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DATA EVALUATION RECORD - SUPPLEMENT

XDE-570 (FLORASULAM)

Study Type: OPPTS 870.5100 [§84-2]; Bacterial Reverse Gene Mutation Assay

Work Assignment No. 4-01-128 R (MRID 46808240)

Prepared for
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Template version 02/06

DATA EVALUATION RECORD

STUDY TYPE: *In vitro* Bacterial Gene Mutation (*Salmonella typhimurium/ E. coli*)/ mammalian activation gene mutation assay; OPPTS 870.5100 [§84-2]; OECD 471 (formerly OECD 471 & 472).

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: Lawlor, T.E. (1995) Mutagenicity test on XDE-570 in the *Salmonella*/mammalian-microsome reverse mutation assay (Ames test) pre-incubation method with a confirmatory assay. Corning Hazleton Inc., Vienna, VA. Laboratory Project Study ID: CHV Study No. 16246-0-422R; Dow Study No. DR-0312-6565-016, December 28, 1995. MRID 46808240. Unpublished.

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

EXECUTIVE SUMMARY - In two independent trials of a reverse gene mutation assay in bacteria (MRID 46808240), *Salmonella typhimurium* strains TA98, TA100, TA1535, and TA1537, and *Escherichia coli* strain WP2*uvr*A were exposed to XDE-570 (Florasulam; 99.2% a.i.; Lot # 930910) in dimethylsulfoxide (DMSO) at concentrations of 0, 0.333, 1, 3.33, 10, 33.3, or 100 µg/plate (*S. typhimurium*) and 0, 10, 33.3, 100, 333, 1000, or 3330 µg/plate (*E. coli*) both in the presence and absence of S9-activation. The S9 fraction was derived from the livers of male Sprague-Dawley rats induced with Aroclor 1254. The pre-incubation method was used in both the initial and confirmatory assays. Standard strain-specific mutagens served as positive controls.

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XDE-570 was tested up to cytotoxic concentrations, as indicated by the reduced numbers of revertants at 33.3µg/plate and above in the *S. typhimurium* strains and at 3333 µg/plate in the *E. coli* strain. There were no marked increases in the mean number of revertants/plate in any strain. The positive controls induced the appropriate response in all strains in the presence and absence of S9-activation. **There was no evidence of induced mutant colonies over background.**

The study is classified as **acceptable/guideline** and satisfies the guideline requirement for Test Guideline OPPTS 870.5100; OECD 471 for *in vitro* mutagenicity (bacterial reverse gene mutation) 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 reverse gene mutation 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.

46808240.PMRA.Der .pdf

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Reviewer: Tom Morris , **Date** April 13, 2000

STUDY TYPE: Bacterial system, *Salmonella typhimurium; E. coli* / mammalian activation gene mutation assay; OPPTS 870.5100[1]; OECD 471 (formerly OECD 471 & 472).

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

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

CITATION: Lawlor, T. E. December 28, 1995. **Mutagenicity Test on XDE-570 in the Salmonella / Mammalian-Microsome Reverse Mutation Assay (Ames Test) Pre-incubation Method with a Confirmatory Assay.** Performing Laboratory: Corning Hazleton inc., 9200 Leesburg Pike, Vienna, Virginia, 22182. Laboratory Project Study ID: CHV Study No. 16246-0-422R; Dow Study No. DR-0312-6565-016. Unpublished

SPONSOR: Dow AgroSciences Canada Inc. (DAS).

EXECUTIVE SUMMARY: In a reverse gene mutation assay in bacteria, strains of *Salmonella typhimurium* (TA98, TA100, TA1535 and TA1537) and *Escherichia coli* (WP2uvrA) were exposed to XDE-570 (Purity - 99.2%) in dimethylsulfoxide (DMSO) at concentrations of 0, 0.333, 1.00, 3.33, 10.0, 33.3 or 100 µg/plate for *Salmonella typhimurium* and 0, 10, 33.3, 100, 333, 1,000 or 3,330 µg/plate for *Escherichia coli*, in both the presence and absence of S9 mammalian metabolic activation (S9 fraction derived from Aroclor 1254-induced rat livers). An independent confirmatory trial was performed. A pre-incubation method (20 ±2 minutes at 37 ± 2 °C) was used for both the initial and confirmatory assays.

XDE-570 did not show a dose-related or biologically relevant increase in the number of revertant colonies/plate over the negative control with any of the *Salmonella typhimurium* or *Escherichia coli* tester strains in either the initial or independent confirmatory trial in the presence or absence of S9 metabolic activation. The positive controls induced the appropriate responses in the corresponding strains. **There was no evidence of induced mutant colonies over background.**

This study is classified as acceptable / guideline. This study satisfies the requirement for Test Guideline OPPTS 870.51001; OECD 471 for *in vitro* mutagenicity (bacterial reverse gene mutation) data.

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

[1]870.5100 - Reverse mutation *E. coli* WP2uvrA; *S. typhimurium* TA98, TA100, TA1535, TA1537

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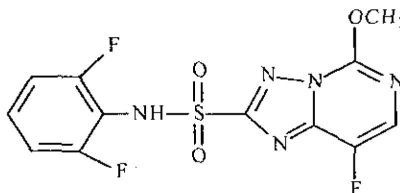
I. MATERIALS AND METHODS

A. MATERIALS:

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

Description: White powder, stored at room temperature
Lot/Batch #: Lot # 930910 (Date received: 05/24/94)
Purity: 99.2 % a.i (determined by HPLC).
CAS #: 145701-23-1
Structure:



Solvent Used: Dimethylsulfoxide (DMSO)

2. **Control Materials:**

Negative: DMSO (CAS # 67-68-5, Lot 83H0557 and Lot 64H1007, purity: 99+%).
Solvent (final conc'n): The vehicle was plated, using a 50 µL aliquot of vehicle along with a 100 µL aliquot of the appropriate tester strain and a 500 µL aliquot of S9 mix (or phosphate buffer, where appropriate) on selective agar. At 100 mg/mL, the most concentrated stock solution prepared, the test article formed a clear, colourless solution.
Positive: Nonactivation:

Sodium azide 20 µg/plate TA100, TA1535
2-Nitrofluorene 1.0 µg/plate TA98
ICR-191 2.0 µg/plate TA1537
4-nitroquinoline-N-oxide 1.0 µg/plate WP2*uvr*A

Activation:

2-Aminoanthracene (2-anthramine) 2.5 µg/plate TA98, TA100, TA1535 and TA1537
2-Aminoanthracene (2-anthramine) 25.0 µg/plate WP2*uvr*A

3. **Activation**: S9 derived from

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

Liver microsomal enzymes (S9 mix) were purchased from Molecular Toxicology, Inc., Annapolis, MD., Batch 0515 (40 mg of protein/mL). The homogenate was prepared from male Sprague-Dawley rats injected (*i.p.*) with Aroclor 1254 (200 mg/mL in corn oil) at 500 mg/kg bw as described by Ames. A 1.0 mL volume of S9 mix consisted of H_2O (0.70 mL); 1M NaH_2PO_4/Na_2HPO_4, pH 7.4 (0.10 mL); 0.25 M glucose-6-phosphate (0.02 mL); 0.10 M NADP (0.04 mL); 0.825 M KCl/0.2 M $MgCl_2$ and S9 homogenate (0.1 mL). The S9 mix was prepared immediately prior to its use in any experimental procedure. Prior to first use, each batch was checked for sterility and metabolising capacity by using reference mutagens and appropriate activity was demonstrated.

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4. **Test organisms:** *S. typhimurium* strains

TA97	X TA98	X TA100	TA102	TA104
X TA1535	X TA1537	TA1538	list any others	

Test organism: *Escherichia coli* strains

X WP2*uvr*A

Properly maintained?	X Yes	No
Checked for appropriate genetic markers (*rfa* mutation, pKM101 plasmid R factor)	X Yes	No

Characteristic number of spontaneous revertants was determined for both the *Salmonella typhimurium* and *Escherichia coli* strains.

5. **Test compound concentrations used**:

Dose range-finding study:

Nonactivated conditions:
- with *S. typhimurium* strains (TA100 only): 0, 6.67, 10.0, 33.3, 66.7, 100, 333, 667, 1,000, 3,330 or 5,000 µg/plate
- with *Escherichia coli* strains (WP2*uvr*A): 0, 6.67, 10.0, 33.3, 66.7, 100, 333, 667, 1,000, 3,330 or 5,000 µg/plate

Activated conditions:
- with *S. typhimurium* strains (TA100 only): 0, 6.67, 10.0, 33.3, 66.7, 100, 333, 667, 1,000, 3,330 or 5,000 µg/plate
- with *Escherichia coli* strains (WP2*uvr*A): 0, 6.67, 10.0, 33.3, 66.7, 100, 333, 667, 1,000, 3,330 or 5,000 µg/plate

Main study:

Nonactivated conditions:
- with *S. typhimurium* strains: 0, 0.333, 1.00, 3.33, 10.0, 33.3 and 100 µg/plate
- with *Escherichia coli* strains: 0, 10.0, 33.3, 100, 333, 1,000 and 3,330 µg/plate

Activated conditions:
- with *S. typhimurium* strains: 0, 0.333, 1.00, 3.33, 10.0, 33.3 and 100 µg/plate
- with *Escherichia coli* strains: 0, 10.0, 33.3, 100, 333, 1,000 and 3,330 µg/plate

All doses of test substance, vehicle controls and positive controls were plated in triplicate.

B. TEST PERFORMANCE

1. **Type of *Salmonella* assay:**

__ standard plate test
X pre-incubation (20 ± 2 minutes at 37 ± 2 °C)
__ "Prival" modification *(i.e. azo-reduction method)*
__ spot test
__ other (describe)

2. **Protocol** The assay conditions are summarized in Table 1. The test substance, tester strains and the S9 mix (or phosphate buffer, where appropriate) were mixed and then incubated at 37 ± 2 °C for 20 ± 2 minutes in an orbitally

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shaking incubator. Following the incubation period, 2.0 mL of top agar (maintained in a molten state at 45 °C) was rapidly distributed into the culture tubes. Each tube of this mixture was then distributed evenly over the surface of 25 mL of minimal bottom agar contained in a petri dish. The agar and the pre-incubation reaction mixture were mixed and then overlaid onto a minimal agar plate. After the overlay had solidified, the plates were inverted and incubated for 48 ± 8 hours at 37 ± 2 °C and revertant colonies were counted immediately following the incubation period. Plates which were not evaluated immediately were held at 5 ± 3 °C until such time that colony counting and bacterial background lawn evaluation could take place. The test was performed both with and without S9 metabolic activation. These procedures were used in both the dose range-finding study and the main mutagenicity study.

Table 1: Cytotoxicity / Mutagenicity Assay

Component	(+) Metabolic Activation System	(-) Metabolic Activation System
test, negative-solvent control or positive control substance	0.05 mL	0.05 mL
0.1 mL of 10-hour bacterial cell culture (0.5 x 10^9 cells/mL)	0.1 mL	0.1 mL
0.1 M sodium phosphate buffer (pH 7.4)	-	0.5 mL
S-9 mix	0.5 mL	-

The condition of the bacterial lawn was evaluated for evidence of cytotoxicity and test substance precipitate. Cytotoxicity was scored relative to the vehicle control plate and was recorded along with the revertant colony counts for all plates. The number of revertant colonies per plate for the vehicle controls and all test article plates were counted manually. The number of revertant colonies per plate for the positive controls were counted by automated colony counter.

Criteria for a valid assay: The following criteria were used to determine a valid assay:

(1) Tester strain integrity: *salmonella typhimurium*
- *rfa* Wall mutation, pKM101 plasmid
- characteristic number of spontaneous revertants, acceptable ranges (revertants/plate) for vehicle controls were as follows: TA98 - 8 to 60; TA100 - 60 to 240; TA1535 - 4 to 45 and TA1537 - 2 to 45.

(2) Tester strain integrity; *Escherichia coli*
- characteristic number of spontaneous revertants, acceptable range (revertants/plate) for WP2*uvr*A was 10 to 50.

(3) Tester strain culture density
- to demonstrate that appropriate numbers of bacteria were plated, the density of tester strain cultures were greater than or equal to 0.5 x 10^9 bacteria/mL and/or had reached a target level of turbidity demonstrated to produce cultures with a density of greater than or equal to 0.5 x 10^9 bacteria/mL.

(4) Positive control values
- positive control values (-) S9 mix - the mean value of a positive control for a respective strain exhibited at least a 3-fold increase over the mean value of the vehicle control for that strain.
- positive control values (+) S9 mix - the mean value of a positive control for a respective strain exhibited at least a 3-fold increase over the mean value of the vehicle control for that strain (an acceptable positive control in the presence of S9 mix for a specific strain was evaluated as having demonstrated both the integrity of the S9 mix and the ability of the tester strain to detect a mutagen).

(4) A minimum of three non-toxic doses were required to evaluate assay data.

3. Statistical Analysis: For all replicate plating, the mean revertants per plate and the standard deviation were calculated.

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4. **Evaluation Criteria:** For the test article to be considered positive it had to produce at least a 3-fold increase in the mean revertants/plate of at least one tester strain over the mean revertants per plate of the appropriate control. This 3-fold or greater increase in the mean number of revertants per plate had to be observed at more than one dose and had to be accompanied by a dose response to increasing concentration of the test article. In addition, the observed dose-responsive increase had to be shown to be reproducible. An observed response which did not meet all three criteria (magnitude, dose-responsiveness, reproducibility) was not evaluated as positive.

II. REPORTED RESULTS

A. **Dose range-finding assay** With the *Salmonella typhimurium* tester strain, TA100, cytotoxicity was observed at 33.3 µg/plate and above in both the presence and absence of S9 metabolic activation as evidenced by a reduction in the number of revertants/plate. With the *Escherichia coli* tester strain, WP2*uvr*A, cytotoxicity was observed at the 3,330 µg/plate dose and above in both presence and absence of S9 metabolic activation as evidenced by a reduction in the number of revertants/plate. The dose levels for the main study for the *Salmonella typhimurium* and *Escherichia coli* tester strains were selected based on these findings.

B. **Mutagenicity assay** - XDE-570 appeared to produce cytotoxic effects at the higher doses (≥ 33.3 µg/plate in the *Salmonella typhimurium* tester strains and at 3,330 µg/plate in the Escherichia coli tester strain) as indicated by reduced numbers of revertant colonies. No positive increases in number of revertant colonies/plate were observed with any of the tester strains in either the presence or absence of S9 metabolic activation. Evaluation of individual dose groups with respect to relevant assessment parameters (dose effect and reproducibility) revealed no biologically relevant difference in the revertant colonies/plate as compared to the corresponding negative controls in either the presence or absence of S9 metabolic activation. The results in the initial assay were confirmed by the results in the confirmatory assay. Thus, XDE-570 was considered to be non-mutagenic under the conditions of this microbial gene mutation assay. All bacterial strains responded to the mutagenic action of the appropriate positive control substance in the presence or absence of S9 metabolic activation; therefore, the sensitivity of the assay and the metabolic activity of the liver S9 mix were adequately demonstrated. Results of the initial and confirmatory mutagenicity assays are summarized in Table 2 (*Salmonella typhimurium* tester strain -S9 Mix), Table 3 (*Salmonella typhimurium* tester strain +S9 Mix) and Table 4 (*Escherichia coli* tester strain ± S9 Mix).

Table 2: *Salmonella typhimurium* tester strain (-)S9 Mix (expressed as # of revertant colonies/plate). (a)

Dose (µg/plate)	Initial Trial				Confirmatory Trial			
	TA98	TA100	TA 1535	TA1537	TA98	TA100	TA1535	TA1537
0	16 ± 1	86 ± 4	8 ± 6	4 ± 1	15 ± 6	88 ± 13	10 ± 2	6 ± 1

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Dose (µg/plate)	Initial Trial				Confirmatory Trial			
	TA98	TA100	TA 1535	TA1537	TA98	TA100	TA1535	TA1537
0.333	16 ± 8	85 ± 8	11 ± 4	4 ± 1	19 ± 3	79 ± 9	11 ± 4	7 ± 3
1.00	12 ± 2	86 ± 6	10 ± 1	4 ± 2	16 ± 4	76 ± 2	12 ± 2	5 ± 1
3.33	15 ± 3	78 ± 2	6 ± 4	4 ± 4	15 ± 2	74 ± 6	6 ± 2	4 ± 2
10.0	8 ± 1	79 ± 5	4 ± 2	5 ± 3	7 ± 2	76 ± 7	4 ± 2	4 ± 3
33.3	4 ± 3	70 ± 9	2 ± 1	2 ± 2	5 ± 1	48 ± 9	3 ± 2	2 ± 1
100	1 ± 1	6 ± 3	0 ± 0	1 ± 1	1 ± 1	8 ± 5	0 ± 0	0 ± 1
Na-azide	-	791 ± 60 *	582 ± 35 *	-	-	743 ± 47 *	587 ± 14 *	-
2-NF	212 ± 19 *	-	-	-	309 ± 20 *	-	-	-
ICR-191	-	-	-	2014 ± 89 *	-	-	-	1900 ± 384 *

(a): Data extracted from pages 30-35 of study report.
* Mutagenic effect
2-NF - 1.0 µg/plate nitrofluorene   Na-azide - 2.0 µg/plate sodium azide   2.0 µg/plate ICR-191

Table 3: *Salmonella typhimurium* tester strain (+) S9 Mix (expressed as # of revertant colonies/plate). (a)

Dose (µg/plate)	Initial Trial				Confirmatory Trial			
	TA98	TA100	TA 1535	TA1537	TA98	TA100	TA1535	TA1537
0	21 ± 2	115 ± 7	15 ± 1	6 ± 2	22 ± 5	110 ± 11	10 ± 2	10 ± 3
0.333	17 ± 2	101 ± 7	8 ± 2	5 ± 0	23 ± 2	103 ± 12	10 ± 1	7 ± 3
1.00	15 ± 1	102 ± 7	13 ± 2	5 ± 1	13 ± 3	98 ± 10	12 ± 4	6 ± 3
3.33	20 ± 8	94 ± 8	7 ± 3	3 ± 2	22 ± 2	102 ± 2	12 ± 3	7 ± 1
10.0	10 ± 3	86 ± 20	2 ± 2	3 ± 1	9 ± 6	83 ± 7	4 ± 3	4 ± 2
33.3	4 ± 2	34 ± 10	1 ± 1	2 ± 2	2 ± 2	16 ± 15	0 ± 1	2 ± 0
100	0 ± 0	1 ± 1	0 ± 1	2 ± 2	0 ± 0	1 ± 1	0 ± 0	1 ± 1
2-AA	1295 ± 91	1546 ± 49 *	152 ± 2 *	224 ± 4 *	1220 ± 129 *	1362 ± 79 *	225 ± 49 *	193 ± 3 *

(a): Data extracted from pages 30-35 of study report.
* Mutagenic effect.
2-AA - 2.5 µg/plate 2-aminoanthracine

Table 4: *Escherichia coli* tester strain (-/+) S9 Mix (expressed as # of revertant colonies/plate). (a)

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Dose (µg/plate)	Initial Trial		Confirmatory Trial	
	(-) S9 Mix	(+) S9 Mix	(-) S9 Mix	(+) S9 Mix
0	13 ± 3	20 ± 3	14 ± 6	16 ± 2
10.0	13 ± 4	17 ± 6	15 ± 5	18 ± 8
33.3	13 ± 3	16 ± 4	16 ± 3	11 ± 1
100	11 ± 9	17 ± 4	17 ± 2	14 ± 6
333	15 ± 2	15 ± 6	15 ± 3	19 ± 2
1,000	9 ± 1	14 ± 4	4 ± 1	10 ± 6
3,330	0 ± 0	0 ± 0	0 ± 0	1 ± 1
4-NQNO	1154 ± 152 *	-	629 ± 271 *	-
2-AA	-	546 ± 19 *	-	374 ± 24 *

(a): Data extracted from pages 30-35 of study report.
* Mutagenic effect.
2-AA - 25.0 µg/plate 2-aminoanthracine 4-NQNO - 1.0 µg/plate 4-nitroquinoline-N-oxide

III. REVIEWER'S DISCUSSION/CONCLUSIONS:

A. This study is classified as acceptable and satisfies the requirement for Test Guideline OPPTS 870.51001; OECD 471 for in vitro mutagenicity (bacterial reverse gene mutation) data. The test substance did not show a dose-related or biologically relevant increase in the number of revertant colonies/plate over the negative control with any of the *Salmonella typhimurium* or *Escherichia coli* tester strains in either the initial or independent confirmatory trial in the presence or absence of S9 metabolic activation. The response of all bacterial strains to the appropriate non-activated and S9 activated positive control substances adequately demonstrated the sensitivity of the test to detect mutagenesis. The study provided acceptable evidence to conclude that, under the conditions of this microbial gene mutation assay, XDE-570 was considered to be non-mutagenic.

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

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