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UNITED STATES ENVIRONMENTAL PROTECTION AGENCY

WASHINGTON, DC 20460

007827

MAR 21 1990

OFFICE OF
PESTICIDES AND
TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: REVIEW OF STUDIES REQUIRED IN THE CONDITIONAL REGISTRATION OF SULFLURAMID - DERMAL SENSITIZATION STUDY IN GUINEA PIGS AND RAT PRIMARY HEPATOCYTE UNSCHEDULED DNA SYNTHESIS ASSAY

TO: MICHAEL MENDELSOHN
PRODUCT MANAGER (17)
REGISTRATION DIVISION (H7505C)

FROM: LINDA L. TAYLOR, PH.D. Linda Lee Taylor 3/12/90
TOXICOLOGY BRANCH II, SECTION II
HEALTH EFFECTS DIVISION (H7509C)

THRU: K. CLARK SWENTZEL K. Clark Swentzel 3/16/90
SECTION II HEAD, TOXICOLOGY BRANCH II
HEALTH EFFECTS DIVISION (H7509C)

AND

MARCIA VAN GEMERT, PH.D. concurrence 3/19/90
CHIEF, TOXICOLOGY BRANCH/HFAS/HED (H7509C)

REGISTRANT:	GRIFFIN CORPORATION
CHEMICAL:	SULFLURAMID
SYNONYM:	GX-071
PROJECT:	0-0117
CASWELL NO.:	454E
RECORD NO.:	254401
IDENTIFYING NO.:	1812-327
MRID NO.:	412510-01 & 412510-02
ACTION REQUESTED:	PLEASE REVIEW STUDIES SUBMITTED IN RESPONSE TO CONDITIONAL REGISTRATION.

COMMENT: IN RESPONSE TO THE NOTICE OF PESTICIDE REGISTRATION FOR SULFLURAMID (DATED MARCH 23, 1989), A DERMAL SENSITIZATION STUDY AND AN UNSCHEDULED DNA SYSTHESIS ASSAY WERE SUBMITTED BY THE REGISTRANT.

UNDER THE CONDITIONS OF THE DERMAL SENSITIZATION STUDY, SULFLURAMID WAS NOT SHOWN TO BE A SKIN SENSITIZER. THE STUDY IS CLASSIFIED AS CORE: SUPPLEMENTARY, PENDING CLARIFICATION OF THE PROCEDURE USED FOR TEST MATERIAL APPLICATION AND IDENTIFICATION OF THE METHOD (REFERENCE) UTILIZED.

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WITH REGARD TO THE UDS ASSAY, UNDER THE CONDITIONS OF THE ASSAY, FOUR DOSES OF SULFLURAMIDE (GX-071) DID NOT INDUCE AN APPRECIABLE INCREASE IN THE NET NUCLEAR GRAIN COUNTS OF TREATED RAT HEPATOCYTES. CYTOTOXICITY WAS CLEARLY DEMONSTRATED AT CONCENTRATIONS $\geq$ 1.5 UG/ML. IT IS CONCLUDED, THEREFORE, THAT GX-071 IS NOT GENOTOXIC IN THE PRIMARY RAT HEPATOCYTE UNSCHEDULED DNA SYNTHESIS ASSAY. HOWEVER, SINCE INFORMATION ON THE TEST MATERIAL PURITY AND ANALYTICAL DATA TO VERIFY ACTUAL TEST MATERIAL CONCENTRATIONS USED WERE NOT PROVIDED, THE STUDY IS CLASSIFIED UNACCEPTABLE.

REVIEWED BY: LINDA L. TAYLOR, PH.D. Linda Lee Taylor 1/3/90
SECTION II, TOX. BRANCH II (H7509C)
SECONDARY REVIEWER: K. CLARK SWENTZEL K. Clark Swentzel 1/4/90
HEAD SECTION II, TOX. BRANCH II (H7509C)

007821

DATA EVALUATION REPORT

STUDY TYPE: DELAYED CONTACT HYPERSENSITIVITY GUINEA PIGS TOX CHEM NO: 454E

MRID NO: 412510-01

TEST MATERIAL: SULFLURAMID

SYNONYMS: GX-071

STUDY NUMBER: LABORATORY PROJECT ID 246722

SPONSOR: GRIFFIN CORPORATION

TESTING FACILITY: EXXON BIOMEDICAL SCIENCES, INC. TOXICOLOGY LABORATORY EAST MILLSTONE, NJ 08875-2350

TITLE OF REPORT: (SULFLURAMID)-DERMAL SENSITIZATION TEST IN GUINEA PIGS.

AUTHORS: GARY W. TRIMMER

REPORT ISSUED: AUGUST 25, 1989

CONCLUSIONS: UNDER THE CONDITIONS OF THE STUDY, SULFLURAMID WAS NOT SHOWN TO BE A SKIN SENSITIZER.

CLASSIFICATION: CORE SUPPLEMENTARY, PENDING CLARIFICATION OF TEST MATERIAL ADMINISTRATION AND IDENTIFICATION OF THE METHOD USED.

A. MATERIALS:

1. TEST COMPOUND: SULFLURAMID
   DESCRIPTION: WHITE POWDER
   BATCH #: AN-90042; EXXON ID# MRD-89-467 (BATCHES I & II)
   PURITY: [redacted] ISOMERS

2. TEST ANIMALS
   SPECIES: GUINEA PIG
   STRAIN: HARTLEY ALBINO
   AGE: APPROXIMATELY 5 WEEKS AT STUDY INITIATION
   WEIGHT: 300-350 G (FEMALES)
   SOURCE: HAZLETON RESEARCH PRODUCTS, INC. DENVER, PA

MANUFACTURING PROCESS INFORMATION IS NOT INCLUDED

STUDY DESIGN: THE METHOD USED WAS NOT IDENTIFIED. BODY WEIGHTS WERE OBTAINED FOR EACH ANIMAL PRIOR TO TREATMENT AND ON DAYS 7, 14, 21, 28, 35, AND AT TERMINAL SACRIFICE. THE ANIMALS WERE ASSIGNED TO THEIR GROUPS USING A COMPUTER GENERATED, BODY-WEIGHT SORTING, PROGRAM. THE ANIMALS HAD FREE ACCESS TO FOOD (AGWAY PROLAB CERTIFIED GUINEA PIG DIET) AND WATER. CLINICAL OBSERVATIONS (NATURE, ONSET, SEVERITY, AND DURATION) OF TOXICOLOGICAL SIGNS WERE MADE AFTER DOSING ON DAY 0 AND ON DAYS 7, 14, 21, 28, 35, AND PRIOR TO TERMINAL SACRIFICE. VIABILITY CHECKS WERE PERFORMED DAILY. THE ANIMALS WERE DOSED AS FOLLOWS.

GROUP	# OF ANIMALS	TEST MATERIAL	INDUCTION DOSE	CHALLENGE DOSE
TREATED	10	SULFLURAMID (100%)	0.3 G	0.3 G
IRRITATION CONTROL	5	SULFLURAMID (100%)	NONE	0.3 G
POSITIVE CONTROL	10	DNCB (0.1%)	0.4 ML	0.4 ML

THE TEST MATERIAL (SULFLURAMID) WAS A POWDER AND WAS USED AS RECEIVED (100%), SINCE AN IRRITATING DOSE WAS NOT FOUND IN THE PRIMARY IRRITANCY TEST. THE TEST MATERIAL WAS MOISTENED WITH PHYSIOLOGICAL SALINE PRIOR TO DERMAL APPLICATION. THE VEHICLE FOR THE POSITIVE CONTROL, 1 CHLORO-2,4-DINITROBENZENE (DNCB), WAS 70% ETHANOL FOR THE INDUCTION PHASE (DNCB WAS DISSOLVED IN ACETONE EQUAL TO ABOUT 2% OF THE TOTAL MIXTURE WEIGHT AND THEN TOPPED OFF WITH 70% ETHANOL) AND ACETONE FOR THE CHALLENGE PHASE.

INDUCTION PHASE - THE SCAPULAR REGION OF EACH TREATED ANIMAL WAS CLIPPED ONE DAY PRIOR TO EACH TOPICAL INDUCTION OF TEST MATERIAL. THE IRRITATION CONTROL ANIMALS WERE CLIPPED ONCE PRE-TEST IN THE FLANK AREA. THE TEST MATERIAL (0.3 GRAMS) WAS STATED TO BE TOPICALLY APPLIED TO THE DORSAL SURFACE UNDER OCCLUSIVE BANDAGING; THE IRRITATION CONTROL ANIMALS REMAINED UNTREATED. AFTER APPROXIMATELY 6 HOURS OF EXPOSURE, THE BANDAGES WERE REMOVED FROM THE TREATED ANIMALS, AND THE SKIN WAS GENTLY WIPED FREE OF REMAINING TEST MATERIAL WITH DRY SURGICAL GAUZE. DERMAL RESPONSES WERE EVALUATED APPROXIMATELY 24 AND 48 HOURS AFTER EACH INDUCTION (ALTHOUGH THERE IS NO MENTION OF REPEATED INDUCTION EXPOSURES AND THE TIME INTERVAL BETWEEN EACH, EXCEPT IN THE SUMMARY OF THE STUDY IT IS MENTIONED THAT THERE WERE 9 INDUCTION EXPOSURES).

NOTE: THERE IS SOME CONFUSION AS TO HOW THE TEST MATERIAL WAS APPLIED. AT THE TOP OF PAGE 16, IT IS STATED THAT THE POWDER WAS MOISTENED WITH PHYSIOLOGICAL SALINE PRIOR TO DERMAL APPLICATION. HOWEVER, ON THE SAME PAGE UNDER ADMINISTRATION OF TEST MATERIAL, IT IS STATED THAT THE TEST MATERIAL WAS TOPICALLY APPLIED TO THE PREVIOUSLY CLIPPED AREA AND MOISTENED WITH PHYSIOLOGICAL SALINE, AND FURTHER ON IN THE SAME PARAGRAPH IT IS STATED THAT THE TEST MATERIAL WAS APPLIED ON A 37 X 40 MM REDI-BANDAGE AND FIRMLY SECURED TO THE TORSO WITH ELASTIC ADHESIVE BANDAGING. ADDITIONALLY, THERE WAS NO MENTION OF HOW THE POSITIVE CONTROL ANIMALS WERE TREATED IN THE EXPERIMENTAL DESIGN SECTION, ALTHOUGH IN APPENDIX E IT IS STATED THAT THE DNCB TREATED GROUP WAS HANDLED IN A MANNER SIMILAR TO THAT FOR THE TEST MATERIAL ANIMALS.

CHALLENGE PHASE - THE FUR IN AN AREA ON THE RIGHT FLANK IN THE ABDOMINAL REGION WAS CLIPPED APPROXIMATELY 4.5 HOURS PRIOR TO THE CHALLENGE DOSE OF THE TEST MATERIAL. A NON-IRRITATING CONCENTRATION OF TEST MATERIAL (FROM THE PRIMARY IRRITANCY TEST) WAS TOPICALLY APPLIED TO BOTH TREATED AND IRRITATION CONTROL ANIMALS 14 DAYS AFTER THE FINAL INDUCTION (DAY 33 AFTER THE INITIAL TOPICAL INDUCTION. ON PAGE 17 AT THE TOP, IT STATES THAT THE TEST MATERIAL WAS TOPICALLY APPLIED TO THE CLIPPED AREA; IN THE NEXT SENTENCE IT STATES THAT THE TEST MATERIAL WAS APPLIED ON A HILLTOP CHAMBER AND FIRMLY SECURED TO THE TORSO WITH ELASTIC ADHESIVE BANDAGING. THE BANDAGING WAS REMOVED AFTER APPROXIMATELY 6 HOURS. ON DAY 34, APPROXIMATELY 21 HOURS AFTER REMOVAL OF THE CHALLENGE PATCH, THE CHALLENGE AREA WAS CLEANED WITH PHYSIOLOGICAL SALINE AND THE SITE WAS CLIPPED. DERMAL RESPONSES WERE EVALUATED AT APPROXIMATELY 24, 48, AND 72 HOURS AFTER REMOVAL OF THE CHALLENGE PATCH ACCORDING TO THE DRAIZE METHOD. ALL ANIMALS WERE WEIGHED, SACRIFICED, AND DISCARDED WITHOUT FURTHER EXAMINATION AFTER THE LAST DERMAL OBSERVATIONS.

STATISTICS - GROUP MEANS AND STANDARD DEVIATIONS OF THE BODY WEIGHTS WERE CALCULATED.

RESULTS: ALL ANIMALS SURVIVED TO TERMINATION, AND ALL DISPLAYED AN OVERALL INCREASE ON BODY WEIGHT. IT IS TO BE NOTED THAT THE TEST MATERIAL ANIMALS DISPLAYED A MEAN OVERALL GAIN THAT WAS 26 GRAMS LESS THAN THAT OF THE IRRITATION CONTROL ANIMALS, WHILE THE POSITIVE CONTROL ANIMALS DISPLAYED A 6 GRAM MEAN INCREASE OVER THE IRRITATION CONTROL VALUE (SEE BELOW).

	Body-Weight Gain		
Interval	Sulfluramid	Irritation Control	Positive Control
Pre-test - Day 0	10.1	13.6	8.8
Day 0 - Day 7	35.1	36.8	35.6
Day 7 - Day 14	19.1	31.0	36.7
Day 14 - Day 21	50.2	49.4	43.2
Day 21 - Day 28	21.6	49.8	40.4
Day 28 - Day 35	39.3	15.6	34.1
Day 35 - Term.	2.5	6.6	10.3
Starting body weight	320.9	321.4	321.3
Terminal body weight	498.8	524.4	530.4

THERE WERE NO DIFFERENCES REPORTED IN THE CLINICAL SIGNS AMONG THE GROUPS. NO SIGNS OF IRRITATION WERE OBSERVED DURING EITHER THE INDUCTION OR CHALLENGE PHASE OF THE STUDY IN EITHER THE TEST-MATERIAL OR IRRITATION-CONTROL ANIMALS. THE POSITIVE CONTROL ANIMALS DISPLAYED DERMAL IRRITATION AFTER 4 INDUCTION DOSES AND DURING THE CHALLENGE PHASE ALL ANIMALS DISPLAYED SIGNS OF DERMAL SENSITIZATION. AFTER THE CHALLENGE DOSING, ERYTHEMA SCORES RANGED FROM VERY SLIGHT TO SEVERE IN ALL ANIMALS, AND EDEMA SCORES RANGED FROM VERY SLIGHT TO SLIGHT IN 8 OF THE ANIMALS.

CONCLUSION

UNDER THE CONDITIONS OF THE STUDY, SULFLURAMID WAS NOT SHOWN TO BE A SKIN SENSITIZER. THE STUDY IS CLASSIFIED AS CORE: SUPPLEMENTARY, PENDING CLARIFICATION OF TEST MATERIAL ADMINISTRATION AND IDENTIFICATION OF (REFERENCE) THE METHOD USED.

EPA No.: 68D80056
DYNAMAC No.: 264-A
TASK No.: 2-64A
March 12, 1990

007827

DATA EVALUATION RECORD

SULFLURAMIDE (GX 071)

Mutagenicity--Rat Primary Hepatocyte Unscheduled DNA Synthesis Assay

APPROVED BY:

Robert J. Weir, Ph.D.
Program Manager
Dynamac Corporation

Signature: Robert J. Weir

Date: 3/12/90

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EPA No.: 68D80056
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TASK No.: 2-64A
March 12, 1990

DATA EVALUATION RECORD

SULFLURAMIDE (GX 071)

Mutagenicity--Rat Primary Hepatocyte Unscheduled DNA Synthesis Assay

REVIEWED BY:

Nancy E. McCarroll, B.S.
Principal Reviewer
Dynamac Corporation

Signature: Nancy E. McCarroll
Date: 3-9-90

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Date: 3-9-90

APPROVED BY:

Roman J. Pienta, Ph.D.
Department Manager
Dynamac Corporation

Signature: Roman J. Pienta
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Linda L. Taylor, Ph.D.
EPA Reviewer, Section II
Toxicology Branch II

Signature: Linda Lee Taylor
Date: 3-12-90

K. Clark Swentzel
EPA Section Head, Section II
Toxicology Branch II

Signature: K. Clark Swentzel
Date: 3/16/90

DATA EVALUATION RECORD

CHEMICAL: Sulfluramide (GX 071).

STUDY TYPE: Mutagenicity--Rat primary hepatocyte unscheduled DNA synthesis assay.

MRID NUMBER: 412510-02.

TEST MATERIAL: Sulfluramide.

SYNONYMS: None listed.

SPONSOR: Griffin Corporation, Valdosta, GA.

TESTING FACILITY: Hazleton Laboratories America, Inc., Kensington, MD.

TITLE OF REPORT: Mutagenicity Test on Sulfluramide (GX 071) in the Rat Primary Hepatocyte Unscheduled DNA Synthesis Assay.

AUTHOR: Cifone, M. A.

STUDY NUMBER: 10549-1-447.

REPORT ISSUED: September 13, 1989.

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CONCLUSIONS - Executive Summary: Under the conditions of the assay, four doses of sulfluramide (GX 071) (0.5, 0.75, 1.0, and 1.25 μg/mL) did not induce an appreciable increase in the net nuclear grain counts of treated rat hepatocytes. Cytotoxicity was clearly demonstrated at concentrations ≥1.5 μg/mL. It is concluded, therefore, that sulfluramide (GX 071) is not genotoxic in the primary rat hepatocyte unscheduled DNA synthesis (UDS) assay. However, the study was incomplete because information on test material purity and analytical data to verify actual test material concentrations used in the assay were not reported.

Study Classification: The study is currently unacceptable, but can be upgraded if the missing test material information is furnished by the study author and/or sponsor.

A. MATERIALS:

1. Test Material:

Name:	Sulfluramide (GX 071)
Description:	White crystalline powder
Batch #:	AN-80271
Purity:	Not reported
Contaminants:	None listed
Solvent used:	Dimethylsulfoxide (DMSO)
Other comments:	The test material, at a concentration of 2.5 mg/mL, formed a clear colorless solution in DMSO. Stock solutions were prepared immediately prior to use.

2. Indicator Cells: Primary rat hepatocytes were obtained by the in situ perfusion of the liver of an adult male Fischer 344 rat (150-300 g) purchased from Harlan Sprague Dawley Laboratories, Inc.

3. Positive Control: The positive control chemical used to demonstrate assay sensitivity was 2-acetylaminofluorene (2-AAF) at a concentration of 0.10 μg/mL.

B. STUDY DESIGN:

1. Cell Preparation:

a. Perfusion Technique: The liver was perfused with Hanks' balanced salts containing 0.5 mM EGTA and HEPES buffer, pH 7.2, for 4 minutes and with 50 to 100 U/mL collagenase for 10 minutes. The liver was excised, removed to a culture dish containing Williams' Medium E (WME) and collagenase, and mechanically dispersed to release the hepatocytes.

b. Hepatocyte Harvest/Culture Preparation: Recovered cells were centrifuged, resuspended in WME containing

serum, L-glutamine, and antibiotics (WMEI), counted, and aliquoted (0.5×10^6 cells/3 mL WME) onto plastic coverslips. The cultures were placed in a humidified, 37°C, 5% CO_2 incubator for a 1.5- to 2-hour attachment period. Unattached cells were removed; viable cells were refed and established as monolayer cultures.

2. Dose Selection: Initially, 10 concentrations of the test material were assayed. When the viability estimate was obtained (20 hours after treatment initiation), at least four of these doses were chosen for analysis of nuclear labeling, starting with the highest dose that resulted in a sufficient number of survivors with intact morphologies and proceeding to successively lower doses.

3. UDS Assay:

   a. Treatment: Five replicate, monolayer cultures were exposed to the selected doses of the test material or negative or positive controls for 18 to 20 hours in WMEI containing 5 μCi/mL [^{3}H]thymidine. Treated monolayers were washed twice with WMEI, two of the five replicates for each treatment group were used to determine cytotoxicity. These cultures were refed, reincubated, and monitored for cytotoxicity at 20 to 24 hours posttreatment by trypan blue exclusion.

   b. UDS Slide Preparation: The remaining cultures were washed with media containing 1 mM thymidine. Treated hepatocytes, attached to coverslips, were exposed to 1% sodium citrate for 8 to 10 minutes, fixed in acetic acid:ethanol (1:3), dried, and mounted.

   c. Preparation of Autoradiographs/Grain Development: Slides were coated with Kodak NTB2 emulsion, dried for 7 to 10 days at 4°C in light-tight desiccated boxes, developed in Kodak D-19, fixed, stained with Williams' modified hematoxylin and eosin, coded, and counted.

   d. Grain Counting: The nuclear grains of 150 morphologically normal cells for each test dose and negative and positive controls were counted microscopically. Net nuclear grain counts were determined by subtracting the nuclear grain counts of each cell from the average cytoplasmic grain count of three nuclear-sized areas adjacent to each nucleus.

4. Evaluation Criteria:

   a. Assay Validity: For the assay to be considered valid, the following criteria must be satisfied: (1) hepatocytes recovered from the perfusion step and

monolayer cultures used for the assay must show ≥70% viability; (2) the solvent controls should have net nuclear grain counts within the range of -5.0 to 1.0; (3) the positive control must demonstrate the sensitivity of the test system to detect UDS; (4) data must be obtained from at least two replicate cultures/dose; and 5) the highest dose must show cytotoxicity, the limit of solubility, or reach the maximum recommended dose for this assay (5 mg/mL).

b. Positive Response: The assay was considered positive if (1) an increase in the mean nuclear grain count was ≥6 grains/nucleus above the concurrent negative control value, or (2) the percent of nuclei with ≥6 grains exceeded 10% of the negative control population.

C. REPORTED RESULTS:

Ten doses of the test material ranging from 0.25 to 2.5 μg/mL were assayed. Doses ≥1.5 μg/mL were excessively cytotoxic. Percent cell survival for the five highest test doses ranged from 19.1% at 2.5 μg/mL to 55.5% at 1.5 μg/mL; below these concentrations, the test material was noncytotoxic. Accordingly, four doses (0.5, 0.75, 1.0, and 1.25 μg/mL) were scored for UDS.

As shown in Table 1, nuclear counts for the selected doses were well below the minimum criteria of ≥6 grains per nucleus over the solvent control value to conclude a positive effect. By contrast, the positive control, 2-AAF, induced marked increases in net nuclear grain counts and the percent of nuclei with ≥6 grains.

Based on these results, the study author concluded the following: "The test material, sulfluramide, did not induce significant changes in the nuclear labeling of rat primary hepatocytes for an applied concentration range of 1.25 μg/mL to 0.500 μg/mL."

D. REVIEWERS' DISCUSSION AND INTERPRETATION OF STUDY RESULTS:

We assess that the study was conducted properly, and that the author's interpretation of the data was correct. None of the doses induced an appreciable increase in UDS. The cytotoxic effect demonstrated at doses ≥1.5 μg/mL showed that the test substance entered the hepatocytes and that the lack of a genotoxic response was not due to the inability of the test

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TABLE 1. Representative Results of the Unscheduled DNA Synthesis Assay with Sulfluramide (GX 071)

Treatment	Dose/mL	Cells Scored	Percent Survival (20 hours)	Average Nuclear Grain Count	Average Percent Nuclei w/ ≥6 Grains
Solvent Control					
Dimethyl-sulfoxide	--	150	100	-1.83	0.0
Positive Control					
2-Acetyl-aminofluorene	0.1 μg	150	94.8	12.79*	83.3*
Test Material					
Sulfluramide	1.25 μg[a]	150	74.2	-0.58	0.7

[a]Highest dose scored; higher concentrations were excessively cytotoxic. Results for lower doses (0.5, 0.75, or 1.0 μg/mL) did not exhibit a genotoxic effect, falling well within range of a negative response.

*Fulfills reporting laboratory's criteria for a positive effect.

material to penetrate the cell wall. The ability of the test system to detect UDS was clearly shown by the results from the positive control (2-AAF, 0.1 µg/mL). However, the lack of test material purity information and analytical data to verify actual concentrations used in the assay preclude full acceptance of the study. The study can, however, be upgraded if the study author can provide the missing test material data.

E. QUALITY ASSURANCE: A quality assurance statement was signed and dated September 13, 1989.

F. CBI APPENDIX: Appendix A, Materials and Methods, CBI pp. 12-18.

APPENDIX A

Materials and Methods
CBI pp. 12-18