2

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HEALTH EFFECTS DIVISION
SCIENTIFIC DATA REVIEWS
EPA SERIES 361

OFFICE OF
PREVENTION, PESTICIDES, AND
TOXIC SUBSTANCES

MEMORANDUM

DATE: May 23, 2007

SUBJECT: Cancer Assessment Review Committee Meeting on PYROXSULAM

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

TO: Addressees

Attached for your review is a package on PYROXSULAM prepared by Kimberly Harper, RAB2.

A meeting to review the carcinogenicity classification of this chemical is scheduled for Wednesday, June 6, 2007, at 9 am in Room 10100, PY1.

Addressees:
G. Akerman (S-11831)
K. Bailey (S-10341)
L. Brunsman (S-11924)
W. Burnam (S-11838)
M. Copley (S-11936)
V. Dellarco (S-10780F)
K. Farwell (S-10873)
K. Harper
J. Kidwell (S-11923)
R. Kent (S-10813)
A. Levy (S-10344)
M. Manibusan (S-10314)
N. McCarroll (S-11826)
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J. Pletcher (Mail)
E. Rinde (S-11927)
J. Rowland (S-11913)
Y. Woo (EPAEast, 6308NN)

CANCER ASSESSMENT DOCUMENT
FOR COMMITTEE DELIBERATION

EVALUATION OF THE CARCINOGENIC POTENTIAL OF *PYROXSULAM/XDE-742*

Date of the Report: June 6, 2007
Submitted by: Kimberly Harper

CANCER ASSESSMENT REVIEW COMMITTEE
HEALTH EFFECTS DIVISION
OFFICE OF PESTICIDE PROGRAMS

2

CONTENTS

3

I. INTRODUCTION

On June 6, 2007, the Cancer Assessment Review Committee of the Health Effects Division of the Office of Pesticide Programs met to evaluate the carcinogenic potential of pyroxsulam.

II. BACKGROUND INFORMATION

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.

(N-(5,7-dimethoxy[1,2,4]triazolo[1,5-a]pyrimidin-2-yl)-2-methoxy-4-(trifluromethyl)pyridine-3-sulfonamide), acts by inhibiting acetolactate synthase. This product is being developed as a systemic post-emergence broad-spectrum grass and broadleaf herbicide for use in wheat production systems (including durum). Dow AgroSciences is submitting two formulations containing pyroxsulam for registration in the U.S. GF-1674 is a 30 g/L oil dispersion with a 3:1 ratio of cloquintocet-mexyl to pyroxsulam; Dow states that this formulation will be for use on Spring wheat. GF-1274 is a 75 g/kg wettable granule that has a 1:1 ratio of cloquintocet-mexyl to pyroxsulam. The PC code is 108702.

III. EVALUATION OF CARCINOGENICITY STUDIES

1. Combined Chronic Toxicity/Carcinogenicity Study with XDE-742 in F-344 Rats

Reference: Stebbins, K. E., and K. J. Brooks (02 November 2005). 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

A. Experimental Design

The study design allocated groups of 65 Fischer 344 rats to nominal dose levels of 0, 10, 100 and 1000 mg/kg/day of Pyroxsulam for 104 weeks. Ten rats/sex/dose were necropsied at the 12-month mark; an additional five rats/sex/dose were necropsied at 12 months for neurologic evaluation.

B. Discussion of Tumor Data:

There were no significant increases in tumors in the treated groups compared to controls.

B. Non-Neoplastic Lesions

4

There was a decrease in the incidence of basophilic foci of altered cells in hepatocytes of the high dose females; the decrease was not considered toxicologically adverse.

Table 1. Selected non-neoplastic histological findings

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*

*Statiscally identified by Yate's Chi-square test, alpha = 0.05, two-sided.

D. Adequacy of the Dosing for Assessment of Carcinogenicity:

There were no treatment related adverse effects on mortality, clinical signs, ophthalmology, hematology, clinical chemistry, histopathology. Body weights were minimally reduced in the high dose females (4-7%) throughout the study, and body weight gains were decreased 8-10% thoughout the study. The differences in BW/BWG as compared to controls were not more pronounced as the study progressed. The mean absolute and relative liver weights in females given 1000 mg/kg/day at 24 months were increased 6.1% and 10.9%, respectively. However, there were no corresponding histopathology findings that would explain the increased weights. The decrease incidence of basophilic foci of alteration is not generally associated with toxicity. Although there weres few treatment related findings in this study, the highest dose tested was the limit dose of 1000 mg/kg/day.

2. Carcinogenicity Study in Mice

Reference: Johnson, K.A., D.V.M., Ph.D. ; M. D. Dryzga, B.S. ; B. L. Yano, D.V.M., Ph.D. (15 December 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

A. Experimental Design

The study design allocated groups of 50 mice to nominal dose levels of 0, 10, 100 and 1000 mg/kg/day of Pyroxsulam for 79 weeks. Actual doses were 0, 10, 100 and 932 mg/kg/day for males.

The was no increased mortality in either sex of the treated groups compared to control. There were no compound-related tumors in the females so only analyses of the males are presented in this document.

5

B. Discussion of Survival and Tumor Data

There were no statistically significant incremental changes in mortality with increasing doses of Pyroxsulam in male mice (Table 2).

Male mice had a statistically significant trend for liver carcinomas at $p < 0.05$. There were statistically significant pair-wise comparisons of the 10 and 1000 mg/kg/day dose groups with the controls for liver adenomas, and liver adenomas and/or carcinomas combined, all at $p < 0.05$. The statistical analyses of the tumors in the male mice were based upon Fisher's Exact Test for pair-wise comparisons and the Exact Test for trend (Table 3).

Historical control data is presented in Table 4. Out of 200 male CD-1 mice, 23 had a liver adenoma (incidence 12%); the range for the individual studies was 2-8 mice with liver adenomas per 50 mice or an incidence rate of 4-16%. Out of 200 male mice, 5 had a liver carcinoma (incidence rate 3%); the range of individual animals with a liver carcinoma (with or without metastasis) was 1-3 per 50 mice (incidence rate 2-6%). The overall incidence rate for combined liver tumors was 27/200 or 14%. The range for the individual studies was 3-10 per 50 mice or an incidence rate of 6-20%.

Table 2. Pyroxsulam – CD-1 (Crl:CD1(ICR)) Mouse Study (MRID 46908406)

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

	Weeks			
Dose (mg/kg/day)	1-26	27-52	53-79[f]	Total
0	0/50	1/50	9/49	10/50 (20)
10	1/50	3/49	6/46	10/50 (20)
100	0/50	2/50	8/48	10/50 (20)
1000	0/50	2/50	10/48	12/50 (24)

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

[f]Final sacrifice at week 79.

( )Percent.

Note: Time intervals were selected for display purposes only.
Significance of trend denoted at control.
Significance of pair-wise comparison with control denoted at dose level.
If *, then $p < 0.05$. If **, then $p < 0.01$.

6

Table 3. Pyroxsulam – CD-1 (Crl:CD1(ICR)) Mouse Study (MRID 46908406)
Male Liver Tumor Rates
and Fisher's Exact Test and Exact Test for Trend Results

	Dose (mg/kg/day)			
	0	10	100	1000
Adenomas (%)	5/49 (10)	13/46 (28)	9[a]/49 (18)	14/48 (29)
p =	0.06696	0.02300*	0.19363	0.01716*
Carcinomas (%)	1/49 (2)	0/46 (0)	2[b]/49 (4)	4/48 (8)
p =	0.02622*	1.00000	0.50000	0.17451
Combined (%)	6/49 (12)	13/46 (28)	10[c]/49 (20)	15[d]/48 (31)
p =	0.05737	0.04462*	0.20651	0.02067*

+Number of tumor bearing animals/Number of animals examined, excluding those that died before week 52.
[a]First adenoma observed at week 53, dose 100 mg/kg/day.
[b]First carcinoma observed at week 73, dose 100 mg/kg/day.
[c]One animal in the 100 mg/kg/day dose group had both an adenoma and a carcinoma.
[d]Three animals in the 1000 mg/kg/day dose group had both an adenoma and a carcinoma.

Note: Significance of trend denoted at control.
Significance of pair-wise comparison with control denoted at dose level.
If *, then $p < 0.05$. If **, then $p < 0.01$.

Table 4. 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.

C. Non-Neoplastic Lesions

Males in the high dose group had greater numbers of mice with foci of altered cells and a greater incidence and multiplicity of hepatocellular tumors – adenomas and/or carcinomas (summary in Table 4).

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. Male mice given 1000 mg/kg/day that had hepatic foci of altered cells tended to have a multiplicity of the effects considered related to treatment, *i.e.*, either more than one subtype of focus of altered cells or a focus along with one or more hepatocelluar adenoma(s) and/or carcinoma(s). However, multiplicity was also found for one control male (#03A1389) which had three basophilic foci and one mixed cell focus of altered cells along with six hepatocellular adenomas. 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.

8

Table 5. Non-neoplastic lesion in the liver of mice exposed to 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	1	2	2	0	2	0	1
- clear, one or more	0	0	0	7*	0	0	0	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

* 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.

D. Adequacy of the Dosing

There were no effects on survival, clinical or ophthalmic examinations, body weights and body weight gains, food consumption, hematology, or clinical chemistry. The only effects related to treatment were increased absolute and relative liver weights in males of the high dose group (26.4% and 31.6%, respectively). Gross pathology

examination showed an increased in the number of liver masses in the male mice of the high dose group. Microscopic examination of the tissues showed an increased number of foci of altered hepatocytes and an increased incidence of tumors. There were no treatment related effects in female mice. It does not appear that the MTD was exceeded; the highest dose tested was the limit dose.

IV. TOXICOLOGY

1. Metabolism

Rat (MRID 46908412): 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

10

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.

This metabolism study is classified acceptable/guideline and satisfies the guideline requirements for a metabolism study (OPPTS 870.7485 and OECD 417) in rats.

Mouse (MRID 46908413): 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 $t_{½}$ (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.

^{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.

2. Mutagenicity:

There is no mutagenic concern for XDE-742 at this time. This assessment of the **parent compound** is based on the following five acceptable/guideline genetic toxicology studies:

1. In an *in vitro* reverse gene mutation test with *Salmonella typhimurium* strains TA1535, TA100, TA1537, TA98 and *Escherichia coli* strain WP2 *urv*A at concentrations up to 5000 µg/plate (limit concentration). XDE-742 was not mutagenic with or without metabolic activation (MRID 46908414).

2. In an *in vitro* mammalian cell gene mutation assay in Chinese hamster ovary cells at the HGPRT locus at concentrations up to 200 µg/mL (limit of solubility), XDE-742 was not mutagenic with or without metabolic activation (MRID 46908408).

12

3. In an *in vitro* chromosomal aberration assay using rat lymphocytes at concentrations up to 200 µg/ml (limit of solubility) in the presence and absence of metabolic activation, there was no evidence of increased chromosomal aberrations induced above background (MRID 46908409).

4. In an *in vivo* micronucelus assay performed in CD-1 mice, no increase in micronucleated polychromatic erythrocytes was seen following dosing up to the limit dose of 2000 mg/kg bw (MRID 46908410). No mortality was present at any dose.

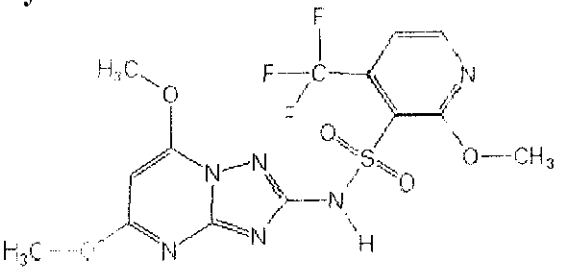
5. In an *in vivo/in vitro* measurement of unscheduled DNA sysnthesis using CD-1 mouse hepatocytes, no induction of UDS was observed at doses up to the limit dose of 2000 mg/kg bw. (MRID 47022001).

13

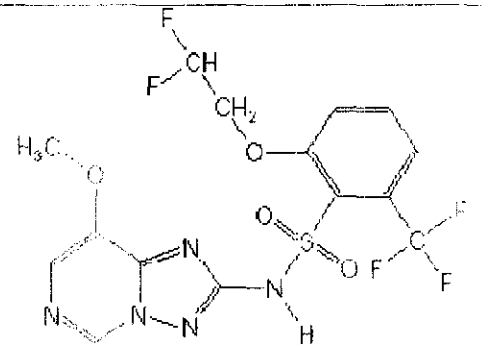
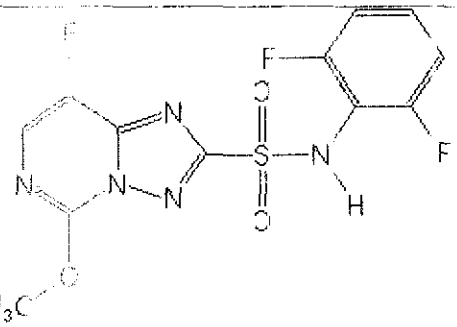
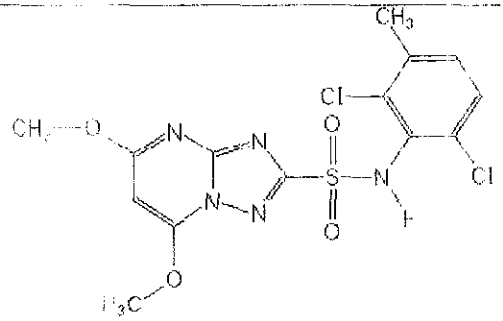
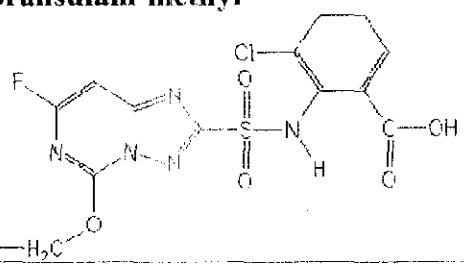
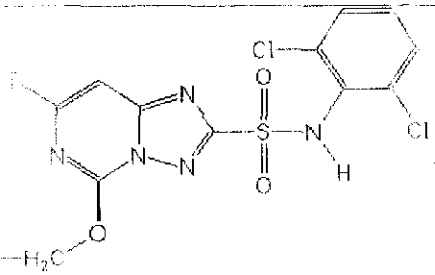
3. **Structure-Activity Relationship:**

Pyroxsulam belongs to the triazolopyrimidine sulfonamide class of chemicals. There are six other chemicals in this class, five of which are registered in the US. None of the chemicals is considered mutagenic; and 4 of the 5 are considered "not likely to be carcinogenic to humans" or "evidence of non-carcinogenicity to humans." Penoxsulam appears to be its closest congener; its' cancer classification is "suggestive evidence but not enough to evaluate human significance," due to an increase in large granular lymphocytic leukemia compared to control.

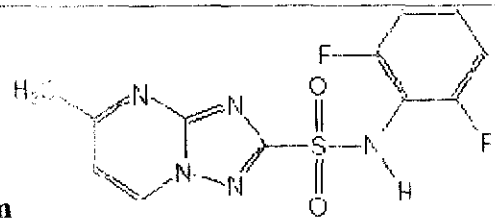
Pyroxsulam:



14

Chemical/ Pesticide Class Structure	PC Code/ CAS#	Cancer Classification	Tumor Types	Muta.
 **Penoxsulam**	119031/ 219714-96-2	Suggestive evidence but not enough to evaluate human significance	Large granular lympho-cytic leukemia	Negative
 **Florasulam**	129108/ 145701-23-1	Not likely	None	Negative
 **Metosulam** not yet registered in US	NA/ 139528-85-1	NA	None	NA
Cloransulam-methyl 	129116/ 147150-35-4	Not likely	None	Negative
 **Diclosulam**	129122/ 145701-21-9	Not likely	None	Negative

15

Chemical/ Pesticide Class Structure	PC Code/ CAS#	Cancer Classification	Tumor Types	Muta.
 **Flumetsulam**	129016/ 98967-40-9	Group E – Evidence of non-carcinogenicity to humans	None	Negative

4. Subchronic and Chronic Toxicity

a) Subchronic Toxicity

28-Day Rats (MRID 46908349): 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 dose levels of 0, 10, 100, 500, or 1000 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/guideline; it is a range-finding study for the 90-day and 2-year rat studies.

90-Day Rats (MRID 46908350): 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

16

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.

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.

90-Day Mice (MRID 46908351): 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/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.

17

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] CD-1 Mice; OPPTS 870.3100 (rodent); OECD 408, EEC, Part B.26, JMAFF (Subchronic Oral Toxicity Study).

b) Chronic Toxicity

Rats (MRID 46908407): This study 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 previously reported.

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 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.

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.

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.

Mice (MRID 46908406): 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. 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/day, with the mean absolute and relative liver weights increased by 26.4% and 31.6%, respectively, increased incidence of liver masses at necropsy, histopathologically increased incidence of foci of altered cells (hepatocytes), and increased incidence and numbers of hepatocellular adenomas and carcinomas, although the tumor incidences were not statistically identified. There was a tendency of affected mice to have both foci of altered cells and multiple tumors (adenomas and/or carcinomas).

Male mice given 10 mg/kg/day had a slightly increased incidence and number of liver adenomas that was not considered dose related because it was not accompanied by increased organ weight or alterations in histopathology as was the high dose group. There were no effects on males or females at 100 mg/kg/day.

The **LOAEL is 1000 mg/kg/day**, based on the increase in mean absolute and relative liver weights, increased incidence of foci of altered cells (hepatocytes), and increased incidence and numbers of hepatocellular adenomas and/or carcinomas. The **NOAEL is 100 mg/kg/day.**

At the doses tested, there was a treatment related increase in tumor incidence in male mice with regards to hepatocellular adenomas and/or carcinomas when 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.

However, the incidence of hepatocellular tumors in the high dose males did exceed the historical control range and half the male mice had multiple adenomas, which may indicate a treatment effect. Although there was little overt toxicity in either sex other than the increase in tumor incidence, dosing is considered adequate because the highest dose tested (1000 mg/kg/day) is the limit dose for chronic/carcinogenicity studies.

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

5. Mode of Action Studies:

No mode of action studies were submitted for this chemical. A short discussion of the mouse liver tumors and human relevance was provided by the registrant; it has been included in the CARC package.

VIII. Bibliography

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22

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

OFFICE OF
PREVENTION, PESTICIDES, AND
TOXIC SUBSTANCES

TXR No. 0054586

MEMORANDUM

DATE: May 18, 2007

SUBJECT: **Pyroxsulam:** Qualitative Risk Assessment Based On CD-1 (Crl:CD1(ICR)) Mouse Carcinogenicity Dietary Study

P.C. Code: 108702

TO: Kimberly Harper, Toxicologist
Registration Action Branch 2
Health Effects Division (7509P)

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

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

BACKGROUND

A carcinogenicity study in CD-1 (Crl:CD1(ICR)) mice was conducted by Dow Chemical Company, Midland, Michigan, for Dow AgroSciences, Indianapolis, Indiana, and dated December 15, 2005 (Study ID No. 031015, MRID No. 46908406).

The study design allocated groups of 50 mice per sex to nominal dose levels of 0, 10, 100 and 1000 mg/kg/day of Pyroxsulam in the diet for 79 weeks. Actual doses were 0, 10, 100 and 932 mg/kg/day for males. There were no compound-related tumors in the females so only analyses of the males are presented in this document.

ANALYSES

Survival Analyses

There were no statistically significant incremental changes in mortality with increasing doses of Pyroxsulam in male mice (Table 1).

Tumor Analyses

Male mice had a statistically significant trend for liver carcinomas at $p < 0.05$. There were statistically significant pair-wise comparisons of the 10 and 1000 mg/kg/day dose groups with the controls for liver adenomas, and liver adenomas and/or carcinomas combined, all at $p < 0.05$. The statistical analyses of the tumors in the male mice were based upon Fisher's Exact Test for pair-wise comparisons and the Exact Test for trend (Table 2).

24

Table 1. Pyroxsulam – CD-1 (Crl:CD1(ICR)) Mouse Study (MRID 46908406)

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

	Weeks			
Dose (mg/kg/day)	1-26	27-52	53-79[f]	Total
0	0/50	1/50	9/49	10/50 (20)
10	1/50	3/49	6/46	10/50 (20)
100	0/50	2/50	8/48	10/50 (20)
1000	0/50	2/50	10/48	12/50 (24)

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

[f]Final sacrifice at week 79.

( )Percent.

Note: Time intervals were selected for display purposes only.
Significance of trend denoted at control.
Significance of pair-wise comparison with control denoted at dose level.
If *, then $p < 0.05$. If **, then $p < 0.01$.

Table 2. Pyroxsulam – CD-1 (Crl:CD1(ICR)) Mouse Study (MRID 46908406)

Male Liver Tumor Rates[+]
and Fisher's Exact Test and Exact Test for Trend Results

	Dose (mg/kg/day)			
	0	10	100	1000
Adenomas (%)	5/49 (10)	13/46 (28)	9[a]/49 (18)	14/48 (29)
p =	0.06696	0.02300*	0.19363	0.01716*
Carcinomas (%)	1/49 (2)	0/46 (0)	2[b]/49 (4)	4/48 (8)
p =	0.02622*	1.00000	0.50000	0.17451
Combined (%)	6/49 (12)	13/46 (28)	10[c]/49 (20)	15[d]/48 (31)
p =	0.05737	0.04462*	0.20651	0.02067*

+Number of tumor bearing animals/Number of animals examined, excluding those that died before week 52.

[a]First adenoma observed at week 53, dose 100 mg/kg/day.
[b]First carcinoma observed at week 73, dose 100 mg/kg/day.
[c]One animal in the 100 mg/kg/day dose group had both an adenoma and a carcinoma.
[d]Three animals in the 1000 mg/kg/day dose group had both an adenoma and a carcinoma.

Note: Significance of trend denoted at control.
Significance of pair-wise comparison with control denoted at dose level.
If *, then $p < 0.05$. If **, then $p < 0.01$.

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_108702 (Pyroxsulam) 5-23-27 Cancer Assessment Meeting on Pyroxsulam – Various MRID #'s

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Pages __28___ through __33___ are not included in this copy.

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33

EPA Reviewer: Kimberly Harper Signature:
RAB2, Health Effects Division (7509C) Date:
EPA Secondary Reviewer: Alan Levy Signature:
RAB2, Health Effects Division (7509C) Date:

Template version 02/06

TXR#: 0053347

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, X66 742

CITATION: Stebbins, K. E., and K. J. Brooks (02 November 2005). 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 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) in 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 previously reported.

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 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 decrements 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 3 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.

34

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.

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.

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.

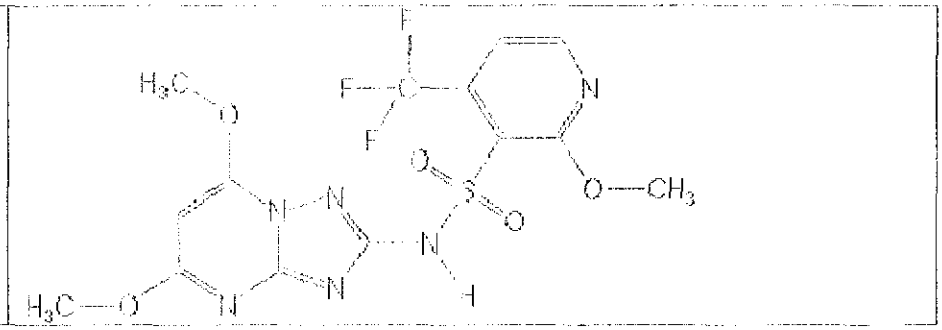
United States Environmental Protection Agency, Health Effects Test Guidelines, OPPTS 870.4300 (Combined Chronic Toxicity/Carcinogenicity) EPA712-C-98-212, August 1998, with the exception that ten oncogenicity animals were evaluated monthly for the first 12 months and quarterly from 12-24 months (rather 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. J. 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; ESN105826
	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

35

	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	
	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

36

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. 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 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.

6.. RESULTS:

38

4. **Observations:**

4a. **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.

4b. **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.

4c. **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).

5. **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.

6. **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:

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

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.

39

$$MI = \frac{(\text{feed consumption}\left(\frac{g}{day}\right) * (1000\,mg/g) * \frac{(\%\text{ of test material in feed})}{100}}{\left(\frac{\text{Current BW [g]} + \text{Previous BW [g]}}{2}\right)} \cdot \frac{1}{1000\,g/kg}$$

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. 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 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 sacrificed on schedule were subjected to 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.

41

	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	Lungs*+	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.

42

5. **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.

43

Table 1. Body Weights of Fischer 344 Rats Given XDE-742 – Selected Intervals

Test Day/Week	Dose Level (mg/kg/day)				
	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	463.3 ± 21.7	457.8 ± 27.4	452.8 ± 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 ± 6.3	150.9 ± 7.1	148.4 ± 6.9*
92/13	188.3 ± 9.9	175.6 - 190.3	188.6 ± 8.9	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)
260/57	219.7 ± 11.2	208.9 - 221.5	218.3 ± 11.3	216.6 ± 10.9	209.7 ± 10.7* (↓5)
365/92	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 ± 23.6	165.6 ± 20.7	151.4 ± 17.6 (↓10)
BWG W1-104	189.8 ± 25.7	NR	188.2 ± 24.1	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 colony 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 24. 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

44.

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 incidences 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.

45

Table [illegible] 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[a]	8.60*	8.62	8.51*
12 months	9.06	8.70 – 8.87[b]	8.87	8.88	8.75
18 months	8.52	8.11 – 8.66[b]	8.73	8.56	8.33
24 months	6.87	6.65 – 7.80[b]	8.48	7.00	7.65
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[a]	7.87	7.85	7.52*
12 months	8.50	7.68 – 8.11[b]	8.48	8.38	8.26
18 months	7.99	7.80 – 8.24[b]	7.75	8.19	7.95
24 months	8.22	7.15 – 7.91[b]	7.92	8.16	7.38
Hematocrit (%)	Males Dose (mg/kg/day)			
	0	Historical Controls	10	100	1000
3 months	40.2	38.6 – 46.3[a]	40.1	40.4	39.9
6 months	43.0	39.7 – 46.2[a]	41.9	42.3	41.9
12 months	46.9	43.0 – 44.5[b]	45.9	46.2	45.7
18 months	42.5	40.2 – 43.1[b]	43.5	42.8	41.9
24 months	41.1	37.7 – 42.6[b]	48.1	40.2	43.1
Hematocrit (%)	Females Dose (mg/kg/day)			
	0	Historical Controls	10	100	1000
3 months	38.7	37.7 – 43.7[a]	38.9	38.4	38.0
6 months	40.7	39.7 – 41.4[a]	41.5	41.2	39.6*
12 months	45.9	41.2 – 43.4[b]	46.0	45.7	44.9
18 months	42.0	39.8 – 42.4[b]	41.3	42.9	41.5
24 months	47.1	39.9 – 42.5[b]	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.

4. **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.

46

TABLE 5. Clinical Chemistry Parameters

Alkaline Phosphatase (U/l)	Males Dose (mg/kg/day)				
	0	Historical Controls	10	100	1000
3 months	54	52 - 73[a]	61	66	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 - 105[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]	181*	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 three studies conducted between 2001 and 2005.

[b] Range from four 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 (5.1%) 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.

47

Table - 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)	2.506	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 concomitant 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	13	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.
*Statistically 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	0	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	2	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.

49

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 ([illegible].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 (6-7%) 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

50

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.

C. STUDY DEFICIENCIES:

None

DER may not be final. 51

EPA Reviewer: Kimberly Harper **Signature:** _____
RAB2, Health Effects Division (7509P) **Date:** _____
EPA Secondary Reviewer: Alan Levy **Signature:** _____
RAB2, Health Effects Division (7509P) **Date:** _____

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. (15 December 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

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. 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/day, with the mean absolute and relative liver weights increased by 26.4% and 31.6%, respectively, increased incidence of liver masses at necropsy, histopathologically increased incidence of foci of altered cells (hepatocytes), and increased incidence and numbers of hepatocellular adenomas and

52

carcinomas, although the tumor incidences were not statistically identified. There was a tendency of affected mice to have both foci of altered cells and multiple tumors (adenomas and/or carcinomas).

Male mice given 10 mg/kg/day had a slightly increased incidence and number of liver adenomas that was not considered dose related because it was not accompanied by increased organ weight or alterations in histopathology as was the high dose group. There were no effects on males or females at 100 mg/kg/day.

The **LOAEL is 1000 mg/kg/day,** based on the increase in mean absolute and relative liver weights, increased incidence of foci of altered cells (hepatocytes), and increased incidence and numbers of hepatocellular adenomas and/or carcinomas. The **NOAEL is 100 mg/kg/day.**

At the doses tested, there was a treatment related increase in tumor incidence in male mice with regards to hepatocellular adenomas and/or carcinomas when 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. However, the incidence of hepatocellular tumors in the high dose males did exceed the historical control range and half the male mice had multiple adenomas, which may indicate a treatment effect. Although there was little overt toxicity in either sex other than the increase in tumor incidence, dosing is considered adequate because the highest dose tested (1000 mg/kg/day) is the limit dose for chronic/carcinogenicity studies.

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.

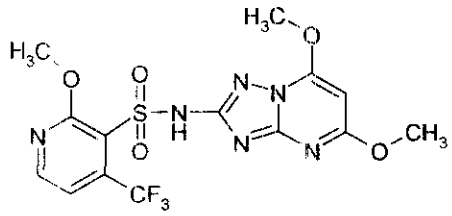
United States Environmental Protection Agency, *Health Effects Test Guidelines*, OPPTS 870.4200 (Carcinogenicity) EPA712-C-98-211, August 1998 with the exception, that the frequency and number of animals having detailed clinical observations were modified. Ten mice/sex/dose level were evaluated monthly 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).

53

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.

54

	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

55

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. 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

56

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

57

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:

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

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 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.

58

	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.

59

	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

60

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
204	-	-	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	-	-
225	0	1	0	0
260	-	-	1	1
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.

61

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

62

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.

   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.

63

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	3	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: Males in the high dose group had greater numbers of mice with foci of altered cells and a greater incidence and multiplicity of hepatocellular tumors – adenomas and/or carcinomas (summary in Table 5).

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. Male mice given 1000 mg/kg/day that had hepatic foci of altered cells tended to have a multiplicity of the effects considered related to treatment, *i.e.*, either more than one subtype of focus of altered cells or a focus along with one or more hepatocelluar adenoma(s) and/or carcinoma(s). However, multiplicity was also found for one control male (#03A1389) which had three basophilic foci and one mixed cell focus of altered cells along with six hepatocellular adenomas. 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.

65

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	1	2	2	0	2	0	1
- clear, one or more	0	0	0	7*	0	0	0	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.

66

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 11 on page 38 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 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. As noted above, one control 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 #03A1487). Multiplicity of hepatocellular tumors is relatively uncommon in control male CD-1 mice in our 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.

67

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.

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.

68

Table 9. 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.

III. DISCUSSION AND CONCLUSIONS:

A. **INVESTIGATORS' CONCLUSIONS**: 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, increased incidence of liver masses at necropsy, histopathologically increased incidence of foci of altered cells (hepatocytes), and increased incidence and numbers of hepatocellular adenomas and carcinomas, although the tumor incidences were not statistically identified. There was a tendency of affected mice to have both foci of altered cells and/or multiple adenomas and/or carcinomas.

The **LOAEL is 1000 mg/kg/day,** based on the increase in mean absolute and relative liver weights, increased incidence of foci of altered cells (hepatocytes), and increased incidence and numbers of hepatocellular adenomas and/or carcinomas. The **NOAEL is 100 mg/kg/day.**

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

DER may not be final.

70

EPA Reviewer: Kimberly Harper **Signature:** _____
RAB2, Health Effects Division (7509P) **Date:** _____
EPA Secondary Reviewer: Alan Levy **Signature:** _____
RAB2, Health Effects Division (7509P) **Date:** _____

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 408.

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. (16 August 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 dose levels of 0, 10, 100, 500, or 1000 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.

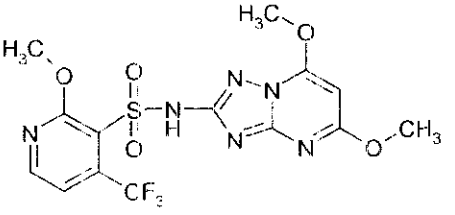
This 28-day oral toxicity study in the rat is acceptable/guideline; it is a range-finding study for the 90-day and 2-year rat studies.

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

71

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)

72

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.	
	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:**	21.8-22.1 °C
		Humidity:	45.6-54.3%
		Air changes:	12-15 times/hour
		Photoperiod:	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 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. 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:

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

75

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.

76

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, re-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.

77

	DIGESTIVE SYSTEM		CARDIOVASC./HEMAT.		NEUROLOGIC
X	Tongue	X	Aorta	XX	Brain+
X	Salivary glands	XX	Heart+	X	Peripheral nerve
X	Esophagus	X	Bone marrow	X	Spinal cord (3 levels)
X	Stomach	X	Lymph nodes	X	Pituitary
X	Duodenum	XX	Spleen*+	X	Eyes (optic nerve)
X	Jejunum	XX	Thymus*+		GLANDULAR
X	Ileum			XX	Adrenal gland+
X	Cecum		UROGENITAL	X	Lacrimal gland
X	Colon	XX	Kidneys+	X	Parathyroid
X	Rectum	X	Urinary bladder	X	Thyroid
XX	Liver+	XX	Testes+		OTHER
X	Gall bladder (not rat)	XX	Epididymides+	X	Bone (sternum and/or femur)
X	Bile duct (rat)	X	Prostate	X	Skeletal muscle
X	Pancreas	X	Seminal vesicles	X	Skin
	RESPIRATORY	X	Ovaries*+	X	All gross lesions and masses
X	Trachea	X	Uterus+	X	Auditory sebaceous glands
X	Lung	X	Mammary gland		
X	Nose	X	Coagulating glands		
X	Pharynx				
X	Larynx				

+ Organ weights required for rodent studies.
X = Tissues examined grossly at necropsy.
XX = Tissues examined grossly 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.

78

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

DER may not be final

80

EPA Reviewer: Kimberly Harper **Signature:** _____
RAB2, Health Effects Division (7509C) **Date:** _____
EPA Secondary Reviewer: Alan Levy **Signature:** _____
RAB2, Health Effects Division (7509C) **Date:** _____

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. MRID46908350. 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 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.**

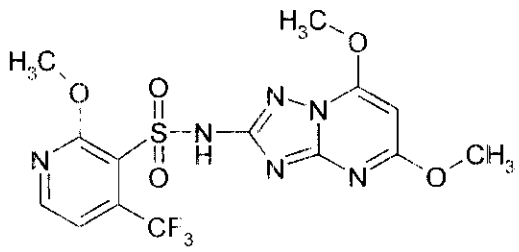
This 90-day oral toxicity study in the rat is acceptable/guideline and satisfies the guideline requirement for a 90-day oral toxicity study (OPPTS 870.3100; OECD 408) in rat.

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.

82

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:**	20.5-22.4 °C
		Humidity:	47.2-60.3%
		Air changes:	12-15 times/hr
		Photoperiod:	12 hrs dark/12 hrs light

83

	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.

84

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:

85

Feed consumption (g/day) = (initial weight of feed container - final weight of feed container) / (# 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:

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

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*

86

	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.

Histopatholology: 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

87

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.

88

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

89

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.

DER may not be final

92

EPA Reviewer: Kimberly Harper **Signature:** _____
RAB2, Health Effects Division (7509P) **Date:** _____
EPA Secondary Reviewer: Alan Levy **Signature:** _____
RAB2, Health Effects Division (7509P) **Date:** _____

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. (16 April 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/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.

93

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

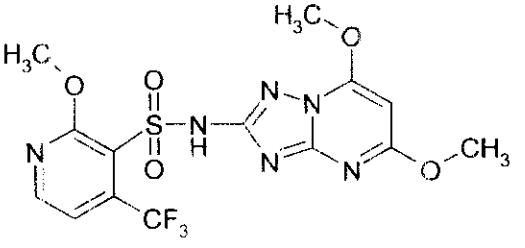
This study is acceptable and satisfies the guideline requirement for a Subchronic Oral Toxicity [feeding] CD-1 Mice; OPPTS 870.3100 (rodent); OECD 408, EEC, Part B.26, JMAFF (Subchronic Oral Toxicity Study).

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.

94

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:**	21.0-22.3 °C
	Humidity:	45.5-56.8%
	Air changes:	12-15/hr
	Photoperiod:	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 / 10.3	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 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.

95

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. 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).

96

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).

97

	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 (0)
		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. 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 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 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 4/8 and 5/10 cholesterol levels in males and females, respectively, 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.

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**	**138**	**139**	**170**
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**	**78**	**88**	**100***
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 increased 18.3% compared to controls while the relative liver weight 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	35.8	36.7	38.3
Historical Controls	37.1 – 42.7			
Liver (g)	1.986	1.902	1.997	2.349* (↑18)
Historical Controls	1.93 – 2.097			
Relative Liver (g/100 g bw)	5.483	5.322	5.439	6.155* (↑12)
Historical Controls	4.756 – 5.627			
	Females			
Body Weight (g)	27.9	27.9	28.3	28.7
Historical Controls	29.6 – 32.3			
Liver (g)	1.400	1.434	1.446	1.508 (↑8)
Historical Controls	1.447 – 1.578			
Relative Liver (g/100 g bw)	5.007	5.127	5.106	5.257 (↑5)
Historical Controls	4.736 – 4.892			

Data comes from Text Table 3 and text on page 27 of the study report.
*indicates statistical significance at $p<0.05$

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:

None

DER may not be final

103

EPA Reviewer: Paul Chin **Signature:** _____
Reregistration Branch 1, Health Effects Division (7509C) **Date:** _____
EPA Secondary Reviewer: Kimberly Harper **Signature:** _____
Registration Action Branch 2, Health Effects Division (7509C) **Date:** _____

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.

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.

105

This metabolism study is classified **acceptable/guideline** and satisfies the guideline requirements for a metabolism study (OPPTS 870.7485 and OECD 417) in rats.

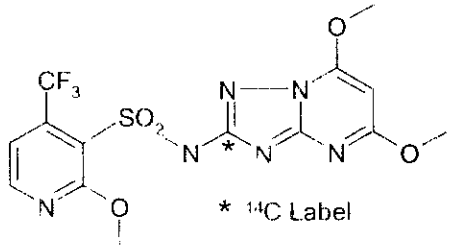
COMPLIANCE: Signed and dated GLP, Quality Assurance, and Data Confidentiality statements were provided.

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

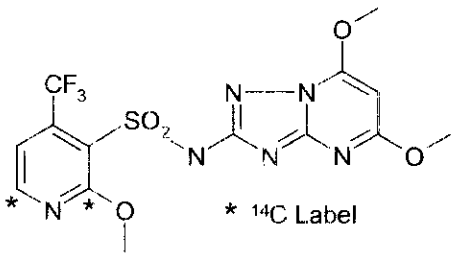


XDE-742-Het-2-^{14}C (triazole)

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

106



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

107

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 200 – 260g	
	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 was 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:**	19-24°C
		Humidity:	45-68%
		Air changes:	12-15/hour
		Photoperiod:	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	

108

		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.

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-labeled 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 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.

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 animals (not quantifiable to 0.01% of the administered dose), 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

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)		

110

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 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 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:
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 ($t_{½α}$; 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 ($t_{½β}$; 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 ($t_{½α}$; 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 ($t_{½β}$; 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:**

112

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.

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 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 radioavtive 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.

14

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

	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 (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.

117

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 E	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.

118

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 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 46908609) 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.

DER may not be final.

120

EPA Reviewer: Paul Chin **Signature:** _____
Reregistration Branch 1, Health Effects Division (7509C) **Date:** _____
EPA Secondary Reviewer: Kimberly Harper **Signature:** _____
Registration Action Branch 2, Health Effects Division (7509C) **Date:** _____

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.

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

122

This metabolism study is classified **acceptable/guideline** and satisfies the guideline requirements for a metabolism study (OPPTS 870.7485 and OECD 417) in rats.

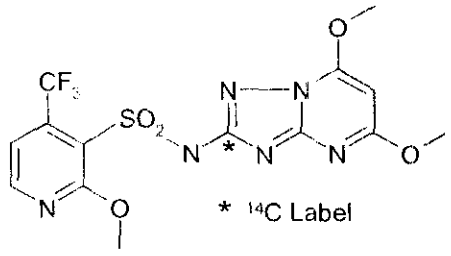
COMPLIANCE: Signed and dated GLP, Quality Assurance, and Data Confidentiality statements were provided.

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

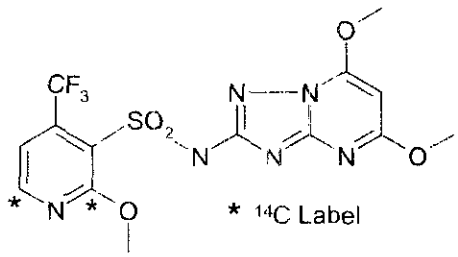


XDE-742-Het-2-^{14}C (triazole)

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

123



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

124

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 200 – 260g	
	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 was 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:**	19-24°C
		Humidity:	45-68%
		Air changes:	12-15/hour
		Photoperiod:	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	

125

		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.

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-labeled 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 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.

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 animals (not quantifiable to 0.01% of the administered dose), 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.

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 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 is calculated from actual values and calculated QL values and presented as mean standard deviation ($X \pm SD$), 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:
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 ($t_{½α}$; 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 ($t_{½β}$; 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 ($t_{½α}$; 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 ($t_{½β}$; 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 from 1-1.3 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.

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 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.

130

TABLE 4. Distribution of radioactivity in rat tissues/organs after administration of ^{14}C-XDE-742 (expressed as ppm equivalent of radioavtive dose administered).

	Group 1[ab]	Group 2[ab]	Group 3[ab]	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[j]	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.

131

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).

132

TABLE 5. Recovery of radioactivity in tissues and excreta of rats after administration of ^{14}C-XDE-742

	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)[f]	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.

133

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 (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.

134

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 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 46908609) 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.

CANCER BRIEFING PACKAGE

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