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

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OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

TOX Chem No.: 454E

SUBJECT: GX-071 Technical [N-ethyl perfluoro-octane-sulfonamide] - I. Review of Mutagenicity Studies Submitted Under MRID Nos. 40915801, 40863201, and 40863202
EPA ID 1812-GET. TB Project Nos.: 9-0508/9-0292
RD Record Nos.: 235799/234298

II. Additional Information on Acute Toxicity Studies Previously Submitted
EPA ID 1812-GEO/1812-GEI. TB Project Nos.: 9-0600/9-0602
RD Record Nos.: 237282/237284

FROM: Irving Mauer, Ph.D., Geneticist JWH for I. Mauer 2/8/89
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TO: Phil Hutton/M. Mendelsohn, PM Team 17
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THRU: Judith W. Hauswirth, Ph.D., Chief Judith W. Hauswirth 2/8/89
Toxicology Branch I, Insecticide-Rodenticide Support
Health Effects Division (TS-769C)

Registrant: Griffin Corporation
Valdosta, GA

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Requests:

I. Review and evaluate the following three mutagenicity studies:

Study 1 - Sister Chromatid Exchange (SCE) in Chinese Hamster Ovary Cells with GX-071 (MRID No. 40612614). ADDENDUM TO THE FINAL REPORT (MRID No. 40915801), by Toxicon Corporation (Laxman S. Desai), Study No. 86G-002, ADDENDUM dated November 23, 1988.

Study 2 - Mutagenicity Test on GX-071 (Sulfluramide) in the Ames Salmonella/Microsome Reverse Mutation Assay, performed by Hazleton Labs America (HLA), HLA Study No. 10549-0-401, dated October 5, 1988 (MRID No. 40863201).

Study 3 - Mutagenicity Test on Sulfluramide (GX-071) in the Rat Primary Hepatocyte Unscheduled DNA Synthesis Assay, performed by Hazleton Labs America (HLA), HLA Study No. 10549-0-447, dated October 21, 1988 (MRID No. 40863202).

II. By letters of December 19, 1988, registrant has submitted the correct names and formulations for Raid Roach Controller II (1812-GEO, containing 1.020% ai) and for Raid Ant Controller II (1812-GEI, containing 0.5102% ai), information requested in our review of the acute oral LD_{50} rat studies (December 1, 1988).

TB Conclusions

I. Mutagenicity

Study 1 (No. 86G-002, SCE in vitro) has been reviewed previously (evaluation attached to memorandum: Taylor to Mendelsohn, date-stamped September 9, 1988, TB Doc. No. 006862), but declared UNACCEPTABLE, because the highest dose tested (1000 ug/mL) did not result in at least 50 percent reduction in second-mitotic cells (presumed evidence of cytotoxicity, by cell cycle delay). In response to this deficiency, the testing laboratory argued that solubility limitations precluded testing at higher concentrations; precipitation of compound was evident at 500 ug/mL, and at 1000 ug/mL, the chemical was stated to be only "partially soluble" (memorandum: Taylor to Mendelsohn, dated October 19, 1988). The registrant was requested to submit the solubility data as an addendum to the Final Report, in order to support this contention. Further, we pointed out that Tables I and III of the original report had typographical errors.

This ADDENDUM to Study 1 submitted (MRID No. 40915801) consists of three attachments:

Attachment I: Corrected Report Tables I and III, changing the cell number "x 10^{-4}" to 10^4 (I); and identifying the final assay as activated, rather than nonactivated (III).

Attachment II: A copy of the raw data sheet attesting that GX-071 was partially soluble at 1000 ug/mL (initial assessment).

Attachment III: Report and data sheet from a solubility recheck documenting increasing precipitation from 100 ug/mL ("slight") to 500 and 1000 ug/mL ("partially soluble"); concentrations of 50 ug/mL and below were stated to be "completely soluble."

This information and the additional data submitted satisfy the deficiencies noted in the initial review of this study, and it is upgraded to ACCEPTABLE in demonstrating negative results for inducing SCE in vitro (CHO cells).

Detailed reviews and evaluations of Studies 2 and 3 are attached to this memorandum. In brief, our assessments of these studies are as follows:

Study (MRID No.)	Type	Reported Results	TB Evaluation
2 (40863201)	Ames	Negative up to the limits of solubility (627 ug/ plate and above), and dosing (tested up to 10,000 ug/plate)	Acceptable
3 (40863202)	UDS	Negative for increased grain counts (UDS) at doses of 0.025 to 1.00 ug/mL. Insufficient dose tested since HDT resulted in 84 percent cell survival.	Unacceptable

II. Addenda to Acute Oral Rat LD_{50} Studies

We acknowledge receipt of the additional information requested in our review of these studies, specifically identification of the products and their purity used in the acute oral LD_{50} rat studies (G2.11 and G2.12).

These studies are now acceptable (upgraded to Core-Minimum).

Attachments

Reviewed By: Irving Mauer, Ph.D., Geneticist, JWA for DM 2/8/89
Toxicology Branch I - IRS (TS-769C)
Secondary Reviewer: Judith W. Hauswirth, Ph.D., Chief, JWA 2/8/89
Toxicology Branch I - IRS (TS-769C)

DATA EVALUATION REPORT

I. SUMMARY

TB Project No.: 9-0292
Caswell No.: 454E
MRID No.: 40863201
Shaughnessy No.: (N/A)

Study Type: Mutagenicity - Gene mutation in bacteria (Ames Assay)

Chemical: GX-071 [N-ethyl perfluoro-octanesulfonamide]

Synonyms: Sulfluramide

Sponsor: Griffin Corporation
Valdosta, GA

Testing Facility: Hazleton Labs America (HLA)
Kensington, MD

Title of Report: Mutagenicity Test on GX-071 (Sufluramide) in the Ames Salmonella/Microsome Reverse Mutation Assay.

Author: D.R. Jagannath

Study Number: HLA No. 10549-0-401

Date of Issue: October 5, 1988

TB Conclusions:

The test article was negative for increasing revertents (i.e., nonmutagenic) in the standard set of five Ames strains of Salmonella typhimurium treated up to 10,000 ug/plate (at which dose heavy precipitation occurred), both in the absence and presence of metabolic activation.

Classification (Core-Grade): ACCEPTABLE

II. DETAILED REVIEW

A. Test Material - GX-071 Technical

Description: White crystalline powder
Batch (Lot): AN-80271
Purity (%): (N/A)
Solvent/carrier/diluent: Dimethylsulfoxide (DMSO)

B. Test Organism - Bacterial cultures

Species: Salmonella typhimurium
Strains: TA1535, TA1537, TA1538, TA98, and TA100 (all his^-)
Source: Dr. B.N. Ames, University of California

C. Study Design (Protocol) - A formal protocol was not included, but a list of authoritative references was appended.

Statements attesting to adherence to both GLPs and a Quality Assurance audit were included in the Final Report.

D. Procedures/Methods of Analysis - Following preliminary range-finding with strain TA100, cultures of all five strains of bacteria were exposed by plate incorporation for 48 to 72 hours to graded concentrations of test material, and the number of his^+ revertents counted by machine (Biotran II Automatic Colony Counter), or manually (in the presence of excessive test material precipitation).

The test material was evaluated for genetic activity using triplicate plates per dose level, without and with the addition of a metabolic activation system consisting of the microsomal fraction in the S9 supernatant prepared from the liver of a Sprague-Dawley adult male rat induced with Aroclor 1254, plus NADPH-generating cofactors (S9 mix). Concurrent negative and positive controls were run with the solvent, DMSO ($\pm$ S9 mix), and with mutagens appropriate to each strain and activation condition.

All strains were checked regularly for genotypic characteristics, as well as the presence of plasmid.

Statistical analysis of the data was not employed. Instead, criteria for a positive response were based on finding dose-related increases in revertents over a minimum of three test concentrations, with the greatest increase at least three times (for TA1535, TA1537, and

TA1538) or twice (in the cases of TA98 and TA100) the solvent control value.

Only one complete assay was run.

Results:

In the preliminary range-finding test, TA100 cells were exposed to 14 concentrations of test material, ranging from 1.22 ug/plate to 10,030 ug/plate (Report Table 1). No toxicity was found at any dose, since the background lawn appeared to be unaffected, but increasing precipitation was recorded at 627 ug and above (1254, 2508, 5015, and 10,030 ug/plate). The doses selected for the main assay ranged from 100 to 10,000 ug/plate.

In the main mutagenicity assay, no increase in revertents was recorded at any dose in any strain, with or without S9 activation (Summary Report Table 3, attached here). Additionally, the test material was nontoxic since no significant decrease in revertents (or background lawn) was observed (individual plate counts were presented as Report Table 2).

By contrast, positive controls induced large increases in revertents, 12 to 130 times solvent controls.

The author concluded that GX-071 did not exhibit genetic activity in S. typhimurium under the conditions of the assay.

TB Evaluation:

ACCEPTABLE. Although only one complete assay was conducted, the results from triplicate cultures per dose point were consistent, and in all other respects, this study was conducted according to recognized procedures. We agree that the test material was not mutagenic in Ames testing up to limits of dosing and solubility.

Attachment

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Reviewed By: Irving Mauer, Ph.D., Geneticist, JMA for IM 2/8/89
Toxicology Branch I - IRS (TS-769C)
Secondary Reviewer: Judith W. Hauswirth, Ph.D., Chief,
Toxicology Branch I - IRS (TS-769C) JMA 2/8/89

DATA EVALUATION REPORT

I. SUMMARY

TB Project No.: 9-0292
Caswell No.: 454E
MRID No.: 40863202
Shaughnessy No.: (N/A)

Study Type: Mutagenicity - DNA repair in rat hepatocytes (HPC/UDS)

Chemical: GX-071 [S-ethyl perfluoro-octanesulfonamide]

Synonyms: Sulfluramide

Sponsor: Griffin Corporation
Valdosta, GA

Testing Facility: Hazleton Labs America (HLA)
Kensington, MD

Title of Report: Mutagenicity Test on Sulfluramide (GX-071) in the Rat Primary Hepatocyte Unscheduled DNA Synthesis Assay.

Authors: M.A. Cifone

Study Number: HLA 10549-0-477

Date of Issue: October 21, 1988

TB Conclusions:

The test article was tested in a single assay, with no increased grain counts (a measure of unscheduled DNA synthesis-repair) recorded at a dose range of 0.025 to 1.00 ug/mL, which generated relative cell survivals of 84 percent and greater.

Classification (Core-Grade):

UNACCEPTABLE, since the material was not tested to the limit of toxicity or solubility.

II. DETAILED REVIEW

A. Test Material - Sulfluramide

Description: White crystalline powder
Batch (Lot): AN-80271
Purity (%): (N/A)
Solvent/carrier/diluent: Dimethylsulfoxide (DMSO)

B. Test Organism - Primary mammalian cultures

Species: Rat
Strain: Hepatocytes from Fischer 344 males
Age: N/A
Weights - Males (only): 150 to 300 g
Source: Charles River

C. Study Design (Protocol) - No formal protocol was presented, but the author indicated the assay was based on referenced (published) procedures described by expert practitioners.

Statements attesting to adherence to GLPs as well as Quality Assurance audits were included.

D. Procedures/Methods of Analysis - After preliminary range-finding, freshly perfused hepatocytes from an adult male F-344 rat (HPC) were allowed to attach to plastic coverslips in small culture dishes, then treated with media containing 5 uCi/mL tritiated (^{3}H)-thymidine (20 Ci/mole) and one of six concentrations of test material (5 cultures per treatment, 2 of which were for cytotoxicity). After 18 to 20 hours, treatment was terminated, and coverslips with cells of three cultures per treatment were harvested under 1 percent sodium citrate (to swell nuclei) and acetic-ethanol fixation, then prepared for radioautography according to standard procedures. The remaining two cultures from each treatment were reincubated for up to 4 hours, then viable cell counts were determined (by trypan blue exclusion) to estimate cell survival relative to negative control.

After 7 to 10 days under refrigeration, radioautographic coverslip preparations were developed in standard photographic solutions and stained. A measure of unscheduled DNA synthesis (UDS) was made by counting silver grains over nuclei and background in 50 cells per coverslip, calculating net nuclear grain count, and comparing the mean count from the three coverslips per treatment to the mean control value.

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Concurrent cultures were treated with solvent (DMSO) or with 2-acetylaminofluorene (2AAF, 0.10 ug/mL), a mutagen known to induce UDS in rat HPC.

Stringent criteria for assay acceptance were followed in order to optimize validity of the results. These included suitable initial viability of hepatocytes collected by perfusion (should be at least 50%, preferably 70%); viability of monolayer cultures after attachment (90%), and after treatment (not less than 50%); a range of net nuclear labeling in controls of -5 to +1, with less than 10 percent of cells containing 6 or more grains; and a suitable response of positive control (e.g., with 2-AAF, 14.89 mean net grains, and > 90 percent of cells with 6 grains or more). In addition, the highest analyzable dose must approach the level of excessive toxicity, or be insoluble, or be at the limit, 5000 ug/mL (or 5000 mL/mL).

To be considered positive for inducing UDS, a test substance must:

i. Increase the mean net nuclear grain count by 6 per nucleus above the concurrent negative (solvent) value, and/or

ii. Increase the percent of nuclei that have 6 or more net grains to at least 10 percent of the population above concurrent control.

It is considered also desirable to obtain a dose-related response at at least two consecutive doses.

These criteria were employed in lieu of statistical treatment of the data.

Results:

The test material was stated to be "excessively toxic" at 2.51 ug/mL and above (but no data were presented), and declared to be precipitate at concentrations above 5 ug/mL. Cell survival increased at 1 ug/mL, and was no different from control at 0.5 ug/mL and below (Report Table 1, attached to this DER). Hence, the six treatments analyzed for grain counts ranged from 0.025 to 1.00 ug/mL.

None of these treatments with GX-071 caused nuclear labeling significantly different from control, in contrast to the response to 2-AAF (Table 1).

The author concluded that the test material, sulfluramide, did not induce significant nuclear labeling in rat HPC at a dose range 0.025 to 1.00 ug/mL.

TB Evaluation:

UNACCEPTABLE. The investigator did not honor her own criteria for dose selection. No data of a preliminary range-finding toxicity test were presented, and no data to support "excessive toxicity" at 2.51 ug/mL or precipitation at 5.01 ug/mL. The entire survival selection for analyzable cultures rested with the single UDS assay itself. Thus, the test material was not assayed to the limits specified by the lab's own criteria for assay acceptability, and certainly not ours.

Attachment