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DATA EVALUATION RECORD - SUPPLEMENT

XDE-570 (FLORASULAM)

Study Type: OPPTS 870.5375 [§84-2]; *In Vitro* Chromosomal Aberration Assay in Rat Lymphocytes

Work Assignment No. 4-01-128 O (MRID 46808237)

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

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

DATA EVALUATION RECORD

STUDY TYPE: *In vitro* Mammalian Cytogenetics (Chromosomal Aberration Assay in Rat Lymphocytes) OPPTS 870.5375 [§84-2]; OECD 473.

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: Linscombe, V.A., D.W. Okowitt, and B.E. Kropscott (1995) Evaluation of XDE-570 in an *In Vitro* chromosome aberration assay utilizing rat lymphocytes. Health and Environmental Sciences, The Toxicology Research Laboratory, Midland, MI. Laboratory Project Study ID: DR-0312-6565-007, January 23, 1995. MRID 46808237. Unpublished.

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

EXECUTIVE SUMMARY - In two independent trials of a mammalian cell cytogenetics assay (chromosome aberration; MRID 46808237), primary rat lymphocyte cultures were exposed to XDE-570 (Florasulam; 99.2% a.i.; Lot # 930910) in dimethylsulfoxide (DMSO) for 4 hours in the presence of S9 and 24 hours in the absence of S9 at concentrations of 0, 3, 10, 30, 100, 300, 1000, or 3000 μg/mL (Trial 1, +/-S9); 0, 30, 100, or 300 μg/mL (Trial 2, –S9); and 0, 300, 1000, or 3000 μg/mL (Trial 2, +S9). Cells were harvested at 24 hours after initiation of treatment in Trial 1 and at 24 and 48 hours after initiation of treatment in Trial 2. The S9 fraction was derived from the livers of male Sprague-Dawley rats induced with Aroclor 1254. The positive controls were mitomycin C (-S9) and cyclophosphamide (+S9).

It was stated that XDE-570 was tested up to the limit of solubility (3000 μg/mL). Based on the observed cytotoxicity (as indicated by reduced mitotic index), cultures at concentrations of 30, 100, and 300 μg/mL (-S9, both trials, 24 hours); 300, 1000, and 3000 μg/mL (+S9, both trials, 24 hours); 300 μg/mL (-S9, Trial 2; 48 hours); and 3000 μg/mL (+S9, Trial 2, 48 hours) were selected for evaluation of chromosomal aberrations. No relevant increases in the number of metaphases with aberrations (excluding gaps) were observed at any concentration at the 24 or 48 hour harvest time in either the presence or absence of S9. The positive controls induced the appropriate response in the presence and absence of S9. **There was no evidence of chromosome aberrations induced over background in the presence or absence of S9-activation.**

This study is classified as **acceptable/guideline** and satisfies the guideline requirement for Test Guideline OPPTS 870.5375; OECD 473 for *in vitro* mutagenicity (chromosome aberration) 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 *In vitro* Mammalian Cytogenetics 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.

46808237.PMRA.Der
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Reviewer: Tom Morris , Date April 18, 2000.

STUDY TYPE: *In vitro* Chromosomal Aberration Assay (rat lymphocytes); OPPTS 870.5375; OECD 473.

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

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

CITATION: Linscombe, V. A., Okowitt, D. W. and Kropscott, B. E. January 23, 1995. **Evaluation of XDE-570 in an *In Vitro* Chromosomal Aberration Assay Utilizing Rat Lymphocytes.** Performing Laboratory: Health and Environmental Sciences, The Toxicology Research Laboratory, 1803 Bldg., Midland, MI, 48674. Laboratory Project Study ID: DR-0312-6565-007. Unpublished

SPONSOR: Dow AgroSciences Canada Inc. (DAS).

EXECUTIVE SUMMARY: In a mammalian cell cytogenetics assay [Chromosomal aberration], primary rat lymphocyte cultures were exposed to XDE-570 (Purity - 99.2%) in dimethylsulfoxide (DMSO at a final concentration of 1% v/v) at concentrations of 0, 3, 10, 30, 100, 300, 1,000 or 3,000 µg/mL in both the presence and absence of S9 metabolic activation (S9 fraction derived from Aroclor 1254-induced rat livers). The positive controls were mitomycin C (without S9 metabolic activation) and cyclophosphamide (with S9 metabolic activation).

The highest concentration tested, 3,000 µg/mL, represented the highest concentration of the test substance still soluble in the treatment medium. In the presence of S9 metabolic activation no cytotoxicity was observed. In the absence of S9 metabolic activation cytotoxicity was observed at 1,000 and 3,000 µg/mL and possibly at 300 µg/mL. When compared to controls, there were no biologically relevant or statistically significant increases in the number of metaphases with aberrations at any concentration level at the 24 or 48 hour harvest time in either the presence or absence of S9 metabolic activation. Positive controls induced the appropriate response. **Under the conditions of this study, there was no evidence of chromosomal aberrations induced over background.**

This study is classified as acceptable / guideline. This study satisfies the requirement for Test Guideline: *In vitro* mammalian cytogenetics [chromosomal aberration] OPPTS 870.5375; OECD 473 for *in vitro* cytogenetic mutagenicity data.

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

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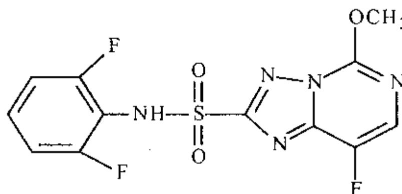
I. MATERIALS AND METHODS

A. MATERIALS:

1. **Test Material:** XDE-570 as named in the study. Chemical Name (CA nomenclature): N-(2,6-difluorophenyl)-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: Dimethylsulfoxide (DMSO)

2. **Control Materials:**

Negative control: DMSO was used as the negative control treatment for both activation/non-activation.
Solvent control (final conc'n): DMSO at a final concentration of 1% (v/v) for both activation/non-activation.
Positive control: Nonactivation: Mitomycin C (MMC, CAS # 50-07-7, Sigma) at a final concentration of 0.05 and 0.075 µg/mL.
Activation: Cyclophosphamide (CP, CAS # 6055-52-3, Sigma) at a final concentration of 0.6 µg/mL.

3. **Activation**: S9 derived from

X	induced	X	Aroclor 1254	X	Rat	X	Liver
	non-induced		Phenobarbital		Mouse		Lung
			None		Hamster		Other
			Other		Other		

S9 liver homogenates prepared from Aroclor-1254 treated (500 mg/kg bw) male Sprague-Dawley rats were purchased from SITEK Research Laboratories, Rockville, MD., and stored at approximately -100 °C or below. Thawed S9 was reconstituted at a final concentration of 10% (v/v) in a mix consisting of 10 mM $MgCl_2$-6 H_2O, 5 mM glucose-6-phosphate, 4 mM nicotinamide adenine dinucleotide phosphate (NADP), 10 mM $CaCl_2$, 30 mM KCl and 50 mM sodium phosphate (pH 8.0). The reconstituted S9 was added to the culture medium to obtain the desired final concentration of 2% (v/v) S9.

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4. **Test cells:** Blood samples were collected from male Sprague-Dawley rats (outbred Crl:CD BR strain, purchased from Charles River, Kingston, NY), aged approximately 13 and 15 weeks. The samples were collected via cardiac puncture, following euthanasia with CO_2, from the animals and pooled.

	V79 cells (Chinese hamster lung fibroblasts)
X	Rat lymphocytes
	Chinese hamster ovary (CHO) cells

Media: Whole blood cultures were set up in RPMI 1640 medium (with 25 mM HEPES) supplemented with 10% heat-inactivated fetal bovine serum, antibiotics and antimycotics (Fungizone 0.25 µg/mL; Penicillin G, 100 U/mL and streptomycin sulfate, 0.1 mg/mL), 20 µg/mL PHA and an additional 2mM L-glutamine.

Properly maintained?	X	Yes	No
Periodically checked for *Mycoplasma* contamination?	X	Yes	No
Periodically checked for karyotype stability?	X	Yes	No

5. **Test compound concentrations used:**

Nonactivated conditions: 0, 3, 10, 30, 100, 300, 1,000 or 3,000 µg/mL.

Activated conditions: 0, 3, 30, 100, 300, 1,000 or 3,000 µg/mL.

The highest concentration tested, 3,000 µg/mL, represented the highest concentration of the test substance still soluble in the treatment medium (RPMI 1640 without serum and the PHA).

B. TEST PERFORMANCE

1. Preliminary Cytotoxicity Assay No preliminary assay performed.

2. Cytogenetic Assay: Cultures were initiated by inoculating approximately 0.5 mL of whole blood/5 mL of culture medium. Cultures were set up in duplicate at each dose level in sterile plastic tissue culture flasks and incubated at 37 °C. Approximately 48 hours after initiation of the cultures, the solvent, positive control chemical and the test substance were added directly to the culture flasks.

a. **Cell exposure time:**	Test Material	Solvent Control	Positive Control
Non-activated:	24 h	24 h	24 h
Activated:	4 h	4 h	4 h

b. **Spindle inhibition**

Inhibition used/concentration: Colcemid at a final concentration of 0.2 µg/mL.

Administration time: at approximately 3 hours prior to cell harvest

c. **Cell harvest time after initiation of treatment:**	Test Material	Solvent Control	Positive Control
Non-activated: Activated:	at 24 h for assay 1 and at 24 and 48 hours for assay 2 for both non-activated and activated.	at 24 h for assay 1 and at 24 and 48 hours for assay 2 for both non-activated and activated.	at 24 h only for both non-activated and activated.

d. Details of slide preparation: The cells were swollen by hypotonic treatment (0.075 M KCl), fixed with methanolic:acetic acid (3:1), dropped on microscope slides and stained in Giemsa.

e. Metaphase analysis

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<u>Number of cells examined per dose</u>: Mitotic Index was determined on at least 1,000 cells/concentration/culture. Structural and numerical aberrations were determined on at least 2 x 100 cells/concentration/culture (2 x 25 cells/culture for positive controls). Only those metaphases that contained 42 centromeres were scored with exception of cells with multiple aberrations, in which case accurate counts of the chromosomes were not possible. Those cells with 5 or more aberrations/cell were classified as cells with multiple aberrations. Gaps were not included in calculations of total cytogenetic aberrations.

Scored for structural?	**X**	Yes - Chromatic gaps, chromosome gaps, chromatid breaks, chromatid exchanges, chromosome breaks and chromosome exchanges.	No
Scored for numerical?	**X**	Yes	No
Coded prior to analysis?	**X**	Yes	No

f. Evaluation criteria: For a test to be acceptable, the chromosomal aberration frequency in the positive control cultures should be significantly higher than the negative controls. The aberration frequency in the negative control should be within reasonable limits of the historical control values for the performing laboratory. A test substance is considered positive in this assay if it induces a significant dose-related and reproducible increase in the frequency of cells with aberrations.

g. Statistical analysis: The frequencies of cells with aberrations (excluding gaps) were compared by the following statistical methods. At each dose level, data from the replicates were pooled. A 2-way contingency table was constructed to analyse the frequencies of cytogenetic abnormalities. An overall Chi-square statistic based on the table, was partitioned into components of interest. Specifically, statistics were generated to test two global hypothesis of (1) no difference in average number of cells with aberrations among the dose groups, and (2) no linear trend of increasing number of cells with aberrations with increasing dose. An ordinal metric (0, 1, 2, etc.) was used for doses in the statistical evaluation. If either statistic was found to be significant at $\alpha = 0.01$ vs a one-sided increasing alternative, pair-wise test (i.e., control vs treatment) was performed at each dose level and evaluated at $\alpha = 0.01$ again vs a one-sided alternative.

II. REPORTED RESULTS The analytically detected concentrations of the test substance in the stock solution varied from 93 to 108% in assay 1 and 95 to 101% in assay 2.

A. <u>Preliminary cytotoxicity assay</u>: No preliminary assay performed.

B. <u>Cytogenetic assay</u>: In assay 1 cultures were harvested at 24 hours only. In the absence of S9 metabolic activation, cultures treated with 1,000 and 3,000 µg/mL could not be scored due to excessive cytotoxicity of the test substance (0.4 and 0% mitotic index, respectively). Cultures treated at 300 µg/mL exhibited a reduction in mitotic index and a reduction in the number of cells on the slides suggesting increased cytotoxicity. The remaining cultures were unaffected by treatment. In the presence of S9 activation, the test substance was not toxic to the cultures and the mitotic index was higher in the treated cultures at all dose levels compared to the negative control.

In assay 2 cultures were harvested at 24 and 48 hours. In the absence of S9 metabolic activation, the highest concentration tested (300 µg/mL) showed a slight reduction in mitotic index (↓ approximately 25%) in cultures harvested at 24 hours. At the 48 hour harvest, only 1 replicate was available for scoring at 300 µg/mL. In the presence of S9 metabolic activation, mitotic index was decreased compared to controls at 1,000 and 3,000 µg/mL at 24 hours and at all dose levels at 48 hours, however, there was no clear dose-response relationship. Mitotic index data are summarized in Table 1.

TABLE 1. Mitotic Index. (a)

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Dose Level (μg/mL)	Cells Harvested 24 hrs After Treatment				Cells Harvested 48 hrs After Treatment			
	(-) S9		(+) S9		(-) S9		(+) S9	
	Assay 1	Assay 2	Assay 1	Assay 2	Assay 1	Assay 2	Assay 1	Assay 2
Negative control	8.6	6.1	2.3	6.1	-	6.5	-	6.6
3	6.5	-	10.9	-	-	-	-	-
10	8.4	-	9.8	-	-	-	-	-
30	6.8	5.8	6.7	-	-	4.8	-	-
100	12.0	5.1	4.3	-	-	5.8 (b)	-	-
300	6.0	4.6	7.5	7.3	-	4.5 (b)	-	5.0
1,000	0.4	-	6.3	5.3	-	-	-	6.1
3,000	0.0	-	3.5	5.6	-	-	-	4.0
0.05 μg/mL MMC	3.4	3.0	-	-	-	-	-	-
0.075 μg/mL MMC	2.1	3.4	-	-	-	-	-	-
6 μg/mL CP	-	-	1.8	1.5	-	-	-	-

(a) Data obtained from pages 21 to 30 of the study report.
(b) Data from only 1 culture due to second culture being lost due to technical error.
MMC - Mitomycin C. CP - 6 μg/mL cyclophosphamide Negative control 1% DMSO

$$\textbf{Mitotic Index} = \frac{\text{number of metaphases}}{\text{number of lymphocytes}} \times 100$$

When compared to controls, there were no biologically relevant or statistically significant increases in the number of metaphases with aberrations at any concentration level at the 24 or 48 hour harvest time in either the presence or absence of S9 metabolic activation in either assay 1 or assay 2. In contrast, the positive control chemicals, mitomycin C (without S9 activation) and cyclophosphamide (with S9 activation), induced statistically significant and biologically relevant increases in the frequency of cells with aberrations and confirmed the adequacy of the experimental conditions for detecting clastogenic potential and the metabolic activity of the S9 mix. Chromosomal aberration assay data are summarized in Table 2 (24 hour harvest) and Table 3 (48 hour harvest).

TABLE 2. Chromosomal aberration assay 24 hours after treatment. (a)

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Dose Level (µg/mL)	Total Aberrations (excluding gaps)		Number of Cells With Aberrations (excluding gaps)		Cell With Multiple Aberrations (5 or more aberrations)	
	Assay 1	Assay 2	Assay 1	Assay 2	Assay 1	Assay 2
(-) S9 Metabolic Activation						
Negative control	5 (2.5%)	9 (4.5%)	5 (2.5%)	8 (4.0%)	0	0
30 µg/mL	1 (0.5%)	8 (4.0%)	1 (0.5%)	8 (4.0%)	0	0
100 µg/mL	4 (2.0%)	3 (1.5%)	4 (2.0%)	3 (1.5%)	0	0
300 µg/mL	3 (1.5%)	10 (5.0%)	4 (2.0%)	10 (5.0%)	1	0
1,000 µg/mL	-	-	-	-	-	-
3,000 µg/mL	-	-	-	-	-	-
MMC	12 * (24%)	11 * (22%)	12 * (24%)	11 * (22%)	4	1
(+) S9 Metabolic Activation						
Negative control	6 (3.0%)	4 (2.0%)	6 (3.0%)	4 (2.0%)	0	0
30 µg/mL	-	-	-	-	-	-
100 µg/mL	-	-	-	-	-	-
300 µg/mL	2 (1.0%)	6 (3.0%)	2 (1.0%)	6 (3.0%)	0	0
1,000 µg/mL	1 (0.5%)	2 (2.0%)	1 (0.5%)	2 (1.0%)	0	0
3,000 µg/mL	5 (2.5%)	6 (3.0%)	5 (2.5%)	6 (3.0%)	0	0
CP	11 * (22%)	20 * (49%)	15 * (20%)	25 * (61%)	6	15

(a) Data obtained from pages 21 to 30 of the study report.
MMC - 0.05 µg/mL Mitomycin C for assay 1 and 0.075 µg/mL Mitomycin C for assay 2. CP - 6 µg/mL cyclophosphamide
Negative control 1% DMSO
* Significantly different from negative control, p ≤ 0.01.

TABLE 3. Chromosomal aberration assay 48 hours after treatment (assay 2 only). (a)

Dose Level (µg/mL)	Total Aberrations (excluding gaps)		Number of Cells With Aberrations (excluding gaps)		Cell With Multiple Aberrations (5 or more aberrations)	
	(-) S9	(+) S9	(-) S9	(+) S9	(-) S9	(+) S9
Negative control	3 (1.5%)	5 (2.5%)	3 (1.5%)	5 (2.5%)	0	0
300 µg/mL	5 (5.0%) (b)	-	5 (5.0%) (b)	-	0 (b)	-
3,000 µg/mL	-	9 (4.5%)	-	9 (4.5%)	-	0
CP	-	-	-	-	-	-
MMC	-	-	-	-	-	-

(a) Data obtained from pages 21 to 30 of the study report.
(b) Data from one culture only (100 cells examined), second culture lost due to technical error.
MMC - 0.05 or 0.075 µg/mL Mitomycin C. CP - 6 µg/mL cyclophosphamide
Negative control 1% DMSO

III. REVIEWER'S DISCUSSION/CONCLUSIONS:

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A. This study is classified as acceptable and satisfies the requirement for Test Guideline: In vitro mammalian cytogenetics [chromosomal aberration] OPPTS 870.5375; OECD 473 for in vitro cytogenetic mutagenicity data. The highest concentration tested, 3,000 µg/mL, represented the highest concentration of the test substance still soluble in the treatment medium. The positive responses of the positive control substances, mitomycin C (without S9 metabolic activation) and cyclophosphamide (with S9 metabolic activation), adequately demonstrate the sensitivity of the assay and the metabolic activity of the liver S9 mix. In the presence of S9 metabolic activation no cytotoxicity was observed. In the absence of S9 metabolic activation cytotoxicity was observed at 1,000 and 3,000 µg/mL and possibly at 300 µg/mL. When compared to controls, there were no biologically relevant or statistically significant increases in the number of metaphases with aberrations at any concentration level at the 24 or 48 hour harvest time in either the presence or absence of S9 metabolic activation. Under the conditions of this study, there was no evidence of chromosomal aberrations induced over background.

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

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