9-9-88

Guideline Series 84 : MUTAGENICITY

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Date: September 6, 1988

DATA EVALUATION REPORT

CHEMICAL: N-Ethyl Perfluorooctanesulfonamide Tox. Chem. No.: 454E

STUDY TYPE: Salmonella/mammalian activation gene mutation assay

MRID No.: 406126-13

SYNONYMS/CAS No.: GX-071

SPONSOR: Griffin Corporation

TESTING FACILITY: Toxikon Corporation, Norwood, MA

TITLE OF REPORT: Ames Test (Bacterial Mutagenesis assay)
Plate Assay of GX-071

AUTHOR(S): Laxman S. Desia, D. Sc.

STUDY NUMBER(S): PROJECT NO.: 85G-0030

REPORT ISSUED: October 3, 1985; amended March 21, 1988

Classification: Unacceptable.

CONCLUSION(S) - Executive Summary: The assay system was not responsive to the positive control chemicals under non-activated conditions and, therefore, the negative results observed with the test material cannot be interpreted. Poor copying also made it impossible to determine what some of the intermediate concentrations were. Also, when one experiment is performed, one generally should use at least triplicate cultures (instead of duplicate as in this study). Overall, this Salmonella assay is unacceptable, based on the above inadequacies.

SALMONELLA

A. MATERIALS

1. Test Material: Name: N-ethyl perfluorooctanesulfonamide; Description: white needle crystals, insoluble in water; Batch #: not provided; Purity: 99+%; Solvent used: DMSO;

2. Control Materials:
Negative: A spontaneous control and a solvent (DMSO) control are listed in the results' tables.

Solvent/final concentration: DMSO was used at the maximum volume used to administer the highest dose of test article.

Positive:	Solvent	Concentration	Strain
Non-activation:			
Sodium azide	water	10 ug/plate	TA100, TA1535
2-Nitrofluorene	DMSO	10 ug/plate	TA98, TA1538
9-Aminoacridine	ethanol	50 ug/plate	TA1537
Activation:			
2-Amino-anthracene	DMSO	2.5 ug/plate	All strains

3. Activation:

The S9 homogenate was obtained commercially. The 9000 x g supernatant was prepared from adult male rat liver (strain not given) induced by Aroclor 1254. The S9 fraction contained the following:

Components	Concentration/ml S9 mix
NADP (sodium salt)	4 umoles
D-glucose-6-phosphate	5 umoles
$MgCl_2$	8 umoles
KCl	33 umoles
Sodium phosphate	100 umoles
S 9 fraction	100 uliters

4. Test organisms: S. typhimurium strains
___ TA97 _X_ TA98 _X_ TA100 ___TA102 ___TA104
_X_ TA1535 _X_ TA1537 _X_ TA1538
Properly maintained? (Y)N (circle one)
Single colony reisolates were checked for their genotypic characteristics (his, rfa,uvrB, bio) and for the presence of the plasmid.

5. Test compound concentrations used:
The same doses (which were listed only in the Results' table) were used for both the non-activated and the activated conditions. The table lists: 0.8, ?.0, 2.2, ?.6, ?.?, and 10.0 mg/plate. The magnitude of these three doses could not be ascertained by this reviewer due to the poor quality of the study report submitted.

B. TEST PERFORMANCE

1. Type of Salmonella assay used was the standard plate test. Protocol: Assays were performed according to the method of Ames, et al. (1975).

Comment: The report is poorly written. There is no indication in the text of the report of what doses were utilized in the study and whether more than one assay plate was run per dose (Table indicates duplicate cultures). With regard to dose selection, it is stated that doses were selected based on information in Section 9.0 (b) and Table 1, and dose selection assays were performed as stated in the sponsor approved protocol (which was not provided).

a) Section 9.0 (b) is "0.025-0.150 ml of solution of the test compound to yield the appropriate dose."
b) Table 1 lists: 0.8, ?.3, 2.2, ?.6, ?.?, and 10.0 mg/plate.

2. Preliminary cytotoxicity assay

No information was provided on the levels tested, at what dose levels growth inhibition was observed, and cytotoxicity indices (effect on background lawn, reduction in revertants) were not listed. The highest concentration used in the mutagenicity assay was 10 mg/plate, which is adequate.

3. Mutagenicity assay

GX-071 was tested at 6 dose levels (from Table 1) in duplicate with 5 tester strains of Salmonella both with and without metabolic activation (S-9). The results are listed in Table 1, attached. It would appear that some of the positive controls do not meet the criteria for mutant chemicals, as listed in the study text. For example, under EVALUATION CRITERIA, it is stated:

a. Strains TA1535, TA1538, TA1537 - If the solvent control value is within the normal range, a test material producing a positive response equal to three times the solvent control value is considered mutagenic. The ranges of revertants for solvent controls (from this laboratory) generally considered acceptable for these three tester strains and the positive controls under non-activated and activated conditions were presented as follows:

		Positive Control	
Range of Revertants		Non-activated	Activated
TA1535	8-30	sodium azide (SA)	2-amino-anthracene (2-AA)
TA1538	10-35	2-nitrofluorene (2-NF)	2-amino-anthracene
TA1537	4-30	9-aminoacridine (9-AA)	2-amino-anthracene

b. Strains TA-98 and TA100 - If the solvent control value is within the normal range, a test material producing a positive response equal to twice the solvent control value for these strains is considered mutagenic. The acceptable ranges of revertants and the positive controls (from this laboratory) for these tester strains under non-activated and activated conditions were presented as follows:

		Positive Control	
Range of Revertants		Non-activated	Activated
TA-98	20-75	2-nitrofluorene	2-amino-anthracene
TA100	80-250	sodium azide	2-amino-anthracene

The results listed in Table 1 indicate that in several instances, shown below, the positive control failed to attain an adequate value for a positive response under non-activated conditions.

Tester strain	Chemical name	Positive control	Solvent control	3X solvent control value
TA1535	SA	55	20	60
TA1538	2-NF	69	58	174
TA1537	9-AA	54	26	78

Tester strain	Chemical name	Positive control	Solvent control	2X solvent control value
TA100	SA	127	122	244

C. CONCLUSION

The assay system was not responsive to the positive control chemicals under non-activated conditions and, therefore, it is difficult to interpret the negative results observed with the test material as evidence of negative mutagenic potential in the Salmonella assay. Poor copying also made it impossible to determine what some of the intermediate concentrations were. Also, when one experiment is performed, one generally should use at least triplicate cultures (instead of duplicate as in this study). Overall, this Salmonella assay is unacceptable, based on the above inadequacies.

Sulfluramid

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