DATA EVALUATION RECORD - SUPPLEMENT

XDE-570 (FLORASULAM)

Study Type: OPPTS 870.5300 [§84-2]; CHO Cells/Mammalian Activation Gene Forward Mutation Assay at the HGPRT Locus

Work Assignment No. 4-01-128 P (MRID 46808238)

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DATA EVALUATION RECORD

STUDY TYPE: *In vitro* Mammalian Cell Gene Mutation Assay in Chinese Hamster Ovary (CHO) Cells; OPPTS 870.5300 [§84-2]; OECD 476.

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 the Chinese hamster ovary cell/hypoxanthine-guanine-phosphoribosyl transferase (CHO/HGPRT) forward mutation assay. Health and Environmental Sciences, The Toxicology Research Laboratory, Midland, MI. Laboratory Project Study ID: DR-0312-6565-006, January 23, 1995. MRID 46808238. Unpublished.

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

EXECUTIVE SUMMARY - In two independent trials of a mammalian cell gene mutation assay at the HGPRT locus (MRID 46808238), Chinese hamster ovary (CHO) cells cultured *in vitro* were exposed to XDE-570 (Florasulam; 99.2% a.i.; Lot # 930910) in dimethylsulfoxide (DMSO) at concentrations of 0, 187.5, 375, 750, 1500, or 3000 µg/mL (+/-S9) for 4 hours. The S9 fraction was derived from the livers of male Sprague-Dawley rats induced with Aroclor 1254. The positive controls were ethyl methanesulfonate (-S9) and 20-methylcholanthracene (+S9).

XDE-570 was tested up to the limit of solubility (3000 µg/mL). No evidence of cytotoxicity was observed at any concentration in either trial in the presence or absence of S9-activation. No marked increase in mutant frequency was observed in any trial in the presence or absence of S9-activation. The positive controls induced the appropriate response in both trials (+/-S9). **There was no evidence of induced mutant colonies 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 870.5300; OECD 476 for *in vitro* mutagenicity (mammalian forward 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 gene mutation assay. A detailed DER by the Canadian Pest Management Regulatory Agency (PMRA) is attached.

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

46808238.PMRA.der
.pdf

Reviewer: Tom Morris , **Date** April 17, 2000

STUDY TYPE: Mammalian cells in culture gene mutation assay in the Chinese Hamster Ovary Cell / hypoxanthine-guanine-phosphoribosyl transferase (CHO/HGPRT). OPPTS 870.5300; OECD 476.

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 the Chinese Hamster Ovary Cell / Hypoxanthine-Guanine-Phosphoribosyl Transferase (CHO/HGPRT) For ward Mutation Assay.** Performing Laboratory: Health and Environmental Sciences, The Toxicology Research Laboratory, 1803 Bldg., Midland, MI, 48674. Laboratory Project Study ID: DR-0312-6565-006. Unpublished

SPONSOR: Dow AgroSciences Canada Inc. (DAS).

EXECUTIVE SUMMARY: In a mammalian cell gene mutation assay at the hypoxanthine-guanine-phosphoribosyl transferase (HGPRT) locus, Chinese hamster ovary (CHO) cells cultured *in vitro* were exposed to XDE-570 (Purity - 99.2%) at concentrations of 0, 187.5, 375, 750, 1,500 or 3,000 µg/mL in the presence and absence of S9 mammalian metabolic activation. S9 liver homogenates were prepared from Aroclor-1254 treated (500 mg/kg bw) male Sprague-Dawley rats.

XDE-570 (florasulam) was tested up to its limit of solubility under culture conditions. No treatment-related effect on cloning efficiency or relative cell survival was observed in either the presence or absence of S9 metabolic activation. The test substance did not induce a significant, dose-related, reproducible increase in mutation frequency compared to the solvent controls in the presence or absence of S9 metabolic activation. The positive controls, ethyl methanesulfonate (without S9 mix) and 20-methylcholanthracene (with S9 mix), induced the appropriate response. **There was no evidence of induced mutant colonies over background; therefore, under the conditions of this *in vitro* mammalian cell gene mutation assay, XDE-570 was considered to be non-mutagenic.**

This study is classified as acceptable / guideline. This study satisfies the requirement for Test Guideline OPPTS 870.5300, OECD 476 for *in vitro* mutagenicity (mammalian forward gene mutation) 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

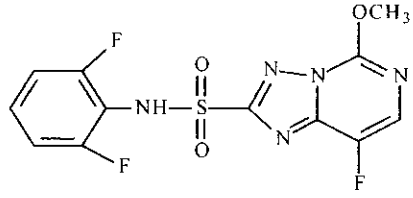
Description: White powder, stored at room temperature

Lot/Batch #: Batch # TSN100298 / Lot # 930910

Purity: 99.2 % a.i (determined by HPLC).

CAS #: 145701-23-1

Structure:



Solvent Used: Dimethylsulfoxide (DMSO)

2. **Control Materials:**

Negative control: DMSO was used as the negative control treatment.

Solvent control: (final conc'n) DMSO for both activation/non-activation final concentration was 1% (v/v)

Positive control: Nonactivation: Ethyl methanesulfonate (EMS, CAS # 62-50-0, Sigma) at a final concentration of 621 µg/mL.
Activation: 20-methylcholanthracene (20-MCA, CAS # 56-49-5, Sigma) at a final concentration of 4 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.

4. **Test cells:** mammalian cells in culture

	mouse lymphoma L5178Y cells		V79 cells (Chinese hamster lung fibroblasts)
X	Chinese hamster ovary (CHO) cells		list any others

CHO-K_1-BH_4 cells were obtained from Dr. Abraham Hsie, Oak Ridge National Laboratory, Oak Ridge , TN. Stock cultures were stored at ≈-100 °C or below.

Media: The cells were maintained in Ham's F-12 nutrient mix (Gibco) supplemented with 5% (v/v) heat-inactivated (56 °C, 30 min) dialyzed fetal bovine serum , 25 mM HEPES and antibiotics-antimycotics (Fungizone, 25 µg/mL; Penicillin G, 100 units/mL and streptomycin sulfate, 0.1 mg/mL).

Properly maintained?	X	Yes	No
Periodically checked for Mycoplasma contamination?	X	Yes	No
Periodically checked for karyotype stability?	X	Yes	No
Periodically "cleansed" against high spontaneous background?	X	Yes	No

5. **Locus Examined:**

	Thymidine kinase (TK)		Hypoxanthine-guanine-phosphoribosyl transferase (HGPRT)	Na^+/K^+ ATPase
Selection agent:	bromodeoxyuridine (BrdU)		8-azaguanine (8-AG)	ouabain
	fluorodeoxyuridine (FdU)	X	6-thioguanine (6-TG) (concentration not provided)	
	trifluorothymidine (TFT)			
Other:				

6. **Test compound concentrations used:**

Nonactivated conditions:	Preliminary cytotoxicity test: 0, 23.4, 46.9, 93.8, 187.5, 375, 750, 1,500 or 3,000 µg/mL.
	Gene mutation assay: 0, 187.5, 375, 750, 1,500 or 3,000 µg/mL.
Activated conditions:	Preliminary cytotoxicity test: 0, 23.4, 46.9, 93.8, 187.5, 375, 750, 1,500 or 3,000 µg/mL.
	Gene mutation assay: 0, 187.5, 375, 750, 1,500 or 3,000 µg/mL.

B. TEST PERFORMANCE

1. Cell treatment:

a. Cells were exposed to test compound, negative/solvent or positive controls for 4 hours (non-activated) 4 hours (activated). Cell density for the cytotoxicity assay was 1 x 10^6/5 mL and for the gene mutation assay was 3 x 10^6/10 mL. Incubation temperature was 37 °C for both non-activated and activated assays.

b. After washing, cells were cultured for 7 days (expression period) before cell selection.

c. After expression, 2 x 10^5 cells/dish (10 dishes/ group) were cultured for 7-9 days in selection medium to determine numbers of mutants and 200 cells/dish (3 dishes/group) were cultured for 7-9 days without selective agent to determine cloning efficiency.

2. Statistical Methods: The frequency of mutants/10^6 clonable cells were statistically evaluated using a weighted analysis of variance; weights are derived from the inverse of the mutation frequency variance. The actual plate counts are assumed to follow a poisson distribution therefore, the mean plate count is used as an estimate of variance. A linear trend test and lack of fit test were employed as an omnibus test to compare treated groups to the negative control. If there is a significant increasing trend or a significant lack of fit, a Dunnett's t-test was conducted, comparing each treated group and positive control to the negative control. An additional comparison of

the positive controls to the negative controls was conducted using a linear contrast statement.

3.Evaluation Criteria: For an assay to be acceptable, the mutation frequency in the positive controls should be significantly higher than the negative controls. An additional criteria is that the mutation frequency in the negative controls should be within reasonable limits of the laboratory historical control values and literature values. The test substance is considered positive if it induces a statistically significant, dose-related, reproducible increase in mutation frequency. The final interpretation of the data also takes into consideration such factors as mutation frequency and cloning efficiencies in the negative controls.

II. REPORTED RESULTS The analytically observed concentrations of the test substance ranged from 86-89% of targeted concentrations in assay 1 and 101-107% of the targeted concentrations in assay 2.

A. Preliminary cytotoxicity assay In the preliminary cytotoxicity assay, the test substance was assayed at concentration ranging from 23.4 to 3,000 µg/mL. The highest concentration was based on the limitations imposed by the solubility of the test substance in the culture medium. In cultures treated in the absence of S9 metabolic activation, the relative cell survival (RCS) showed no toxicity (Table 1). In cultures treated in the presence of S9 metabolic activation, only slight toxicity was observed at the three highest dose levels based on slightly reduced RCS values compared to controls (Table 1). Based on these results, dose levels of 187.5, 375, 750, 1,500 and 3,000 µg/mL were selected for both the presence and absence of metabolic activation in the mutagenicity assay.

Table 1. Summary of preliminary cytotoxicity assay. (a)

Dose Level (µg/mL)	Relative Cell Survival (%) (b)	
	Non-activated (-S9 mix)	Activated (+S9 mix)
0 (c)	100.0	100.0
23.4	122.3	91.2
46.9	105.6	94.0
93.8	119.8	93.0
187.5	107.8	94.7
375	104.3	97.7
750 (d)	93.0	81.2
1,500 (d)	100.8	84.7
3,000 (d)	103.2	78.7

(a) Data obtained from page 20 of the study report.
(b) Values expressed as Relative Cell Survival (%):

$$\text{Relative Cell Survival (\%)} = \frac{\text{Mean \# of colonies/dish in the treated}}{\text{Mean \# of colonies/dish in negative control}} \times 100$$

(c) 1% (v/v) DMSO
(d) Test substance precipitated when added to treatment medium but cleared upon sonication.

B. Mutagenicity assay No treatment-related effect on cloning efficiency or relative cell survival was observed in either the presence or absence of S9 metabolic activation. There was no statistically significant increase in mutation

frequency over the concurrent vehicle controls in the presence or absence of S9 metabolic activation at any dose level. However, at the high dose, 3,000 µg/mL, a slightly increased mutant frequency was observed in experiment 1 in both the presence and absence of S9 metabolic activation. This was not statistically significant, was not confirmed in the second experiment and was within the range of the historical background values of 6-thioguanine resistant mutation frequency in CHO cells from this laboratory (1.5-27.9 per 10^6 clonable cells). Therefore, under conditions of this study, XDE-570 was not mutagenic. The spontaneous mutant frequencies in the solvent-treated controls were within the historical control data range. In both assays, the positive controls induced significant increases in the mutant frequencies and this data confirmed the adequacy of the experimental conditions for detecting induced mutations. Results of the gene mutagenicity assay are summarized in Table 2.

Table 2. Summary of mutagenicity assays. (a)

Dose Level (µg/mL)	Relative Cell Survival (% of vehicle control)		Cloning Efficiency (%)		Mutant Frequency/10^6 Clonable Cells	
	Exp 1	Exp 2	Exp 1	Exp 2	Exp 1	Exp 2
(-) S9 Mix						
0 (DMSO)	100.0	100.0	55.2	74.0	4.3	2.0
187.5	125.0	116.9	49.1	78.4	6.7	3.8
375	110.3	110.2	50.7	59.4	5.5	3.0
750	106.5	96.1	na	64.8	na	3.5
1,500	129.6	102.2	67.8 (b)	62.3	8.1 (b)	1.6
3,000	128.8	96.8	53.3	63.0	10.1	1.2
EMS	39.0	50.4	24.5 (b)	27.1	449 * (b)	106.2 *
(+) S9 Mix						
0 (DMSO)	100.0	100.0	55.5	72.4	3.8	0.3
187.5	102.5	106.2	60.2	72.0	6.3	3.4
375	85.8	106.2	64.7	67.2	3.9	1.1
750	96.2	101.2	58.8	64.3	0.9	5.8
1,500	94.3	86.4	56.9	65.8	3.6	2.0
3,000	16.0 ©	98.4	51.7 (c)	61.4	11.7 (c)	1.3
20-MCA	70.9	94.2	50.0	50.0	82.7 *	60.0 *

(a) Data obtained from pages 21 to 24 of study report.
(b) Only one replicate present (insufficient cells).
(c) Precipitation of test substance in medium, but cleared upon sonication.
EMS - Ethyl methanesulfonate. 20-MCA - 20-methylcholanthracene.
na - Inadequate number of cells for plating in the first subculture.
* Significantly higher than concurrent negative control value, $p \leq 0.05$.

III. REVIEWER'S DISCUSSION/CONCLUSIONS:

A. This study is classified as acceptable and satisfies the requirement for Test Guideline OPPTS 870.5300, OECD 476 for in vitro mutagenicity (mammalian forward gene mutation) data. The spontaneous mutant frequencies in the solvent-treated controls were within the historical control data range. In both assays, the positive controls induced significant increases in the mutant frequencies. This data confirmed the adequacy of the experimental conditions for detecting induced mutations. The test substance did not induce a significant, dose-related, reproducible increase in mutation frequency compared to the solvent controls; therefore, under the conditions of this *in vitro* mammalian cell gene mutation assay, XDE-570 was considered to be non-mutagenic.

B. **Study deficiencies** - There are no deficiencies which would impact on the outcome of the study.