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UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

SEP - 9 1988

MEMORANDUM:

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

SUBJECT: Acute Studies Submitted in Support of a Registration

TO: Michael Mendelsohn
Product Manager (17)
Registration Division (TS-767C)

FROM: Linda L. Taylor, Ph.D. Linda Lee Taylor 9/9/88
Toxicology Branch II, Section II
Hazard Effects Division (TS-769C)

Thru: Marcia van Gemert, Ph.D. M. van Gemert 9/9/88
Acting Section Head, Section II, TB II
Hazard Effects Division (TS-769C)

And

William Burnam, Ph.D.
Acting Chief, TB II, HED (TS-769C) W. Burnam 9/9/88

Registrant: Griffin Corporation
Chemical: GX-071; N-ethyl perfluorooctanesulfonamide
Project: 8-0995
Caswell No.: 454E
Record No.: 225107

Action Requested: Reveiw of acute studies submitted in support of GX-071.

Comment: Griffin Corporation is requesting registration of GX-071, a technical of the toxicant N-ethyl perfluorooctanesulfonamide, for use in formulating end-use products in child-resistant bait stations for indoor cockroach control (per Note To Rick Tinsworth).

Seven acute oral, one acute dermal, primary dermal and eye irritation, a delayed contact hypersensitivity, and three mutagenicity studies were submitted on GX-071 in support of this request. These studies have been reviewed and the DER's are attached.

1) Acute oral LD_{50} in rats - UGA 002-2, dated April 21, 1986 (amended April 20, 1988). The combined LD_{50} was calculated as 543.48 mg/kg (607.14 mg/kg -males; 507.09 mg/kg-females). No target organ was identified.

2) Acute oral LD_{50} in rats - Project No. 86-1169, dated June 6, 1986 (amended April 20, 1988). The dose of 5000 mg/kg is above the LD_{50}. The liver and kidneys are the apparent target organs. Three males and four females died during the study.

3) Acute oral LD_{50} in rats - Project No. 85G-0037, dated October 31, 1985 (amended April 20, 1988). More than half of the animals died within 14 days of treatment with 5000 mg/kg.

4) Acute oral LD_{50} in rats - Project No. 85G-0034, dated September 30, 1985 (amended April 20, 1988). The LD_{50} for both sexes exceeded 817 mg/kg (HDT). No deaths or clinical signs of toxicity were observed.

5) Acute oral LD_{50} in rats - Project No. 86G-0031, dated October 23, 1986 (amended April 20, 1988). No LD_{50} was determined.

6) Acute oral LD_{50} in rats - UGA 003, dated November 20, 1985 (amended April 20, 1988). All animals died within 10 days following the administration of GX-071 at a dose level of 5 grams/kg (only dose tested).

7) Acute oral LD_{50} in rats - Project No. 86G-0001, dated February 6, 1986 (amended April 20, 1988). Only one animal died during the study. No LD_{50} was calculated. Under the conditions of the study, the LD_{50} for GX-071 would appear to be in excess of 6.0 grams/kg.

8) Single dose dermal toxicity in rabbits - Project No. 85G-0032, dated September 18, 1985 (amended March 21, 1988). The single dose dermal LD_{50} is greater than 2000 mg/kg (Tox. Category III).

9) Primary eye irritation in rabbits - Project No. 85G-0033, dated September 30, 1985 (amended March 21, 1988). GX-071 did not produce ocular irritation and was not considered an eye irritant.

10) Primary dermal irritation in rabbits - Project No. 85G-0031, dated September 26, 1985 (amended March 21, 1988). GX-071 was reported to be a potential mild skin irritant, based on a Primary Dermal Irritation Score of 0.13.

11) Delayed contact hypersensitivity in guinea pigs - SLS 3159.3, dated October 29, 1986 (amended March 24, 1988). No positive control was used in this study; therefore, the study is inadequate for use in the assessment of the potential of GX-071 to elicit a delayed contact hypersensitivity response in guinea pigs.

12) In vitro transformation of BALB/3T3 cells - Project No. 86G-0007, dated July 25, 1986 (amended March 21, 1988). Due to inadequacies in the study, this assay cannot be used to adequately assess the carcinogenic potential of GX-071 via its ability to transform BALB/3T3 cells in vitro.

13) Salmonella/mammalian activation gene mutation assay - Project No. 85G-0030, dated October 3, 1985 (amended March 21, 1988). The assay system was not responsive to the positive control chemicals under non-activated conditions and, therefore, the negative results observed with GX-071 cannot be interpreted.

14) Sister chromatid exchange in Chinese hamster ovary cells - Project No. 86G-0002, dated July 25, 1986 (amended March 21, 1988). The study should be repeated using higher dose levels since the top dose did not result in at least a 50% reduction in the second mitosis (significant cell cycle delay).

The seven acute oral studies gave conflicting results (see Summary Table), which may be explained as due to the differences in the vehicle used and/or dosage regimen and/or volume administered. The test material, GX-071, is said to be insoluble in water, soluble in acetone and alcohol (neither of these was used in any of the studies), and its solubility in oil is less than 1%. The vehicles utilized in these acute oral studies were corn oil and soybean oil; one study utilized a gelatin suspension. The range of LD_{50} values obtained from the submitted studies is approximately 500 mg/kg to greater than 6.0 grams/kg. Using the lowest value obtained, GX-071 falls into Toxicity Category III. However, a question (raised by one of the study authors) regarding the availability of the test material for absorption, in light of GX-071's limited solubility, was not addressed. A more accurate expression of acute oral toxicity would probably have been determined if GX-071 had been tested in a vehicle in which it is soluble.

With regard to the mutagenicity studies submitted, the choice of studies provided fulfills the requirement for a battery of tests, since they address the three required categories of genetic effects; i.e., gene mutations; structural chromosomal aberrations; and other mechanisms of mutagenicity. Since all three of the submitted mutagenicity studies will have to be repeated (see above), the Registrant may choose to perform the same or different tests to provide data addressing these three categories.

Since the technical grade of GX-071 and the manufacturing use product are the same thing (as per our conversation on September 9, 1988), separate toxicity studies on the TGAI and the MP are not required. It is to be noted that toxicity data comparable to the toxicity data submitted on GX-071 will be required for an end-use product.

The request for registration of GX-071 for use in bait station roach traps for use in the home is not supported by the available toxicity data, since all of the mutagenicity studies and the delayed contact hypersensitivity study are inadequate.

CONCLUSION

The request for registration of GX-071 is not supported by adequate toxicity data. None of the mutagenicity studies is adequate. The delayed contact hypersensitivity study is also inadequate, based on the lack of a positive control. Additionally, one acute oral LD_{50} study (MRID # 406126-20) is classified as supplementary, pending submission of the body-weight data.

ACUTE ORAL TOXICITY SUMMARY TABLE FOR GX-071

Laboratory - Toxikon 9/30/85 MRID No. 406126-05
Vehicle - Corn oil
Dose Regimen - Single dose, 1.5 ml total
Species/Supplier - Sprague-Dawley rat/Charles River
LD_{50}: 817 mg/kg

Dose level (mg/kg)	Mortality Males	Females	Total
817	0/5	0/5	0/10
495	0/5	0/5	0/10
300	0/5	0/5	0/10

Laboratory - University of Georiga 4/21/86 MRID No. 406126-02
Vehicle - Soybean oil
Dose Regimen - Triple-divided, 1.5 hours apart, 2 ml/portion
Species/Supplier - Sprague-Dawley rat/Harlan
LD_{50}: 543 mg/kg (472-762)

Dose level (mg/kg)	Mortality Males	Females	Total
600	2/5	4/5	6/10
500	2/5	2/5	4/10
400	1/5	1/5	2/10
300	0/5	0/5	0/10

Laboratory - Toxikon 10/23/86 MRID No. 406126-06
Vehicle - Soybean oil
Dose Regimen - Triple-divided, 1.5 hours apart, 2 ml/portion
Species/Supplier - Sprague-Dawley rat/Charles River
LD_{50}: unable to calculate

Dose level (mg/kg)	Mortality Males	Females	Total
6000	4/5	1/5	5/10
2000	2/5	3/5	5/10
500	4/5	2/5	6/10
500	1/5	2/5	3/10
200	0/5	0/5	0/10
60	1/5	0/5	1/10

ACUTE ORAL TOXICITY SUMMARY TABLE FRO GX-071
(cont'd)

Laboratory - University of Georgia 11/20/85 MRID No. 40626-07
Vehicle - Soybean oil
Dose Regimen - Double-divided, 2.0 hours apart, total volume 4 ml
Species/Supplier - Sprague-Dawley rat/Harlan
LD_{50}: unable to calculate

	Mortality		
Dose level (mg/kg)	Males	Females	Total
5000	5/5	5/5	10/10

Laboratory - Toxikon 6/6/86 MRID No. 406126-03
Vehicle - Soybean oil
Dose Regimen - Triple-divided, 1.5 hours apart, 2 ml/portion
Species/Supplier - Sprague-Dawley rat/Charles River
LD_{50}: unable to calculate

	Mortality		
Dose level (mg/kg)	Males	Females	Total
5000	3/5	4/5	7/10

Laboratory - Toxikon 10/31/85 MRID No. 406126-04
Vehicle - Corn oil
Dose Regimen - Double-divided, 4.0 hours apart, total 5 ml
Species/Supplier - Sprague-Dawley rat/Charles River
LD_{50}: unable to calculate

	Mortality		
Dose level (mg/kg)	Males	Females	Total
5000	2/5	4/5	6/10

Laboratory - Toxikon 2/6/86 MRID No. 406126-20
Vehicle - Gelatin suspension
Dose Regimen - Single dose hardened gelatin
Species/Supplier - Sprague-Dawley rat/Charles River
LD_{50}: unable to calculate

	Mortality		
Dose level (mg/kg)	Males	Females	Total
6000	0/5	1/5	1/10
3000	0/5	0/5	0/10
1500	0/5	0/5	0/10

Reviewed by: Linda L. Taylor, Ph.D. Linda Lee Taylor 9/15/88
Section III, Tox. Branch (TS-769C)
Secondary Reviewer: Marcia van Gemert, Ph.D. M. van Gemert 9/9/88
Head, Section III, Tox. Branch (TS-769C)

DATA EVALUATION REPORT

STUDY TYPE: Acute oral LD_{50} - rats TOX CHEM NO: 454E

MRID NO: 406126-20

TEST MATERIAL: N-Ethyl Perfluorooctanesulfonamide

SYNONYMS: GX-071

STUDY NUMBER: Project No.: 86G-0001

SPONSOR: Griffin Corporation Valdosta, GA

TESTING FACILITY: Toxikon Corporation

TITLE OF REPORT: Acute Oral LD_{50} Toxicity Study GX-071

AUTHORS: R.A. Adams, Ph. D., D.A.B.T.

REPORT ISSUED: February 6, 1986; amended April 20, 1988

CONCLUSIONS: Only one animal (high-dose female) died during this study. No LD_{50} was calculated. Under the conditions of this study, the LD_{50} for GX-071 appears to be in excess of 6.0 g/kg.

CLASSIFICATION: Core supplementary, pending receipt of body-weight data.

A. MATERIALS:

1. Test Compound: GX-071
   Description: white needle crystals
   Batch #: not specified
   Purity: 99+%

2. Test Animals:
   Species: rats, both sexes
   Strain: Sprague-Dawley
   Age: 8-12 weeks old
   Weight: 215-226 grams
   Source: Charles River Breeding Laboratories, Wilmington, Del.

Study Design: Five (fasted) rats per sex per group were administered 1.5, 3.0, or 6.0 grams of test material per kg in a 10% gelatin suspension. It is stated that the dose was given in 4-5 mls of the suspension (flavored with a few drops of anise flavoring), which was allowed to harden, and that all animals were dosed on the same day within a 2-3 hour period. The control was administered 4-5 mls of the gelatin; it is not stated whether the control also received anise flavoring. The

animals were observed for 14 days following dosing; body weights were measured and days 7 and 14 following dosing, and a gross necropsy was performed on all animals at termination.

Results: It is stated that the data indicate a treatment-related (females - dose-related) inhibition in weight gain, with an absolute weight loss noted among the high-dose females. The body-weight effects were said to be accompanied by a treatment-related thinning of the fur. There was one (female) death, which was attributed to an apparent impairment in food consumption. No LD_{50} was calculated, but it was suggested that when GX-071 is administered as in this study, the LD_{50} would be in excess of 6.0 grams/kg. It is to be noted that the Appendix referred to in the RESULTS section was not included in the final report. Additionally, on page 11, the dose is incorrectly listed as mg/kg in stead of gm/kg.

Conclusion: This study is classified as supplementary, pending receipt of the body weight data.

Discussion: It is noted that the study results obtained here are in sharp contrast to a study (MRID # 406126-07), in which there was 100% death at 5 g/kg. The vehicle used in these studies differed, suggesting that availability of GX-071 for absorption may depend on the vehicle used. The current study utilized a 10% gelatin suspension; MRID # 406126007 utilized soybean oil. In a third study (MRID # 406126-06), in which a dose of 6 g/kg was tested (vehicle-soybean oil), 4 out of 5 males and 1 of 5 females died. The differences between the two studies testing 6 g/kg were the number of portions the dose was divided into (Study-20 had one portion; Study-06 had three) and the vehicle (gelatin and soybean suspensions, respectively). A fourth study (MRID # 406126-04) also used a 5 g/kg dose, and two males and four females died within 48 hours of dosing. The vehicle was corn oil and the dose was divided into two portions.

Guideline Series 84 : MUTAGENICITY

Reviewed by: Linda L. Taylor, Ph.D. Linda Lee Taylor 9/6/88
Section: III, Tox. Branch (TS-769C)
Secondary reviewers: Marcia van Gemert, Ph.D. Marcia Gemert 9/9/88
Section: III, Tox. Branch (TS-769C)
Kerry Dearfield, Ph.D.
Hazard Evaluation Division (TS-769C) Kerry Dearfield 9.9.88
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.

TOXIKON PROJECT # 85G-0030

TABLE I

Ames Bacterial Assay Results Griffin Corporation/White Needle Crystals/GX-071 with and Without Microsomal Activation (S-9)

CHEMICALS	NON-ACTIVATION TA98	TA100	TA1535	TA1537	TA1538	ACTIVATION TA98	TA100	TA1535	TA1537	TA1538
Spontaneous Control	50	128	16	19	29	37	228	28	38	38
DMSO Vehicle Control	40	122	20	26	58	40	212	44	28	39
2-Amino-Anthracene 2.5 ug/plate	88	193	60	35	65	114	670	156	173	178
2-Nitro-Fluorene 1 ug/plate	89	170	52	30	69	89	227	44	34	101
Sodium Azide 10 ug/plate	67	127	55	37	96	98	352	95	44	52
9-Amino-Acridine 50 ug/plate	79	204	40	54	38	59	382	59	148	66
TEST ARTICLE										
0.8 mg/plate	41	138	35	24	39	36	182	39	44	29
.3 mg/plate	50	128	24	22	28	44	247	50	24	36
2.2 mg/plate	32	125	25	21	21	32	185	27	55	37
.6 mg/plate	45	143	23	24	22	67	231	27	29	27
.0 mg/plate	44	133	20	20	22	44	220	31	53	32
10.0 mg/plate	35	135	34	20	33	34	175	33	48	33

Each value represents the number of histidine$^+$ revertants per plate and are the average of duplicate assay plates. The test material was dissolved in DMSO to obtain the appropriate dilution.

Guideline Series 84 : MUTAGENICITY

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Section: III, Tox. Branch (TS-769C)
Kerry Dearfield, Ph.D.
Hazard Evaluation Branch (TS-769C) Kerry Dearfield 9.9.88
Date: September 6, 1988

DATA EVALUATION REPORT

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

STUDY TYPE: In Vitro Transformation of BALB/3T3 Cells - GX-071

MRID NUMBER: 406126-12

SYNONYMS/CAS No.: GX-071

SPONSOR: Griffin Corporation

TESTING FACILITY: Toxikon Corporation, Norwood, MA

TITLE OF REPORT: In Vitro Transformation of BALB/3T3 Cells - GX-071

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

STUDY NUMBER(S): 86G-0007

REPORT ISSUED: July 25, 1986; amended March 21, 1988

Classification: Unacceptable.

CONCLUSION(S) - Executive Summary: This mutagenicity assay is inadequate in that there are too few flasks scored per test material dose level. Although the Methods' section of the report states that 15 flasks were used per control and test groups, and the Assay Acceptance Criteria state that more than eight flasks per test condition were available for analysis, the tables listing the results show only 2-3 dishes (flasks) per test-dose group. This assay cannot be used to adequately assess the carcinogenic potential of GX-071 via its ability to transform BALB/3T3 cells in vitro.

I. Materials and Methods: GX-071 (Purity-99+%; Lot/Batch No. not provided; white needle crystals) was said to be insoluble in the growth medium, and therefore, DMSO was used as the solvent. BALB/3T3 Clone A_{31} mouse cells were used in the transformation assays. Stocks were said to be checked periodically to ensure the absence of mycoplasma contamination. Routine cultures were grown and passaged weekly in Eagle's Minimum Essential Medium (EMEM) supplemented with 5% calf serum. The assay was performed under activated and non-activated conditions.

Negative controls: 1- Assay procedures performed on untreated cells; and 2- A solvent control (DMSO; final concentration of 0.3% in the growth medium). Fifteen flasks of the negative controls were prepared for each assay.

Positive control: A known carcinogen, 3-methylcholanthrene (MCA), which can be metabolized to the active carcinogen by this mouse cell line, was used in the assay. Fifteen flasks were treated with 5 ug MCA/ml medium.

In the activated assay, S9 reaction mixture was added to the growth medium, together with the test material and incubated for 2 hours, with the exposure period terminated by washing cells twice with saline. The cells were treated subsequently as were those in the non-activated assay (see below). The activated system utilized the S9 fraction derived from the liver of Fischer 344 male rats induced with Aroclor 1254. The S9 fraction contained 1.4 mg NADP, 2.7 mg isocitrate, and 15 ul per growth medium of 2.5% serum. It was stated that commercially available S9 fraction was used whenever found feasible. Note: In a 1983 review of cell transformation by chemical agents *, no validated metabolic activation system was said to be available.

Transformation assay: Twenty-four hours prior to treatment, a series of flasks were seeded with 10^4 cells/flask and incubated at 37° C. Fifteen flasks were treated for each of the following conditions: (a) five preselected doses of test article; (2) positive control; (3) negative control, and the flasks were incubated for 3 days. Following washing, the cells were fed with test-article-free medium and incubated for 4 weeks, with re-feeding twice a week. Termination of the assay was made by fixing the cell monolayers with methanol and staining with Giemsa. The number of transformed foci were scored on the basis of the criteria described in the protocol (copy attached). The flasks were read "blind".

II. Results:

The doses used in the transformation assay were 500 ug to 31.25 ug/ml, based on the results of a range-finding study. The results were presented in two tables (copies attached). Note: Table III, which the study report identifies as the results of the activated assay, is mis-labeled.

It is stated that the test article did not induce a significant increase in the frequency of transformed foci, relative to controls, in either the presence or absence of microsomal activation. Under the conditions of the assay, the author concluded that GX-071 was not mutagenic in the transformation assay. Comment: Metabolic activation had no effect on the 3-MCA response.

III. Conclusion:

TB cannot concur with the author's conclusion. In the Method's section of the report, 15 flasks were to be used for each of the doses of test material and each control. Additionally, the Assay Acceptance Criteria indicate that more than eight flasks per test condition were available for analysis (should have 15-20 flasks). However, the results, as presented in Tables II and III, give results for only 2-3 flasks per test group. This is an inadequate number of samples for analysis.

* Heidelberger, C., Freeman, A.E., Pienta, R.J., Sivak, A., Bertram, J.S., Casto, B.C., Dunkel, V.C., Francis, M.W., Kakunaga, T., Little, J.B., and Schechtman, L.M. Cell Transformation by Chemical Agents - A Review and Analysis of the Literature. Mutation Research 114, 283-385 (1983).

TABLE I

DOSE SELECTION STUDY RESULTS WITHOUT MICROSOMAL ACTIVATION

Test Material: GX-07i Test Date: 5/29/86

Sponsor: Griffin Corporation Project #: 86G-0007

CONTROLS	COLONIES/DISH*
Spontaneous	129±10.5
DMSO (0.3%)	134± 8.0
GX-07i ug/ml	
0.061	138±18.5
0.122	128±13.5
0.244	127± 8.5
0.488	132± 6.8
0.976	137±11.2
1.95	138±20.5
3.90	133± 5.9
7.81	132± 5.0
15.62	131± 6.6
31.25	134± 7.2
62.50	133± 6.4
125	126± 5.1
250	119± 2.1
500	99±10.0
1000	36± 8.2

*Values represent the mean ± S.D. of triplicate culture dishes.

TABLE II

TRANSFORMATION ASSAY OF TEST ARTICLE GX-071 WITHOUT MICROSOMAL ACTIVATION

Test Material: GX-071 Test Date: 6/6/86

Sponsor: Griffin Corp. Project #: 86G-0007

CONTROLS	TOTAL/TOTAL FOCI/DISHES	TRANSFORMED* FOCI/DISHES	STATISTICAL ANALYSIS**
Spontaneous	20/14	1.43±1.7	
DMSO 0.3%)	18/14	1.28±1.4	
3-MCA(5ug/ml)	1503/15	107 ± 17	c > 61***
GX-071 (ug/ml)			
31.25	3/3	1.0±1.0	c < 24 (NS)
62.50	5/3	2.7±0.6	c < 24 (NS)
125	4/3	1.3±1.5	c < 24 (NS)
250	5/2	2.5±2.1	c < 34 (NS)
500	4/2	2.0±2.0	c < 34 (NS)

* Values represent the mean ± S.D. of transformed foci score' per flask. (n=14-15 for controls; n=3 for the test material).

**The Kastenbaum and Bowman Tables were used to determine the significance of the transformed foci scored per dose level relative to solvent (DMSO) controls (2).

***Significant: $\alpha = 0.01$

TABLE III

TRANSFORMATION ASSAY OF TEST ARTICLE GX-071 WITHOUT MICROSOMAL ACTIVATION

Test Material: GX-071 Test Date: 6/6/86

Sponsor: Griffin Corp. Project #: 86G-0007

CONTROLS	TOTAL/TOTAL FOCI/DISHES	TRANSFORMED* FOCI/DISHES	STATISTICAL ANALYSIS**
Spontaneous	26/15	1.73±1.6	
DMSO 0.3%)	14/15	1.08±1.2	
3-MCA(5ug/ml)	1663/15	111 ± 12	c > 63***
GX-071 (ug/ml)			
31.25	1/3	0.3±0.6	c < 27 (NS)
62.50	3/3	1.0±1.7	c < 27 (NS)
125	6/3	2.0±1.7	c < 27 (NS)
250	5/3	1.7±2.9	c < 27 (NS)
500	4/3	1.3±1.5	c < 27 (NS)

* Values represent the mean ± S.D. of transformed foci scored per flask. (n=14-15 for controls; n=3 for the test material).

**The Kastenbaum and Bowman Tables were used to determine the significance of the transformed foci scored per dose level relative to solvent (DMSO) controls (2).

***Significant: α =0.01

Guideline Series 84 : MUTAGENICITY

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Kerry Dearfield, Ph.D.
Hazard Evaluation Division (TS-769C) Kerry Dearfield 9.9.88
Date: September 6, 1988

DATA EVALUATION REPORT

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

STUDY TYPE: Sister Chromatid Exchange - Chinese Hamster Ovary (CHO) Cells

MRID NUMBER: 406126-14

SYNONYMS/CAS No.: GX-071

SPONSOR: Griffin Corporation

TESTING FACILITY: Toxikon Corporation, Norwood, MA

TITLE OF REPORT: Sister Chromatid Exchange (SCE) In Chinese Hamster Ovary Cells - GX071

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

STUDY NUMBER(S): 86G-0002

REPORT ISSUED: July 25, 1986; amended March 21, 1988

Classification: Unacceptable.

CONCLUSION(S) - Executive Summary: Under the conditions of this assay, GX-071 was found to be negative for induction of sister chromatid exchange in Chinese hamster ovary cells, both with and without metabolic activation, at concentrations up to 1000 ug/ml. However, this top concentration did not result in at least a 50% reduction in the second mitosis, thus indicating that a higher dose should have been utilized. The study should be repeated using higher dose levels.

SISTER CHROMATID EXCHANGE

I. Materials and Methods: GX-071 (Lot # not provided) was described as white needle crystals with a purity of 99+%. The Chinese hamster ovary cells (CHO) were obtained from the American Type Culture Collection (Repository No. CCL61), which is a permanent cell line with an average cycle time of 10 to 12 hours, a model chromosome number of 20, and a plating efficiency of approximately 90%. The cells were grown in "complete" growth medium (Minimal Essential Medium (MEM) supplemented with 10% fetal calf serum. There were two negative controls: assay procedures were performed on untreated cells and dimethyl sulfoxide (DMSO) served as the solvent control. The assays were conducted with and without metabolic activation. The activated systems utilized the S9 fraction derived from the liver of male Fischer 344 rats induced with Aroclor 1254. The S9 reaction mixture contained S9 15 ul/ml, NADP 1.4 mg/ml, and isocitric acid 2.7 mg/ml growth medium with 2.5% serum. The positive control agents used in the assays were Ethylmethane sulfonate (EMS) for the non-activation series and Dimethylnitrosamine (DMN) in the metabolic activation series.

Forty second division cells (M2) were scored for the frequency of SCEs per cell (20 cells each from duplicate cultures per concentration). The proportions of cells in the first, second, and third divisions (i.e., M1, M2, and M3 cells) were also determined by scoring 50 cells. Scoring of slides was performed "blind". A quality assurance statement was provided.

II. Results:

A. Solubility, Stability, and Dose Determination - The test article was insoluble in water. A solvent control was utilized since the test article was insoluble in the growth medium. DMSO was the solvent used and the concentrations tested were 10, 50, 100, 500, and 1000 ug/ml.

B. Sister Chromatid Exchange Assay Without Metabolic Activation - There was some toxicity demonstrated at the top dose level of test material (slight delay in cell cycle time). Results are shown in Table II, attached. No significant increase in SCE was observed at any of the dose levels. GX-071 was considered negative under non-activation conditions for inducing SCE.

C. Sister Chromatid Exchange Assay With Metabolic Activation - Very slight cell cycle delay was reported at the top dose level. There was no significant increase in SCE reported at the concentrations tested (Table III, attached). The test article was considered to be negative for inducing SCE under the conditions of metabolic activation.

TABLE I

TOXICITY AND DOSE SELECTION STUDY RESULTS WITHOUT MICROSOMAL ACTIVATION

Test Material: GX-07i **Test Date:** 6/16/86

Sponsor: Griffin Corporation **Project #:** 86G-0002

CONTROLS	Cell Number ($\times 10^4$)	% of Cells in M1, M2, M3**		
Spontaneous	2.2 ±0.3*	2	93	5
DMSO (0.3%)	2.0 ±0.4	3	96	1
GX-07i (ug/ml)				
1	2.1 ±0.3	6	93	1
5	2.2 ±0.1	2	95	3
10	2.0 ±0.1	6	90	4
50	2.5 ±0.4	8	89	2
100	2.0 ±0.4	8	90	2
500	1.9 ±0.2	10	88	1
1000	1.3 ±0.3	32	68	0 ←

* Values represent the mean ± S.D. of CHO cells counted from triplicate 25cm^2 plates.

** Values represent the percentages of cells in the first, second, third-division (i.e., M1, M2, and M3 cells, respectively). A total of 50 cells were scored.

TABLE II

SCE ANALYSIS OF TEST MATERIAL GX-071 TREATED CHO CELLS WITHOUT MICROSOMAL ACTIVATION

Test Material: GX-071 Test Date: 6/26/86

Sponsor: Gritrin Corporation Project #: 86G-0002

CONTROLS	SCE/M2 Cell*	(p value)	SCE/ chromosome	% of Cells in M1, M2, M3**		
Spontaneous	10.0±2.14		0.50	1	95	4
DMSO (0.3%)	10.2±2.34		0.51	2	97	1
EMS (0.3ul/ml)	74.6±5.13	<0.001	3.74	11	87	1
GA-071 (ug/ml)						
10	10.1±2.43	0.846	0.51	3	94	3
50	10.0±2.82	0.730	0.50	7	92	1
100	10.2±2.53	0.995	0.51	8	89	2
500	10.3±2.56	0.850	0.52	6	93	1
1000	10.2±2.47	0.995	0.51	24	76	0 ←

* Slides were prepared from duplicate culture flasks for each condition (20 M2 cells were scored from each flask for a total of 40 cells). Values represent the mean ± S.D. of SCEs scored.

**Values represent the percentages of cells in the first, second, and third division metaphase (i.e., M1, M2, and M3 cells, respectively). A total of 50 cells were scored.

TABLE III

SCE ANALYSIS OF TEST MATERIAL GX-071 TREATED CHO CELLS WITHOUT MICROSOMAL ACTIVATION

Test Material: GX-071 **Test Date:** 6/26/86

Sponsor: Griffin Corporation **Project #:** 86G-0002

CONTROLS	SCE/M2 Cell*	(p value)	SCE/ chromosome	% of Cells in M1, M2, M3**		
Spontaneous	10.3±2.46		0.52	2	96	2
DMSO (0.3%)	10.1±2.96		0.51	1	97	2
DMN (0.3ul/ml)	101.3±5.30	<0.001	5.05	13	84	3
GX-071 (ug/ml)						
10	9.55±2.53	0.688	0.49	3	97	0
50	10.2±2.27	0.854	0.51	6	93	1
100	9.87±2.01	0.688	0.49	4	95	1
500	9.92±2.47	0.765	0.49	8	92	0
1000	10.1±2.43	0.995	0.51	16	84	0 ←

* Slides were prepared from duplicate culture flasks for each condition (20 M2 cells were scored from each flask for a total of 40 cells). Values represent the mean ± S.D. of SCEs scored.

**Values represent the percentages of cells in the first, second, and third division metaphase (i.e., M1, M2, and M3 cells, respectively). A total of 50 cells were scored.

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DATA EVALUATION REPORT

STUDY TYPE: Delayed Contact Hypersensitivity Guinea Pigs TOX CHEM NO: 454E

MRID NO: 406126-11

TEST MATERIAL: N-Ethyl Perfluorooctanesulfonamide

SYNONYMS: GX-071

STUDY NUMBER: SLS 3159.3

SPONSOR: Griffin Corporation

TESTING FACILITY: Springborn Life Sciences, Inc.-Toxicology and Human Safety Division

TITLE OF REPORT: Delayed Contact Hypersensitivity Study in Guinea Pigs with GX-071

AUTHORS: Joseph C. Siglin, B.A.

REPORT ISSUED: October 29, 1986; amended March 24, 1988

CONCLUSIONS: This study is inadequate for use in the assessment of the potential of GX-071 to elicit a delayed contact hypersensitivity response in guinea pigs. No positive control was utilized.

CLASSIFICATION: Core supplementary.

A. MATERIALS:

1. Test Compound: GX-071
   Description: not provided
   Batch #: Lot # 10; SIB ID S86.005.3159
   Purity: not specified

2. Test Animals:
   Species: guinea pigs
   Strain: Hsd:(HA)BR Hartley derived albino
   Age: young adult
   Weight: 300-500 g
   Source: Harlan Sprague Dawley, Inc.

Study Design: There were two phases to this study: an induction phase and a challenge phase, as well as an irritation screen. Body weights were recorded one day prior to each phase/screen and at termination.

Irritation Screen: GX-071 was tested for irritation potential to determine the appropriate concentrations to use in the sensitation study. Closed patches at various concentrations of GX-071 were applied to the animals (one patch per level, four levels per animal) as follows:

1- 0.4 ml of either a 75, 50, or 25% concentration of GX-071 in acetone was placed on a Webril patch or 0.3 grams of test material as supplied was placed in a Hilltop chamber.

2- the animal was placed in a restrainer and the patch(es) applied to the clipped surface of skin.

3- The patch(es) were occluded with a rubber dental dam pulled taut and fastened to the bottom of the restrainer with clips.

About 6 hours after dosing, the dental dam and patches were removed and the animals were returned to their cages. Twenty hours after patch removal, the exposure sites were depilated with a depilatory, which was subsequently washed off and the animals dried and returned to their cages.

After a minimum of two hours after dipilatation, the sites were graded on a scale of 0 to 3 (0=no reaction; ± slight patchy erythema; 1= slight, but confluent or moderate, patchy erythema; 2=moderate erythema; 3=severe erythema with or without edema). An additional grading was performed 24 hours later.

a) induction phase - dermal application of 0.3 g od GX-071 (as supplied) was applied to each animal's back, under the Hill Top Chamber (patch) , as described above. Approximately 6 hours after dosing, the dental dam and patch were removed and the animals were returned to their cages. This procedure was repeated once a week for 3 consecutive weeks, for a total of three 6-hour treatments with test material. After the third induction exposure, the animals were rested for 12-16 days before the primary challenge. No vehicle control was utilized. The protocol stated that the positive control would be dissolved in ethanol for induction and acetone for challenge. The positive control animals were to be induced with three treatments of 0.3% dinitrochlorobenzene (DNCB) and challenged with 0.2 and 0.02% DNCB.

b) challenge phase - animals previously exposed during the induction phase, as well as the previously untreated controls were challenged to the test material after the rest phase. They were treated as described in the irritation screen by applying the 0.3 g aliquot

of test material to an unexposed test site. Approximately 20 hours after patch removal, the exposure sites were debilitated as described previously. After a minimum of two hours after depilitation, the test sites were graded as described previously. All animals were discarded at study termination.

Results: Following a range-finding study in which 100%, 75% and 50% concentrations produced no dermal irritation, the test material was used as received. It was reported that the test material produced no dermal irritation during the induction phase, although no data were provided. Two control animals had slight patchy erythema at 24 hours after the challenge phase. There were no responders reported in the group treated with 100% GX-071 and challenged with 100% GX-071. Although the protocol and MATERIAL AND METHODS section describe the positive control, no mention is made of the results, and no raw data on the positive control were provided.

Comment: This report consists of:

1- the protocol
2- a MATERIAL AND METHODS section
3- raw data sheets
4- a one-page summary giving the PURPOSE, RESULTS (Table), and a CONCLUSION.

Conclusion: This study is inadequate in that there was no positive control utilized to demonstrate that a sensitizer could be identified under the conditions of the study. This study is classified as supplementary.

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DATA EVALUATION REPORT

STUDY TYPE: Primary Dermal Irritation - rabbits TOX CHEM NO: 454E

ACCESSION NUMBER: MRID NO: 406126-10

TEST MATERIAL N-Ethyl Perflourooctanesulfonamide

SYNONYMS: GX-071

STUDY NUMBER: PROJECT NO.:85G-0031

SPONSOR: Griffin corporation

TESTING FACILITY: Toxikon Corporation

TITLE OF REPORT: Primary Dermal Irritation Study Of GX-071 In Rabbits

AUTHORS: Richard Adams , Ph. D.

REPORT ISSUED: September 26, 1985; amended March 21, 1988

CONCLUSIONS: GX-071 was reported to be a potential mild skin irritant, based on a Primary Dermal Irritation Score of 0.13.

CLASSIFICATION: Core minimum

A. MATERIALS:

1. Test Compound: GX-071
   Description: white needle crystals
   Batch # not specified
   Purity: 99+%

2. Test Animals:
   Species: male and female rabbits
   Strain: New Zealand White
   Age: young adult (6-9 months)
   Weight: 2.5-3.9 kilograms
   Source: Peter Mazzoleni, Taunton, MA

Study Design: The application sites were prepared by clipping the skin of the trunk free of hair (time period before test substance application was not stated). There were two application sites per animal. The test substance (0.5 grams of solid) was introduced under gauze patches that were applied to the skin and secured with adhesive tape. The animals were immobilized (method not specified). The entire trunk of the animal was then wrapped with an impervious, nonreactive rubberized elastic band material. Exposure was for 4 hours. Following exposure, the wrapping was removed and the skin was wiped to remove any test substance remaining. Animals were observed for signs of erythema and edema at 4, 24, 48, and 72 hours after exposure. Body weights were recorded prior to study initiation and at the end of the observation period. Observations were scored according to the "Draize Scale for Scoring Skin Reactions" (FDA, 1965).

Results: None of the animals displayed clinical signs that could be attributed to the test substance. All animals either maintained their body weight or gain weight during the study. Very slight erythema (value=1) was reported in 3 of 6 test animals, with no evidence of edema at the four-hour observation period. No other effects were noted at any other time point. The final Primary Dermal Irritation Score for GX-071 was reported as 0.13, and was considered to have the potential of a mild irritant.

Conclusion: GX-071 was reported as a potential mild irritant.

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DATA EVALUATION REPORT

STUDY TYPE: Primary Eye Irritation - rabbits TOX CHEM NO: 454E

ACCESSION NUMBER: MRID NO: 406126-09

TEST MATERIAL N-Ethyl Perflourooctanesulfonamide

SYNONYMS: GX-071

STUDY NUMBER: PROJECT NO.:85G-0033

SPONSOR: Griffin corporation

TESTING FACILITY: Toxikon Corporation

TITLE OF REPORT: Primary Ocular Irritation Study Of GX-071 In Rabbits

AUTHORS: Richard Adams , Ph. D.

REPORT ISSUED: September 30, 1985; amended March 21, 1988

CONCLUSIONS: The test material did not produce ocular irritation. GX-071 is not considered an eye irritant.

CLASSIFICATION: Core minimum.

A. MATERIALS:

1. Test Compound: GX-071
   Description: white needle crystals
   Batch # not specified
   Purity: 99+%

2. Test Animals:
   Species: male and female rabbits
   Strain: New Zealand White
   Age: 11 months of age
   Weight: 3.0-3.7 kilograms
   Source: registered commercial breeding laboratory

Study Design: GX-071 (100 mg of solid test material) was introduced into the eye of 6 rabbits by placement on the lower lid of the left eye everted by gently pulling it away from the eyeball to form a cup into which the test material was dropped. The upper and lower lids were gently held together for an instant. The right eye remained untreated (control). Three rabbits also received the test substance in the left eye (as above) and after 30 seconds, the eye was flushed for one minute. The right eye served as control. The animals were examined for evidence of eye irritation at approximately 1, 24, 48, and 72 hours after treatment for macroscopic findings. Fluorescein sodium opthalmic solution was used at the 24, 48, and 72 hour observation periods. Body weights were recorded at 72 hours

Results: There was no evidence reported of corneal opacity, iris abnormality, or conjunctival chemosis at any time point (see attached tables). Animals in the treated-unwashed group exhibited slight conjunctival redness and discharge following instillation of the test substance. Slight fluorescein staining was noted in one animal of the group. One animal in the treated-washed group exhibited slight conjunctival redness. No fluorescein staining was noted in this group.

Conclusion: The test material was not shown to be an eye irritant. No rabbit exhibited a positive response and all changes were reversible.

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DATA EVALUATION REPORT

STUDY TYPE: Single Dose Dermal Toxicity-rabbits TOX CHEM NO: 454E

MRID NO: 406126-08

TEST MATERIAL: N-Ethyl Perfluorooctanesulfonaimid

SYNONYMS: GX-071

STUDY NUMBER: 85G-0032

SPONSOR: Griffin Corporation

TESTING FACILITY: Toxikon Corporation

TITLE OF REPORT: Single Dose Dermal Toxicity Study of GX-071 in Rabbits Limit Test

AUTHORS: Richard Adams, Ph.D.

REPORT ISSUED: September 18, 1985; amended March 21, 1988

CONCLUSIONS: The single dose dermal LD_{50} for GX-071 is greater than 2000 mg/kg; Toxicity Category III.

CLASSIFICATION: Core minimum.

A. MATERIALS:

1. Test Compound: GX-071
   Description: White needle crystals
   Batch # not specified
   Purity: 99+%

2. Test Animals:
   Species: male and female rabbits
   Strain: New Zealand White
   Age: young adult; 6 and 8 months
   Weight: 2.7-3.2 kilograms
   Source: Peter Mazzoleni, Taunton,.MA

Study Design: The site of application was prepared by clipping the skin of the trunk free of hair. The length of time prior to dosing was not specified. The test material was applied to the skin under gauze patches placed directly on the skin (area of 10% of the body surface) of 10 animals. There were no controls. The animals were

immobilized with patches secured in place by adhesive tape. The entire trunk was then wrapped with an imperious, nonreactive rubberized elastic band material to keep the test material in contact with the skin for 24 hours. The wrappings were removed after 24 hours, excessive test material removed by wiping off the material remaining on the skin. The animals were observed for signs of erythema and edema and weighed on days 7 and 14 and observed for clinical signs of toxicity (daily) for 14 days. All animals were subjected to a gross necropsy at the end of the study. Note: The criteria for the selection of the animals for testing was not described.

Results: All rabbits survived the 14-day study period. None of the animals were reported to have exhibited any clinical signs that could be attributed to the test material. Four of the 10 rabbits exhibited very slight erythema after the 24-hour exposure period.

The animals either maintained or gained weight during the observation period (males +0.132 kg; females +0.126 kg). Note: The final body weight for the males was incorrectly reported as 3.07 kg; it should read 3.03 kg.

There were no significant lesions reported for any of the animals.

Conclusion: The acute dermal LD_{50} in rabbits is greater than 2000 mg/kg.

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DATA EVALUATION REPORT

STUDY TYPE: Acute oral LD_{50} - rats TOX CHEM NO: 454E

MRID NUMBER: 406126-07

TEST MATERIAL: N-Ethyl Perfluorooctanesulfonamide

SYNONYMS: GX-071

STUDY NUMBER: Project No.: UGA 003

SPONSOR: Griffin Corporation Valdosta, GA

TESTING FACILITY: Department of Pathology College of Veterinary Medicine
The University of Medicine University - Georgia

TITLE OF REPORT: Acute Oral Limit Toxicity Test In Rats GX-071 (LOT 4)

AUTHORS: Willie L. Chapman, D.V.M., Ph. D.

REPORT ISSUED: November 20, 1985; amended April 20, 1988

CONCLUSION: All animals died within 10 days of GX-071 administration at a dose level of 5 grams/kg.

CLASSIFICATION: Core minimum.

A. MATERIALS:

1. Test Compound: GX-071
   Description: not specified
   Batch #: EN 8406191; Lot 4
   Purity: 99+%

2. Test Animals:
   Species: rats, both sexes
   Strain: Sprague-Dawley
   Age: 46-68 days old
   Weight: males-205grams; females-189 grams
   Source: Harlan Sprague Dawley, Inc., Indianapolis, IN

Study Design: Five (fasted) rats per sex were given single (5000 mg/kg) doses of test material by intragastric intubation (suspended in soybean oil) in two divided portions given two hours apart. Animals were weighed prior to dosing and at 7 days after treatment. Twice-daily observations were made for 10 days following dosing. No pathological examinations were performed.

Results: According to the study text, abnormal clinical signs observed in the treated animals included weight loss, emaciation, inappetance, ruffled hair coat, depression, and convulsions. All treated animals were dead within 7-10 days following dosing. Fifty percent were dead within 24 hours (3 males, 2 females) and 80% (4/sex) within 48 hours. On days 4 and 5 after dosing, one animal per sex displayed ruffled hair coats, hunched posture, and convulsed when provoked by auditory stimuli.

Conclusion: All animals administered 5000 mg/kg died within 10 days of test material administration. No LD_{50} was attained. The study is classified as core minimum.

Discussion: In a similar study in which a dose of 5000 mg/kg was tested (MRID # 406126-04), two males and four females died within 48 hours. The vehicle in this latter study was corn oil (dose divided into 2 portions, four hours apart), in contrast to soybean oil used in the current study. Two other studies in which doses of 6000 mg/kg were tested (MRID #s 406126-06 and 406126-20) also had dissimilar results. In MRID # 406126-06, four males and one female died (out of 5/sex). The vehicle was soybean oil and the dose was divided into 3 portions. In MRID # 406126-20, only one female died. The test substance was administered in a gelatin suspension (one portion).

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DATA EVALUATION REPORT

STUDY TYPE: Acute oral LD_{50} - rats TOX CHEM NO: 454E

MRID NUMBER: 406126-06

TEST MATERIAL: N-Ethyl Perfluorooctanesulfonamide

SYNONYMS: GX-071

STUDY NUMBER: Project No.: 86G-0031

SPONSOR: Griffin Corporation Valdosta, GA

TESTING FACILITY: Toxikon Corporation Norwood, MA

TITLE OF REPORT: Acute Oral LD_{50} Toxicity Study GX-071

AUTHORS: Joseph V. Rutkowski, Ph. D.

REPORT ISSUED: October 23, 1986; amended April 20, 1988

CONCLUSION: No LD_{50} was determined.

CLASSIFICATION: Core supplementary.

A. MATERIALS:

1. Test Compound: GX-071
   Description: white needle crystals
   Batch #: not specified
   Purity: 99+%

2. Test Animals:
   Species: rats, both sexes
   Strain: Sprague-Dawley
   Age: 8-12 weeks old
   Weight: 200-400 grams
   Source: Charles River Breeding Laboratories, Wilmington, Del.

Study Design: Five (fasted) rats per sex per group were administered a triple-divided dose of the test material by intragastric intubation (suspension in soybean oil). There were two phases to this study - animals were dosed with 6, 2, and 0.5 g/kg on one day and the remainder were dosed at 0.06, 0.2, and 0.5 g/kg a month later. No information was given as to when the control group was dosed. The animals were observed daily for 14 days after dosing. Individual body weights were recorded on days 0, 7, and 14, and a gross necropsy was performed on each animal at

termination.

Results: According to the study text, the determination of the LD_{50}, probit slope, and 95% confidence interval for the LD_{50} was not possible because the data are significantly heterogeneous. It was also stated that the results suggest a need for further study. It was suggested also that the limited solubility of the test material interferes with an LD_{50} determination by limiting the absorption of the test material from the gastrointestinal tract. An assessment of the bioavailability of the test material following oral administration was suggested as appropriate, although no such data were submitted.

	MORTALITY	
DOSE (g/kg)	MALES (%)	FEMALES (%)
6.0	4/5 (80)	1/5 (20)
2.0	2/5 (40)	3/5 (60)
0.5	4/5 (80)	2/5 (40)
0.5	1/5 (20)	2/5 (40)
0.2	0/5 (0)	0/5 (0)
0.06	1/5 (20)	0/5 (0)

No signs of toxicity were reported for the 0.06 g/kg animals. At all other dose levels, emaciated appearance, nasal discharge, rough hair coat, alopecia near the genito-urinary area, diarrhea, and staining of the hair with feces/urine were reported. A viscous fluid in the digestive tract and a pink color of the lungs was noted at necropsy to various degrees among the groups.

Conclusion: An LD_{50} was not determined. The results at the 0.5 g/kg dose level, which was used in both parts of this study, were not reproducible in the male animals. This study is classified as core supplementary.

Discussion: In another study (MRID # 406126-20) in which 6 g/kg GX-071 was tested, only one female (out of 5; o/5 males) died. The differences between these two studies are the number of portions the dose was divided into (Study-20 had one portion; Study-06 had three) and the vehicle used (gelatin and soybean suspensions, respectively).

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Head, Section III, Tox. Branch (TS-769C)

DATA EVALUATION REPORT

STUDY TYPE: Acute oral LD_{50} - rats TOX CHEM NO: 454E

MRID NUMBER: 406126-05

TEST MATERIAL: N-Ethyl Perfluorooctanesulfonamide

SYNONYMS: GX-071

STUDY NUMBER: Project No.: 85G-0034

SPONSOR: Griffin Corporation Valdosta, GA

TESTING FACILITY: Toxikon Corporation Norwood, MA

TITLE OF REPORT: Acute Oral LD_{50} Toxicity Study GX-071

AUTHORS: Richard A. Adams, Ph.D., D.A.B.T.

REPORT ISSUED: September 30, 1985; amended April 20, 1988

CONCLUSIONS: LD_{50} for male and female (fasted) rats exceeds 817 mg/kg. No deaths or clinical signs of toxicity were observed. Gross pathological examination revealed no specific organ toxicity.

CLASSIFICATION: Core minimum.

A. MATERIALS:

1. Test Compound: GX-071
   Description: white needle crystals
   Batch #: not specified
   Purity: 99+%

2. Test Animals:
   Species: rats, both sexes
   Strain: Sprague-Dawley
   Age: 8-12 weeks old
   Weight: approximately 200 grams
   Source: Charles River Breeding Laboratories, Wilmington, Del.

Study Design: Five (fasted) rats per sex per group were given a single dose of test material by intragastric intubation (suspension in corn oil) at dose levels of 0, 300, 495, or 817 mg/kg. Controls received corn oil at a dose of 5 g/kg. The animals were observed daily for 14 days. Individual body weights were recorded on days 0, 7, and 14. A gross necropsy was performed on each animal at termination.

Results: According to the study text, no mortalities or clinical signs of toxicity were observed. It is stated that all controls and all males showed significant weight gain during the observation period and females "did not experience a normal weight gain." There were no data provided to evaluate this effect. Gross pathological examination revealed no evidence of effect. Note: The total volume administered did not exceed 1.54 ml.

Conclusion: It was concluded by the author that the LD_{50} exceeds 817 mg/kg for both sexes of fasted rat. In another acute oral study at lower dose levels, an LD_{50} was calculated to be 607.14 mg/kg for males and 507.09 mg/kg for females. These two studies differed in the the latter study used soybean oil as the vehicle in contrast to corn oil in the current study. Additionally, the latter study dose was divided into three portions (2 ml each).

Reviewed by: Linda L. Taylor, Ph.D. Linda Lee Taylor 9/18/88
Section III, Tox. Branch (TS-769C)
Secondary Reviewer: Marcia van Gemert, Ph.D. M van Gemert 9/9/88
Head, Section III, Tox. Branch (TS-769C)

DATA EVALUATION REPORT

STUDY TYPE: Acute oral LD_{50} - rats TOX CHEM NO: 454E

MRID NUMBER: 406126-04

TEST MATERIAL: N-Ethyl Perfluorooctanesulfonamide

SYNONYMS: GX-071

STUDY NUMBER: Project No.: 85G-0037

SPONSOR: Griffin Corporation Valdosta, GA

TESTING FACILITY: Toxikon Corporation Norwood, MA

TITLE OF REPORT: Acute Single Dose Oral Toxicity Limit Test In Rats - GX-071

AUTHORS: Richard A. Adams, Ph.D., D.A.B.T.

REPORT ISSUED: October 31, 1985; amended April 20, 1988

CONCLUSIONS: More than half of the animals died within 14 days of treatment (dose level - 5000 mg/kg). It was concluded (by the author) that an additional study in the lethal range was needed.

CLASSIFICATION: Core minimum.

A. MATERIALS:

1. Test Compound: GX-071
   Description: white needle crystals
   Batch #: not specified
   Purity: 99+%

2. Test Animals:
   Species: rats, both sexes
   Strain: Sprague-Dawley
   Age: 8-12 weeks old
   Weight: 177.5-203.3/205.3 grams (listed in deviation statement/text)
   Source: Charles River Breeding Laboratories, Wilmington,.MA

Study Design: Five (fasted) rats per sex were given doses (5000 mg/kg) of test material by intragastric intubation (suspended in 0.2 g/ml corn oil) in two portions 4 hours apart (no controls). The observation period was 14 days. Body weight was recorded on days 7 and 14 after dosing, and a gross necropsy was performed on all animals dying on test and at termination.

Results: Deaths occurred within 48 hours of dose administration. Two males and four females died. Both males and three of the females died within 24 hours. Death was accompanied by signs of extreme lethargy and indifferent temperament prior to death. Bloody exudate around the eyes was noted in both males and one female. No gross pathological changes were reported for any of the animals. Note: Although the dose was divided into two portions administered 4 hours apart, the volume was 4.5-5.0 ml, and one would have liked to see a control administered a comparable volume.

Conclusion: Since more than half of the animals died, the author concluded that the test article is toxic and further study at the lethal range was suggested. A comparable study (MRID # 406126-07) at 5000 mg/kg resulted in 100% mortality within 10 days following dosing in soybean oil (compared to corn oil in the current study). The total volume administered in the comparable study was 4 ml.

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DATA EVALUATION REPORT

STUDY TYPE: Acute oral LD_{50} - rats TOX CHEM NO: 454E

MRID NUMBER: 406126-03

TEST MATERIAL: N-Ethyl Perfluorooctanesulfonamide

SYNONYMS: GX-071

STUDY NUMBER: Project No.: 86-1169

SPONSOR: Griffin Corporation Valdosta, GA

TESTING FACILITY: Toxikon Corporation Norwood, MA

TITLE OF REPORT: Acute Oral Toxicity Limit Test Triple Divided Dose In Soybean Oil GX-071

AUTHORS: Laxman S. Desai, D.Sc.

REPORT ISSUED: June 6, 1986; amended April 20, 1988

CONCLUSIONS: The dose of 5000 mg/kg is above the LD_{50}. The liver and kidneys are the apparent target organs.

CLASSIFICATION: Core minimum

A. MATERIALS:

1. Test Compound: GX-071
   Description: white needle crystals
   Batch #: 5090720096
   Purity: 99%

2. Test Animals:
   Species: rats, both sexes
   Strain: Sprague-Dawley
   Age: 8-12 weeks old
   Weight: 200-400 grams
   Source: Charles River Breeding Laboratories, Wilmington, MA

Study Design: Five (fasted) rats per sex were given a dose (5000 mg/kg) of test material (or vehicle) by intragastric intubation (soybean oil-vehicle), which was divided into three portions (2 ml each). These were administered at 1.5-hour intervals. Clinical examinations were made once daily, more frequently in the early part of the observation period to determine onset and severity of toxic signs. Individual body weights were determined on the fasted animals prior to dosing, on days 7 and 14 of the observation period, and at death. All survivors were weighed

before sacrifice. A gross necropsy was performed on all animals dying on test, and all gross pathology changes were recorded.

Results: Three males and four females of the treated groups died. Death in the males was said to be accompanied by signs of anorexia, dehydration, edema, internal hemorrhaging, and an abnormal appearance of the liver and kidneys. In females, death was accompanied by all of these signs except edema.

Conclusion: The author concluded that the dose of 5 mg/kg is above the LD_{50}, and that the female is more sensitive than the male. Although the summary stated that the liver and kidney of some of the treated animals was abnormal in appearance, no specific data were presented. It is assumed that these two organs are the target organs.

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DATA EVALUATION REPORT

STUDY TYPE: Acute oral LD_{50} - rats TOX CHEM NO: 454E

MRID NUMBER: 406126-02

TEST MATERIAL: N-Ethyl Perfluorooctanesulfonamide

SYNONYMS: GX-071

STUDY NUMBER: UGA 002-2

SPONSOR: Griffin Corporation

TESTING FACILITY: Department of Pathology - College of Veterinary Medicine
The University of Medicine/University of Georgia

TITLE OF REPORT: Acute Oral Limit Toxicity Test In Rats GX-071 (Lot 4)

AUTHORS: Willie L. Chapman, D.V.M., Ph.D.

REPORT ISSUED: April 21, 1986; amended-April 20, 1988

CONCLUSIONS: LD_{50} was calculated as 607.14 mg/kg for males and 507.09 mg/kg for females. The combined LD_{50} was 543.48 mg/kg. No target organ was identified.

CLASSIFICATION: Core minimum

A. MATERIALS:

1. Test Compound: GX-071 N-ethylperfluorooctanesulfonamide, BN 8406191
   Description: not provided
   Batch #: LOT # 4
   Purity: 99+%

2. Test Animals:
   Species: rats, both sexes
   Strain: Sprague-Dawley
   Age: 46-49 days (males); 56-68 days (females)
   Weight: 174-207 grams
   Source: Harlan Sprague-Dawley, Inc. Indianapolis, IN

Study Design: Five rats per sex per dose (fasted 12 hours) were given single doses of test material by intragastric intubation (suspension in soybean oil) at dose levels of 0, 300, 400, 500, and 600 mg/kg. The dosages were divided into three equal portions (each dose in 2 ml of vehicle). The animals were observed twice daily (once on Saturday and Sunday) for 14 consecutive days.

Results: Clinical signs reported at doses of 400 mg/kg and higher included weight loss, bloody nasal discharge, emaciation, inappetance, ruffled hair coat, depression, and convulsions. No deaths occurred at 300 mg/kg for either sex. One animal/sex died at 400 mg/kg, 2/sex at 500 mg/kg, and 2 males and 4 females at 600 mg/kg. No gross pathological examinations were performed (apparently), and no target organ was identified. Additionally, there were no details provided regarding which animals had convulsions.

Note: The heading for the last column in both Tables 1A and 2A (pages 21 and 22) is confusing. It should be mg/actual body weight.

Conclusion: LD_{50} was calculated to be 607.14 mg/kg and 507.09 mg/kg for male and female rats, respectively. No target organ was identified.