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

JAN 16 1997

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Suttocide A: Review of two mutagenicity studies

DP BARCODE: D225714
P.C. CODE: 128972
MRID No.: 43970501 & 43970502

SUBMISSION CODE: S504485
TOX. CHEM. NO.: Unknown

CHEMICAL (synonym): Sodium hydroxymethylamino acetate

TO: Steve Robbins
RCAB / HED (7509C)
&
V. Goncarovs/M. Johnson, PM Team 31
Registration Division (7505C)

FROM: Whang Phang, Ph.D. 1/10/97
Pharmacologist
Tox. Branch II/ HED (7509C)

THROUGH: James Rowe, Ph.D. James N. Rowe 1/15/97
Section Head, Section III
and
Mike Ioannou, Ph.D. J.M. Ioannou 1/15/97
Acting Branch Chief
Tox. Branch II/ HED (7509C)

The registrant, Sutton Laboratories, Inc., submitted a revised mutagenicity study (MRID 43970501) and an UDS assay in primary rat hepatocytes following in vivo exposure (MRID 43970502). The newly submitted reports were reviewed by Nancy McCarroll; the citation and conclusion of each study are presented sequentially as follows:

1. Stankowski, L.F. (1995) Revised rat hepatocyte primary culture/DNA repair test on Suttocide A. Supplement to : "Rat hepatocyte primary culture/DNA repair test on Suttocide A" MRID No. 41980432. Author, Juan R. SanSebastian. Pharmakon Research Internal, Inc., Waverly, PA. Study No. PH 311-SU-002-90. Study Date: Sep. 13, 1990. Revised report date: Jan. 19, 1995. (Unpublished) MRID 43970501.

In the previous report (MRID 41980432), the data on single scorable slide prepared from hepatocytes treated with 40 μg/ml were missing in the report. The study was classified as **unacceptable** (Tox. Doc. No. 010598). However, there was general agreement with the conclusion of the study author that Suttocide A did not induce genotoxic effect over a dose range of 2.5-20 μg/ml and that concentrations ≥60 μg/ml were severely cytotoxic. The failure to provide the grain count data for the scorable slide at 40 μg/ml was assessed by EPA reviewers as a study deficiency that could be corrected if the mean net nuclear counts from the dose group in question were furnished.

In response, the registrant has submitted the missing information. Based on the re-evaluation of the data and in the consideration of the additional information, it was concluded that Suttocide A, when tested up to a severely cytotoxic dose (40 μg/ml), was negative for the induction of UDS in cultured rat hepatocytes. The study is upgraded and reclassified as **Acceptable**, and it satisfies the guideline requirements [§84-2] for an unscheduled DNA synthesis assay.

2. San, R.H.C. and Raabe, H.A. (1994) In vivo/in vitro rat hepatocyte unscheduled DNA synthesis assay. Unpublished study conducted by Microbiological Associates, Inc.; Study No. TD994.381. April 28, 1994. MRID 43970502.

In an In vivo/in vitro rat hepatocyte unscheduled DNA synthesis (UDS) assay, groups of 10 male Fischer 344 rats were administered single oral gavage dose of 200, 700, or 2000 mg/kg Suttocide A/Integra 44 (50%) prepared in deionized water. An additional 3 male rats were added to the high dose group. Dosing solutions were adjusted to 100% active ingredient. The test animals (5/group) were sacrificed at 2-4 and 12-18 hours post-treatment and hepatocytes recovered from 3 rats/group/sacrificed time were scored for UDS.

The results showed that 7/13 high-dose rats died; lethargy was also seen at 700 and 2000 mg/kg. Cytotoxicity for the hepatocytes was not apparent at any dose. The results obtained with the positive controls confirmed the sensitivity of the test system to detect UDS. There was no evidence that the test material induced a genotoxic response at any dose or sacrifice time. The study is classified as **Acceptable**, and satisfies the guideline requirements for an unscheduled DNA synthesis assay (§84-2).

SUTTOCIDE A **IN VIVO UDS (84-2)**

Principal Reviewer: Nancy E. McCarroll
Review Section III, Toxicology Branch
II/HED (7509C)
Signature: Nancy E. McCarroll
Date: 5/23/96

Secondary Reviewer: Byron T. Backus, Ph.D.
Review Section II,
Toxicology Branch II/HED (7509C)
Signature: Byron T. Backus
Date: 5/23/96

DATA EVALUATION REPORT

STUDY TYPE: Mutagenicity: In vivo/in vitro unscheduled DNA synthesis assay in primary rat hepatocytes following in vivo exposure; OPPTS 870.5550 [§84-2]

DP BARCODE: D225714 SUBMISSION NO.: S504485

PC CODE: 128972 TOX. CHEM. NO.: MRID NO: 43970502

TEST MATERIAL (PURITY): Suttocide A/Integra 44 (50%)

SYNONYM(S): Sodium hydroxymethylglycinate (active ingredient)

CITATION: San, R.H.C. and Raabe, H.A. (1994) In Vivo-In Vitro Rat Hepatocyte Unscheduled DNA Synthesis Assay; Microbiological Associates, Inc., Bethesda/Rockville, MD; Report No. TD994.381; Study Completion Date: April 28, 1994. (Unpublished) MRID NUMBER: 43970502

SPONSOR: Sutton Laboratories, Chatham, NJ

EXECUTIVE SUMMARY: In an in vivo-in vitro rat hepatocyte unscheduled DNA synthesis (UDS) assay (MRID No: 43970502), groups of five male Fischer 344 rats per sacrifice time were received single oral gavage administrations of 200, 700 or 2000 mg/kg Suttocide A/Integra 44 (50%) prepared in deionized water. A satellite group consisting of three males also received the high dose and were held in reserve in the event of unscheduled deaths in the primary group. Dosing solutions were adjusted to 100% active ingredient (a.i.). Animals were sacrificed at 2-4 and 12-18 hours post-treatment and hepatocytes recovered from three rats per group per sacrifice time were scored for UDS.

Seven of 13 high-dose rats (including rats in a satellite group) died; lethargy was also seen at 2000 and 700 mg/kg. Cytotoxicity for the hepatocytes was not apparent at any dose. The results obtained with the positive controls confirmed the sensitivity of the test system to detect UDS. **There was, however, no evidence that the test material induced a genotoxic response at any dose or sacrifice time.**

The study is classified as Acceptable and satisfies the guideline requirements for an unscheduled DNA synthesis assay.

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

I. MATERIALS AND METHODS

A. MATERIALS:

1. Test Material: Suttocide A/Integra 44

Description: Clear liquid
Lot/batch number: SA-152
Purity: 50%
Receipt date: November 2, 1993
Stability: Based on analytical data (see Study Report pp 36-38), dose solutions were stable at room temperature for at least 4 months.
CAS number: 70161-44-3
Structure:
Vehicle used: Deionized water
Other provided information: The test material was stored at room temperature, protected from light. Dosing solutions were adjusted to 100% a.i and were prepared on the day of use. Analytical determinations were performed on dosing formulations used in the main study.

2. Control Materials:

Vehicle control/concentration/route of administration: Deionized water was administered at a dosing volume of 10 mL/kg.

Positive controls/concentration/route of administration:

- Methyl methanesulfonate (MMS) was administered at 200 mg/kg; hepatocytes were recovered 2-4 hour post-treatment.
- 2-Acetlyaminofluorene (2-AAF) at 100 mg/kg was used for the 12-18 hour harvest.

3. Medium: WME: Williams' Medium E supplemented with 10 mM HEPES buffer, 2 mM L-glutamine and antibiotics; WMEC: WME + 10% fetal bovine serum.

4. Test Compound:

Route of administration: Once by oral gavage (dosing volume = 10 mL/kg).

Dose levels:
Dose range-finding studies:

Trial I: 50, 150, 500, 1500 and 5000 mg/kg (5 ♂/group)
Trial II: 2000, 3000 and 4000 mg/kg (5 ♂/group)

UDS Assay: 200, 700 and 2000 mg/kg

5. Test Animals:

(a) Species: Rat; Strain: Fischer 344; Age (at arrival): ≈9-10 weeks; Sex: Males
Weight range (at randomization):
·Dose ranging-finding test I: 227.8-275.0 g
·Dose ranging-finding test II: 198.8-251.5 g
·UDS assay: 204.4-248.3 g
Source: Harlan Sprague Dawley, Inc. Frederick, MD

(b) Number of animals/dose:

Dose range-finding tests: 5 ♂/group

UDS assay (sacrifice at 2-4 and 12-18 hours postexposure):

- Treatment groups: 10 males (5/sacrifice time)
- Vehicle control: 10 males (5/sacrifice time)
- Positive controls: 10 males (5/positive control)

Note: Hepatocytes were prepared from three animals per group. An additional group of three animals received the high dose and were held in reserve in the event of unscheduled deaths in the primary group.

(c) Properly maintained? Yes.

B. TEST PERFORMANCE

1. UDS Assay:

(a) Perfusion techniques/hepatocyte harvest: At ≈2-4 and 12-18 hours postdosing, animals in the appropriate test material, vehicle or positive control groups were anesthetized with metofane and livers were perfused with a 0.5 mM EGTA solution and with an 80-100 IU/mL solution of collagenase. Livers were removed and hepatocytes were released by stirring in cold collagenase solution. Separated hepatocytes were centrifuged, resuspended in WMEC and assessed for viability by trypan blue exclusion. Approximately $5x10^5$ viable cells/dish, were seeded onto coverslips placed in culture dishes; six coverslips were made per suspension. Cultures were allowed to attach at 37°C with 5% CO_2 for ≈1.5-2.5 hours. Unattached cells were removed and viable cells were refed WME containing ^{3}H-thymidine (10 μCi/mL) for 4 hours. Cells were washed and reincubated ≈17-20 hours in WME with unlabeled thymidine (0.25 mM).

(b) Slide preparation: Hepatocytes attached to coverslips were washed, swollen in 1% sodium citrate, fixed in glacial acetic acid:ethanol, dried and mounted.

(c) Preparation of autoradiographies/grain development: At least three of six slides per animal were coated with Kodak NTB2 emulsion, exposed at 2-6°C in the dark for 10 days, developed in Kodak D-19, fixed, and stained with hematoxylin-sodium acetate-eosin. All slides were coded prior to analysis.

(d) Grain counting: Hepatocytes harvested from animals that were sacrificed at 2-4 and 12-18 hours postexposure were used to determine UDS. The grains of 150 morphologically normal cells (50/slide/animal) were counted. To determine the net nuclear grains (NNG), grains in one nuclear-size cytoplasmic area adjacent to each nucleus were counted and subtracted from the nuclear grain count. The percentage of cells in repair (i.e., cells having ≥ 5 NNG) was also determined. Means and standard deviations of the NNG counts were calculated.

(e) Statistical methods: The data were not evaluated for statistical significance.

2. Evaluation Criteria:

(a) Assay validity: The assay was considered acceptable if: (1) the percentage of cells in repair in the vehicle control group was <20% and (2) ≥5 NNG counts over the vehicle control were obtained in the positive control groups.

(b) Positive response: The assay was considered positive if the mean NNG count for any treatment group was ≥5 and the effect was dose related.

C. REPORTED RESULTS:

1. Dose Range-finding Studies:

(a) Trial I: Single oral gavage doses ranging from 50 to 5000 mg/kg were administered to groups of five male rats. Animals were observed for mortality and other clinical signs after dosing and daily, thereafter, for 7 days. Body weights were recorded prior to dosing and at 1, 3 and 7 days postadministration. All high-dose animals succumbed to treatment within 3 days of compound administration. No deaths or other clinical signs were reported for the lower treatment groups. Since the available data could not be used to establish an LD_{50}, a second trial was performed.

(b) Trial II: Trial II was conducted as described for Trial I with experimental doses of 2000, 3000 and 4000 mg/kg of the test material. Deaths occurred in all rats receiving 4000 mg/kg, 4/5 rats in the 3000-mg/kg group and 1/5 at 2000 mg/kg within 4 hours of dosing. By day 1 posttreatment, the remaining rat treated with 3000 mg/kg and two additional low-dose rats were found dead. Lethargy was noted prior to death and also in the animals that

survived exposure to the low dose. Based on these results, the LD_{50} was estimated to be 2080 mg/kg. Accordingly, the UDS assay was conducted with 200, 750 and 2000 mg/kg.

2. UDS Assay:

a. Analytical determinations: Dose formulations prepared for the 2-4 and 12-18 hour exposures were initially analyzed and found to contain appreciably higher levels of the test material than intended (≈40-55% higher). The study authors indicated that following discussions with the Sponsor's chemist, problem areas were identified and corrected. Reevaluation of the samples revealed that all dosing solutions were within ±14% of the intended concentrations.

b. Animal observations: Seven of the 13 rats exposed to 2000 mg/kg did not survive until the scheduled sacrifice. Lethargy was also recorded in the high-dose group and in 3/10 rats at 700 mg/kg.

c. Hepatocyte analysis: Data from hepatocyte harvests at 2-4 and 12-18 hours postexposure to Suttocide A/Integra 44 are summarized in Study Report Tables 3 and 4, pp. 20 and 21, respectively (see Attachment). As shown, neither the NNG counts nor the percentage of cells in repair for hepatocytes recovered from rats treated with 200, 700 or 2000 mg of the test substance and harvested at either 2-4 or 12-18 hours postexposure were appreciably increased compared to the vehicle control values. By contrast, the two positive controls (200 mg/kg MMS at 2-4 hours and 100 mg/kg 2-AAF at 12-18 hours) caused marked increase in UDS

From the overall results, the study authors concluded that Suttocide A/Integra 44 was not genotoxic in this whole animal UDS assay.

D. REVIEWERS' DISCUSSION/CONCLUSIONS: We assess that the study authors correctly interpreted the data. Suttocide A/Integra 44 was assayed up to a severely toxic dose (2000 mg/kg) but failed to increase the frequency of UDS in the treated rats. There was, however, no evidence of test material/target cell interaction. Results with the positive controls (200 mg/kg MMS and 100 mg/kg 2-AAF) demonstrated that the assay was sufficiently sensitive to detect genotoxicity. We conclude, therefore, that the study provided acceptable evidence that Suttocide A/Integra 44 was negative in this in vivo/in vitro rat hepatocyte UDS assay.

E. STUDY DEFICIENCIES: NONE

ATTACHMENT

STUDY REPORT TABLES 3 and 4, PP. 20 and 21

TABLE 3
SUMMARY OF UDS ASSAY WITH Suttocide A/Integra 44
2 – 4 Hours Post Exposure Harvest

TREATMENT	ANIMAL NO.	SLIDE CODE	NO. OF NUCLEI COUNTED	AVERAGE NET GRAINS PER NUCLEUS		S.D.	MEAN PER ANIMAL		S.D.	CELLS IN REPAIR	MEAN PER GROUP		S.D.#
Sterile Deionized Water (Vehicle Control)													
10.0 ml/kg	27	21A	50	-3.2	+/-	1.8	-3.0	+/-	2.0	0%	-2.8	+/-	0.3
		21B	50	-2.9	+/-	2.2							
		21C	50	-2.8	+/-	2.0							
	28	23A	50	-2.8	+/-	2.3	-2.9	+/-	2.4	0%			
		23B	50	-2.4	+/-	2.2							
		23C	50	-3.3	+/-	2.6							
	29	6A	50	-2.2	+/-	2.1	-2.5	+/-	2.2	0%			
		6B	50	-2.6	+/-	2.2							
		6C	50	-2.6	+/-	2.3							
Suttocide A/Integra 44													
200 mg/kg	31	8A	50	-2.2	+/-	2.0	-2.3	+/-	1.7	0%	-2.8	+/-	0.8
		8B	50	-2.6	+/-	1.5							
		8C	50	-2.0	+/-	1.6							
	32	26A	50	-3.2	+/-	2.3	-3.5	+/-	2.7	0%			
		26B	50	-3.7	+/-	3.1							
		26C	50	-3.5	+/-	2.5							
	33	13A	50	-2.6	+/-	1.8	-2.6	+/-	2.0	0%			
		13B	50	-2.7	+/-	2.5							
		13C	50	-2.5	+/-	1.5							
700 mg/kg	36	24A	50	-2.9	+/-	2.1	-2.9	+/-	2.0	0%	-2.5	+/-	0.4
		24B	50	-3.1	+/-	1.8							
		24C	50	-2.7	+/-	2.0							
	38	1A	50	-2.1	+/-	2.1	-2.1	+/-	1.8	0%			
		1B	50	-1.8	+/-	1.8							
		1C	50	-2.3	+/-	1.8							
	39	11A	50	-2.8	+/-	2.1	-2.6	+/-	2.2	0%			
		11B	50	-2.2	+/-	1.9							
		11C	50	-2.7	+/-	2.7							
2000 mg/kg	41	18A	50	-2.8	+/-	2.1	-2.6	+/-	2.2	0%	-2.6	+/-	0.4
		18B	50	-2.7	+/-	2.1							
		18C	50	-2.7	+/-	2.3							
	42	17A	50	-2.8	+/-	2.1	-2.9	+/-	2.0	0%			
		17B	50	-3.1	+/-	1.9							
		17C	50	-2.8	+/-	2.1							
	43	7A	50	-2.2	+/-	2.1	-2.2	+/-	2.0	0%			
		7B	50	-2.0	+/-	1.9							
		7C	50	-2.4	+/-	2.1							
MMS (Positive Control)													
200 mg/kg	47	4A	50	6.7	+/-	4.4	8.8	* +/-	4.8	82%	7.8	* +/-	7.7
		4B	50	8.3	+/-	4.2							
		4C	50	8.5	+/-	5.7							
	48	16A	50	15.5	+/-	6.2	14.6	* +/-	6.4	86%			
		16B	50	12.7	+/-	6.1							
		16C	50	15.5	+/-	6.8							
	50	22A	50	-0.5	+/-	3.8	-0.8	+/-	3.6	7%			
		22B	50	-0.6	+/-	3.9							
		22C	50	-0.7	+/-	3.0							

* Significant (see protocol Section 8.0, Evaluation of Test Results)

S.D.#: Standard deviation reflecting animal to animal variation

TABLE 4
SUMMARY OF UDS ASSAY WITH Suttocide A/Integra 44
12 – 18 Hours Post Exposure Harvest

TREATMENT	ANIMAL NO.	SLIDE CODE	NO. OF NUCLEI COUNTED	AVERAGE NET GRAINS PER NUCLEUS		S.D.	MEAN PER ANIMAL		S.D.	CELLS IN REPAIR	MEAN PER GROUP		S.D.#
Sterile Deionized Water (Vehicle Control)													
10.0 ml/kg	1	29A	50	-2.6	+/-	2.0	-3.0	+/-	2.1	0%	-2.6	+/-	0.4
		29B	50	-3.0	+/-	2.2							
		29C	50	-3.5	+/-	2.2							
	2	6A	50	-2.3	+/-	2.1	-2.5	+/-	1.8	0%			
		6B	50	-2.8	+/-	1.7							
		6C	50	-2.4	+/-	1.8							
	3	15A	50	-2.4	+/-	2.0	-2.3	+/-	2.0	0%			
		15B	50	-2.6	+/-	2.0							
		15C	50	-1.8	+/-	1.9							
Suttocide A/Integra 44													
200 mg/kg	6	5A	50	-1.3	+/-	1.6	-2.0	+/-	2.0	1%	-2.6	+/-	0.6
		5B	50	-2.2	+/-	2.1							
		5C	50	-2.8	+/-	2.2							
	7	30A	50	-3.0	+/-	2.3	-3.1	+/-	2.1	0%			
		30B	50	-3.0	+/-	2.2							
		30C	50	-3.2	+/-	1.8							
	8	20A	50	-2.8	+/-	2.6	-2.8	+/-	2.3	0%			
		20B	50	-2.6	+/-	2.0							
		20C	50	-2.9	+/-	2.1							
700 mg/kg	11	27A	50	-2.2	+/-	2.4	-2.8	+/-	2.2	0%	-2.8	+/-	0.6
		27B	50	-3.1	+/-	2.1							
		27C	50	-2.4	+/-	2.0							
	12	18A	50	-3.1	+/-	2.0	-3.3	+/-	1.9	0%			
		18B	50	-3.3	+/-	1.6							
		18C	50	-3.4	+/-	1.9							
	13	12A	50	-2.6	+/-	2.2	-2.4	+/-	2.2	0%			
		12B	50	-2.4	+/-	2.2							
		12C	50	-2.2	+/-	2.2							
2000 mg/kg	19	2A	50	-2.3	+/-	2.1	-2.1	+/-	2.1	0%	-2.5	+/-	0.5
		2B	50	-2.0	+/-	1.9							
		2C	50	-2.1	+/-	2.3							
	51	16A	50	-2.7	+/-	2.4	-2.3	+/-	2.2	0%			
		16B	50	-2.1	+/-	2.3							
		16C	50	-2.0	+/-	1.7							
	44	25A	50	-3.1	+/-	2.1	-3.0	+/-	2.1	0%			
		25B	50	-2.9	+/-	2.2							
		25C	50	-2.9	+/-	2.0							
2-AAF (Positive Control)													
100 mg/kg	21	26A	50	4.8	+/-	4.4	6.0	* +/-	5.2	58%	7.3	* +/-	2.0
		26C	50	7.4	+/-	6.0							
		26F	50	6.9	+/-	5.0							
	22	3A	50	10.6	+/-	5.7	8.6	* +/-	5.6	61%			
		3B	50	9.0	+/-	6.4							
		3C	50	8.3	+/-	4.8							
	23	14A	50	5.7	+/-	5.9	6.3	* +/-	5.5	56%			
		14B	50	6.7	+/-	5.4							
		14C	50	6.4	+/-	5.3							

* Significant (see protocol Section 8.0, Evaluation of Test Results)

S.D.#: Standard deviation reflecting animal to animal variation

SUTTOCIDE A **IN VITRO UDS (84-2)**

Principal Reviewer: Nancy E. McCarroll
Review Section III, Toxicology Branch
II/HED (7509C)
Secondary Reviewer: Byron T. Backus, Ph.D.
Review Section II,
Toxicology Branch II/HED (7509C)

Signature: Nancy E. McCarroll
Date: 5/23/96
Signature: Byron T. Backus
Date: 5/23/96

DATA EVALUATION REPORT

STUDY TYPE: Mutagenicity: In vitro unscheduled DNA synthesis assay in primary rat hepatocytes; OPPTS 870.5550 [§84-2]

DP BARCODE: D225714 SUBMISSION NO.: S504485

PC CODE: 128972 TOX. CHEM. NO.: MRID NOs: 43970500,01/41980432[1]

TEST MATERIAL (PURITY): Suttocide A® (50%)

SYNONYM(S): Sodium hydroxymethylglycinate (active ingredient)

CITATION: Stankowski, L.F. (1995) Revised Rat Hepatocyte Primary Culture/DNA Repair Test on Suttocide A Supplement to: "Rat Hepatocyte Primary Culture/DNA Repair Test on Suttocide A," MRID No. 41980432, Data Requirement: Guideline Reference No. 84-4, Author, Juan R. SanSebastian, Study No. PH 311-SU-002-90; Study Date: September 13, 1990; Pharmakon Research International, Inc., Waverly, PA; Revised Study Completion Date: January 19, 1995, (Unpublished) MRID NUMBER: 43970501

SPONSOR: Sutton Laboratories, Chatham, NJ

EXECUTIVE SUMMARY: Comments and additional data were received from the sponsor (MRID Nos. 43970500 and 01, respectively) regarding the EPA toxicology review, completed August 24, 1993 of an in vitro primary rat hepatocyte unscheduled DNA synthesis (UDS) assay with Suttocide A®[2].

EPA reviewers considered the study unacceptable because data were not provided from the single scorable slide prepared from hepatocytes treated with 40 µg/mL. There was general agreement with the conclusions of the study author that Suttocide A did not induce a genotoxic effect over a dose range of 2.5-20 µg/mL and that concentrations ≥60 µg/mL were severely cytotoxic. However, the failure to provide grain count data for the scorable slide at 40 µg/mL was assessed by EPA reviewers as a study deficiency that could be corrected if the mean net nuclear grain counts from the dose group in question were furnished. In response

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[1]Original report: SanSebastian, J.R. (1990); Rat Hepatocyte Primary Culture/DNA Repair Test on Suttocide A, (1990); prepared for Sutton Laboratories; Inc., Chatham by Pharmakon Research International, Inc., Waverly, PA; Study No. PH 311-SU-002-90; dated September 13, 1990(unpublished) MRID NO. 41980432.

[2]IBID.

to the Agency's position, the Sponsor's representative has submitted the requested information.

Based on a reevaluation of the data and in consideration of the additional information, we conclude that Suttocide A®, when tested up to a severely cytotoxic dose (40 µg/mL), was negative for the induction of UDS in cultured rat hepatocytes. **The study is, therefore, upgraded and reclassified as Acceptable.**

The study satisfies the guideline requirements [§84-2] for an unscheduled DNA synthesis assay. A detailed analysis of the additional data required for upgrading the study is presented below:

REVIEW OF ADDITIONAL DATA:

Summarized results from the scoring of 150 cells on the only slide with analyzable hepatocytes recovered from cultures treated with 40 µg/mL Suttocide A® are presented in Amended Study Report Table 1, p.17 (see Attachment I). The original Data Evaluation Report (DER) is attached as an addendum (see Attachment II). As shown in Amended Study Report Table 1, a slight increase in net nuclear grain counts (1.1 ± 11.4) was obtained at 40 µg/mL; the increase was, however, less than required for a positive response (i.e., ≥5 net nuclear grains). When evaluated in conjunction with the high percentage of cells in repair (29.3%), the findings could be construed as suggestive of a weak positive response[3]. However, we tend to agree with the study author's assessment that the increased percentage of cell in repair was probably associated with the severe cytotoxicity occurring at this dose level. The wide variation in the data as indicated by the high standard deviation, the lack of a response at 20 µg/mL and the clear evidence of cell lethality at 40 µg/mL supports this position. Similarly, the negative results of the in vivo/in vitro rat hepatocyte UDS assay conducted with Suttocide A® (see MRID No. 43970502) add additional strength to the argument. Based on these considerations, we conclude that Suttocide A® has been adequately tested in the primary rat hepatocyte UDS assay and found to be negative in this in vitro test system. The study is upgraded to Acceptable.

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[3]Barfknecht, T.R., Naismith, R.W. and Kornbrust, D.J. (1987). Variations on the Standard Protocol Design of the Hepatocyte DNA Repair Assay. Cell Biol. and Tox. 3:193-207.

ATTACHMENT I

AMENDED STUDY REPORT TABLE 1, P. 17

TABLE 1. Autoradiographic Analysis of DNA Repair Induced by Suttocide A®

Test or Control Article	Concentration (μg/mL)	Net Nuclear Grains[a] ($\bar{x}$ ± 1 SD)	Cells in Repair (%)[b]
Untreated	0	-3.1 ± 3.2	0
2AAF	0.1[c]	9.8 ± 4.9*	95
Suttocide A®[d]	2.5	-3.3 ± 2.6	0
Suttocide A®	7.5	-4.2 ± 2.2	0
Suttocide A®	10	-2.7 ± 2.7	0
Suttocide A®	20	-3.7 ± 3.3	2
Suttocide A®	40[e]	1.1 ± 11.4	29.3

[a]As described in the text, 150 cells were scored for nuclear incorporation of ^{3}H-thymidine (50/triplicate coverslip).
[b]Percent cells with a NNG value ≥5.
[c]μM.
[d]All doses of Suttocide A® are corrected for active ingredient.
[e]Only one coverslip contained sufficient scorable cells due to severe toxicity. Thus, the 150 cells scored came from a single culture.
*Positive response (average NNG ≥5).

ATTACHMENT II

DATA EVALUATION RECORD MRID NO. 41980432

FINAL

DATA EVALUATION REPORT

SUTTOCIDE® A

Study Type: Mutagenicity: Unscheduled DNA Synthesis (UDS) Assay in Primary Rat Hepatocytes

Prepared for:

Health Effects Division
Office of Pesticide Programs
Environmental Protection Agency
1921 Jefferson Davis Highway
Arlington, VA 22202

Prepared by:

Clement International Corporation
9300 Lee Highway
Fairfax, VA 22031-1207

Principal Reviewer _____ Date 8/13/93
Dean Walton, Ph.D.

Independent Reviewer _____ Date 8/13/93
Nancy E. McCarroll, B.S.

QA/QC Manager _____ Date 8/13/93
Sharon Segal, Ph.D.

Contract Number: 68D10075
Work Assignment Number: 2-99
Clement Number: 282
Project Officer: Caroline Gordon

MUTAGENICITY STUDIES

EPA Mutagenicity Reviewer: Byron T. Backus, Ph.D.
Review Section II,
Toxicology Branch II/HED H7509C
EPA Reviewer: Whang Phang, Ph.D.
Review Section III,
Toxicology Branch II/HED H7509C
EPA Section Head: James Rowe, Ph.D.
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Toxicology Branch II/HED H7509C

Signature: Byron T. Backus
Date: 8/27/93

Signature: W. Phang
Date: 8/24/93

Signature: James N Rowe
Date: 8/24/93

DATA EVALUATION REPORT

CHEMICAL: Suttocide® A

Tox Chem Number: 128972

P.C. Code:

STUDY TYPE: Mutagenicity: Unscheduled DNA synthesis assay in primary rat hepatocytes.

MRID Number: 419804-32

SYNONYM/CAS No.: Sodium hydroxymethylglycinate (active ingredient)

SPONSOR: Sutton Laboratories, Inc., Chatham, NJ

TESTING FACILITY: Pharmakon Research International, Inc., Waverly, PA

TITLE OF REPORT: Rat Hepatocyte Primary Culture/DNA Repair Test on Suttocide A

AUTHOR: J.R. SanSebastian

STUDY NUMBER: PH 311-SU-002-90

REPORT ISSUED: September 13, 1990

CONCLUSIONS-EXECUTIVE SUMMARY: Negative for inducing unscheduled DNA synthesis (UDS) in primary rat hepatocytes treated with doses of the test material up to 20 µg/mL. Concentrations ≥60 µg/mL were severely cytotoxic. At 40 µg/mL, 1/3 coverslips were reported to be scorable, but no grain counts were reported for this coverslip.

STUDY CLASSIFICATION: Unacceptable. This study does not satisfy the requirements for FIFRA Test Guideline 84-4 for a UDS study (other genotoxic effects). This study can be upgraded to acceptable if mean net nuclear grain

count data from at least 50 cells are provided from the one 40-µg/mL coverslip reported as scorable.

A. MATERIALS:

1. Test Material: Suttocide® A

Description: Clear, colorless solution
Lot number: PL1-4D
Purity: Reported to be a 50% (w/v) aqueous solution
Receipt date: February 15, 1990
Stability: Not reported
Contaminants: Not reported
Solvent used: Williams' Medium E (reported as Williams' Medium A in the report summary)
Other provided information: The test material was stored at ambient temperature in its shipping container. The material was analyzed to verify the targeted concentrations; however the solvent interfered with the analysis and the results were inconclusive. Dosing solutions were prepared the day of the assay. "A stock solution of 20,000 µg/mL of Suttocide® A (50% w/v) was dissolved in WME serum-free medium to obtain a 100% pure solution of 10,000 µg/mL Suttocide® A."

2. Indicator Cells: Rat hepatocytes, collected from a 229-g adult male rat (Strain: Fischer-344, Taconic Farms).

3. Control Substances:

- The positive control was $1x10^{-7}$ M (=0.025 µg/mL) 2-Acetamidofluorene (2AAF)
- Williams' Medium E (WME) served as the negative control

4. Medium: WME; WME+: WME plus 10% calf serum

5. Test Compound Concentrations Used:

- 0.75, 2.5, 5, 7.5, 10, 20, 40, 60, 80, and 100 µg/mL were tested; cells exposed to 2.5, 7.5, 10, and 20 µg/ml were scored.

B. STUDY DESIGN:

1. Cell Preparation:

(a) Perfusion technique: The liver was perfused with media A [0.5 mM ethylene-glycol-bis-(B-aminoethyl ether) N-N'-2-tetraacetic acid (EGTA) in Ca^{++}-Mg^{++} free Hank's balanced salt solution buffered with 10 mM N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid (HEPES) followed by media B [collagenase 100

units/mL in WME buffered with 10 mM HEPES, pH 7.35]. After perfusion, the liver was excised, placed in WME, rinsed and transferred to fresh medium B; the hepatocytes were dispersed.

(b) Hepatocyte harvest/culture preparation: Cells were dispensed into tubes with WME+ and allowed to flocculate for 10 minutes. Cell viability was determined by trypan blue exclusion and $\approx 1 \times 10^5$ viable cells were inoculated into each well of replicate cluster culture dishes containing WME+ and plastic coverslips. Cells were incubated for a 2-hour attachment period.

4. UDS Assay:

(a) Treatment: The UDS assay was initiated by adding the selected dose of the test material or positive control, and [^{3}H]-thymidine (10 µCi/mL; 50-80 µCi/mmol) to the prepared monolayers. The hepatocytes were incubated for 18-20 hours. Cells were washed three times with phosphate buffered saline. The cells were swollen with 1% sodium citrate and fixed in ethanol:acetic acid (3:1). The coverslips were rinsed and mounted onto slides. Each slide was dipped in NTB photographic emulsion in the dark and dried overnight.

(b) Preparation of autoradiographs/grain development: Slides were exposed for 7 days at 4°C in light-proof boxes containing desiccant, developed and stained with hematoxylin-eosin. Slides were coded prior to analysis.

(c) Grain counting: The nuclear and cytoplasmic grains of 150 cells (50/coverslip) per treatment were counted. The net nuclear grain counts were quantitated by subtracting the highest cytoplasmic grain count of three nuclear-sized areas adjacent to each nucleus from the nuclear grain count. Cells in S-phase were not scored.

5. Evaluation Criteria:

(a) Assay validity: The assay was considered valid if: (1) the solvent and/or the untreated controls had a net nuclear grain count of ≤ 1; and (2) the positive control induced a mean net nuclear grain count of ≥ 5 with 70-100% of the cells in repair.

(b) Positive response: The test material was considered to be positive if: (1) a mean net nuclear grain count of ≥ 5 grains/nucleus was consistently observed in triplicate wells; and (2) the response was dose related.

6. Statistical Methods: The data were not analyzed for statistical significance.

C. REPORTED RESULTS: Ten doses, ranging from 0.75 µg/mL to 100 µg/mL of the test material were examined in a parallel cytotoxicity and UDS assay. A review of the primary data accompanying the study report indicated that doses ≥60 µg/mL were severely cytotoxic. At 40 µg/mL, one of three coverslips was reported as scorable, with very few cells present on the two remaining coverslips. Accordingly, hepatocytes treated with 2.5, 7.5, 10, or 20 µg/mL of the test material were scored for the incorporation of tritiated thymidine. Representative findings presented in Table 1 show that the selected doses of Suttocide® A did not induce a genotoxic effect. By contrast, marked increases in the net nuclear grain counts and the percentage of cells in repair were observed in hepatocytes exposed to the positive control (10^{-7} M 2AAF).

Based on the overall results, the study author concluded that Suttocide® A was not genotoxic in this test system.

D. REVIEWERS' DISCUSSION/CONCLUSIONS: We agree with the study author's conclusions that Suttocide® A did not induce genotoxic effects at the concentrations (2.5-20 µg/mL) evaluated in this rat hepatocyte DNA repair assay. In addition, the sensitivity of the test system to detect UDS was adequately demonstrated by the results obtained with the positive control (10^{-7} M 2AAF). However, one of the three coverslips prepared at 40 µg/mL was reported to be scorable, but mean net nuclear grain count data were not provided.

E. QUALITY ASSURANCE MEASURES: Was the test performed under GLPs? Yes. (A quality assurance statement was signed and dated March 26, 1991.)

CORE CLASSIFICATION: Unacceptable. This study does not satisfy the requirements for FIFRA Test Guideline 84-4 for other genotoxic effects. This study can be upgraded to acceptable if the mean net nuclear grain count data are provided from the one 40-µg/mL exposure level coverslip reported as being scorable.

TABLE 1. Results of the Unscheduled DNA Synthesis (UDS) Rat Hepatocyte Assay with Suttocide® A

Treatment	Dose/mL	Number of Cells Scored/ Group	Net Nuclear Grains[a]	Percent of Cells in Repair (≥5 Net Nuclear Grains)
Negative Control				
Culture medium	--	150	-3.1±3.2	0
Positive Control				
2-Acetamidofluorene	$1x10^{-7}$ M	150	9.8±4.9[b]	95
Test Material				
Suttocide® A	10 µg[c]	150	-2.7±2.7	0
	20 µg	150	-3.7±3.3	2

[a]Mean and standard deviations of net nuclear grain counts for 150 cells; 50 cells from each of three slides per group were analyzed.
[b]Fulfills the reporting laboratory's criteria for an acceptable positive control (i.e., mean net nuclear grain count ≥5 with >70% of the cells in repair)
[c]Lower doses (2.5 or 7.5 µg/mL) did not suggest a genotoxic effect.

Note: Data were extracted from the study report p. 14.