DATA EVALUATION RECORD - SUPPLEMENT

XDE-570 (FLORASULAM)

Study Type: OPPTS 870.5395 [§84-2]; Micronucleus Assay in Mice

Work Assignment No. 4-01-128 Q (MRID 46808239)

Prepared for
Health Effects Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
2777 South Crystal Drive
Arlington, VA 22202

Prepared by
Pesticides Health Effects Group
Sciences Division
Dynamac Corporation
1910 Sedwick Road, Bldg 100, Ste B.
Durham, NC 27713

Primary Reviewer
David A. McEwen, B.S.

Signature: David A McEwen
Date: 12/19/06

Secondary Reviewer
Stephanie E. Foster, M.S.

Signature: Stephanie E. Foster
Date: 12/19/06

Program Manager:
Michael E. Viana, Ph.D., D.A.B.T.

Signature: Michael E Viana
Date: 12/19/06

Quality Assurance:
Mary L. Menetrez, Ph.D.

Signature: Mary L Menetrez
Date: 12/20/06

Disclaimer

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

EPA Reviewer: Karlyn J. Bailey, M.S. **Signature:** [signature] 5/31/07
Registration Action Branch 2, Health Effects Division (7509P) **Date:**
EPA Secondary Reviewer: Myron Ottley, Ph.D. **Signature:** [signature] 5/31/07
Registration Action Branch 3, Health Effects Division (7509P) **Date:**
Template version 02/06

DATA EVALUATION RECORD

STUDY TYPE: *In Vivo* Mammalian Cytogenetics - Erythrocyte Micronucleus Assay in Mice; OPPTS 870.5395 [' 84-2]; OECD 474.

PC CODE: 129108 **DP BARCODE:** D331116
TXR#: 0054348

TEST MATERIAL (PURITY): XDE-570 (Florasulam; 99.2% a.i.; Lot # 930910)

SYNONYMS: XR-570, XRD-570, DE-570, N-(2,6-diflurophenyl)-8-fluoro-5-methoxy(1,2,4)triazolo(1,5-*c*)pyrimidine-2-sulfonamide

CITATION: Lick, S.J., B.B. Gollapudi, and B.E. Kropscott (1995) Evaluation of XDE-570 in the mouse bone marrow micronucleus assay. Health and Environmental Sciences, The Toxicology Research Laboratory, Midland, MI. Laboratory Project Study ID: DR-0312-6565-013, March 10, 1995. MRID 46808239. Unpublished.

SPONSOR: Dow AgroSciences Canada, Inc., 2100- 450 1 St. SW, Calgary, AB, Canada

EXECUTIVE SUMMARY - In a bone marrow micronucleus assay (MRID 46808239), young adult CD-1 mice (5/sex/dose/harvest time) were treated once via gavage (20 mL/kg) with XDE-570 (Florasulam; 99.2% a.i.; Lot # 930910) in corn oil at doses of 0, 1250, 2500, or 5000 mg/kg. Bone marrow cells were harvested at 24, 48, and 72 hours after dosing. Cyclophosphamide (120 mg/kg) served as the positive control.

No treatment-related clinical signs of toxicity were observed during the study. At 5000 mg/kg, two females died on Day 2; however, the cause of death and association with the test substance was not established. Both the MPCE frequency and the PCE:NCE ratio were comparable between vehicle controls and all treated groups at all sampling times in both sexes. Although there were no clinical signs and no apparent effect on marrow toxicity, dosing was considered to be adequate as XDE-570 was tested up to more than twice the limit dose of 2000 mg/kg. The positive control induced the appropriate response. **There was no significant increase in the frequency of micronucleated polychromatic erythrocytes in bone marrow after any treatment time.**

This study is classified as **acceptable/guideline** and satisfies the guideline requirement for Test Guideline OPPTS 870.5395; OECD 474 for *in vivo* cytogenetic mutagenicity data.

COMPLIANCE - Signed and dated Data Confidentiality, GLP Compliance, and Quality

Assurance statements were provided.

NOTE: This DER summarizes EPA conclusions regarding effects observed in the mouse micronucleus assay. A detailed DER completed by the Canadian Pest Management Regulatory Agency (PMRA) is attached.

COMMENTS: EPA concurs with the PMRA toxicology evaluation, no conclusions have been changed.

46808239.PMRA.der
.pdf

Reviewer: Tom Morris , **Date** April 20, 2000.

STUDY TYPE: *In vivo* mammalian cytogenetics - micronucleus assay in (mice); OPPTS 870.5395; OECD 474.

TEST MATERIAL (PURITY): XDE-570 (Purity - 99.2%)

SYNONYMS: XR-570, XRD-570, DE-570, florasulam.

CITATION: Lick, S. J., Gollapudi, B. B. and Kropscott, B. E. March 10, 1995. **Evaluation of XDE-570 in the Mouse Bone Marrow Micronucleus Test.** Performing Laboratory: Health and Environmental Sciences, The Toxicology Research Laboratory, 1803 Bldg., Midland, MI, 48674. Laboratory Project Study ID: DR-0312-6565-013. Unpublished

SPONSOR: Dow AgroSciences Canada Inc. (DAS).

EXECUTIVE SUMMARY: In a CD-1 (ICR) BR mouse bone marrow micronucleus assay, 5 animals/sex/dose/ sampling time received a single dose of 0, 1,250, 2,500 or 5,000 mg/kg bw of XDE-570 (Purity - 99.2%) in cornoil via oral gavage. Bone marrow cells were harvested at 24, 48 and 72 hours post-treatment. The vehicle/negative control substance was corn oil (20 mL/kg bw). The positive control substance was cyclophosphamide (120 mg/kg bw).

There were no clinical signs of toxicity during the study. Two females at 5,000 mg/kg bw died spontaneously on day 2, however, the cause of death and association with the test substance was not established. The frequencies of micronucleated polychromatic erythrocytes (MN-PCE) and the ratio of polychromatic erythrocytes to normochromic erythrocytes (%PCE) were comparable between the negative control group and the treated groups at all sacrifice times for both sexes. There was no treatment-related effect on cell division and no evidence of induced chromosomal or other damage leading to micronucleus formation. XDE-570 was tested at an adequate dose since the high dose of 5,000 mg/kg bw exceeded the limit dose of 2,000 mg/kg bw as indicated in OPPTS 870.5395. The positive control induced the appropriate response. **There was not a significant increase in the frequency of micronucleated polychromatic erythrocytes in bone marrow after any treatment time.**

This study is classified as acceptable / guideline. This study satisfies the requirement for Test Guideline OPPTS 870.5395; OECD 474 for *in vivo* cytogenetic mutagenicity data.

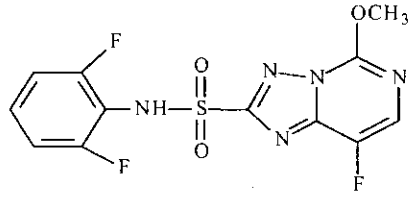
COMPLIANCE: Signed and dated GLP, Quality Assurance, and Data Confidentiality statements were provided.

I. MATERIALS AND METHODS

A. MATERIALS:

1. **Test Material:** XDE-570 as named in the study. Chemical Name (CA nomenclature): N-(2,6-diflurophenyl)-8-fluoro-5-methoxy(1,2,4)triazolo(1,5-c)pyrimidine-2-sulfonamide

Description: White powder, stored at room temperature
Lot/Batch #: Batch # TSN100298 / Lot # 930910
Purity: 99.2 % a.i (determined by HPLC).
CAS #: 145701-23-1
Structure:



Solvent Used: The test substance was mixed with corn oil and homogenized for approximately 5 minutes. The positive control, cyclophosphamide, was mixed with distilled water for dosing.

2. **Control Materials:**

Negative control :	Corn oil	**Final Volume:** 20 mL/kg bw	**Route:** oral gavage
Vehicle:	Corn oil	**Final Volume:** 20 mL/kg bw	**Route:** oral gavage
Positive control :	Cyclophosphamide	**Final Dose(s):** 120 mg/kg bw (administered in aliquots of 10 mL/kg bw)	**Route:** oral gavage

Freshly prepared solutions were used for dosing the animals. The concentration of the test substance in the dosing solutions were verified by high pressure liquid chromatography (HPLC). Homogeneity analysis was also performed on selected dosing suspensions.

3. **Test animals:**

Species:	Male and female mice
Strain:	CD-1 (ICR) BR
Age/weight at study initiation:	At study initiation the animals were ≈9 weeks of age with a body weight range of 31.8 to 38.2 g for males and 22.7 to 28.8 g for females.
Source:	Charles River Laboratories Inc., Wilmington, MA.
No. animals used per dose	5 animals/sex/dose/sampling time (sacrificed at 24, 48 and 72 hrs post-treatment).
Properly Maintained?	Yes

4. **Test compound administration:**

	Dose Levels	Final Volume	Route
Preliminary:	5,000 mg/kg bw (limit dose)	20 mL/kg bw	single, oral gavage
Main Study:	0, 1,250, 2,500 or 5,000 mg/kg bw	20 mL/kg bw	single, oral gavage

B. TEST PERFORMANCE

1. Treatment and Sampling Times:

a. Test compound:

Dosing:	X	once		twice (24 hrs apart)				Other		
Sampling (after last dose):		6 hr		12 hr	X	24 hr	X	48 hr	X	72 hr
Other:										

b. Negative and/or vehicle control:

Dosing:	X	once		twice (24 hrs apart)				Other		
Sampling (after last dose):		6 hr		12 hr	X	24 hr	X	48 hr	X	72 hr
Other:										

c. Positive control:

Dosing:	X	once		twice (24 hrs apart)				Other		
Sampling (after last dose):		6 hr		12 hr	X	24 hr		48 hr		72 hr
Other:										

2. Tissues and Cells Examined:

Bone marrow :	both femurs
No. of polychromatic erythrocytes (PCE) examined per animal:	1,000
No. of normochromatic erythrocytes (NCE; more mature RBCs) examined per animal:	1,000
Other:	

3. Details of slide preparation: At the end of the specified intervals following dosing, the animals were sacrificed by cervical dislocation. Bone marrow samples were obtained in the following way. The distal end of the femur was severed to expose the marrow cavity. A 25-gauge needle was used to aspirate the bone marrow into a 3 mL disposable plastic syringe containing 0.5 mL of fetal bovine serum. After aspiration, the contents of the syringe were transferred into a 1.5 mL centrifuge tube containing 0.5 mL of serum and centrifuged at 1000 rpm for approximately 5 minutes. The supernatant was discarded leaving a small amount of serum covering the pellet after which the pellet was resuspended. Wedge smears were prepared on microscope slides using small portions of the cell suspension. The slides were allowed to air dry and stained with Wright-Giemsa using a Hematek automated slide stainer. The slides were coded and scored blind by a single investigator.

4. Evaluation Criteria: One thousand polychromatic erythrocytes (PCE) were examined from each animal and the number of micronucleated polychromatic erythrocytes (MN-PCE) were recorded. Micronuclei were identified as darkly stained bodies with smooth contours and varying shapes such as round, almond, or ring. The ratio of polychromatic erythrocytes;normochromatic erythrocytes (PCE-NCE) in the bone marrow was determined by examining 1,000 erythrocytes. The ratio was expressed as PCE x 100/PCE + NCE. Criteria for determining a positive response was if the mean number of MN-PCE was statistically different from controls as determined by Dunnett's t-test ($p \leq 0.01$). The final interpretation of biological significance of the responses was based on both statistical outcome and scientific judgement.

5. Statistical methods: The raw data on the counts of MN-PCE for each animal were first transformed by adding 1 to each count and then taking the natural log of the adjusted number. The transformed MN-PCE data and the data on the percent PCE were analysed by 3-way ANOVA (sex, dose and time), assuming the 3-way interaction to be zero. From this initial analysis, the 2-way interactions were reviewed for significance. Depending on this review, the data were analysed by either 1-, 2- or 3-way ANOVA looking only at main effects. Pair-wise comparison of treated vs negative control groups were done , if necessary, by Dunnett's t-test, 1-sided (upper) for MN-PCE and 2-sided for percent PCE. The alpha level at which all the tests were conducted was 0.01.

II. REPORTED RESULTS The observed concentration for the preliminary toxicity assay was 128% of the targeted value (Table 1). The observed concentrations for the micronucleus assay ranged from 108% to 115% of the

targeted values (Table 1). The observed concentrations of the 1250 and 5000 mg/kg bw dose levels were the average of the homogeneity results. Although the dose solutions in the micronucleus test appeared to be somewhat non-homogenous, the average dose administered at these dose levels was within 8-9% of the target.

TABLE 1: Concentration and homogeneity check. (a)

Test	Dose (mg/kg bw)	Target Concentration (mg/mL)	Observed Concentration (b) (mg/mL)	% of Target Concentration	Homogeneity Results (c) (mg/mL)
Range-Finding	5000	250	319	128	324 (top) 322 (middle) 312 (bottom)
Micronucleus	5000	250	273	109	16.7 (top) 309.0 (middle) 344.0 (bottom)
	2500	125	144	115	
	1250	62.5	67.3	108	55.3 (top) 68.8 (middle) 77.9 (bottom)

(a) Data obtained from pages 16 and 17 of the study report.
(b) Observed concentrations are the means of the homogeneity results.
(c) Three samples were taken from each dose level at approximately the top, middle and bottom of the suspension.

A. Preliminary toxicity assay: The survival of the treated mice was monitored for 4 days. There were no positive signs of toxicity or mortalities observed during the range-finding assay. Based upon these results, the limit dose of 5,000 mg/kg bw was selected to be the highest dose for the micronucleus assay.

B. Micronucleus assay Two females at 5,000 mg/kg bw died spontaneously on study day 2. One appeared normal the other exhibited decreased activity prior to death. The cause of death and association with the test substance was not established. Both females had small amounts of ingesta in the pharynx, however it was not typical of choke syndrome. One of the females had slight visceral congestion that was considered a terminal event. Additional observations included roughened coat (1 female at 2,500 mg/kg bw) and perineal soiling (1 female at 5,000 mg/kg bw and 1 male at 2,500 mg/kg bw). Body weight was unaffected by treatment.

The frequencies of micronucleated polychromatic erythrocytes (MN-PCE) and the ratio of polychromatic erythrocytes to normochromic erythrocytes (%PCE) were comparable between the negative control group and the treated groups at all sacrifice times for both sexes (Table 2). There was no treatment-related effect on cell division and no evidence of induced chromosomal or other damage leading to micronucleus formation. Under identical test conditions, cyclophosphamide (120 mg/kg bw) produced a significant increase in the frequency of micronucleated polychromic erythrocytes (MN-PCE) and a significant decrease in percent polychromatic erythrocytes (%PCE). The positive response of the positive control substance adequately demonstrated the sensitivity of the assay.

TABLE 2. Summary of frequencies of micronucleated polychromatic erythrocytes (MN-PCE) and % polychromatic erythrocytes (%PCE) in mice. (a)

Dose Level (mg/kg bw)	# of Animals	Sacrifice (hrs)	Frequency of MN-PCE		% PCE	
			Males	Females	Males	Females
Negative Control	5	24	0.2 ± 0.4	0.4 ± 0.5	63.8 ± 2.6	69.0 ± 1.3
	5	48	0.8 ± 1.1	1.6 ± 0.9	63.9 ± 7.8	66.1 ± 6.3
	5	72	0.2 ± 0.4	1.0 ± 0.7	62.9 ± 3.3	68.3 ± 3.6
1250	5	24	0.4 ± 0.5	1.0 ± 1.2	64.3 ± 3.4	70.6 ± 5.2
	5	48	1.2 ± 0.8	0.6 ± 0.5	64.2 ± 2.8	67.9 ± 1.9
	5	72	1.0 ± 0.7	0.0 ± 0.0	62.9 ± 3.9	68.6 ± 3.6
2500	5	24	0.8 ± 0.8	1.4 ± 1.5	68.4 ± 3.4	65.7 ± 7.3
	5	48	0.6 ± 0.9	0.2 ± 0.4	67.9 ± 2.0	64.5 ± 7.5
	5	72	0.6 ± 0.9	0.8 ± 0.8	66.6 ± 5/1	69.0 ± 5.8
5000	5	24	0.6 ± 0.9	0.5 ± 0.6	65.1 ± 5.0	67.0 ± 2.6
	5	48	0.2 ± 0.4	0.8 ± 1.3	65.9 ± 5.2	59.5 ± 9.1
	5	72	0.8 ± 1.3	1.0 ± 0.0	65.2 ± 5.7	69.5 ± 4.4
Positive Control	5	24	53.4 ± 8.3 *	45.4 ± 9.9 *	53.7 ± 3.7 *	57.3 ± 6.0 *

(a) Data obtained from pages 18 and 19 of the study report.
Positive control - 120 mg/kg bw cyclophosphamide
Negative control - 20 mL/kg bw cornoil

$$\%PCE = \frac{PCE}{PCE + NCE} \times 100$$

* Statistically significant from negative control value, $p \leq 0.01$.

III. REVIEWER'S DISCUSSION/CONCLUSIONS:

A. This study is classified as acceptable and satisfies the requirement for Test Guideline OPPTS 870.5395; OECD 474 for *in vivo* cytogenetic mutagenicity data. The positive response of the positive control substance, cyclophosphamide, adequately demonstrates the sensitivity of the assay. The frequencies of micronucleated polychromatic erythrocytes (MN-PCE) and the ratio of polychromatic erythrocytes to normochromic erythrocytes (%PCE) were comparable between the negative control group and the treated groups at all sacrifice times for both sexes. There was no treatment-related effect on cell division and no evidence of induced chromosomal or other damage leading to micronucleus formation. XDE-570 (Purity - 99.2%) was tested at an adequate dose since the high dose of 5,000 mg/kg bw exceeded the limit dose of 2,000 mg/kg bw as indicated in OPPTS 870.5395. Under the conditions of this study, there was not a significant increase in the frequency of micronucleated polychromatic erythrocytes in bone marrow after any treatment time.

B. **STUDY DEFICIENCIES** - There were no deficiencies that would impact on the outcome of the study.