose levels of 0, 10, 100, or 1000 mg/kg bw/day for 12 months. (mg/kg bw/day is alternately referred to as mg/kg/day) Neurobehavioral assessment [functional observational battery (FOB) and motor activity testing] and determinations of grip strength (fore- and hindlimb), rectal temperature, and landing foot splay were performed in 10 rats/sex/group pre-exposure to establish a baseline, at the end of the first month, and subsequently at 3, 6, 9, and 12 months. At study termination, 5 randomly-selected rats/sex/group were perfused for neuropathological examination. Of the perfused animals, the control and high-dose rats were subjected to histopathological evaluation of central and peripheral nervous system tissues.

There were no treatment-related deaths. There was a treatment-related increase in the incidence of perineal urine soiling in both sexes at 1000 mg/kg/day, with females displaying the more consistent response. Additionally, there was an increase in urination during the open-field activity in the treated females, but there was no dose-response. A slight decrease in body weight was observed in both sexes at the high-dose level compared to the controls at 12 months (96%-97% of control), and body-

weight gains were slightly lower throughout the study (overall males 96%/females 94% of control) at this dose level.

No treatment-related effects were observed on any parameter monitored to assess neurotoxic potential, including FOB, motor activity, grip strength, landing foot splay, and histopathology of the central and peripheral nervous systems. **The NOAEL for neurotoxicity for pyroxsulam is 1000 mg/kg/day, the highest dose tested.**

The study is classified as acceptable (guideline), and it satisfies the guideline requirement for a subchronic neurotoxicity study in rats (870.6200b). It is noted that there is not a guideline for a chronic neurotoxicity study.

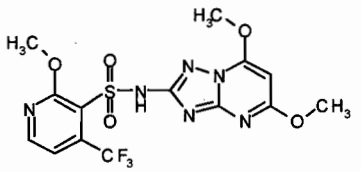
COMPLIANCE: Signed and dated GLP, Quality Assurance, and Data Confidentiality statements were provided.

I. MATERIALS AND METHODS:

A. MATERIALS:

1. Test material: XDE-742

Description:	white powder (from template; in found in report)
Lot/Batch #:	E0952-52-01/TSN103826
Purity:	98.0 % a.i.
Compound Stability:	not provided; template states that previous 28-day study w/ Fischer 344 rats demonstrated stable for at least 36 days in feed
CAS # of TGAI:	422556-08-9
Structure:	



2. Vehicle and/or positive control: Lot/Batch #; Purity (LabDiet® Certified Rodent Diet #5002M)

3. Test animals:

Species:	Rat	
Strain:	Fischer 344	
Age/weight at study initiation:	6-7 weeks; males:	
Source:	Charles River Laboratories Inc. NC	
Housing:	2/cage for first 12 months	
Diet:	LabDiet® Certified Rodent Diet #5002M (meal form) *ad libitum*; PMI Nutrition International	
Water:	municipal *ad libitum*	
Environmental conditions:	**Temperature:**	22±1°C
	Humidity:	40-70%
	Air changes:	12-15/hr
	Photoperiod:	12 hrs dark/12 hrs light
Acclimation period:	approximately 2 weeks	

B. STUDY DESIGN:

1. In life dates: Start: April 3, 2003; End: April 16, 2004

2. Animal assignment and treatment: Animals were randomly assigned to the test groups noted in Table 1 (stratified by body weight). Test substance was administered *via* the diet for up to 12 months. Dose levels were chosen based on the results of the 90-day dietary rat study [MRID 46908350; separate DER]. The animals in this one-year chronic neurotoxicity study are a subgroup of the 2-year chronic toxicity/carcinogenicity/neurotoxicity study [MRID 46908407; separate DER]. Ten rats/sex were preselected [5/sex shared with those preselected for the chronic toxicity (12 month) phase] to evaluate potential chronic neurotoxic effects up to 12 months.

TABLE 1. Study design				
Experimental parameter	Dose group (mg/kg bw/day)			
	Control	10	100	1000
Total number of animals/sex/group	10/sex	10/sex	10/sex	10/sex
Behavioral testing (FOB, Motor activity)	10/sex	10/sex	10/sex	10/sex
Neuropathology	5/sex	5/sex	5/sex	5/sex

3. **Test Substance preparation and analysis:** Diet was prepared (periodically throughout the study based on stability data) by serially diluting a concentrated test material-feed mixture with ground feed. The concentrations of the test material in the feed were adjusted weekly for the first 13 weeks and at 4-week intervals thereafter, based upon the most recent body weight and feed consumption data. Analyses of the test material included measurements of homogeneity and concentration. Stability of the test material in feed was determined prior to study initiation.

Results: Data were not provided in the study report. However, since this study is a subset of the rat chronic toxicity/carcinogenicity/neurotoxicity study, the DER for MRID 46908407 should be consulted for details.

4. **Statistics:** Statistical analyses were conducted on body weights (at FOB time points), grip strength (average of 3 grip performance trials), landing foot splay (average of 3 trials), motor activity (counts reported as their square roots), and FOB observations. For overall FOB summarization, ranked scores for each FOB observation (for males and females at each dose level) were converted into average scores for that observation; average scores were descriptive only, and were not quantitatively analyzed. The next, more detailed level of summarization was incidence tables of FOB observations; *i.e.,* the number of rats/sex and dose with a particular observation. FOB incidence scores were statistically analyzed by the z-test of proportions comparing each treated group to the control (factors of sex, severity level, time point, dose level comparisons).

   Means and standard deviations were calculated for all continuous data and homogeneity of variance was evaluated with the Bartlett's test. Body weights, rectal temperature, forelimb and hindlimb grip strength, landing foot splay, and motor activity were analyzed by a factorial repeated-measures design, the multivariate approach, with factors of sex and treatment and the repeated factor of time. Motor activity also had the repeated factor of epoch (within time) in the model. The inclusion of pre-exposure data in the analysis made relevant only the analyses that included factors of both treatment and time since the treatment-by-time interaction assessed the true effect of treatment.

   The report states that the overall approach is consistent with the recommendations proposed by Tukey *et al.,* (1985), Mantel (1980), and USEPA (1991).

C. METHODS / OBSERVATIONS:

1. **Mortality and clinical observations:** According to the template [MRID 46908608], a cage-side

(general) clinical examination was conducted at least once a day and was designed to detect significant clinical abnormalities that were clearly visible upon a limited examination, and to monitor the general health of the animals. Animals were observed twice daily for mortality and moribundity.

2. **Body weight:** According to the template, body weights were recorded during the pre-exposure period, weekly during the first 13 weeks, and then at monthly intervals. In the chronic neurotoxicity study report, only the body weights taken prior to each FOB session were examined and reported.

3. **Food consumption:** Food consumption was measured for the first 13 weeks and then monthly thereafter. Test material intake was calculated using test material concentrations in the feed, actual body weights, and feed consumption.

4. **Cholinesterase determination:** Cholinesterase activity was not determined.

5. **Neurobehavioral assessment:**

   a. **Functional observational battery (FOB):** A FOB was conducted at pre-exposure (baseline) and during 1, 3, 6, 9, and 12 months of treatment. The FOB was conducted under red light conditions by the same observer on all rats, at approximately the same time each test day. Cage-side, hand-held, and open-field observations, and measurements of body weight, rectal temperature, fore- and hindlimb grip performance, and landing foot splay were included. The FOB was conducted on rats randomly selected and presented to the observer who was blind to the treatment status of the rat.

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	X	Rearing+
X	Convulsions*	X	Salivation*	X	Arousal/ general activity level*
X	Tremors*		Piloerection*	X	Convulsions*
X	Abnormal Movements*or behavior	X	Fur appearance	X	Tremors*
X	Palpebral closure*	X	Palpebral closure*	X	Abnormal movements*
X	Feces consistency	X	Respiratory rate+	X	Urination / defecation*
X	Resistance to removal from cage		Red/crusty deposits*		Grooming
X	SENSORY OBSERVATIONS	X	Mucous membranes /eye /skin color	X	Gait abnormalities / posture*
X	Approach response+	X	Eye prominence*		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*				
	Eyeblink response	X	PHYSIOLOGICAL OBSERVATIONS	X	NEUROMUSCULAR OBSERVATIONS
	Forelimb extension	X	Body weight*	X	Hindlimb extensor strength
	Hindlimb extension	X	Body temperature+	X	Forelimb grip strength*
	Air righting reflex+			X	Hindlimb grip strength*
	Olfactory orientation			X	Landing foot splay*

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		X	OTHER OBSERVATIONS		Rotarod performance

*Required parameters; +Recommended parameters

b. Locomotor activity: Locomotor activity was evaluated using an automated system for data collection. Each test session consisted of six 8-minute epochs, totaling 48 minutes of testing per rat per test session. Activity counts for each epoch were recorded. Each beam break that lasted more than 100 msec, following at least 100 msec since the last beam break, constituted an activity count. These minimum durations were set to minimize counting activities such as tail-flicking, head-bobbing, etc. Rats were allocated to the motor activity cages in such a way that the counterbalancing of treatment groups and sexes across cages and test times was maximized. The data presented were total counts (square root of total counts) and mean counts per epoch. The time of the MA assessment in relation to the time of the FOB performance was not indicated. NOTE: Motor Activity System Positive Control Report was provided as Appendix C, pages 299-372.

c. Ophthalmology: The eyes of all rats were examined pre-exposure and prior to the scheduled necropsy using indirect ophthalmoscopy. Eyes were also examined during necropsy using a moistened glass slide pressed to the cornea.

6. Sacrifice and pathology: Five randomly, pre-selected, rats/sex/group were evaluated for neuropathologic effects. Rats submitted alive for necropsy were given an intraperitoneal injection of heparin approximately 10 minutes prior to perfusion. Rats were anesthetized by the inhalation of isoflurane vapors. While under deep anesthesia, the heart was exposed, the left ventricle cannulated, and the right atrium was incised. Rats were perfused by gravity pressure with phosphate buffer containing sodium nitrite followed by a phosphate-buffered solution of glutaraldehyde-formaldehyde. Tissues were examined for gross pathologic alterations. The brain, head, spinal column with spinal cord, fore- and hindlimbs, and tail were trimmed to remove excess skin and muscle; muscles from the hindlimbs were reflected to further expose the nerves. All tissues were immersed in the glutaraldehyde/formaldehyde fixative. Thoracic and abdominal viscera were collected and preserved in the same fixative.

The CHECKED (X) tissues were evaluated. Tissues for neuropathologic evaluation were prepared from all rats in the control and high-dose groups. Nine cross-sections of the brain were prepared from the following structures: olfactory bulb, cerebrum (frontal, parietal, temporal, and occipital lobes), thalamus/hypothalamus, midbrain, pons, cerebellum, and medulla oblongata. Additionally, sections were prepared from the trigeminal ganglion and nerve, pituitary gland, eyes with optic nerve, spinal cord (cervical and lumbar), olfactory epithelium, and skeletal muscles (gastrocnemius and anterior tibial). These tissues were processed by standard histologic procedures (embedded in paraffin, sectioned ~6-µm thick, stained with hematoxylin and eosin). Spinal nerve roots (cervical and lumbar), dorsal root ganglia (cervical and lumbar), and peripheral nerves (sciatic, tibial {proximal and distal – at knee and calf muscle branches} and sural) were osmicated, embedded in epoxy resin, sectioned ~2-3 µm thick and stained with toluidine blue.

X	CENTRAL NERVOUS SYSTEM	X	PERIPHERAL NERVOUS SYSTEM
	BRAIN		**SCIATIC NERVE**
	Forebrain		Mid-thigh
X	Olfactory bulb		Sciatic Notch
X	Cerebrum, frontal, parietal, temporal, occipital	X	Proximal
X	Midbrain		
X	Cerebellum		**OTHER**
X	Pons	X	Sural Nerve
X	Medulla oblongata (including nucleus gracilis/cumeatus)	X	Tibial Nerve (proximal and distal)
X	Thalamus/Hypothalamus	X	Peroneal Nerve (saved)
	SPINAL CORD	X	Caudal Nerve (saved)
X	Cervical swelling	X	Lumbar dorsal root ganglion
X	Lumbar swelling	X	Lumbar dorsal root fibers
	Thoracic swelling	X	Lumbar ventral root fibers
	OTHER	X	Cervical dorsal root ganglion
	Gasserian ganglion	X	Cervical dorsal root fibers
X	Trigeminal ganglia with nerve	X	Cervical ventral root fibers
	Optic nerve	X	Optic (longitudinal section only)
X	Eyes		
	Gastrocnemius muscle		

7. **Positive controls:** Positive control data on *d*- amphetamine and chlorpromazine were provided for motor activity. Marable, B. R. and Andrus, A. K. (2001). Motor Activity Validation Study Using Positive Controls: Effects of Amphetamine and Chlorpromazine. Toxicology & Environmental Research and Consulting. The Dow Chemical Company, Midland, Michigan. Laboratory Project Study ID: 001189. The motor activity system was shown to be capable of detecting increases and decreases in motor activity and was able to detect motor activity habituation. Positive control data for neuropathology were provided in an abbreviated report of Neuropathology Proficiency Demonstration Study Using Acrylamide and Trimethyltin in the Fischer 344 Rat (B. L. Yano).

II. RESULTS:

A. OBSERVATIONS:

1. **Clinical signs:** There was a treatment-related increase in the incidence of perineal urine soiling in both sexes at 1000 mg/kg/day, with females displaying the more consistent response.

TABLE 2. Clinical observations

Observation	Dose level (mg/kg bw/day)			
	Control	10	100	1000
Males				
Soiling, perineal, urine				
baseline	-	-	-	-
1 month	0	0	0	0
3 month	0	0	0	4
6 month	0	0	0	1
9 month	1	0	0	1
12 month	1	1	0	2
Females				
Soiling, perineal, urine				
baseline	-	-	-	-
1 month	0	0	0	2
3 month	0	0	0	6
6 month	1	0	0	5
9 month	0	0	0	7
12 month	0	1	0	6

Data were extracted from Tables 14-19, pages 64-76 of the report
Numbers represent the number of animals with the observation; n=10

2. **Mortality:** One male at 100 mg/kg/day was reported to have died spontaneously prior to necropsy.

B. **BODY WEIGHT AND BODY WEIGHT GAIN:** Body-weight data are summarized in Table 3. Detailed body-weight data can be found in the DER for the combined chronic toxicity/carcinogenicity/neurotoxicity study [MRID 46908407]. There was a slight decrease in body weight at the high-dose level compared to the controls at 12 months (96%-97% of control), and body-weight gains were slightly lower at the high-dose level throughout the study (overall males 96%/females 94% of control) for both sexes.

TABLE 3. Body weight and body weight gain (g ±s.d.)				
Observation (g ± s.d.)	Dose level (mg/kg bw/day)			
	Control	10	100	1000
Body weight-Males				
Baseline	83.3±5.4	83.6±4.6	83.8±5.5	83.5±5.4
Month 1	222.7±13.6	226.9±12.1	224.8±12.1	218.3±17.8 (97)
Month 6	383.9±26.3	390.7±19.7	381.7±23.3	369.3±27.8 (96)
Month 12	441.7±26.7	450.0±22.6	437.3±26.5 {9}	427.7±32.4 (96)
Body weight-Females				
Baseline	77.6±5.0	77.4±6.0	77.8±7.3	78.8±6.2
Month 1	147.0±7.3	142.0±6.4	143.4±6.8	145.4±6.4
Month 6	212.6±15.4	206.5±10.8	207.6±13.1	205.3±10.8
Month 12	237.9±11.8	234.9±13.5	232.2±18.1	229.7±14.6 (97)
Body weight gain-Males♪				
Baseline-Month 1	139.4	143.3	141.0	134.8 (97)
Month 1-Month 3	105.6	105.7	103.3	99.3 (94)
Month 3-Month 6	55.6	58.1	53.6	51.7 (93)
Baseline-Month 12	358.4	366.4	353.5	344.2 (96)
Body weight gain-Females♪				
Baseline-Month 1	69.4	64.6	65.6	66.6
Month 1-Month 3	43.0	41.8	43.3	38.9 (90)
Month 3-Month 6	22.6	22.7	20.9	21 (93)
Baseline-Month 12	160.3	157.5	154.4	150.9 (94)

Data were extracted from Table 5, page 55 of the report
Values represent mean ± s.d. (% of control); ♪ calculated by reviewer using mean data
n=10 unless {}

C. **FOOD CONSUMPTION**: Food consumption data were not provided. Detailed food consumption data can be found in the DER for the combined chronic toxicity/carcinogenicity/neurotoxicity study [MRID 46908407]. Actual compound intake is shown in Table 4.

TABLE 4. Actual Intake				
Week No.	Intended Dose level (mg/kg bw/day)			
	Control	10	100	1000
Males	0	10.3	101.8	1024
Females	0	10.1	101.3	1014

Data were extracted from page 26 of the report

D. **CHOLINESTERASE ACTIVITIES:** N/A

E. **NEUROBEHAVIORAL RESULTS:**

1. **FOB findings:** An apparent dose-related decrease in the level of open-field activity was reported for males at the 9-month time point, but the decrease was not sustained nor was it supported by motor activity data (Table 5). It is to be noted that a similar dose-response was displayed at 6 months, and the treated male groups all displayed slightly lower levels of activity than the control group throughout the study (except low dose at 1 month). There was an increase in urination during the open-field activity in the treated females, but there was no dose-response.

TABLE 5. Functional observational battery results (average ranked scores)

Observation	Dose level (mg/kg bw/day)			
	Control	10	100	1000
Males				
Level of activity				
Baseline	3.0	3.0	3.0	3.0
Month 1	2.5	2.5	2.4	2.4
Month 3	2.1	2.0	1.7	2.0
Month 6	1.9	1.8	1.6	1.5
Month 9	1.9	1.6	**1.4**	**1.3**
Month 12	1.6	1.3	1.2	1.3
Urination				
Baseline	1.3	1.1	1.4	1.1
Month 1	1.6	1.3	1.5	1.7
Month 3	1.5	1.2	1.6	1.8
Month 6	2.0	1.7	1.8	1.7
Month 9	1.7	2.0	1.8	1.8
Month 12	2.1	2.2	2.2	2.1
Females				
Level of activity				
Baseline	3.0	3.0	3.0	3.0
Month 1	2.9	2.9	2.8	2.9
Month 3	2.4	2.4	2.5	2.3
Month 6	2.1	2.0	1.8	1.8
Month 9	2.0	1.9	1.7	1.9
Month 12	1.8	1.7	2.0	1.8
Urination				
Baseline	1.0	1.1	1.1	1.2
Month 1	1.2	1.3	1.2	1.1
Month 3	1.0	1.3	1.1	1.4
Month 6	1.3	1.4	1.4	1.5
Month 9	1.3	1.4	**1.8**	1.2
Month 12	1.1	**1.7**	**2.0**	**1.6**

Data were extracted from Tables 8-13, pages 58-63 of the study report
N = 10; **bolded values** indicate difference from control was 0.5 rank or greater

2. **Motor activity:** No adverse effects were observed in either sex at any dose level (Table 6).

TABLE 6. Motor Activity Data (mean±s.d.)

Observation	Dose level (mg/kg bw/day)			
	Control	10	100	1000
Males				
Square root of total counts				
Baseline	11.52±1.40	10.44±2.07	11.42±1.13	11.72±1.68
Month 1	13.96±1,26	12.56±2.15	12.98±1.41	12.87±1.04
Month 3	14.56±1.44	13.84±2.04	12.81±2.67	14.36±1.39
Month 6	13.07±1.36	13.53±1.25	12.58±1.78	13.69±1.18
Month 9	11.19±1.97	12.23±1.48	11.43±1.96	11.89±1.58
Month 12	11.29±1.73	11.58±0.92	10.77±1.15	10.77±1.68
Motor activity epoch data baseline				
Epoch 1	7.26±0.63	6.40±1.69	6.74±0.49	6.85±0.88
Epoch 2	5.70±1.03	4.98±0.99	5.55±0.82	6.03±0.94
Epoch 3	4.07±1.21	3.12±2.46	4.67±0.92	5.23±1.33
Epoch 4	4.17±1.27	3.08±2.19	3.27±1.92	3.55±0.66
Epoch 5	2.25±2.27	2.59±2.02	2.52±2.21	2.59±1.73
Epoch 6	0.56±1.15	1.46±1.51	2.14±1.74	1.13±1.64
Motor activity epoch data 6 months				
Epoch 1	8.15±0.87	8.26±1.14	7.64±0.93	8.11±1.00
Epoch 2	6.31±1.03	6.31±0.82	6.16±0.94	6.58±0.79

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TABLE 6. Motor Activity Data (mean±s.d.)

Observation	Dose level (mg/kg bw/day)			
	Control	10	100	1000
Epoch 3	5.18±1.18	5.16±0.92	4.37±1.17	5.11±1.43
Epoch 4	3.95±0.81	3.30±1.73	3.82±1.19	4.09±1.69
Epoch 5	3.56±1.65	4.71±0.96	2.51±1.91	4.25±1.04
Epoch 6	1.80±1.51	2.59±2.08	4.01±1.22	3.05±1.73
Motor activity epoch data 12 months			n=9	
Epoch 1	7.37±1.31	7.25±1.11	7.05±0.89	7.06±1.30
Epoch 2	4.95±1.09	5.16±1.02	5.11±1.23	5.17±1.03
Epoch 3	3.40±1.92	4.23±0.93	3.41±0.66	3.47±1.17
Epoch 4	3.65±1.36	3.75±1.40	3.29±1.06	3.69±1.27
Epoch 5	2.75±1.48	2.71±1.62	3.01±1.49	2.06±1.43
Epoch 6	2.41±1.94	2.71±1.55	1.72±1.29	1.40±1.84
Females				
Square root of total counts				
Baseline	12.44±1.42	11.71±1.66	13.16±2.89	12.15±1.33
Month 1	13.63±1.68	14.49±1.75	14.41±1.64	15.18±1.48
Month 3	13.27±2.29	13.56±1.73	14.87±2.65	14.04±1.01
Month 6	13.15±2.09	11.89±1.62	14.28±1.53	13.36±1.29
Month 9	12.53±1.80	11.37±2.09	13.33±1.88	12.03±1.83
Month 12	11.44±1.51	10.91±1.57	12.41±1.95	11.56±1.22
Motor activity epoch data baseline				
Epoch 1	7.26±0.63	7.24±0.61	7.38±1.05	7.41±0.59
Epoch 2	6.04±0.60	5.83±0.53	6.04±0.92	5.42±0.99
Epoch 3	5.27±0.57	4.56±1.37	5.93±1.83	4.98±0.99
Epoch 4	3.66±2.18	4.04±1.40	4.07±2.17	3.58±2.26
Epoch 5	3.28±2.60	1.83±2.25	2.95±2.22	3.01±1.84
Epoch 6	1.37±1.61	1.07±1.82	2.81±3.00	2.27±1.92
Motor activity epoch data 6 month				
Epoch 1	7.88±0.93	7.80±1.03	8.80±0.43	7.82±1.20
Epoch 2	5.98±1.15	4.89±1.13	6.59±0.95	5.79±1.07
Epoch 3	4.75±1.56	4.82±0.97	4.95±1.16	5.39±0.91
Epoch 4	3.69±1.97	3.73±1.66	4.56±1.37	4.24±1.58
Epoch 5	4.11±1.56	2.14±1.86	3.93±2.10	3.72±2.12
Epoch 6	3.20±2.57	2.26±2.02	3.651.89	3.78±0.93
Motor activity epoch data 12 months				
Epoch 1	7.38±0.77	7.51±1.11	7.63±1.01	7.08±1.07
Epoch 2	4.90±1.17	4.29±1.69	5.10±1.22	4.65±1.73
Epoch 3	3.73±1.17	3.10±1.77	4.41±1.19	4.70±1.36
Epoch 4	3.81±1.05	3.34±1.37	4.09±1.69	3.35±1.76
Epoch 5	2.79±1.42	2.62±1.46	3.44±1.15	3.24±1.39
Epoch 6	3.20±1.63	2.35±1.88	3.86±1.81	2.82±1.40

Data were extracted from Tables 29,32, and 34, pages 86, 89, and 91 of the study report
Epoch data values represent mean activity counts ± s. d.; n=10

3. <u>Grip Performance</u>: There were no treatment-related alterations in grip strength in either sex at any time point (Table 7).

TABLE 7. Grip Performance (grams)				
Observation	**Dose level (mg/kg bw/day)**			
	Control	**10**	**100**	**1000**
Males				
Hindlimb grip				
Baseline	314±46	321±54	331±43	318±37
Month 1	591±60	555±96	611±69	544±84 (92)
Month 3	741±126	658±96	715±95	699±101
Month 6	835±77	895±136	852±139	862±93
Month 9	809±146	872±141	748±95	906±174
Month 12	765±123	854±110	835±118	844±125
Forelimb grip				
Baseline	406±65	428±74	415±71	394±83
Month 1	719±83	726±112	754±113	738±80
Month 3	865±142	886±95	881±133	889±166
Month 6	954±170	943±108	954±98	1010±114
Month 9	900±156	867±147	899±156	945±217
Month 12	993±75	933±108	960±104	1023±141
Females				
Hindlimb grip				
Baseline	316±61	351±58	325±63	329±41
Month 1	454±81	478±60	419±45	471±76
Month 3	506±114	542±40	527±113	536±72
Month 6	613±90	590±46	614±92	670±94
Month 9	618±102	589±65	665±121	609±64
Month 12	589±115	597±83	569±103	586±155
Forelimb grip				
Baseline	375±54	399±49	392±61	411±51
Month 1	605±97	630±75	606±134	666±100
Month 3	735±116	682±112	772±168	708±131
Month 6	805±148	816±202	819±204	797±10
Month 9	672±167	720±138	741±136	751±122
Month 12	778±153	804±189	813±115	804±186

Data were extracted from Tables 20 and 22, pages 77 and 29 of the study report
N = 10; underlined value shows time of peak effect

4. Landing Foot Splay : Landing foot splay was not affected in either sex (Table 8).

TABLE 8. Landing Foot Splay (cm)				
Test day	**Dose level (mg/kg bw/day)**			
	Control	**10**	**100**	**1000**
Males				
Baseline	6.80±1.19	6.55±0.94	6.28±1.01	6.60±1.20
Month 3	6.92±1.02	7.02±0.89	6.95±1.33	6.38±0.98
Month 9	8.01±1.24	7.59±0.79	7.92±1.04	7.14±0.86
Month 12	7.32±0.93	7.83±0.61	7.89±0.96	7.16±0.89
Females				
Baseline	6.18±1.04	6.22±0.57	5.67±0.94	6.36±0.98
Month 3	5.23±0.88	5.29±0.94	5.54±0.75	5.98±0.98
Month 9	5.67±0.85	6.39±0.69	5.68±0.59	6.31±0.75
Month 12	5.86±0.80	5.78±0.41	6.14±0.69	6.11±0.82

Data were extracted from Table 24, page 81 of the study report
Values represent mean ±s.d.; n=10

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F. SACRIFICE AND PATHOLOGY:

1. **Gross pathology:** Two of the five high-dose females displayed decreased size (one left, one right) of the cranial nerve (optic). No other gross lesions were reported in either sex.

2. **Brain weight:** Brain weight data were not provided in the report. However, brain weight data from the main study (12-month sacrifice) show no treatment-related effect (Table 9).

TABLE 9: Absolute and relative brain weights (n=6/sex)

Weights (mg)	Dose level (mg/kg bw/day)			
	Control	10	100	1000
Males				
Body wt	421.4±24.1	426.2±23.6	413.9±21.9	403.2±31.7 (96)
Brain wt	2.069±0.049	2.069±0.023	2.071±0.043	2.069±0.046
Brain/body wt	0.492±0.020	0.487±0.030	0.501±0.025	0.516±0.037
Female				
Body wt	219.5±11.9 {9}	219.5±10.5	216.7±16.4	206.4±9.1 (94)
Brain wt	1.894±0.053	1.857±0.045	1.897±0.038	1.874±0.024
Brain/body wt	0.865±0.047	0.847±0.035	0.879±0.063	0.909±0.041

Data were extracted from MRID 46908407, Tables 98 and 99, pages 206 and 208; n=10 except { }; (% of control)

3. **Neuropathology:** There were no treatment-related histopathologic lesions in the central or peripheral nervous systems in either sex. The most common nervous system lesions observed were degeneration of individual nerve fibers and axonal swellings (Table 10).

TABLE 10. Incidence of neuropathological findings

Lesion	Treatment group			
	Male		Female	
	Control	1000 mg/kg/day	Control	1000 mg/kg/day
Cranial Nerve (optic) n=	(5)	(5)	(5)	(5)
•atrophy, unilateral				
Moderate	0	0	0	2
Brain – cerebrum n=	(5)	(5)	(5)	(5)
•swollen axons, individual nerve fibers, focal				
very slight	1	0	0	0
Brain – medulla oblongata n=	(5)	(5)	(5)	(5)
•swollen axons, gracile nucleus, multifocal				
very slight	4	2	3	3
slight	0	0	1	1
•swollen axons, individual nerve fibers, focal				
very slight	0	0	1	2
•swollen axons, individual nerve fibers, multifocal				
very slight	1	1	3	1
•degeneration, individual fibers, trapezoid body				
very slight	2	2	4	5
Brain – midbrain n=	(5)	(5)	(5)	(5)

TABLE 10. Incidence of neuropathological findings

Lesion	Treatment group			
	Male		Female	
	Control	1000 mg/kg/day	Control	1000 mg/kg/day
•degeneration; individual nerve fibers, focal				
very slight	0	1	0	1
Brain – thalamus/hypothalamus n=	(5)	(5)	(5)	(5)
•swollen axons, individual nerve fibers, focal				
very slight	1	1	1	0
Eye n=	(5)	(5)	(5)	(5)
•mineralization, cornea, unilateral				
very slight	2	1	0	0
•mineralization, cornea, bilateral				
very slight	2	4	5	4
•atrophy, retina, unilateral				
slight	0	0	0	1
moderate	0	0	0	1
Dorsal root ganglion w/ roots, cervical n=				
•degeneration, individual nerve fibers, focal				
very slight	0	0	1	2
Dorsal root ganglion w/ roots, lumbar n=				
•degeneration, individual nerve fibers, focal				
very slight	0	1	1	0
•degeneration, individual nerve fibers, multifocal				
very slight	1	0	0	0
Peripheral nerve – proximal sciatic n=				
•degeneration; individual nerve fibers, focal				
very slight	0	1	0	2
•degeneration; individual nerve fibers, multifocal				
very slight	1	1	1	0

Data were extracted from Table 38, pages 102-106 of the study report

III. DISCUSSION AND CONCLUSIONS:

A. INVESTIGATORS' CONCLUSIONS: Twelve months of dietary exposure up to 1000 mg/kg/day XDE-742 did not affect body weight in either sex of Fischer 344 rats assigned to the neurotoxicity subgroup of the rat chronic toxicity/carcinogenicity/neurotoxicity study. For the FOB, including ranked and categorical observations, only perineal urine soiling in high-dose females was attributed to treatment. Treatment did not affect grip performance, landing foot splay, rectal temperature, motor activity, or ophthalmic observations in either sex. There were no treatment-related gross or histopathologic observations in the central or peripheral nervous systems of rats administered XDE-742. The NOEL for neuropathology was 1000 mg/kg/day for both male and female Fischer 344 rats. Given the absence of neuropathologic findings in the central and peripheral nervous system and the lack of effect on all other parameters suggestive of neurotoxicity, the chronic dietary NOEL for XDE-742 neurotoxicity in male and female Fischer 344 rats was 1000 mg/kg/day, the highest dose tested.

B. REVIEWER COMMENTS: Following dietary exposure to XDE-742 (pyroxsulam) for 12

months at the limit dose (1000 mg/kg/day), no treatment-related effects were observed on any parameter monitored to assess neurotoxic potential. The parameters monitored included a functional observational battery (FOB), determinations of grip strength (fore- and hindlimb), rectal temperature, landing foot splay, and an automated test for motor activity. Additionally 5 rats/sex/dose were perfused, and tissues from the central and peripheral nervous systems of the control and high-dose groups were examined for neuropathology. Although there was a treatment-related increase in the incidence of perineal urine staining in both sexes at the high-dose level, by itself, it is not considered an adverse effect. **The NOAEL for neurotoxicity for pyroxsulam is 1000 mg/kg/day, the highest dose tested.**

The registrant, the Dow Chemical Company, originally prepared this STUDY PROFILE TEMPLATE (STP) (MRID 46908608) in HED's DER format. The HED reviewers may have added minor adjustments/additions to the original STP. The conclusions of the study and assignment of its classification as determined by HED reviewers are in the Executive Summary above.

C. **STUDY DEFICIENCIES:** Brain weight data were not provided or discussed in the study report; however, brain weight data were reported in the main study report [MRID 46908407], and no effect was observed in either sex. Although an assessment of the righting reflex was not performed, the lack of these data does not alter study interpretation.

EPA Reviewer: Paul Chin
Reregistration Branch 1, Health Effects Division (7509C)
EPA Secondary Reviewer: Kimberly Harper
Registration Action Branch 2, Health Effects Division (7509C)

Signature: Paul Chin
Date: 1/3/08
Signature: Kimberly D Harper
Date: 12/12/07

TXR#: 0054347

DATA EVALUATION RECORD

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

DP BARCODE: D332276

P.C. CODE: 108702

TEST MATERIAL (RADIOCHEMICAL PURITY):

Triazole-ring ^{14}C-labeled XDE-742 (99.5% a.i.)
Pyridine-ring ^{14}C-labeled XDE-742 (100% a.i.)
Non-radiolabeled XDE (98.0% a.i.)

SYNONYMS: N-(5,7-dimethoxy[1,2,4]triazolo[1,5-a]pyrimidin-2-yl)-2-methoxy-4-(trifluoromethyl)-3-pyridinesulfonamide, X666742, Pyroxsulam, BAS-770H, XR-742

CITATION: S. C. Hansen, B.S., A.J. Clark, B.S., D.A. Markham, B.S., and A.L. Mendrala, M.S. (2005). XDE-742: Metabolism and Pharmacokinetics of ^{14}C-XDE-742 in Male Fischer 344 Rats Following Single and Repeated Oral Administration. Toxicology & Environmental Research and Consulting, The Dow Chemical Company, Midland, Michigan. Study ID: 041019, 13 December 2005. MRID 46908412. Unpublished.

SPONSOR: Dow AgroSciences LLC, 9330 Zionsville Road, Indianapolis, Indiana 46268

EXECUTIVE SUMMARY: In a rat metabolism study (MRID 46908412), ^{14}C-pyroxsulam (^{14}C-XDE-742; batch no. DAS Inv# 1901; purity 99.5% a.i.; triazole-ring ^{14}C-labeled) was administered as an aqueous METHOCEL™ suspension by oral gavage to groups of three or four male Fischer 344 rats as a single nominal dose of 10 or 1000 mg pyroxsulam (XDE-742) per kg body weight. Another group of four male rats was administered 14 daily 10 mg/kg oral doses of unlabeled XDE-742 followed by a single 10 mg/kg triazole-ring ^{14}C-labeled XDE-742 on day 15. An additional group of four male Fischer 344 rats was administered a single oral nominal dose of 10 mg/kg of pyridine-ring ^{14}C-labeled XDE-742 (batch no DAS Inv# 1905; purity 100% a.i.) to determine if ring separation occurs during metabolism. In order to determine the biliary elimination of ^{14}C-XDE-742, three male rats were administered an intravenous (iv) emulsion of 10 mg/kg triazole-ring ^{14}C-labeled XDE-742.

The data indicate XDE-742 was rapidly absorbed and ^{14}C-XDE-742-derived radioactivity was rapidly excreted. Saturation of absorption was observed between the doses of 10 and 1000 mg

XDE-742/kg leading to a decrease in the bioavailability of XDE-742. Between 85 and 90% of the XDE-742 dosed was essentially unchanged in the urine and feces. One major metabolite found at 4-16% of the administered dose in the urine and feces was 2'-demethyl-XDE-742. Volatile organics and CO_2 were negligible for the low dose groups of both ring ^{14}C-labels of XDE-742 (groups 1 and 5) and group 2 animals (high dose).

Based on the time to peak plasma or RBC radioactivity levels, ^{14}C-XDE-742 was rapidly absorbed and eliminated both by oral and iv routes. Following a single dose of ^{14}C-XDE-742 at 10 mg/kg, a mean peak plasma or RBC concentration was reached at 26-30 minutes and 6 minutes post-dosing for oral and iv routes, respectively. The mean $t_{½}$ of distribution was 1-1.3 hours and the mean $t_{½}$ of elimination was 11-14.5 hours for both oral and iv routes. The AUCs for RBCs were about a tenth of that obtained with plasma, suggesting little binding of XDE-742 with RBCs.

XDE-742 was rapidly excreted *via* the urine and feces with the majority of the radioactivity eliminated by 12 and 24 hr post-dosing, respectively, and with some dose dependency in the routes of excretion. At 48 hours post-dosing between 98 and 110% of the administered dose was recovered in the urine, feces, tissues, carcasses and cage wash for all dose groups. The urine accounted for 57-78% and 30% of the administered dose from all low dose groups and high dose group, respectively, following 48 hours post-dosing. The feces accounted for 45-51% and 69% of the administered dose from all low dose groups (except for iv dose group) and high dose group, respectively. Following the iv administration of XDE-742, the feces accounted for 17% of the administered dose. Based on this, one might conclude that at least 17% of the administered dose would be excreted via the biliary route. For all dose groups, radioactivity recovered in the tissues/carcasses and the cage wash accounted for less than 1% and 1-3% of the administered dose, respectively. Also, no remarkable differences in tissue distribution or bioaccumulation were seen for all dose groups. The data indicate that over 70% of the administered dose was systematically bioavailable.

Volatile organics and CO_2 in expired air were not quantifiable for the low dose groups of both ring ^{14}C-labels of XDE-742 (groups 1 and 5). Group 2 animals (high dose) had <0.005 and 0.001% of the administered dose detected in volatile organics and CO_2.
There were a total of 7 radioactive peaks detected at >0.05% of the administered dose in the excreta from the groups that were analyzed. Only parent XDE-742 and 2'-demethyl-XDE-742 (XDE-742-DM) were detected in all the matrices and ranged from 80-90% and 4-16% of the administered dose, respectively. In the urine, the parent XDE-742 and 2'-demethyl-XDE-742 (XDE-742-DM) ranged from 28-50 and 2-11% of the administered dose, respectively. In the feces, XDE-742 and 2'-demethyl-XDE-742 ranged from 34-62 and 2-7% of the administered dose, respectively. No other peaks accounted for >1.5% of the administered dose/group.
There were essentially no differences in the total radioactivity eliminated in the urine and feces between the two different ring ^{14}C-labels of XDE-742 when they were administered as a single oral dose. Also, there were no differences among the distribution of parent XDE-742 and 2'-demethyl-XDE-742 in the urine and feces. Four major peaks (3 in the urine and 1 in the feces, <1% of the administered dose each) unique to the metabolism of the triazole ^{14}C-labeled XDE-

742 samples would be consistent with minimal ring cleavage occurring during the metabolism of XDE-742.
This metabolism study is classified **acceptable/guideline** and satisfies the guideline requirements for a metabolism study (OPPTS 870.7485 and OECD 417) in rats.

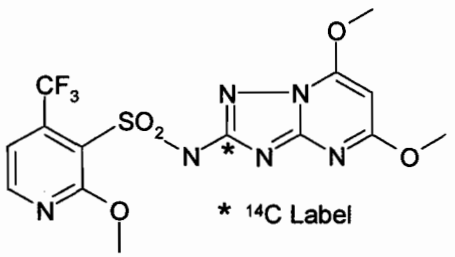
COMPLIANCE: Signed and dated GLP, Quality Assurance, and Data Confidentiality statements were provided.

I. MATERIALS AND METHODS

A. MATERIALS: ^{14}C-XDE-742 was either labeled in the Het (triazole) or pyridine rings. The test material was augmented with non-radiolabeled XDE-742 in dose solution preparation as needed to deliver the intended doses (as mg XDE-742/kg body weight).

1a. 1st Radiolabeled Test Compound:

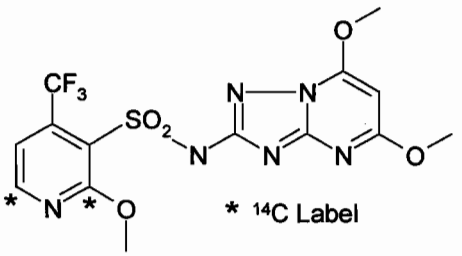
Radiolabeled Test Material:		XDE-742-Het-2-^{14}C (triazole)
	Radiochemical purity	99.5% [determined by HPLC]
	Specific Activity	36.6 mCi/mmol - [Het-2-^{14}C]-XDE-742
	Lot/Batch #:	DAS Inv# 1901



XDE-742-Het-2-^{14}C (triazole)

1b. 2nd Radiolabeled Test Compound:

Radiolabeled Test Material:		XDE-742-pyridine-2,6-^{14}C
	Radiochemical purity	100.0% [determined by HPLC]
	Specific Activity	43.7 mCi/mmol - [Pyridine-2,6-^{14}C]-XDE-742
	Lot/Batch #:	DAS Inv# 1905



XDE-742-pyridine-2,6-^{14}C

1c. Non-radiolabeled Test Compound:

Non-Radiolabeled Test Material:		XDE-742
	Description:	off white to yellow powder
	Lot/Batch #:	TSN103826; Lot# 0952-52-01
	Purity:	98.0% [determined by HPLC/MS]
	Contaminants:	
	CAS #	422556-08-9

2a. Vehicle for oral dosing: 0.5% aqueous methylcellulose

2b. Vehicle for intravenous dosing: The iv dosing emulsion was prepared using a modification of a published method. Cholic acid was dissolved in pH-adjusted Ringer's solution by stirring and slightly heating. L-α-phosphatidylchloine was added to a vial containing the ^{14}C-XDE-742. To this, the corn oil and Ringer's/cholic mixture was added. The iv dose emulsion was homogenized and the pH 8 was verified and the emulsion was stored in the refrigerator until used.

3.	**Test animals:**		
	Species:	Rat	
	Strain:	Fischer 344	
	Age/weight at study initiation:	8-9 weeks; males 203 – 264g	
	Source:	Jugular-vein cannulated (JVC): Taconic Inc., Germantown, New York (Groups 1 and 6) Non-cannulated: Charles River Laboratories Inc., Raleigh, North Carolina (Groups 2, 3, 4, and 7) and Taconic Inc., Germantown, New York (Group 5)	
	Housing:	Individual Roth-type metabolism cages; or stainless steel cages with wire-mesh floors. Cages contained hanging feeders and a pressure activated lixit valve type watering systems.	
	Feed and Water:	Animals were provided LabDiet® Certified Rodent Diet #5002 (PMI Nutrition International, St. Louis, Missouri) in pelleted form. Feed and municipal water were provided *ad libitum* except that access to feed was restricted to one pellet approximately 16 hours prior to the administration of test material to orally dosed rats and was returned about 4 hours post-dosing. Rats dosed intravenously were not fasted prior to dosing. Drinking water obtained from the municipal water source was periodically analyzed for chemical parameters and biological contaminants by the municipal water department.	
	Environmental conditions:	**Temperature:** **Humidity:** **Air changes:** **Photoperiod:**	22±1°C 58±% 12-15/hour 12 hours dark/12 hours light
	Acclimation period:	Group 1 and 5 rats were acclimated to the metabolism cages upon arrival to the laboratory for six days prior to the start of	

		dosing. Rats in Groups 2, 3, 4, and 7 were acclimated to the laboratory for seven days prior to the start of study, including at least two days in metabolism cages. The acclimation period for rats in Group 6 was not stated in the study report.

4. Preparation of dosing solutions: Five separate oral dose suspensions were prepared in 0.5% METHOCEL™. Only non-radiolabeled XDE-742 was added to obtain the nominal dose of 10 mg XDE-742/kg body weight for administration to the repeated dose animals for 14 days. Groups 1-4 and 7 animals were administered with separate radiolabeled dose suspensions made with non-radiolabeled and XDE-742 (Het-2-^{14}C). Group 5 dose suspension was prepared by adding an appropriate amount of non-radiolabeled and XDE-742-pyridine-2,6-^{14}C. An iv dose emulsion was prepared with unlabeled XDE-742 and ^{14}C-XDE-742 (Het).

B. STUDY DESIGN AND METHODS

1. Group Arrangements: Animals were assigned randomly to the test groups noted in Table 1. Animals in groups 1 and 6 (JVC rats) were selected based on the patency of the cannulas, then randomized, if applicable. Animals in groups 2-5 and 7 were stratified by body weight and then randomized by computer.

TABLE 1. Dosing Groups and Study Design

Dosing Groups	Dose of labeled material (mg/kg)	Number of male F-344 rats	Remarks
1. Single low dose gavage	10	4	A, B, C, D, E
2. Single high dose gavage	1000	4	A, C, D, E
3. Single low dose gavage - C_{max}	10	4	A, C, G
4. Single low dose gavage - ½C_{max}	10	4	A, C, G
5. Single low dose gavage	10	3	C, D, E, F
6. iv administration	10	3	A, B, C, E
7. Repeated low dose gavage	10	4	A, C, D, E, H

A. XDE-742-Het-2-^{14}C
B. Plasma ^{14}C-concentration-time course
C. Excreta/tissues, CO_2 and volatile organics (if applicable) were collected and analyzed for radioactivity as described below
D. Selected urine and fecal samples were subjected to chemical analysis
E. This segment of the study continued for 48 hours post-dosing when ≥95% of the administered dose was recovered in excreta
F. XDE-742-pyridine-2,6-^{14}C
G. The sacrifice times were determined from Group 1 data (30 minutes and 2 h post dosing for Groups 3 and 4, respectively).
H. 14 daily oral doses of unlabeled XDE-742 followed by a single oral dose of XDE-742-Het-2-^{14}C

2. Dosing and sample collection: Group 1 and Group 5 animals received a single oral dose of ^{14}C-XDE-742 (Het) or ^{14}C-XDE-742 (pyridine) at a nominal dose level of 10 mg/kg. Group 2 animals received a single oral dose of 1000 mg ^{14}C-XDE-742 (Het)/kg bw. Group 3 (C_{max}) and 4

(½C_{max}) animals received a single oral dose of 10 mg ^{14}C-XDE-742 (Het)/kg. Group 6 animals received a single intravenously administered dose of 10 mg ^{14}C-XDE-742 (Het)/kg. Group 7 animals received 14 daily oral doses of 10 mg non-radiolabeled XDE-742/kg followed by a single oral dose of 10 mg ^{14}C-XDE-742 (Het)/kg on day 15.

a. Pharmacokinetic studies: The rats of Groups 1 (oral) and 6 (iv) were fitted with indwelling jugular vein cannulae and plasma/RBC ^{14}C concentration-time course data was generated to estimate peak (C_{max}) and half-peak (½ C_{max}) plasma/RBC ^{14}C concentrations after oral or iv administration of ^{14}C-XDE-742 (Het). From Group 1 animals, approximately 0.1-0.2 ml blood were collected at 0.25, 0.5, 1, 2, 4, 6, 8, 10, 12, 24, 36, and 48 hour post-dosing. From Group 6 animals, three additional blood samples were collected after iv dosing at 0.05, 0.10, and 0.75 hours post-dosing, while eliminating the 6 h sample.

All urine voided during the study was collected in dry-ice cooled traps with the exception of Groups 3 (C_{max}) and 4 (½C_{max}). The urine traps were changed at 12, 24, and 48 hour post-dosing (terminal sacrifice). The cages were rinsed with water at the time the traps were changed and the rinse collected. Each urine specimen and urine/cage rinse was analyzed for radioactivity by LSS. Equal volume aliquots of urine samples (per group) from the 0 to 12-hour and the 12 to 24-hr collection intervals were pooled and stored at –80 °C for chemical analysis.

Feces from Groups 1, 2, 5, 6, and 7 were collected in dry-ice chilled containers at 24-hour intervals until terminal sacrifice at 48 hours post-dosing. Aqueous homogenates (~25% w/w) were prepared from fecal samples at each collection interval and weighed aliquots of the homogenates were oxidized and quantitated for radioactivity by LSS. Equal volume aliquots of fecal homogenates from each animal were taken from the 0 to 24-hour and 24 to 48-hour collection intervals and pooled (per group). These pooled samples were stored at –80°C pending later chemical analysis.

For Groups 3 and 4, a final cage wash was conducted that included a voided urine and feces.

Upon exiting the cages, the expired air from Group 1, 2, and 5 animals were passed through charcoal to trap expired volatile organics. After exiting the charcoal trap, the expired air of Group 1, 2, and 5 animals was passed through a solution of monoethanolamine:1-methoxy-2-propanol (3:7 v/v) to trap expired CO_2. Due to the absence of radioactivity associated with expired CO_2 from Group 1 and 5 animals (not quantifiable to 0.01% of the administered dose) and the low levels (0.01% of the administered dose in Group 2 animals, CO_2 traps were not used to collect expired air from the other groups.

The following tissues were collected at sacrifice (Table 2), 2 days post-dosing for Groups 1, 2, 5, 6, and 7. The tissues for Group 3 (C_{max}) and Group 4 (½C_{max}) animals were collected at 30 minutes and 2 hours post-dosing, respectively. Animals were sacrificed by exsanguination under CO_2/O_2 anesthesia.

TABLE 2. Tissues Collected at Terminal Sacrifice

Kidney	red blood cells (RBC)	skin
Liver	gastrointestinal (GI) tract [including ingesta]	spleen
perirenal fat		residual carcass
plasma (terminal)		

The GI tract with contents, kidney, and liver were collected and homogenized (~ 33% homogenate). A weighed aliquot was solubilized or oxidized and analyzed for radioactivity by liquid scintillation spectrometry (LSS). Blood was centrifuged to obtain plasma and analyzed for radioactivity by LSS. The skin was removed from the carcass and a representative skin sample was oxidized and analyzed for radioactivity by LSS. The spleen, perirenal fat, and RBC were directly oxidized without homogenization and analyzed for radioactivity by LSS.

Samples with dpm less than twice the concurrently run background (blanks) were considered to contain insufficient radioactivity to reliably quantify. For tissues, when a sample was non-quantifiable (NQ), that sample was assigned the quantitation limit (QL) for calculations and displayed as NQ with the QL in parenthesis. The mean was calculated from actual values and calculated QL values and presented as mean ± standard deviation (X ± SD), unless greater than ½ of the values were presented as NQ, in which case the mean was expressed NQ (X) ± SD. If all tissue values were NQ the mean is presented as NQ (QL) with no SD displayed.

b. Metabolite characterization studies:
Selected urine samples (0-12 hour collection interval) and fecal samples (0-24 and 24-48 hour collection intervals) were pooled by time and dose group. Distribution of metabolites and parent test material in urine and feces was determined in duplicate via high-performance liquid chromatography (HPLC) with in-line radiochemical detection. Identification of parent XDE-742 and metabolite 2'demethyl-XDE-742 in the excreta was accomplished via LC-MS/MS.

3. Statistics: Descriptive statistics were used, *i.e.*, mean ± standard deviation. All calculations in the database were conducted using Microsoft Excel® spreadsheets and databases in full precision mode (15 digits of accuracy). Certain pharmacokinetic parameters were estimated from plasma data, including AUC (area-under-the-curve), C_{max}, $½C_{max}$, and elimination rate constants, using the PK Solutions (Summit Research Services, Montrose, Colorado) pharmacokinetic computer modeling program.

II. RESULTS

A. Pharmacokinetic Studies: From Groups 1 and 6 animals, certain pharmacokinetic parameters were estimated from plasma and red blood cell concentration-time course data, including AUC (area-under-the-curve), C_{max}, $½C_{max}$, and half-lives of elimination (Table 3).

TABLE 3. Plasma and Red Blood Cell Kinetic Parameters Following Administration of ^{14}C-XDE-742 [a]

Plasma

	Group 1 - Oral Dose					Group 6 - Intravenous Dose			
Animal Number	04A3125	04A3126	04A3127	04A3128	Mean ± SD	04A3145	04A3146	04A3147	Mean ± SD
Target Dose (mg/kg)	10.0	10.0	10.0	10.0		10.0	10.0	10.0	
Actual Dose (mg/kg)	6.39	6.43	7.42	6.46	6.7 ± 0.5	9.81	9.92	9.24	9.7 ± 0.4
C_{max} (µg/ml)	14.1	17.4	25.5	22.2	19.8 ± 5.1	44.4	87.1	53.6	61.7 ± 22.4
T_{max} (hours)	0.5	0.5	0.5	0.5	0.50 ± 0.0	0.1	0.05	0.1	0.1 ± 0.0
Half-life Distribution ($t_{½\alpha}$; hours)	1.56	1.28	1.15	1.34	1.33 ± 0.17	1.23	0.79	0.88	1.0 ± 0.2
Half-life Elimination ($t_{½\beta}$; hours)	12.7	10.2	10.5	10.7	11.0 ± 1.1	13.8	15.3	14.3	14.5 ± 0.8
AUC (µg-hr/ml)	61	57.8	63.6	65.2	61.9 ± 3.2	96.5	85.6	75.2	85.8 ± 10.7
Vd (ml)	1925.5	1636.1	1773.8	1532.2	1716.9 ± 170.7	2029.0	2560.7	2526.4	2372.0 ± 297.6
Cl (ml/hr)	104.8	111.3	116.6	99.0	107.9 ± 7.7	101.7	115.9	122.9	113.5 ± 10.8

Red Blood Cells

	Group 1 - Oral Dose					Group 6 - Intravenous Dose			
Animal Number	04A3125	04A3126	04A3127	04A3128	Mean ± SD	04A3145	04A3146	04A3147	Mean ± SD
Target Dose (mg/kg)	10.0	10.0	10.0	10.0		10.0	10.0	10.0	
Actual Dose (mg/kg)	6.39	6.43	7.42	6.46	6.7 ± 0.5	9.81	9.92	9.24	9.7 ± 0.4
C_{max} (µg/ml)	0.8	0.5	0.8	0.6	0.7 ± 0.2	2.9	15.3	6.9	8.4 ± 6.3
T_{max} (hours)	0.50	0.50	0.25	0.5	0.44 ± 0.1	0.1	0.05	0.1	0.1 ± 0.0
Half-life Distribution ($t_{½\alpha}$; hours)	2.94	2.12	1.83	2.03	2.23 ± 0.49	0.94	0.19	0.29	0.5 ± 0.4
Half-life Elimination ($t_{½\beta}$; hours)	48.9	65.3	98.0	208	105.0 ± 71.6	35.0	42.7	29.6	35.8 ± 6.6
AUC (µg-hr/ml)	3.5	3.8	5.8	6.9	5.0 ± 1.6	9.8	10.6	7.3	9.2 ± 1.7

a = Data were obtained from Table 10 on page 50 of MRID 46908412.

Concentration-Time Course of Radioactivity in Plasma (Dose groups 1 and 6)
Following a single dose of ^{14}C-XDE-742 at 10 mg/kg, a mean peak plasma concentration was calculated to be 19.8 µg/ml (occurring at 30 minutes post-dosing) and 61.7 µg/ml (occurring at 6 minutes post-dosing) for oral and iv routes, respectively.

The mean $t_{½}$ of distribution ranged from1-1.3 hours and the mean $t_{½}$ of elimination ranged from 11-14.5 hours for oral and iv routes. The AUC value was 61.9 and 85.8 µg-hr/ml for oral and iv routes, respectively.

Concentration-Time Course of Radioactivity in RBC (Dose groups 1 and 6)
Following a single dose of ^{14}C-XDE-742 at 10 mg/kg, a mean peak RBC concentration was calculated to be 0.7 µg/ml (occurring at 26 minutes post-dosing) and 8.4 µg/ml (occurring at 6 minutes post-dosing) for oral and iv routes, respectively. The mean $t_{½}$ of distribution was 2.2 hours and 30 minutes and the mean $t_{½}$ of elimination was 105 and 35.8 hours for oral and iv routes, respectively. The AUC value was 5 and 9.2 µg-hr/ml for oral and iv routes, respectively. The AUCs were about a tenth of that obtained with plasma, suggesting little binding of XDE-742 with RBCs.

1. **Preliminary experiment:** Not applicable

2. **Absorption:**

The test compound was absorbed rapidly by rats as maximum plasma concentrations being attained within 30 minutes following a single oral or iv dose of ^{14}C-XDE-742 at 10 mg/kg. The urine accounted for 57-78% and 30% of the administered dose from all low dose groups and high dose group, respectively, following 48 hours post-dosing. The amount of radioactivity excreted in the feces via the bile of i.v. dosed rats was 17.4% of the administered dose. Tissues and carcass accounted for 0.58-0.65% and 0.35% of the administered dose for all low dose groups and high dose group, respectively. Following a single oral dose of 10 mg/kg, approximately 78% of the administered dose was absorbed (59.54% excreted in urine, 17.4% excreted in bile, and 0.64% remaining in carcass/tissues).

The ratio calculated from dose-adjusted AUC's of orally dosed animals compared to the dose-adjusted AUC's for iv-dosed animals were used to estimate the fraction of the systemically bioavailable absorbed dose (from oral administration). Based on the AUCs obtained in the plasma, the systemic bioavailability of orally administered ^{14}C-XDE-742 was 72%.

Taken together, these data indicate that over 70% of the administered dose was absorbed following oral dosing with 10 mg/kg of ^{14}C-XDE-742.

3. Tissue distribution:

The mean radioactivity remaining in tissues and carcasses at 48 hours post-dosing was similar for all groups dosed at 10 mg/kg ranging from 0.58-0.75% of the administered dose and the single oral 1000 mg/kg group animals had a mean of 0.35% of the administered dose. The following table summarizes the distribution of radioactivity in rat tissues/organs collected at terminal sacrifice. Values are expressed as ppm equivalent of radioactive dose administered (Table 4).

Low dose tissue disposition (Groups 1, 3, and 4: Het-2-^{14}C)

In the group 1 animals, 48 hours after dosing, liver (0.28 ppm) and GI tract (0.14 ppm) contained relatively higher concentrations than the other tissues, which individually contained less than 0.05 ppm. The animals from the groups 3 and 4 were sacrificed at 30 minutes (Cmax) and 2 hours post- dosing (½Cmax) as determined by data generated from group 1 animals. In groups 3 and 4 animals, liver (18-20 ppm), kidney (7-13 ppm), plasma (17-30 ppm), RBC (5-8 ppm), and GI tract (124-135 ppm) contained relatively higher concentrations of radioactivity; all other tissues contained less than 3 ppm.

TABLE 4. Distribution of radioactivity in rat tissues/organs after administration of ^{14}C-XDE-742 (expressed as ppm equivalent of radioactive dose administered).

	Group 1[ab]	Group 2[ab]	Group 3[abc]	Group 4[abd]	Group 5[be]	Group 6[af]	Group 7[ag]
Carcass	0.011 ± 0.006	0.647 ± 0.459[h]	1.388 ± 0.128	1.863 ± 1.172	0.010 ± 0.001	0.035 ± 0.024	0.009 ± 0.002
GI Tract	0.143 ± 0.078	21.676 ± 10.255	134.778 ± 13.482	123.646 ± 15.351	0.108 ± 0.016	0.201 ± 0.081	0.283 ± 0.142
Kidney	0.040 ± 0.010	8.170 ± 0.632	13.479 ± 2.732	7.240 ± 1.213	0.062 ± 0.005	0.527 ± 0.060	0.120 ± 0.042
Liver	0.283 ± 0.022	16.684 ± 2.008	20.056 ± 0.464	18.185 ± 1.107	0.322 ± 0.025	0.467 ± 0.040	0.441 ± 0.015
Plasma	0.048 ± 0.007	3.426 ± 1.440	30.170 ± 3.327	17.185± 1.890	0.042 ± 0.002	0.089 ± 0.013	0.074 ± 0.004
Perirenal fat	NQ (0.007) ± 0.002[i]	NQ (1.540) ± 0.246[i]	1.021± 0.307	0.824 ± 0.456	0.005 ± 0.000[i]	0.012 ± 0.003[j]	0.008 ± 0.002[k]
RBC	0.014 ± 0.003	0.807 ± 0.440	8.092 ± 3.161	4.529 ± 2.598	0.021 ± 0.001	0.019 ± 0.003	0.015 ± 0.001
Skin	0.038 ± 0.012	2.456 ± 0.207	1.313 ± 0.153	2.760 ± 0.435	0.026 ± 0.004	0.053 ± 0.032	0.028 ± 0.006
Spleen	0.010 ± 0.001	0.639 ± 0.153	1.700 ± 0.206	1.012 ± 0.132	0.014 ± 0.001	0.169 ± 0.026	0.012 ± 0.002
Nominal mg XDE-742/kg	10	1000	10	10	10	10	10
Animals in Group	4	4	4	4	3	3	4

[a] – XDE-742-Het-2-^{14}C
[b] – Single oral dose
[c] – Cmax
[d] – ½Cmax
[e] – XDE-742-pyridine-2,6-^{14}C
[f] – Intravenous (iv) administration
[g] – 14 daily oral doses of unlabeled XDE-742 followed by a single oral dose of ^{14}C- XDE-742 Het-2-^{14}C
[h] – NQ value is an average of three animals and the NQ limits of one animal
[I] – NQ value is an average of one animal and the NQ limits of three animals
[j] – NQ value is an average of one animal and the NQ limits of two animals
[k] – Value is an average of two animals and the NQ limits of two animals
Data were obtained from Table 3 on page 43 of MRID 46908412.

Low dose tissue disposition (Group 5, pyridine-2,6-^{14}C)
In group 5 animals, 48 hours after dosing, liver and GI tract contained relatively higher concentrations of radioactivity (0.1-0.3 ppm) and all other tissues contained less than 0.1 ppm.

Low dose IV tissue disposition (Group 6: Het-2-^{14}C)
In group 6 animals, 48 hours after dosing, kidney, liver, spleen and GI tract contained relatively higher concentrations of radioactivity (0.2-0.5 ppm) and all other tissues contained less than 0.1 ppm.

Repeated Low dose IV tissue disposition (Group 7: Het-2-^{14}C)
In group 7 animals, 48 hours after dosing, kidney, liver, and GI tract contained relatively higher concentrations of radioactivity (0.1-0.4 ppm) and all other tissues contained less than 0.1 ppm.

High dose tissue disposition (Group 2: Het-2-^{14}C)
In group 2 animals, 48 hours after dosing, kidney (8 ppm), liver (17 ppm), plasma (3 ppm), skin (2 ppm), and GI tract (22 ppm) contained relatively higher concentrations of radioactivity and all other tissues contained less than 1 ppm.

4. Excretion:
XDE-742 was rapidly excreted *via* the urine and feces with the majority of the radioactivity eliminated by 12 and 24 hr post-dosing, respectively, and with some dose dependency in the routes of excretion. At 48 hours post-dosing between 95 and 110% of the administered dose was recovered in the urine, feces, tissues, carcasses and cage wash for all dose groups (Table 5). The urine accounted for 57-78% and 30% of the administered dose from all low dose groups and high dose group, respectively. The feces accounted for 45-51% and 69% of the administered dose from all low dose groups (except for iv dose group) and high dose group, respectively. The iv administration of XDE-742 demonstrated 17% excretion in the feces. For all dose groups (except for groups 3 and 4), radioactivity recovered in the tissues/carcasses and the cage wash accounted for less than 1% and 1-3% of the administered dose, respectively.

Volatile organics and CO_2 in expired air were not quantifiable for the low dose groups of both ring ^{14}C-labels of XDE-742 (groups 1 and 5). Group 2 animals (high dose) had <0.005 and 0.01% of the administered dose detected in volatile organics and CO_2, respectively. Volatile organics and CO_2 were not collected for groups 3, 4, 6, and 7.

TABLE 5. Recovery of radioactivity in tissues and excreta of rats after administration of ^{14}C-XDE-742 as Percent of Administered Dose

	Group 1[ab]	Group 2[ab]	Group 3[abc]	Group 4[abd]	Group 5[be]	Group 6[af]	Group 7[ag]
Volatiles	NQ[h]	0.00[i]	NC[j]	NC	NQ	NC	NC
CO_2	NQ	0.01	NC	NC	NQ	NC	NC
Tissues and Carcass	0.64 ± 0.15	0.35 ± 0.11	94.71 ± 4.98	89.96 ± 7.57	0.58 ± 0.01	0.75 ± 0.33	0.65 ± 0.17
Cage wash	2.77 ± 2.95	2.58 ± 3.11	NQ (0.38)[kl]	10.25 ±6.96[km]	0.90 ± 1.13	1.25 ± 1.71	0.65 ± 0.69
Urine (0-12 hours)	54.91 ± 4.06	25.51 ± 4.94			52.43 ± 1.66	72.33 ± 12.23	50.39 ± 4.35
Urine (12-24 hours)	3.30 ± 1.00	3.62 ± 2.32			3.78 ± 1.45	4.85 ± 3.14	8.61 ± 2.00
Urine (24-48 hours)	1.34 ± 0.87	1.16 ± 0.60			1.07 ± 0.83	1.03 ± 0.67	2.19 ± 2.08
Urine[b] Total	59.54 ± 5.02	30.29 ± 5.73	NC[k]	NC[k]	57.29 ± 2.05	78.21 ± 10.32	61.18 ± 5.48
Feces (0-24 hours)	39.49 ± 10.34	47.04 ± 17.08			47.23 ± 3.11	13.95 ± 2.50	41.48 ± 7.31
Feces (24-48 hours)	5.57 ± 2.40	21.65 ± 18.28			4.25 ± 2.26	3.41 ± 0.67	5.38 ± 1.50
Feces Total	45.06 ± 10.02	68.69 ± 2.91	NC	NC	51.49 ± 2.66	17.36 ± 2.59	46.86 ± 6.21
TOTAL	108.01±10.31	101.92 ± 5.83	94.71 ± 4.98	100.21± 1.19	110.26 ± 1.60	97.57 ± 6.34	109.35 ± 0.87
Dose (mg/kg)	10	1000	10	10	10	10	10
No. of Animals	4	4	4	4	3	3	4

[a] – XDE-742-Het-2-^{14}C

[b] – Single oral dose
[c] – Cmax
[d] – ½Cmax
[e] – XDE-742-pyridine-2,6-^{14}C
[f] – Intravenous (iv) administration
[g] – 14 daily oral doses of unlabeled XDE-742 followed by a single oral dose of ^{14}C- XDE-742 Het-2-^{14}C
[h] – Not quantifiable
[i] – contains <0.005% of administered dose
[j] – Not collected
[k] – Any urine/feces voided included in cage wash
[l] – NQ value is a mean of the NQ from 3 animals and a value from 1
[m] – Mean of 3 values and 1 NQ
Data were obtained from Table 2 on page 42 of MRID 46908412.

B. Metabolite characterization studies:

There were a total of 7 radioactive peaks detected at >0.05% of the administered dose in the excreta from groups 1, 2, 5 and 7. Only parent XDE-742 and 2'-demethyl-XDE-742 were detected in all the matrices and ranged from 85-90% and 4-16% of the administered dose, respectively. In the urine, the parent XDE-742 and 2'-demethyl-XDE-742 ranged from 28-50 and 2-11% of the administered dose, respectively. In the feces, XDE-742 and 2'-demethyl-XDE-742 ranged from 34-62 and 2-7% of the administered dose, respectively. No other peaks accounted for >1.5% of the administered dose. The high-dose group's (group 2) pooled urine did not yield any unique metabolites. Relative to the parent, there was less metabolite 2'-demethyl-XDE-742 compared to other groups.

The major metabolite, 2'-demethyl-XDE-742, was formed via O-dealkylation of XDE-742. A proposed metabolic pathway of XDE-742 in rats is presented in Figure 1.

Figure 1. Proposed metabolic pathway of XDE-742 in rats

XDE-742 2-Desmethyl-XDE-742

There were essentially no differences in the total radioactivity eliminated in the urine and feces between the two different ring ^{14}C-labels of XDE-742 when they were administered as a single oral dose. There were also no differences among the distribution of parent XDE-742 and the major metabolite, 2'-demethyl-XDE-742, in the urine and feces. Four major peaks (3 in the urine and 1 in the feces, <1% of the administered dose each) unique to the metabolism of the triazole ^{14}C-labeled XDE-742 samples would be consistent with minimal ring cleavage occurring during the metabolism of XDE-742.

TABLE 6. Combined Distribution of the Radioactive Peaks Detected in the Urine and Feces 48 Hours Post-Dosing as Percent of Administered Dose.

	Percent of administered dose[a]			
	Group 1[bc]	Group 2[bc]	Group 5[cd]	Group 7[be]
Demethyl-XDE-742 (Peak E[f])	15.81	3.76	15.13	15.97
XDE-742 (Peak G[g])	84.77	89.74	88.14	85.70
Total identified	100.58	93.50	103.27	101.67
Unidentified Peak A	0.57	ND[h]	1.29	ND
Unidentified Peak B	0.63	ND	ND	ND
Unidentified Peak C	0.76	ND	ND	ND
Unidentified Peak D	1.48	ND	ND	ND
Unidentified Peak F	0.58	ND	ND	0.79
Total unidentified	4.02	ND	1.29	0.79
Total accounted for[i]	104.60	93.50	105.12	102.45
Amount un-extracted (feces)	ND	5.48	3.65	5.59
TOTAL	104.60	98.98	108.77	108.04
Dose (mg/kg)	10	1000	10	10
Animals in Group	4	4	3	4

[a] – Data extracted from Appendix A
[b] – XDE-742-Het-2-^{14}C
[c] – Single oral dose
[d] – XDE-742-pyridine-2,6-^{14}C
[e] – 14 daily oral doses of unlabeled XDE-742 followed by a single oral dose of ^{14}C- XDE-742 Het-2-^{14}C
[f] – Identified as demethyl-XDE-742 (Appendix A)
[g] – Identified as parent XDE-742 (Appendix A)
[h] – ND – not detected at or above 0.5% of the administered dose
[i] – Total accounted for = (Total identified) + (Total unidentified)
Data were obtained from Table 6 on page 46 of MRID 46908412.

III. DISCUSSION

A. Investigators' conclusions: The data indicate XDE-742 was rapidly absorbed and ^{14}C-XDE-742-derived radioactivity was rapidly excreted. Saturation of absorption was observed between the doses of 10 and 1000 mg XDE-742/kg leading to a decrease in the bioavailability of XDE-742. Between 85 and 90% of the XDE-742 dosed was essentially unchanged in the urine and feces. One major metabolite found at 4-16% of the administered dose in the urine and feces was 2'-demethyl-XDE-742. Volatile organics and CO_2 were negligible for the low dose groups of both ring ^{14}C-labels of XDE-742 (groups 1 and 5) and group 2 animals (high dose). Based on the time to peak plasma or RBC radioactivity levels, ^{14}C-XDE-742 was rapidly absorbed and eliminated both by oral and iv routes. Following a single dose of ^{14}C-XDE-742 at 10 mg/kg, a mean peak plasma or RBC concentration was reached at 26-30 minutes and 6 minutes post-dosing for oral and iv routes, respectively. The mean $t_{½}$ of distribution was 1-1.3 hours and the mean $t_{½}$ of elimination was 11-14.5 hours for both oral and iv routes. The AUCs for RBCs were about a tenth of that obtained with plasma, suggesting little binding of XDE-742 with RBCs.

XDE-742 was rapidly excreted *via* the urine and feces with the majority of the radioactivity eliminated by 12 and 24 hr post-dosing, respectively, and with some dose dependency in the routes of excretion. At 48 hours post-dosing between 98 and 110% of the administered dose was recovered in the urine, feces, tissues, carcasses and cage wash for all dose groups. The urine accounted for 57-78% and 30% of the administered dose from all low dose groups and high dose group, respectively, following 48 hours post-dosing. The feces accounted for 45-51% and 69% of the administered dose from all low dose groups (except for iv dose group) and high dose group, respectively. Following the iv administration of XDE-742, the feces accounted for 17% of the administered dose. Based on this, one might conclude that at least 17% of the administered dose would be excreted via the biliary route. For all dose groups, radioactivity recovered in the tissues/carcasses and the cage wash accounted for less than 1% and 1-3% of the administered dose, respectively. Also, no remarkable differences in tissue distribution or bioaccumulation were seen for all dose groups.

Volatile organics and CO_2 in expired air were not quantifiable for the low dose groups of both ring ^{14}C-labels of XDE-742 (groups 1 and 5). Group 2 animals (high dose) had <0.005 and 0.001% of the administered dose detected in volatile organics and CO_2.

There were a total of 7 radioactive peaks detected at >0.05% of the administered dose in the excreta from the groups that were analyzed. Only parent XDE-742 and 2'-demethyl-XDE-742 (XDE-742-DM) were detected in all the matrices and ranged from 80-90% and 4-16% of the administered dose, respectively. In the urine, the parent XDE-742 and 2'-demethyl-XDE-742 (XDE-742-DM) ranged from 28-50 and 2-11% of the administered dose, respectively. In the feces, XDE-742 and 2'-demethyl-XDE-742 ranged from 34-62 and 2-7% of the administered dose, respectively. No other peaks accounted for >1.5% of the administered dose/group.

There were essentially no differences in the total radioactivity eliminated in the urine and feces between the two different ring ^{14}C-labels of XDE-742 when they were administered as a single

oral dose. Also, there were no differences among the distribution of parent XDE-742 and 2'-demethyl-XDE-742 in the urine and feces. Four major peaks (4 in the urine and 2 in the feces, <1% of the administered dose each) unique to the metabolism of the triazole ^{14}C-labeled XDE-742 samples would be consistent with minimal ring cleavage occurring during the metabolism of XDE-742.

B. Reviewer comments:

These experiments provided data describing the absorption, distribution, biotransformation, and excretion of ^{14}C- XDE-742 by rats following a single oral dose of 10 or 1000 mg/kg or a 14-day repeated oral dose (10 mg/kg) of unlabeled XDE-742 followed by a single oral exposure to 10 mg/kg ^{14}C- XDE-742. This was a well-designed and well-conducted study that describes the metabolism of the test article in rats.

The registrant, the Dow Chemical Company, originally prepared this STUDY PROFILE TEMPLATE (STP) (MRID 46908607) in HED's DER format. The HED reviewers may have added minor adjustments/additions to the original STP. The conclusions of the study and assignment of its classification as determined by HED reviewers are in the Executive Summary above.

C. Study deficiencies:

There were no deficiencies that affected the conduct or outcome of the reviewed studies.

EPA Reviewer: Paul Chin **Signature:** Paul Chin
Reregistration Branch 1, Health Effects Division (7509P) **Date:** 11/3/08
EPA Secondary Reviewer: Kimberly Harper **Signature:** Kimberly D Harper
Registration Action Branch 2, Health Effects Division (7509P) **Date:** 12/12/08

TXR#: 0054347

DATA EVALUATION RECORD

STUDY TYPE: Metabolism - Mouse; non-guideline

DP BARCODE: D332276

P.C. CODE: 108702

TEST MATERIAL (RADIOCHEMICAL PURITY):
Triazole-ring ^{14}C-labeled XDE-742 (100% a.i.)

SYNONYMS: N-(5,7-dimethoxy[1,2,4]triazolo[1,5-a]pyrimidin-2-yl)-2-methoxy-4-(trifluoromethyl)-3-pyridinesulfonamide; X666742; Pyroxsulam; XR-742, BAS-770H, X666742

CITATION: S. C. Hansen, B.S., A. J. Clark, B.S., and S. A. Saghir, M.S.P.H., PhD., D.A.B.T (2006). XDE-742: PHARMACOKINETICS OF ^{14} C-XDE-742 IN CD-1 MICE FOLLOWING SINGLE ORAL GAVAGE ADMINISTRATION. Toxicology & Environmental Research and Consulting, The Dow Chemical Company, Midland, Michigan, 48674. Study ID: 061017, (18 May 2006). MRID 46908413. Unpublished

SPONSOR: Dow AgroSciences LLC, 9330 Zionsville Road, Indianapolis, Indiana 46268

EXECUTIVE SUMMARY: In a mouse metabolism study (MRID 46908413), three groups of 40 male mice were administered a single oral dose of ^{14}C-pyroxsulam (triazole-ring ^{14}C-XDE-742; batch no. DAS Inv. 1901; purity 100% a.i) in a suspension of 0.5% METHOCEL™ at 10, 100, or 1000 mg/kg to provide data on plasma, RBC, and liver ^{14}C-time-course through 72 hours post-dosing. Data from 4 mice per group were obtained at 0.25, 0.5, 1, 2, 4, 6, 12, 24, 48, and 72 hours post-dosing. In addition, limited plasma, RBC, and liver ^{14}C concentrations were generated from 12 female mice following a single oral gavage administration of 100 mg ^{14}C-XDE-742/kg for comparison. From these data, dose-related changes in test material absorption, distribution and elimination were estimated.

Orally administered ^{14}C-XDE-742 was rapidly absorbed without any apparent lag time with an absorption rate constant of 4.4, 2.6, and 0.7 per hour at the 10, 100 and 1000 mg/kg doses, respectively. Both plasma and RBC C_{max} occurred at 0.5, 1, and 1 hour post-dosing and liver C_{max} occurred at 0.5, 1, and 4 hours post-dosing for male mice dosed at 10, 100 and 1000 mg/kg, respectively. ^{14}C-XDE-742 cleared quickly from plasma, RBC and liver

with $t_{½}$ (α phase) of 2, 2, and 3 h for the 10, 100, and 1000 mg/kg groups, respectively. Overall, the plasma, RBC, and liver AUCs increased by a factor of 6 from the 10 to 100 mg/kg dose groups, and by a factor of 4 to 5 from the 100 to the 1000 mg/kg dose groups indicating lower or less efficient absorption at the middle and high doses when compared to the low dose. Although the increases in AUC were less than dose proportional, significantly higher exposure of ^{14}C-XDE-742 with increasing dose was apparent (i.e., up to 30-fold from 10 to 1000 mg/kg).

Elimination of the absorbed radioactivity from plasma, RBC and liver followed a biexponential pattern comprising of a rapid (α) and a slow (β) phase. Most of the absorbed radioactivity was eliminated from the body via α elimination phase which resulted in a $t_{½}$ of 2-3 hours. The remaining radioactivity was eliminated slowly via the β elimination phase resulting in the terminal $t_{½}$ of 22-30 hours in plasma, 62-212 hours in RBC, and 32-307 hours in the liver for the males dosed at 10, 100 and 1000 mg/kg, respectively.

^{14}C-XDE-742 did not accumulate in the carcass or tissues 72 hours post-dosing in any of the dose groups. For all dose groups, radioactivity recovered in the tissues/carcasses and the cage wash accounted for less than 1% and 1-4% of the administered dose, respectively.

XDE-742 was rapidly excreted *via* the urine and feces with the majority of the radioactivity eliminated by 12 and 24 hr post-dosing, respectively, and with some dose dependency in the routes of excretion. At 72 hours post-dosing between 101 and 108% of the administered dose was recovered in the urine, feces, tissues, carcasses and cage wash for all dose groups. The major route of elimination of ^{14}C-XDE-742 was urine (56-61% of the administered dose) for the males dosed at 10 and 100 mg/kg and the females dosed at 100 mg/kg. For the single oral high dose (1000 mg/kg), 26% of the administered dose was eliminated in the urine. Between 77 and 84% of the radioactivity was eliminated in the urine (all groups) within 0-12 hours post-dosing. By 72 hours post-dosing, between 39 and 43% of the administered dose was eliminated in feces for the males dosed at 10 and 100 mg/kg and the females dosed at 100 mg/kg. For the 1000 mg/kg group, 77% of the administered dose was eliminated in the feces.

This metabolism study is classified **acceptable/non-guideline.** This study was conducted to provide data on plasma, RBC, and liver ^{14}C-time-course of ^{14}C-XDE-742 following single oral gavage administrations to male and female mice for comparative purposes. This study was not designed to satisfy a metabolism guideline.

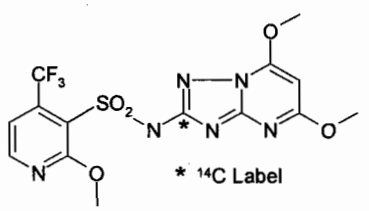
COMPLIANCE: Signed and dated GLP, Quality Assurance, and Data Confidentiality statements were provided.

I. MATERIALS AND METHODS

A. MATERIALS: ^{14}C-XDE-742 was labeled in the Het (triazole) ring. The test material was augmented with non-radiolabeled pyroxsulam (XDE-742) in dose solution preparation as needed to deliver the intended doses (as mg XDE-742/kg).

1a. Radiolabeled Test Compound:

Radiolabelled Test Material:		XDE-742-Het-2-^{14}C
	Radiochemical purity	100%
	Specific Activity	36.6 mCi/mmol
	Lot/Batch #:	DAS INV. 1901, Reference F0981-185-A, FA&PC Number 034003



XDE-742-Het-2-^{14}C (triazole)

1b. Non-radiolabeled Test Compound:

Non-Radiolabelled Test Material:		XDE-742
	Description:	Off-white to yellow powder
	Lot/Batch #:	E0952-52-01, TSN103826, ML-AL MD-2004-000592
	Purity:	98.0%
	Contaminants:	
	CAS #:	422556-08-9

2. Vehicle for oral dosing: 0.5% aqueous METHOCEL

3.	**Test animals:**	
	Species:	Mice
	Strain:	CD-1
	Age/weight at study initiation:	6-8 weeks, 23.0-31.1 g
	Source:	Charles River Laboratories Inc. (Kingston, NY)
	Housing:	Animals were housed one per cage in Roth-type metabolism cages; or stainless steel cages with wire-mesh floors. Cages had wire mesh floors and were suspended above absorbent paper. Cages contained hanging feeders and pressure activated lixit valve type watering systems.
	Feed & Water:	Animals were provided LabDiet® Certified Rodent Diet #5002 (PMI Nutrition International, St. Louis, Missouri) in pelleted form. Animals were not fasted prior to dosing. Drinking water obtained from the municipal water source was periodically analyzed for chemical parameters and biological contaminants by the municipal water department. Feed and water were provided *ad libitum*.

	Environmental conditions:	Temperature: Humidity: Air changes: Photoperiod:	Temperature: 22±1 °C Humidity: 40-70% 12-15/hour 12 hrs dark/12 hours light
	Acclimation period:	The animals sacrificed at 72 hr post-dosing were acclimated to Roth metabolism cages 2 days prior to dosing.	

4. **Preparation of dosing solutions:** Three separate oral dose suspensions were prepared in 0.5% METHOCEL. Appropriate amounts of ^{14}C-labeled and/or non-radiolabeled XDE-742 were added to obtain the target doses of 10, 100, or 1000 mg XDE-742/kg body weight.

B. STUDY DESIGN AND METHODS:

1. **Group Arrangements:** Animals were stratified by body weight and assigned randomly to the test groups noted in Table 1.

TABLE 1: Dosing Groups and Study Design

Single Oral Gavage Dosing Groups	Dose of Labeled Material (mg/kg)	Total Number of Male Mice	Total Number of Female Mice	Remarks
1. Male low dose	10	40	-	A, B, D
2. Male middle dose	100	40	-	A, B, D
3. Male high dose	1000	40	-	A, B, D
4. Female middle dose	100	-	12	B, C, D

A. Plasma, RBC, and liver ^{14}C-concentration-time course as described below
B. Selected tissues were collected and analyzed for radioactivity as described below
C. Plasma, RBC, and liver were collected at plasma C_{max} and ½C_{max} as determined in Group 2 (male middle dose)
D. Excreta were collected from animals that were sacrificed at 72 hours post-dosing (n = 4/dose level) and mass balance of administered dose determined
E.

2. **Dosing and sample collection:** All animals were administered either a single oral dose of 10 or 100 or 1000 mg ^{14}C-XDE-742/kg. The samples for the plasma, RBC, and liver ^{14}C-time course determinations were collected via serial sacrifice (4 animals/time point). Urine, feces, and selected tissues were collected from the animals sacrificed at 72 hours-post-dosing.

   a. **Pharmacokinetic studies:** This study consisted of a plasma, RBC, and liver ^{14}C concentration-time course in male mice to determine C_{max} and ½C_{max} of plasma, RBC, and liver ^{14}C concentrations, limited distribution, and elimination of ^{14}C-XDE-742 following single oral doses of ^{14}C-XDE-742 at three dose levels. Blood and livers from the male animals of Groups 1, 2, and 3 were collected at chosen times (0.25, 0.5, 1, 2, 4, 6, 12, 24, 48, and 72-hours post-dosing). Blood and livers from Group 4 female mice were collected at plasma C_{max} and ½C_{max} (as determined from male animals in Group 2), and 72 hours post-dosing. Urine and feces were collected

in dry-ice traps at 12 (urine only), 24, 48, and 72 hours post-dosing from the animals sacrificed at 72 hours post-dosing, respectively. Organic volatiles and CO_2 were not collected.

Animals were sacrificed by exsanguination under CO_2/O_2 anesthesia. The following tissues were collected at sacrifice for the 72 hours post-dosing animals (Groups 1, 2, 3, and 4):

Kidney	red blood cells (terminal)	skin
Liver	gastrointestinal (GI) tract	spleen
perirenal fat	(including ingesta)	residual carcass
plasma (terminal)		

The carcass and GI tract with contents, kidney, and liver were collected and homogenized (~ 33% homogenate). A weighed aliquot was oxidized and analyzed for radioactivity by liquid scintillation spectrometry (LSS). Blood was centrifuged to obtain plasma and the plasma was analyzed for radioactivity by LSS. The skin was removed from the carcass and a representative skin sample was oxidized and analyzed for radioactivity by LSS. The remaining tissues were directly oxidized without homogenization and analyzed for radioactivity by LSS.

Samples with dpm less than twice the concurrently run background (blanks) were considered to contain insufficient radioactivity to reliably quantify. For tissues, when a sample was non-quantifiable (NQ), that sample was assigned the quantitation limit (QL) for calculations and displayed as NQ with the QL in parenthesis. The mean is calculated from actual values and calculated QL values and presented as mean standard deviation (X ± SD), unless greater than ½ of the values are presented as NQ, in which case the mean is expressed NQ (X) ± SD. If all tissue values are NQ the mean is presented as NQ (QL) with no SD displayed.

b. Metabolite characterization studies: Metabolites were not characterized in excreta or tissues.

3. Statistics: All calculations in the database were conducted using Microsoft® Excel spreadsheets and databases in full precision mode (15 digits of accuracy). Certain pharmacokinetic parameters were estimated from the plasma, blood, and liver ^{14}C concentration-time course including AUC (area-under-curve), C_{max}, $½C_{max}$, absorption/uptake rate constants, half- lives, using PK Solutions (Summit Research Services, Montrose, Colorado), a pharmacokinetic computer modeling program.

II. RESULTS

A. <u>Pharmacokinetic Studies</u>: Certain pharmacokinetic parameters were estimated from plasma and red blood cell concentration-time course data, including AUC (area-under-the-curve), C_{max}, $½C_{max}$, and half-lives of elimination (Table 2). Based on the plasma concentration-time course data, orally administered ^{14}C-XDE-742 was rapidly

absorbed without any apparent lag time with an absorption rate constant of 4.4, 2.6, and 0.7 per hour at the 10, 100 and 1000 mg/kg doses, respectively. Both plasma and RBC C_{max} occurred at 0.5, 1, and 1-2 hours post-dosing and liver C_{max} occurred at 0.5, 1, and 4 hours post-dosing for male mice dosed at 10, 100 and 1000 mg/kg, respectively. ^{14}C-XDE-742 cleared quickly from plasma, RBC and liver with $t_{½}$ (alpha phase) of 2-3 hours for the 10, 100, and 1000 mg/kg groups. Overall, the plasma, RBC, and liver AUCs increased by a factor of 6 from the 10 to 100 mg/kg dose groups and by a factor of 4 to 5 from the 100 to the 1000 mg/kg dose groups indicating lower or less efficient absorption at the middle and high doses when compared to the low dose. Although the increases in AUC were less than dose proportional, significantly higher exposure of ^{14}C-XDE-742 with increasing dose was apparent (i.e., up to 30-fold from 10 to 1000 mg/kg). Elimination of the absorbed radioactivity from plasma, RBC and liver followed a biexponential pattern comprising of a rapid (α) and a slow (β) phase. Most of the absorbed radioactivity was eliminated from the body via α elimination phase which resulted in a $t_{½}$ of 2-3 hours (alpha phase) for the 10, 100 and 1000 mg/kg groups. The remaining radioactivity was eliminated slowly via the β elimination phase resulting in the terminal $t_{½}$ of 22-30 hours in plasma, 62-212 hours in RBC, and 32-307 hours in the liver for male mice dosed at 10, 100 and 1000 mg/kg.

1. **Preliminary experiment:** Not applicable

2. **Absorption:** The test compound was absorbed rapidly by mice as maximum plasma concentrations being attained within 0.5-2 hours post-dosing for each dosing regimen. By 72 hours post-dosing, the major route of elimination of ^{14}C-XDE-742 was urine (56-61% of the administered dose) for the males dosed at 10 and 100 mg/kg and the females dosed at 100 mg/kg. For the males dosed at 1000 mg/kg, 26% of the administered dose was eliminated in the urine.

   Based on the radioactivity recovered in the urine, tissues, carcass and cage wash (from Table 4), and without knowledge of the biliary absorption, approximately 61-65% of the administered dose was absorbed for males from the 10 and 100 mg/kg bw and for females from the 100 mg/kg bw dose groups, while 29% of the administered dose was absorbed for the males from the 1000 mg/kg bw dose group.

3. **Tissue distribution:** In general, all tissues showed an increase of ^{14}C-derived radioactivity with increasing dose at 72 hours post-dosing though not proportional to dose. Relatively higher concentrations of radioactivity were found in carcass, GI tract, and liver than the other tissues sampled. The radioactivity remaining in these tissues ranged from 0.07 to 0.17, 0.6 to1.3, and 4.2 to 7 µg-eq/g in the males dosed at 10, 100, and 1000 mg/kg, respectively (Table 3). In the females dosed at 100 mg/kg, the radioactivity remaining in these tissues ranged from 1 to 2 µg-eq/g. For all dose groups, radioactivity recovered in the tissues/carcasses accounted for less than 1% of the administered dose.

TABLE 2. Plasma, Red Blood Cell, and Liver Kinetic Parameters Following Administration of ^{14}C-XDE-742 [a]

	Plasma			RBC			Liver		
PK parameters	10 mg/kg	100 mg/kg	1000 mg/kg	10 mg/kg	100 mg/kg	1000 mg/kg	10 mg/kg	100 mg/kg	1000 mg/kg
T_{max} (h)	0.5	1.0	2.0	0.5	1.0	1.0	0.5	1.0	4.0
C_{max} (µg g^{-1})	36.6	174.4	258.2	5	35.7	894.6	31.48	182.2	336.4
$AUC_{0\rightarrow t}$ (µg h g^{-1})	118.6	676.2	2562.7	20.4	128.3	610.8	139.2	783.0	3083.7
$K_{absorption/uptake}$ (h^{-1})	4.35	2.6	0.7	5.09	2.1	1.6	10.47	1.8	1.5
$t_{½\,\alpha}$ (h)	1.97	2.1	3.38	1.97	2.0	2.6	2.1	2.1	3.23
$t_{½\,\beta}$ (h)	27.4	29.6	22.4	61.5	82.4	212.0	31.1	31.8	306.8
Vd (ml kg^{-1})	3297	6206	12508	41651	85147	385297	3061	5501	71399
Cl (ml h^{-1} kg^{-1})	83.5	145.3	386.6	469.1	715.8	1259.7	68.1	120.0	161.3
MRT (h)	5.6	6.9	8.0	11.4	20.3	92.4	14.4	15.5	264.1

T_{max} - Time of maximum concentration
C_{max} - Maximum concentration
$AUC_{0\rightarrow t}$ - Area under the curve
$K_{absorption/uptake}$ - Rate constant
$t_{½\,\alpha}$ - Elimination half-life alpha phase
$t_{½\,\beta}$ - Elimination half-life beta phase
MRT - Means residence time

a = Data were obtained from Table 35 on page 75 of MRID 46908413.

TABLE 3. Distribution of radioactivity in mouse tissues/organs 72-hours after administration of ^{14}C-labeled XDE-742[a]

	µg-eq./g Tissue of Radioactive Dose Administered			
	Group 1 10 mg/kg bw	**Group 2 100 mg/kg bw**	**Group 3 1000 mg/kg bw**	**Group 4 100 mg/kg bw**
Sex	Male	Male	Male	Female
Carcass	0.073 ± 0.010	0.579 ± 0.067	4.170 ± 3.731	1.515 ± 0.813
Fat (perirenal)	NQ[a] (0.034) ± 0.087	NQ[b] (0.152)	NQ (1.699)	NQ (0.136)
GI Tract	0.161 ± 0.074	1.261 ± 0.430	4.553 ± 2.058	2.015 ± 0.468
Kidney	0.030 ± 0.013	0.195 ± 0.020	0.786 ± 0.199	0.137 ± 0.024
Liver	0.168 ± 0.022	1.098 ± 0.513	7.041 ± 4.447	1.027 ± 0.211
Plasma	0.031 ± 0.008	0.466 ± 0.035	0.737 ± 0.323	0.216 ± 0.054
RBC	0.010 ± 0.002	0.096 ± 0.016	NQ[a] (0.598) ± 0.166	0.073 ± 0.017
Skin	0.022 ± 0.018[c]	0.133 ± 0.027	NQ[a] (1.718) ± 3.413	0.189 ± 0.249[c]
Spleen	0.009 ± 0.003[d]	0.055 ± 0.004[d]	NQ (0.593)	0.127 ± 0.028[d]

[a] – NQ value is a mean of the NQ from 3 animals and a value from 1
[b] – Not Quantifiable at or above number in parenthesis
[c] – Mean of 3 values and 1 NQ
[d] – Mean of 2 values and 2 NQ

Data were obtained from Tables 7-18 and 23-26 on pages 47-58 and 63-66 of MRID 46908413.

4. Excretion: XDE-742 was rapidly excreted *via* the urine and feces with the majority of the radioactivity eliminated by 12 and 24 hr post-dosing, respectively, and with some dose dependency in the routes of excretion. At 72 hours post-dosing between 101 and 108% of the administered dose was recovered in the urine, feces, tissues, carcasses and cage wash for all dose groups (Table 4). Urine represented the major route of elimination of ^{14}C-XDE-742 (56-61% of the administered dose) for the males dosed at 10 and 100 mg/kg and the females dosed at 100 mg/kg. For the single oral high dose (1000 mg/kg), 26% of the administered dose was eliminated in the urine. By 72 hours post-dosing, between 39 and 43% of the administered dose was eliminated in feces for the males dosed at 10 and 100 mg/kg and the females dosed at 100 mg/kg. For the 1000 mg/kg group, 77% of the administered dose was eliminated in the feces. For all dose groups, radioactivity recovered in the cage wash accounted for 1-4% of the administered dose.

III. DISCUSSION

A. Investigators' conclusions:

Orally administered ^{14}C-XDE-742 was rapidly absorbed without any apparent lag time with an absorption rate constant of 4.4, 2.6, and 0.7 per hour at the 10, 100 and 1000 mg/kg doses, respectively. Both plasma and RBC C_{max} occurred at 0.5, 1, and 1 hour post-dosing and liver C_{max} occurred at 0.5, 1, and 4 hours post-dosing for male mice dosed at 10, 100 and 1000 mg/kg, respectively. ^{14}C-XDE-742 cleared quickly from plasma, RBC and liver with $t_{½}$ (alpha phase) of 2, 2, and 3 h for the 10, 100, and 1000 mg/kg groups, respectively. Overall, the plasma, RBC, and liver AUCs increased by a factor of 6 from the 10 to 100 mg/kg dose groups, and by a factor of 4 to 5 from the 100 to the 1000 mg/kg dose groups indicating lower or less efficient absorption at the middle and high doses when compared to the low dose. Although the increases in AUC were less than dose proportional, significantly higher exposure of ^{14}C-XDE-742 with increasing dose was apparent (i.e., up to 30-fold from 10 to 1000 mg/kg).

Elimination of the absorbed radioactivity from plasma, RBC and liver followed a biexponential pattern comprising of a rapid (α) and a slow (β) phase. Most of the absorbed radioactivity was eliminated from the body via α elimination phase which resulted in a $t_{½}$ of 2-3 hours. The remaining radioactivity was eliminated slowly via the β elimination phase resulting in the terminal $t_{½}$ of 22-30 hours in plasma, 62-212 hours in RBC, and 32-307 hours in the liver for the males dosed at 10, 100 and 1000 mg/kg, respectively.

TABLE 4. Recovery of radioactivity in tissues and excreta of mice after administration of ^{14}C-labeled XDE-742

	Percent of Radioactive Dose Recovered			
	Group 1 10 mg/kg bw	Group 2 100 mg/kg bw	Group 3 1000 mg/kg bw	Group 4 100 mg/kg bw
Sex	Male	Male	Male	Female
Expired Volatiles	NC[a]	NC	NC	NC
CO_2	NC	NC	NC	NC
Tissues	0.38 ± 0.14	0.34 ± 0.08	0.15 ± 0.08	0.49 ± 0.04
Carcass	0.37 ± 0.04	0.33 ± 0.04	0.22 ± .019	0.89 ± 0.46
Cage Wash	3.01 ± 2.29	4.27 ± 2.94	1.75 ± 1.57	3.32 ± 1.13
Urine (0-12 hours)	47.39 ± 6.59	47.20 ± 9.94	22.27 ± 3.96	46.81 ± 10.56
Urine (12-24 hours)	11.60 ± 6.11	7.23 ± 5.47	3.27 ± 1.22	9.41 ± 6.66
Urine (24-48 hours)	1.66 ± 0.69	1.28 ± 0.51	0.68 ± 0.60	2.16 ± 1.57
Urine (48-72 hours)	0.77 ± 0.42	0.41 ± 0.16	0.18 ± 0.10	1.09 ± 0.98
Urine Total	61.42 ± 4.69	56.12 ± 8.08	26.40 ± 3.49	59.47± 8.68
Feces (0-24 hours)	41.43 ± 3.75	38.95 ± 4.47	76.64 ± 3.26	37.54 ± 6.58
Feces (24-48 hours)	1.23 ± 1.25	0.65 ± 0.79	0.74 ± 0.51	0.78 ± 0.17
Feces (48-72 hours)	0.40 ± 0.19	0.23 ± 0.18	0.09 ± 0.02	0.38 ± 0.08
Feces Total	43.06 ± 3.80	39.82 ± 4.90	77.47 ± 2.99	38.710 ± 6.43
Total	108.24 ± 0.28	100.89 ± 0.92	105.99 ± 0.78	102.30 ± 0.93

[a] Not collected
Data were obtained from Tables 23-34 on pages 63-74 of MRID 46908413.

^{14}C-XDE-742 did not accumulate in the carcass or tissues 72 hours post-dosing in any of the dose groups. For all dose groups, radioactivity recovered in the tissues/carcasses and the cage wash accounted for less than 1% and 1-4% of the administered dose, respectively. XDE-742 was rapidly excreted *via* the urine and feces with the majority of the radioactivity eliminated by 12 and 24 hr post-dosing, respectively, and with some dose dependency in the routes of excretion. At 72 hours post-dosing between 101 and 108% of the administered dose was recovered in the urine, feces, tissues, carcasses and cage wash for all dose groups. The major route of elimination of ^{14}C-XDE-742 was urine (56-61% of the administered dose) for the males dosed at 10 and 100 mg/kg and the females dosed at 100 mg/kg. For the single oral high dose (1000 mg/kg), 26% of the administered dose was eliminated in the urine. Between 77 and 84% of the radioactivity was eliminated in the urine (all groups) within 0-12 hours post-dosing. By 72 hours post-dosing, between 39 and 43% of the administered dose was eliminated in feces for the males dosed at 10 and 100

mg/kg and the females dosed at 100 mg/kg. For the 1000 mg/kg group, 77% of the administered dose was eliminated in the feces.

B. Reviewer comments:

These experiments provided data describing the absorption, distribution, and excretion of ^{14}C- XDE-742 by mice following a single oral dose of 10, 100 or 1000 mg/kg. This was a well-designed and conducted study that describes the metabolism of the test article in mice.

The registrant, the Dow Chemical Company, originally prepared this STUDY PROFILE TEMPLATE (STP) (MRID 46908610) in HED's DER format. The HED reviewers may have added minor adjustments/additions to the original STP. The conclusions of the study and assignment of its classification as determined by HED reviewers are in the Executive Summary above.

C. Study deficiencies:

There were no deficiencies that affected the conduct or outcome of the reviewed studies.

13544

R168049

Chemical Name: Pyroxsulam

PC Code: 108702
HED File Code: 13000 Tox Reviews
Memo Date: 12/13/2007
File ID: 00000000
Accession #: 000-00-0130

HED Records Reference Center
3/20/2009