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

009322

EXPEDITE

FEB 27 1992

OFFICE OF
PESTICIDES AND TOXIC
SUBSTANCES

MEMORANDUM

SUBJECT: Chlorothalonil Metabolites SDS-3701 and SDS-46851 - Consideration of the toxicologic basis for inclusion in the chlorothalonil tolerance expression

Caswell No. 215B
HED Project No. 2-0684

FROM: Elizabeth A. Doyle, Ph.D., Section Head
Review Section IV, Tox Branch II (H7509C)

E A Doyle 2/21/92

TO: James Stone/Cynthia Giles-Parker, PM-22
Registration Division (H7505C)

THRU: Marcia van Gemert, Ph.D., Branch Chief
Toxicology Branch II
Health Effects Division (H7509C)

JM Saunders for 2/21/92

Registrant: ISK Biotech

Action Requested: 1) Review of toxicology studies for soil metabolite SDS-46851, and 2) consideration of the toxicological basis for inclusion of SDS-3701 and SDS-46851 in the tolerance expression for chlorothalonil.

Background: The registrant has requested the removal of the rotational crop restriction from the chlorothalonil label. In support of this petition, the registrant has provided toxicology data for a soil metabolite, SDS-46851, which has been found as a residue in crops rotated to soil previously treated with chlorothalonil. This data is provided in conjunction with a petition for exemption from tolerance for this soil metabolite.

Currently, the tolerance expression for chlorothalonil contains plant metabolite SDS-3701 which occurs in many treated crops. Tox Branch, in conjunction with CBTS, has revisited the question of the need for inclusion of this plant metabolite in the tolerance expression based upon the database previously submitted by the registrant.

Data Summary: The data in support of exemption of SDS-46851 from tolerance are summarized below. Four new studies were submitted with the current petition.

1) 28-Day Feeding Study - Mouse
MRID No. 420901-02
Doses: 0, 250, 500, 1000, 5000, 10,000 ppm in feed
No treatment related effects in males or females.
Core - Supplementary (Not a guideline study)

2) 90-Day Feeding Study - Mouse (82-1)
MRID No. 420901-03
Doses: 0, 250, 750, 2200, 7500 ppm
No treatment related effects in males or females.
Core - Supplementary (No clinical chemistry or ophthalmological examinations performed)

3) Metabolism - Rat (85-1)
MRID No. 420901-06
A single oral dose of 10 or 1000 mg/kg of ^{14}C-SDS-46851 was given. Distribution and excretion of radiolabel was monitored for seven days. More than 90% of the radiolabel was excreted in urine and feces during the first 72 hours. No significant accumulation in tissue was reported.
Core - Supplementary (No identification of metabolites; only one treatment regimen was used)

4) Combined Chronic/Oncogenicity - Rat (Interim Report)
MRID No. 420901-04
Doses: 0, 80, 200, 500, 1000 mg/kg/day
No treatment related effects in males or females.
Core - Supplementary (Interim report only)

Although these studies do not meet guideline requirements, they provide sufficient information for consideration of this task. The registrant does not seek registration of SDS-46851.

Data previously reported for soil metabolite SDS-46851 included:

- no evidence of developmental toxicity in rats (NOEL > 2000 mg/kg/day) or rabbits (NOEL > 1000 mg/kg/day).
- a reproductive NOEL = 750 mg/kg/day and an LOEL = 2000 mg/kg/day based on reduced pup weights in rats.
- increased liver weights in rats and dogs given 750 and 50 mg/kg/day, respectively, for 90 days.
- no evidence of mutagenicity in assays from 84-2a, 84-2b or 84-4.

Data previously provided for plant metabolite SDS-3701 included:

- No evidence of carcinogenicity in mice or rats (NOEL > 20 mg/kg/day and 1500 mg/kg/day, respectively).
- No evidence of develcpmental (rat and rabbit) or reproductive toxicity (rats). (NOEL > 1000 mg/kg/day.)

- increased liver weights and decreased body weight gains in rats and mice (rats: NOEL = 3 mg/kg/day, LEL = 10 mg/kg/day; mouse: NOEL < 75 mg/kg/day).

- no evidence of mutagenicity in assays from 84-2a, 84-2b or 84-4.

- less than 30% of the metabolite is absorbed from the gut of rats.

The parent compound, chlorothalonil, is classified as a B2 carcinogen with a $Q^* = 1.1 \times 10^{-2}$ $(mg/kg/day)^{-1}$ based upon renal tumors in mice and rats. The RfD is 0.015 mg/kg/day based upon a chronic study in dogs in which renal tubular vacuolization was observed with an NOEL = 1.5 mg/kg/day and an LEL = 3.0 mg/kg/day.

Recommendation: Based upon the toxicologic data provided by the registrant, Tox Branch has no objections to 1) the exemption of SDS-46851 from tolerance in rotated crops, and 2) the deletion of SDS-3701 from the tolerance expression for chlorothalonil by direct application.

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DATA EVALUATION REPORT

SDS-46851

Study Type: A 28-Day Feeding Study in Mice

Prepared for:

Office of Pesticide Programs
U.S. Environmental Protection Agency
1921 Jefferson Davis Highway
Arlington, VA 22202

Prepared by:

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

Principal Author Jessica Kidwell Date 2/14/92
Jessica Kidwell

Reviewer John Liccione Date 2/14/92
John Liccione

QA/QC Manager Sharon Segal Date 2/14/92
Sharon Segal

Contract Number: 68D10075
Work Assignment Number: 1-70
Clement Number: 91-213
Project Officer: James E. Scott

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EPA Reviewer and Section Head: Dr. Elizabeth Doyle
Review Section IV, Toxicology Branch II/HED

Signature: E. A. Doyle
Date: 2/20/92

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DATA EVALUATION REPORT

STUDY TYPE: A 28-Day Feeding Study in Mice

TEST MATERIAL: SDS-46851

Tox Chem. Number: 215B

SYNONYMS: None located

MRID Number: 420901-02

STUDY NUMBER: 90-0155

SPONSOR: ISK Biotech Corporation, 5966 Heisley Road, P.O. Box 8000, Mentor, OH 44061-8000

TESTING FACILITY: Ricerca, Inc., Department of Toxicology and Animal Metabolism, 7528 Ashburn Road, P.O. Box 1000, Painesville, OH 44077-1000

TITLE OF REPORT: A 28-Day Feeding Study in Mice with SDS-46851

AUTHORS: Maija Mizens, Ph.D., D.A.B.T., and James C. Killeen, Jr., Ph.D.

REPORT ISSUED: Study completed on April 17, 1991

CONCLUSIONS: SDS-46851 was administered via the diet to mice for 28 days at doses of 0, 250, 500, 1000, 5000, and 10,000 ppm. No mortality was observed at any of the dose levels tested. The toxicological significance of hyperplasia of the renal tubular epithelium noted in males is questionable. The finding was considered equivocal. It was present in male mice at 250, 1000, and 10,000 ppm, but not at 500 or 5000 ppm. A dose-response relationship was not established; the severity of the lesion did not increase with the dose, and the lesion was absent in females. No treatment-related effects were observed in female mice at any of the doses tested.

CORE CLASSIFICATION: Core Supplementary. This study provides supplementary information in the establishment of dose levels for a 90-day subchronic oral toxicity study.

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A. MATERIALS, METHODS, AND RESULTS

1. Test Article Description

Name: SDS-46851

Formula: 3-carbamyl-2,4,5-trichlorobenzoic acid

Batch description: SDS-46851-0207; S/N 20381-36-07

Purity: 99.3% (sponsor analysis) prior to study initiation; 99.5% (sponsor analysis) at end of study

Physical Property: Off-white powder

Stability: Stable in diet for at least 14 days

2. Test Article Analyses for Purity and Stability

Using a mortar and pestle, a premix was prepared fresh weekly, for each dietary level, consisting of the appropriate amount of test material and a small amount of basal diet. The premix was then blended with the remainder of the basal diet for each group for a ten minute period in a Patterson-Kelley V-shell mixer. The prepared diets were stored in the dark at room temperature. SDS-46851 was administered to the mice at constant concentrations. The achieved dosages of test material were calculated at the end of each week based on the body weight and food consumption for that week and the dietary concentration.

The homogeneity of the test substance in the freshly prepared diets (250 and 10,000 ppm) was determined prior to the initiation of the study. Five samples at each concentration were analyzed in duplicate. Results of the homogeneity analyses indicated that the test material was homogeneously distributed in the diet. Homogeneity assay results were 95% and 96%, respectively, of the 250 ppm and 10,000 ppm nominal concentrations.

Analyses of the stability of the test material in the 250- and 10,000-ppm diets were conducted. The samples collected after 14 days were analyzed. The mean assay values for the 250- and 10,000-ppm samples stored 14 days under study room conditions were 96% and 102%, respectively, of the mean assay values for the 250 and 10,000 µg/g samples collected on the day of preparation. These results indicate that SDS-46851 was stable in the diet for at least 14 days under study room conditions.

The concentrations of SDS-46851 in prepared diets were measured weekly throughout the study. Samples for assays were collected immediately after diet preparation and analyzed. The mean assay values from analyses of samples collected during the in-life phase of the study were 97%, 94%, 95%, 99%, and 106%, respectively, of the diets containing 250, 500, 1000, 5000, and 10,000 ppm test material. These results indicate that the diets were prepared at the intended concentrations throughout the study.

3. Animals

Mice (140 total, Charles River Crl:CD-1 (ICR)VAF/PLUS) were received on May 16, 1990 from Charles River Breeding Laboratory, Inc., Portage, MI. Forty males and 40 females were assigned to this study. The mice were 27 days old upon receipt and 41 days old at the initiation of the test material administration. Thirty of the males and 30 of the females were designated as experimental animals and the remaining 10 of each sex as prestudy reserves. The mice were housed 3 each in a suspended stainless steel cage for the first 4 days of the acclimation period in order to aid in their acclimation to the automatic watering system. Thereafter, the animals were housed individually. The air flow in the study room was 10 or more fresh air changes per hour. The temperature and relative humidity, measured daily, were in the range of 67-75°F and 40-80%, respectively. An alternating 12-hour light/dark cycle was maintained during the acclimation and study periods. Water was provided ad libitum. All animals were fed Purina® Certified Rodent Chow #5002 ad libitum during the acclimation period. This rodent chow was also used in the preparation of the diets for the study. Feeders designed to minimize soiling and scattering were used and excessive spillage was noted. The feeders were sanitized weekly. Prior to randomization, a physical examination of all animals was conducted to determine the suitability of the animals for placement in the study. The selection and randomization of animals to groups was performed separately for each sex. Mice were randomized by body weight such that the treated group means did not differ from the control group mean by more than 5% and that none differed statistically from the control group mean at the experiment wise error rate of 5%. The number of rats assigned to each group were as follows:

Group	Dietary Level (ppm)	Number/Sex (at start)
1	0	5
2	250	5
3	500	5
4	1000	5
5	5000	5
6	10,000	5

4. Statistical Methods

Body weights, body weight gains, food consumption (absolute and relative to body weight), clinical pathology parameters, organ weights, organ weight relative to body weight, and organ weight relative to brain weight values were analyzed first using Bartlett's test for normality and variance homogeneity. If Bartlett's test was significant at the 5% level of significance, then nonparametric procedures were used: Dunn's test was used to compare the treated groups' means to the control group mean, and Jonckheere's test was used to test for a monotonic trend over the doses. If Bartlett's

test was not significant, then parametric procedures were used: Bonferroni's t-tests were used to compare the treated groups' means to the control group mean, and regression analysis was used to test for a linear trend over the doses.

When comparing group means in both the nonparametric and the parametric cases, significance was reported at the two-sided experiment-wise error rates of 1% and 5%. For the tests for trend, significance was reported only at the (two-sided) 1% level so that the significance level of the test was comparable to that of the individual mean comparisons. However, in parametric cases, the test for a linear trend was performed only if the test for lack-of-fit was not significant at the 5% level. A test for lack-of-fit is not applicable in the nonparametric case.

5. General Observations

(a) Mortality/moribundity/survival

The mice were observed once daily prior to test material administration and twice daily during the dosing period, once in the morning and once in the afternoon.

Summary mortality data were presented. All the mice survived to terminal necropsy.

(b) Clinical observations

The mice were observed once daily prior to test material administration and twice daily during the dosing period, once in the morning and once in the afternoon. A complete physical examination was conducted weekly from 1 week prior to the administration of test material until termination.

No physical observations were noted in any of the mice during the 28-day study period.

(c) Body weights/food consumption/test material intake

Body weights--Individual body weights were recorded weekly from 1 week prior to the administration of the test material to termination.

Tables were provided for summary body weight, body weight gain, individual body weight, and body weight gain data.

No treatment-related effects on body weight or body weight gain were observed in the study for males or females. In males, mean body weights at Week 0 were statistically lower ($p \leq 0.05$) than the controls in Groups 4, 5, and 6 (See Table 1). The study authors indicated that no reason for this was apparent. They did note, however, that all mean body weights were within 5% of the control group and no statistical differences between groups were present when the animals were assigned to the groups. Once test material administration was initiated, no differences ($p \leq 0.05$) were observed in mean body weights

or mean body weight gain between the control and the treated groups for the 4 weeks on study for the males or females (See Tables 1 and 2). Although not statistically different from controls, mean body weights in the 10,000 ppm group were 5-7% lower than the control group at weeks 1, 2, and 3.

TABLE 1

Male Mean Body Weights, grams

Group Number	Dose (ppm)	Week 0	Week 1	Week 4
1	0	27.0	28.8	32.4
2	250	26.4	29.0	32.4
3	500	25.4	27.4	32.2
4	1000	24.6*	26.8	30.8
5	5000	24.4*	26.8	30.2
6	10,000	24.4*	26.8	30.0

*Statistically significant difference from control group at the 0.05 level.

TABLE 2

Male Mean Body Weight Gain, grams

Group Number	Dose (ppm)	Week 0-1	Week 0-4
1	0	1.8	5.4
2	250	2.6	6.0
3	500	2.0	6.8
4	1000	2.2	6.2
5	5000	2.4	5.8
6	10,000	2.5	5.8

Food consumption--Individual food consumption was recorded weekly from 1 week prior to the administration of the test material to termination. Calculations were made for daily food consumption, food consumption relative to body weight, and compound consumption for each individual animal without rounding off. These values were then rounded off prior to calculation of means and standard deviations.

Tables were presented for summary food consumption and individual food consumption data.

No statistically significant effects on absolute food consumption were seen in males or females. Statistically significant increases ($p \leq 0.01$ or $p \leq 0.05$) were seen in food consumption relative to body weight of males in Group 6 (10,000 ppm) at weeks 1, 2, and 3. Although not statistically different from controls, mean body weights in the 10,000-ppm group were 5-7% lower than the control group at

weeks 1, 2, and 3. The study authors reported that the increased relative food consumption suggests that the high dose males were consuming more food than would be expected for their body weight. A statistically significant increasing trend ($p \leq 0.01$) was seen for relative food consumption in males at weeks 1 and 2. No statistically significant differences were seen in the mean relative food consumption for females.

Test material intake--Tables were presented for summary compound consumption and individual compound consumption data. Test article intake (mg/kg/da) was calculated based on individual food consumption and body weight data.

The mean intakes of SDS-46851 for Weeks 1-4 are as follows:

Group Number	Sex	Dose (ppm)	Mean Compound Consumption (mg/kg/day)
2	M	250	46
2	F	250	54
3	M	500	96
3	F	500	108
4	M	1000	188
4	F	1000	217
5	M	5000	963
5	F	5000	1023
6	M	10,000	2028
6	F	10,000	2112

6. Clinical Pathology

Hematology analysis was performed after 28 days of exposure for males and 29 days of exposure for females. Samples of blood were withdrawn under ether anesthesia from the orbital sinus of all mice. The protocol indicated that the blood would be collected from the abdominal aorta. The mice were bled through the orbital sinus rather than the abdominal aorta because a larger sample could be obtained by this route. The study authors indicated that this protocol deviation did not adversely affect the results of the study. Food was withheld 16-20 hours prior to the blood sample collection. The parameters checked (X) below were examined.

Hematology

X Hematocrit (HCT)
X Hemoglobin (HGB)
X Leukocyte count (WBC)
X Erythrocyte count (RBC)
X Platelet count
X Differential leukocyte count
X Mean corpuscular HGB (MCH)
X Mean corpuscular HGB concentration (MCHC)
X Mean corpuscular volume (MCV)

Tables were provided for summary hematology and individual hematology data.

No treatment-related effects were observed in the hematology data for males or females. In female mice, a statistically significant monotonic trend ($p \leq 0.01$) was observed for hemoglobin concentration. The decreases in hemoglobin levels were slight and not dose-related. The study authors did not consider this finding to be test material-related since there were no significant differences between the control and treated groups.

7. Sacrifice and Pathology

After 28 days of compound administration, all the animals on the study were anesthetized with ether, killed by exsanguination through the orbital sinus, and necropsied. The tissues were preserved in 10% neutral buffered formalin. The tissues checked (X) below were examined histologically, and those that are double-checked (XX) were also weighed at necropsy.

Digestive System	Cardiovascular/Hematologic	Neurologic
Tongue	X Thoracic Aorta	XX Brain
X Salivary glands	X Heart	X Peripheral nerve (sciatic nerve)
X Esophagus	X Bone marrow	X Spinal cord (three levels)
X Stomach	X Lymph nodes	X Pituitary
X Duodenum	X Spleen	X Eyes (Optic nerve)
X Jejunum	X Thymus	
X Ileum		Glandular
X Cecum	Urogenital	
X Colon		X Adrenals
X Rectum	XX Kidneys	Lacrimal gland
XX Liver	X Urinary bladder	X Mammary glands
X Gallbladder	XX Testes	X Thyroids
X Pancreas	X Epididymides	X Parathyroids
	X Prostate	Harderian glands
Respiratory	X Seminal vesicles	
	X Ovaries	
X Trachea	X Uterus	
X Lung	X Vagina	
Nasal turbinates	X Cervix	

Other

X Bone (femur)
X Skeletal muscle
X Skin
X All gross lesions
Joint (tibial-femoral)

(a) Macroscopic

Since observations noted at necropsy occurred only sporadically, they were not considered by the study authors to be treatment related. One male in the 10,000-ppm group had numerous red foci

in the submucosa of the glandular stomach and the cecal content was black. This animal was considered to have less than normal mesenteric fat. Other observations included a subcapsular cyst on the right kidney (in one male in the 250-ppm group), an enlarged lymph node (in one male in the 1000-ppm group), and a slightly red cervical lymph node (in one male in the 5000-ppm group). One female in the 1000-ppm group had red ears and one female in the 5000-ppm group had a distended uterus and enlarged ovaries.

(b) Organ weights and body weight ratios

The mean organ weights, organ-to-body weight ratios, and organ-to-brain weight ratios were calculated for the adrenals, brain, kidneys, liver, ovaries, and testes.

No treatment-related effects were seen in the organ weight and body weight ratios for males. A significant increase ($p \leq 0.05$) in the absolute brain weight was observed in females at the lowest dose tested (250 ppm). No other organ weight or organ weight ratio changes were observed in females. The study authors stated that since no dose-response relationship was seen for brain weights and no observations at gross or microscopic examination of brains suggested an effect on this organ, this was not considered a treatment-related finding.

(c) Microscopic

All microscopic lesions were considered common spontaneous lesions in mice except for hyperplasia of the renal tubule epithelium in the kidneys of some male mice. The hyperplasia was considered equivocal. It was observed in one male (1 of 5) in the 250-ppm dose group, in one male (1 of 5) in the 1000-ppm dose group, and two males (2 of 5) in the 10,000-ppm dose group. It was not present in the control group or in the males that received 500 ppm or 5000 ppm SDS-46851 in the diet. The severity of the lesions was slight to mild. The pathologist considered the hyperplasia equivocal since a dose-response relationship was not present and the severity of the lesion did not increase with dose. Hyperplasia of the renal tubule epithelium was not present in female mice.

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The reviewers have no other comments regarding the materials and methods sections.

A description of the statistical analysis employed was included in the report.

A Good Laboratory Practice Compliance Statement was signed and dated by Maija Mizens (Study Director) and Robert Baxter (Sponsor). A Quality Assurance Statement including a list of Quality Assurance Inspections was signed and dated by Gilbert Claudio (Quality Assurance Supervisor).

B. DISCUSSION

Taking into account the fact that there are no guidelines for a 28-day feeding study, the study design was, for the most part, acceptable for a repeated oral dosing study. However, clinical chemistry evaluations were not performed. The summary table data were supported by the individual animal data.

The reviewers agree with the study authors conclusions that no treatment-related effects on body weight, body weight gain, absolute food consumption, and hematology data were observed for males and females. The macroscopic findings and organ weight changes were also not considered to be treatment related. The only finding which was possibly test material related was hyperplasia of the renal tubular epithelium in males. However, this finding is of questionable toxicological significance since it was present in male mice at 250-, 1000-, and 10,000 ppm, but not at 500- or 5000 ppm. Also, the severity of this lesion did not increase with dose, and the lesions were not present in the females.

Since the highest dose level (≈2000 mg/kg body weight) did not result in any apparent toxic effect, it is possible that the animals may have tolerated a higher dose level. Although a rationale for dose selection was not provided, an initial dose level of 2000 mg/kg is reasonable for a dose range-finding study. Additional data may be needed to determine if higher doses should be tested.

In summary, no apparent toxicological effects were noted in rats fed 0-, 250-, 500-, 1000-, 5000- or 10,000 ppm SDS-46851 for 28 days. The hyperplasia of renal tubular epithelium is of uncertain toxicological significance. The results of this repeated oral dosing study provide supplementary information in the establishment of dosages for a 90-day subchronic oral toxicity study.

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DATA EVALUATION REPORT

SDS-46851

Study Type:
Subchronic Oral Toxicity in Mice

Study Title:
A 90-Day Feeding Study in Mice With SDS-46851

Prepared for:

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

Prepared by:

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

February 10, 1992

Principal Reviewer	Betty Shindel, M.P.H.	Date 2-13-92
Independent Reviewer	Wayne Reichardt, M.S.	Date 2-13-92
QA/QC Manager	Sharon Segal, Ph.D.	Date 2/13/92

Contract Number: 68D10075
Work Assignment Number: 1-70
Clement Number: 91-212
Project Officer: James Scott

009322
Guideline 82-1
Subchronic Oral Toxicity Study

EPA Reviewer: Elizabeth Doyle
Review Section IV, Toxicology Branch II
Health Effects Division

Signature: E.A. Doyle
Date: 2/21/92

EPA Section Head: Elizabeth Doyle
Review Section IV, Toxicology Branch II
Health Effects Division

Signature: E.A. Doyle
Date: 2/21/92

DATA EVALUATION REPORT

STUDY TYPE: Subchronic oral toxicity in mice

TEST MATERIAL: SDS-46851

TOX CHEM. NUMBER: 215B

SYNONYMS: 3-carbamyl-2,4,5-trichlorobenzoic acid

PC NUMBER: 81901

STUDY NUMBER: 3853-90-0317-TX-003

MRID NUMBER: 420901-03

SPONSOR: ISK Biotech Corporation, 5966 Heisley Road, P.O. Box 8000, Mentor, Ohio 44061-8000

TESTING FACILITY: Ricera, Inc., Department of Toxicology and Animal Metabolism, 7528 Auburn Road, P.O. Box 1000, Painesville, Ohio 44077

TITLE OF REPORT: A 90-Day Feeding Study in Mice With SDS-46851

AUTHORS: George E. Fillmore, James C. Killeen, Jr., and Maija Mizens

REPORT ISSUED: July 12, 1991

CONCLUSIONS: Oral administration of SDS-46851 to mice for 13 weeks in doses of 250, 750, 2,200, and 7,500 ppm produced no toxicological effects that could be clearly attributed to chemical exposure. The NOEL for subchronic oral exposure achieved in this study was ≥7,500 ppm for males and females. A LOEL was not established for males or females.

CORE CLASSIFICATION: Core Supplementary. This study is classified as Core Supplementary because blood chemistry analyses and ophthalmological examinations were not performed as required by Guideline 82-1.

A. MATERIALS, METHODS, AND RESULTS

1. Test Article Description

Name: SDS-46851

Formula: 3-carbamyl-2,4,5-trichlorobenzoic acid

Batch Number: SDS-46851-0207
S/N 20381-36-07

Physical description: Off-white powder

Source: Fermenta ASC Corporation, 5966 Heisley Road, P.O. Box 8000, Mentor, Ohio 44061-8000

Purity: 99.3% labeled; 99.5% analyzed during study

Stability: Stable for 14 days (Ricerca Document # 3582-90-0155-TX-033; previous range-finding study)

Storage: Stored in dark at room temperature in a tightly closed container

2. Test Article Analyses for Purity and Stability

The purity of the neat test material, as reported by the sponsor, was 99.3%. Measurements made at the testing facility during the study and at study completion indicated purities of 99.5% and 99.6%, respectively.

Test diets of nominal SDS-46851 concentrations of 250, 750, 2,200, and 7,500 ppm were prepared fresh weekly. Using a mortar and pestle, a premix was prepared for each dose level containing the appropriate amount of test article and a small amount of basal diet (Purina Certified Rodent Chow #5002). The premix was blended with additional basal diet for 10 minutes in a Patterson-Kelley V-shell blender to produce the required concentrations, and the prepared diets were stored in the dark at room temperature. Nominal concentrations were kept constant throughout the study, and the achieved dosages (mg/kg/day) were calculated at the end of each week based upon the nominal concentrations and the body weight and food consumption data for that week. Duplicate 100-g samples were collected at each dietary level immediately after diet preparation, and actual dietary concentrations were determined from one sample per level by high-performance liquid chromatography following acetone extraction. Assays were performed weekly for the first 4 weeks and on even-numbered weeks thereafter, and the results indicated that achieved concentrations were close to target concentrations throughout the study. Target concentrations and mean actual concentrations were as follows:

	Target Concentration (ppm)			
	250	750	2,200	7,500
Actual Concentration (ppm)	244	728	2,173	7,519
Standard Deviation (ppm)	6	34	75	220
Percent of Target	98	97	99	100

Test diets were not analyzed for homogeneity and stability. However, the results of analyses conducted during a previous 28-day range-finding study (Study #3582-90-0155-TX-033), in which diets were prepared and stored as described above, indicated acceptable stability and homogeneity of SDS-46851 in the diet at concentrations of 250 and 10,000 ppm. After 14 days of room-temperature storage, the mean stability assay values in that study were 96% and 102% of

preparation-day values at 250 and 10,000 ppm, respectively, and the homogeneity assay results were 95% and 96%, respectively.

3. Animals

Species: Mouse

Strain: Charles River Crl:CD-1 (ICR) VAF/PLUS®

Source: Charles River Laboratories, Inc., Portage, Michigan

Sex and numbers: 50 males, 50 females

Age at study initiation: 41 days

Weight at study initiation: Males, 21-30 g; females, 18-26 g

The mice were acclimated to the animal room for 14 days prior to test article administration. Mice were housed three to a cage for the first 4 days of the acclimation period and were housed individually thereafter. Animals were uniquely identified by an ear tag number and matching cage card. Cages were suspended stainless steel models. Air flow in the animal room was measured once per month throughout the study and was equal to 10 or more fresh air changes per hour. A 12-hour light/dark cycle was maintained in the animal room. Room temperature and relative humidity were recorded daily and were within the ranges of 67-75°F and 40-70%, respectively, except for one day when the humidity was out of range for approximately 4 hours. Tap water was available ad libitum through an automatic watering system during the acclimation and test periods. Mice were fed Purina Certified Rodent Chow #5002 ad libitum during the acclimation period. During the test period, Purina Certified Rodent Chow #5002 was used to prepare the diet containing the test article. Diets were routinely provided on a weekly basis during the test period and as often as needed to ensure an adequate food supply.

Animals were observed once daily during the acclimation period for mortality/moribundity and general signs of health. A physical examination of all animals was performed prior to group assignments and unsuitable animals were removed. The remaining animals were randomly assigned to study groups according to body weight based on a computerized distribution. There were four treatment groups and one control group containing 10 animals of each sex. Following group assignments, there were no statistically significant differences in mean body weights between treatment and control groups.

4. Statistical Analyses

The same statistical procedures were used to analyze body weight, body weight gain, absolute and relative food consumption, clinical pathology parameters, and absolute and relative organ weights. Bartlett's test was used to determine the data normality and variance homogeneity. If Bartlett's test was significant at the 1% level, then nonparametric procedures were employed, including Dunn's test and Jonckheere's test. If significance was not shown by Bartlett's

test, then parametric procedures were used, including the Bonferroni t-test and regression analysis. In the tests for trend, significance was only reported at the 1% level (both parametric and nonparametric cases), and then only if the test for lack-of-fit was not significant at the 1% level (parametric cases only).

5. General Observations

(a) Mortality/moribundity/survival

Observations for mortality/moribundity were made twice daily during the dosing period, and once daily during the acclimation period. No treatment-related mortality was observed during the study.

One female from the 250-ppm group died, according to the study authors, as a result of a caging accident. A second animal from the same group was sacrificed because of moribundity during week 7; clinical signs noted in the moribund animal prior to sacrifice included increased activity, hunched posture, few feces, extreme ano-genital staining, thin appearance, and partial closing of eyes. The study authors did not consider the moribundity to be treatment related because no deaths occurred at higher doses. No remarkable gross or microscopic abnormalities of the tissues were observed in this animal.

(b) Clinical observations

Animals were observed for clinical signs of toxicity once daily during the acclimation period and twice daily during the exposure period. A physical examination was performed weekly beginning one week prior to the start of test article administration. No treatment-related clinical signs of toxicity were observed in the treated animals during the study period.

(c) Body weights/food consumption/test material intake

Body weights: Individual body weights were measured weekly beginning 1 week prior to the start of test article administration and until study termination at week 13. Body weights in the treated groups were statistically similar to those of the controls, with the exception of a statistically significant decrease ($p \leq 0.05$) in mean body weight at week 12 in the male 250-ppm group. The study authors considered this finding to be spurious and not treatment related; no reason was given. There were no statistically significant differences in body weight gain for treatment groups as compared to controls.

Food consumption: Food consumption was measured weekly beginning 1 week prior to the start of test article administration. Excessive spillage for several females was recorded; these data were excluded from the mean calculations.

Statistically significant changes in males included an increase in the relative food consumption of 2,200-ppm males during weeks

11 and 13 ($p \leq 0.05$), an increase in the absolute food consumption of 7,500-ppm males during week 9 ($p \leq 0.05$), and an increase in the relative food consumption of 7,500-ppm males during weeks 8 ($p \leq 0.05$), 9 ($p \leq 0.05$), 11 ($p \leq 0.01$), and 12 ($p \leq 0.01$). The study authors did not consider these changes in food consumption of males to be treatment related because there was no corresponding effect on body weights. In females, a statistically significant decrease ($p \leq 0.05$) was seen in mean absolute food consumption in the 250-ppm group at week 12; however, the decrease appeared to be incidental because no significant trend was observed. In 7500-ppm females, a statistically significant increase ($p \leq 0.05$) was seen at week 1 for mean relative food consumption, and a statistically significant increase ($p \leq 0.05$) in mean relative food consumption was seen at week 7. In females at 2,200 ppm, a statistically significant ($p \leq 0.05$) increase in mean relative food consumption was seen at week 7. Food consumption data for a number of individual female animals were excluded by the authors because of excessive spillage, but these omissions probably did not significantly affect the validity of the group means.

Test article intake: Nominal concentrations were kept constant throughout the study, and the test article intake for each group (mg/kg/day) was calculated weekly based upon the nominal concentrations and the body weight and food consumption data for that week. Weekly compound consumption decreased steadily in all exposure groups throughout the study as relative food consumption decreased. The means for the entire 13-week period were calculated by the authors and presented as the effective dosages for each exposure group. These means were as follows:

	Mean Compound Consumption (mg/kg/day)	
Target Concentration (ppm)	Males	Females
250	41	47
750	122	145
2,200	368	456
7,500	1,270	1,532

(d) Ophthalmoscopic examination

This study deviated from Guideline 82-1 requirements by not performing ophthalmological examinations. Guideline 82-1 states that ophthalmological examinations should be made prior to the administration of the test substance and at the termination of the study, preferably in all animals, but at least in the high dose and control groups.

6. Clinical Pathology

Hematological examinations were conducted on the two animals that died during the study and on all remaining animals at study termination. Blood was withdrawn from the orbital sinus of ether-anesthetized animals after food was withheld for up to 25 hours. The parameters checked (X) were examined.

(a) Hematology

X Hematocrit (HCT)*	X Leukocyte differential count*
X Hemoglobin (HGB)*	X Mean corpuscular HGB (MCH)
X Leukocyte count (WBC)*	X Mean corpuscular HGB concentration (MCHC)
X Erythrocyte count (RBC)*	X Mean corpuscular volume (MCV)
X Platelet count*	

* = Recommended by Subdivision F (November 1984) Guidelines

There were no treatment-related effects on the hematological parameters examined in this study. A statistically significant increase ($p \leq 0.05$) in platelet count was seen in the 250-ppm males but was judged by the study authors to be incidental in nature because there was no dose response. There was a statistically significant decrease ($p \leq 0.05$) in the number of monocytes in the differential leukocyte count for males at 2,200 ppm; however, a dose response was not seen and the decrease appeared to be incidental in nature.

(b) Blood (clinical) chemistry

This study protocol deviated from Guideline 82-1 requirements by not including blood clinical chemistry analyses. Guideline 82-1 states that blood clinical chemistry analyses should be made at the end of the test period. Blood clinical chemistry analyses were not performed in this study and were not specified in the study protocol.

(c) Urinalysis

Urinalysis was not performed in this study. Guideline 82-1 does not require performance of urinalysis on a routine basis. The Guideline states that urinalysis is to be performed only when there is useful data that might by obtained based on expected or observed toxicity.

7. Sacrifice and Pathology

All surviving animals were sacrificed by exsanguination via the orbital sinus at the end of the 90-day exposure period. Gross examinations were performed on all animals at terminal sacrifice and on the two animals that died during the study. Tissues were taken from all animals and preserved in 10% buffered neutral formalin. The tissues checked (X) below were examined histologically in high-dose animals, control animals, and animals that died; the lungs, liver, kidneys, and gross lesions were examined in all animals. The organs that are double-checked (XX) below were also weighed for all animals.

Digestive System	Cardiovascular/Hematologic	Neurologic
Tongue	X Aorta*	XX Brain*
X Salivary glands*	X Heart*	X Peripheral nerve (sciatic nerve)*
X Esophagus*	X Bone marrow*	X Spinal cord (three levels)
X Stomach*	X Lymph nodes*	X Pituitary*
X Duodenum*	X Spleen*	X Eyes
X Jejunum*	X Thymus*	
X Ileum*		
X Caecum*		
X Colon*	**Urogenital**	**Glandular**
X Rectum*		
XX Liver*	XX Kidneys*	X Adrenals*
X Gallbladder*	X Urinary bladder*	Lachrymal glands
X Pancreas*	XX Testes*	X Mammary glands
	X Epididymides	X Thyroids*
Respiratory	X Prostate	X Parathyroids*
	X Seminal vesicle	Harderian glands
X Trachea*	X Ovaries	
X Lung*	X Uterus*	
	X Cervix	

Other

X Bone marrow (sternum and femur)
X Skeletal muscle
X Skin
X All gross lesions and masses*

* = Recommended by Subdivision F (November 1984) Guidelines

(a) Macroscopic

Gross examination revealed no treatment-related changes. The authors note that the most frequent observation was the presence of multiple, white, pinpoint foci on the pancreas of female mice. The authors state that this finding was not treatment related because it occurred with similar frequency in control and treated groups.

(b) Organ weights and body weight ratios

There were no statistically significant changes in absolute organ weights or relative organ weights (organ/body weight ratios, organ/brain weight ratios) in treatment groups as compared to controls.

(c) Microscopic

Renal tubular epithelial hyperplasia was observed in one, two, and four male mice in the 250-, 750-, and 7,500-ppm groups, respectively. The study authors regarded this finding as equivocal because it was not observed in the 2,200-ppm group and

the severity of the lesion did not increase with dose. However, similar findings were also reported in a previous 28-day feeding study (Ricerca document number 3582-90-0155-TX-003) where they were also judged to be equivocal.

No treatment-related histopathological changes were observed in other organs. Some tissues or sections were missing and could not be examined microscopically. Most notable among these omissions was the regular absence of one of the pair of parathyroids. However, no histopathological changes were observed in the parathyroids which were examined, so these data gaps are not considered to be a limitation to the interpretation of the results.

The reviewers have no other comments regarding the materials and methods sections.

A signed Good Laboratory Practice Compliance Statement and a signed Quality Assurance Statement were included.

B. DISCUSSION

Although the data were well presented and the summary table data were supported by the individual animal data, the study design had deviations from Guideline 82-1 that limited the evaluation of the potential toxicity of SDS-46851. These guideline deviations consist of a lack of blood clinical chemistry analyses and ophthalmological examinations. The authors did not provide a rationale for selection of 7,500 ppm (approximately 1,000 mg/kg/day) as the high dose in this study. However, this study was preceded by a 28-day range-finding study in which doses of 250-10,000 ppm SDS-46851 were used. In the range-finding study, no definitive treatment-related effects were observed even at the high dose of 10,000 ppm (approximately 2,000 mg/kg/day), exceeding the limit dose of 1,000 mg/kg/day. The study authors do not state if they believe there is no reasonable potential for human exposure to SDS-46851 at levels exceeding the limit dose of 1,000 mg/kg/day.

There were no mortalities or signs of clinical toxicity that were attributable to treatment. There were no statistically significant changes in body weight or body weight gain for males or females. There were statistically significant increases in relative food consumption for 2,200- and 7,500-ppm males; however, these changes were not considered a toxic effect because they were not accompanied by changes in body weight or body weight gain. In females, there were occasional statistically significant changes in food consumption, but no trend was observed and the changes appeared to be incidental. There were no changes in hematological parameters that were attributable to treatment. Gross examination showed no treatment-related effects, and there were no statistically significant changes in absolute or relative organ weights.

Histopathological examination demonstrated renal tubular epithelial hyperplasia in one, two, and four male mice in the 250-, 750-, and 7,500-ppm groups, respectively. The biological significance of the hyperplasia

is equivocal because it occurred in only one sex and a dose response was not observed with regard to frequency or severity. The study authors stated that renal tubular epithelial hyperplasia was also noted in males in the range-finding study. Blood clinical chemistry analyses and urinalysis may have aided in determining the biological significance of the renal hyperplasia. No treatment-related histopathological changes were observed in other organs.

In summary, no treatment-related toxicological effects were noted in mice fed SDS-46851 for 90 days. The hyperplasia of renal tubular epithelium occasionally observed in males is of uncertain toxicological significance. The NOEL for subchronic oral exposure achieved in this study was ≥7,500 ppm for males and females. A LOEL was not established for males or females. However, the highest dose tested (7,500 ppm; approximately 1,000 mg/kg/day) was a limit dose. Further data are needed to determine if the level of expected human exposure indicates the need for testing higher dose levels.

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DOC920190
FINAL

DATA EVALUATION REPORT

SDS-46851

Study Type: Metabolism in Rats

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 Author Jessica Kidwell Date 2/14/92
Jessica Kidwell

Reviewer Sanju Divan Date 2/14/92
Sanju Divan

QA/QC Manager Sharon Segal Date 2/14/92
Sharon Segal

Contract Number: 68D10075
Work Assignment Number: 1-70
Clement Number: 91-214
Project Officer: James Scott

0024

GUIDELINES 85-1: Metabolism

EPA Reviewer
and Section Head: Elizabeth Doyle, Ph.D. Signature: E. A. Doyle
Review Section IV, Toxicology Branch II/HED Date: 2/20/92

DATA EVALUATION REPORT

STUDY TYPE: Metabolism in rats

EPA IDENTIFICATION NUMBERS:

Tox. Chem. Number: 215B

MRID Number: 420901-06

TEST MATERIAL: SDS-46851

SYNONYMS: 3-Carbamyl-2,4,5-trichlorobenzoic acid

SPONSOR: Ricerca, Inc., Department of Toxicology and Animal Metabolism, P.O. Box 1000, Painesville, OH

TESTING FACILITY: Fermenta ASC Corporation, 5966 Heisley Road, P.O. Box 8000, Mentor, OH

AUTHORS: M. David Ho, Ph.D., Joseph P. Marciniszyn, Ph.D., and James C. Killeen, Jr., Ph.D.

DATE: October 26, 1990

TITLE OF REPORT: Study of the Excretion and Distribution of Radiolabel Following Oral Administration of ^{14}C-SDS-46851 to Sprague-Dawley Rats

STUDY NUMBER: 3589-90-0159-AM-001

CONCLUSIONS: The distribution and excretion of SDS-46851 were studied in groups of male and female Sprague-Dawley rats administered a single oral gavage dose of 10 or 1000 mg/kg ^{14}C-SDS-46851.

SDS-46851 was rapidly and extensively absorbed. The predominant route of excretion following oral administration was in the feces. The urine was the secondary route of elimination. Essentially no radiolabel was excreted in the expired air for either sex in both dose groups. At the end of 7 days, approximately three-quarters of the orally dosed radioactivity in both male and female rats was excreted in the feces (68.48-76.40%), while the other one-quarter of dosed radioactivity was excreted in the urine (16.68-26.37%) (Table 1). There were sex- and dose-related differences in the rate of both urinary and fecal excretion, although the sex-related differences were not statistically significant. During the first 24 hours following dose administration, the animals in the low-dose group excreted more radiolabel in the urine and feces than did the animals in the high-dose group. About 90% of

the total radiolabel in both urine and feces was excreted by both sexes in the low-dose group within 48 hours. However, for animals in the high-dose group, it required 96 hours for urinary excretion and 72 hours for fecal excretion to be greater than 90% completed. The study authors indicated, that the slower urinary excretion of radiolabel by the animals in the high-dose group indicated prolonged absorption of the test material. Tissue content of ^{14}C-SDS-46851 was mostly insignificant (Table 2). Overall, most tissues showed low activities (<0.2% of administered dose). The low tissue levels of radioactivity at both doses demonstrated low bioaccumulation and retention of SDS-46851.

STUDY CLASSIFICATION: Unacceptable

This study was unacceptable. The study did not meet the minimum requirements set forth under Guideline 85-1 (and Addendum 7) for a metabolism study in rats. It was judged to be unacceptable for the following reasons: (1) although the test material was rapidly and extensively absorbed, the authors failed to determine the amounts and rates of absorption at different dose levels; (2) no attempt was made to identify the metabolic pathways or the metabolites of SDS-46851 in the urine and feces; (3) repetitive dosing and I.V. dosing were not used, only a single oral dose was used; (4) the results for the control animals dosed with the vehicle were not presented, thus the potential interference of the dosing vehicle with the kinetics of SDS-46851 is unknown; (5) the study authors' rationale for choosing the high-dose level, 1000 mg/kg, was that it was an effect level; however, according the Combined Chronic Toxicity/Oncogenicity Study in Rats (One Year Interim Report) (MRID# 420901-04), no apparent toxic effects were seen at the 1000-mg/kg dose level.

A. MATERIALS

1. Test Substance

The analytical grade, unlabeled test material (Lot Number 0207) was 3-carbamyl-2,4,5-trichlorobenzoic acid (MW = 268.5). A chemical purity, determined by High Performance Liquid Chromatography (HPLC), of 99.3% was reported. The test material was stored in the dark at 4°C.

The radiolabeled test material was 3-carbamyl-2,4,5-trichlorobenzoic acid-ring-UL-^{14}C. The specific activity was determined to be 3.51 mCi/mmole. The radiochemical purity was 95.2% by HPLC and subsequent Liquid Scintillation Counting. Because the radiochemical purity of the test material was lower than desired, the ^{14}C-SDS-46851 was purified. The purification process was performed prior to the preparation of the dose suspension. The purified SDS-46851 had a radiochemical purity of 98.6% (HPLC/LSC). The test material was stored in the original container at less than -40°C.

2. Test Animals

Twelve male and 15 female Crl:CD Br VAF/Plus® rats were obtained from Charles River Breeding Laboratories, Inc., Portage, MI. At the time of dosing, the weight range for low-dose and high-dose males was 240-154 g and 247-270 g, respectively; the weight range for low-dose and high-dose females was 185-208 g and 186-213 g, respectively. Ten rats of each sex originally received a single oral dose of either 10 or 1000 mg/kg of ^{14}C-SDS-46851 suspended in the vehicle, 0.75% methylcellulose/water (w/v). The study authors indicated that the rationale for the dose selection was that 10 mg/kg was a no-effect level and that 1000 mg/kg was an effect level. Two rats of each sex received a single oral dose of the vehicle and served as controls. Because the percent of administered dose recovered from several females was not satisfactory, three additional females received test material.

B. METHODS

1. Acclimation

The rats were held in quarantine for 6-10 days. The rats were housed individually in stainless steel cages during acclimation and in glass metabolism cages after dose administration. The glass cages were designed for the collection of expired gases. Animals were chosen for the study using a random permutation program. The diet was Purina® Certified Rodent Laboratory Chow® (No. 5002). Feed was available ad libitum at all times except 16-17 hours prior to dose administration and 4 hours after the radiolabeled dosing. Water was available ad libitum at all times during acclimation and throughout the study.

2. Dosing Solution Preparation and Administration

The radiolabeled oral dosing solutions were prepared in 0.75% methylcellulose/water (w/v) without grinding to minimize volatilization. The chemical concentration was approximately 1.0 mg/ml for the low-dose level preparations. The specific activity of the suspension for the male rats at the low-dose level was 2.4 mCi/mmole, or 19.7 DPM/ng. The low dose suspension for the female rats had a specific activity of 3.2 mCi/mmole, or 26.5 DPM/ng. The high-dose level preparation had a chemical concentration of approximately 100 mg/ml. The suspension for the males had a chemical concentration of 99.9 mg/ml, while the concentration for the females was 103.4 mg/ml. The specific activity of the suspension for the male rats at the high-dose level was 0.02 mCi/mmole, or 0.1738 DPM/ng. The high-dose suspension for the female rats had a specific activity of 0.03 mCi/mmole, or 0.2155 DPM/ng. The median particle size of the four dose suspensions ranged from 3.6 to 4.6 µm, which indicated that the dose suspensions were similar with respect to particle size. The purity of the dose suspensions prior to administration ranged from 98.7% to 99.6%. After dose administration, the purity of the suspensions ranged from 98.5% to 99.4%. The nearly identical range of the pre- and post-dose purities indicates that the dose suspensions remained stable. The dose preparations were homogenous.

The male rats at the low-dose level received an average of 10.1±0.1 mg of SDS-46851/kg of body weight, while the females received 9.9±0.1 mg/kg. The amount of radioactivity administered to the male rats was 22.3±0.4 µCi. The female rats received 22.1±0.4 µCi. At the high-dose level, the males received an average of 1038±42 mg of SDS-46851/kg of body weight. The female rats in the high-dose group received 1040±4 mg/kg. The amount of radioactivity administered to the animals was 20.9±0.6 µCi for the male rats and 20.0±1.0 µCi for the female rats.

3. Sample Collection and Analysis

Expired carbon dioxide was collected from each animal using a 10% potassium hydroxide (KOH) solution as a trapping agent at 6, 12, and 24 hours. At 24 hours an analysis showed that no radiolabel was being trapped. A trap containing an activated polymer, Chromosorb® 102 60/80 mesh, followed the 10% KOH solution to catch other expired volatile organics. The polymer was collected and replaced with fresh polymer following the same schedule used for collection of the KOH solution. All of the KOH and Chromosorb® samples were refrigerated until analysis.

Urine samples were collected in receptacles over dry ice from all animals following dosing at 0-6, 6-12, and 12-24 hours, and for every 24-hour interval thereafter until termination at 168 hours.

Sample volume was measured and all samples were stored at <10°C.

Feces were collected in receptacles over dry ice from each animal from 0 to 12 hours after dose administration. Feces were collected for the remainder of the study using the schedule that was being used for urine collection. Each sample was stored frozen to prevent decomposition.

The metabolism cages were rinsed with HPLC-grade water and then methanol to collect any radiolabeled material that may have adhered to the cage. The combined water/methanol rinses were collected following removal of the animals for termination and were stored refrigerated.

Following exsanguination via the dorsal aorta at day 7 post-exposure, blood and major tissues were collected and stored. Blood and blood fractions were stored at 4°C. Selected tissues, including the heart, lungs, kidneys, spleen, adrenals, liver, mesenteric fat, gonads, leg muscle (semimembranosus), brain, bone (femur), and the residual carcass, were collected and then stored at <-10°C to prevent decomposition. All of the collected samples (KOH solution, Chromosorb®, urine, feces, cage washes, and tissues), except the red blood cell fraction, were analyzed for radiolabel content by Liquid Scintillation Counting, either directly or indirectly following combustion by biological oxidation. The red blood cell fraction was not analyzed because the whole blood contained less than 1% of the administered dose.

4. Protocols

The methods section is presented in the appendix of this report (CBI pp. 20-31).

5. Statistics

All calculations were performed using a computer program (RS/1, Release 4.01). The statistical analyses used were simple expressions of variation (i.e., mean and standard deviation) and a Student's t-test to determine differences between groups.

C. REPORTED RESULTS

1. Elimination and Recovery

The mean total recoveries of radioactivity in the urine, feces, tissues, expired air, and cage wash ranged from 90.68% to 98.69% of the administered dose (Table 1). Although there were some sex-related differences in the elimination of SDS-46851 following oral dosing, these differences were not statistically significant. The mean total recoveries of radioactivity in urine and feces ranged from 90.25% to 97.70% after 168 hours (7 days). The major route of elimination was the feces, with a mean percent recovery

of radioactivity ranging from 68.48% to 76.40% of the administered dose at 7 days postexposure. The urine was the secondary route of elimination with mean total recoveries ranging from 16.68% to 26.37% of the administered dose. Essentially no radiolabel (0-0.02% of the administered dose) was excreted in the expired air for either sex in both dose groups.

During the first 24 hours after dosing, the cumulative urinary excretion of radiolabel of the low-dose group was different from that of the high-dose group. During the first 24 hours following dose administration, the animals in the low-dose group excreted more radiolabel in the urine compared to the high-dose group. The males in the low-dose group excreted 18.6% of the administered dose (85% complete) versus 11.2% of the administered dose (42% complete) excreted by the males in the high-dose group. The females in the low-dose group excreted 17.1% of the administered dose (71% complete) versus 8.5% of the administered dose (51% complete) excreted in the high-dose group. Greater than 90% of the radiolabel in the urine was excreted by both sexes in the low-dose group within 48 hours. It required 96 hours for urinary excretion to be greater than 90% complete for the animals in the high-dose group. The study authors indicated that the slower urinary excretion of radiolabel by the animals in the high-dose group indicated prolonged absorption of the test material.

As in the case of the urine, the cumulative excretion of radiolabel in the feces of the low-dose group was different from that of the high-dose group during the first 24 hours after dosing. During the first 24 hours following dose administration, the animals in the low-dose group excreted more radiolabel in the feces compared to the high-dose group. The males in the low-dose group excreted 56.9% of the administered dose (83% complete) versus 17.8% of the administered dose (26% complete) excreted by the males at the high-dose level. The females in the low-dose group excreted 52.8% of the administered dose (72% complete) versus 26.5% of the administered dose (35% complete) excreted by the high-dose group. Between 89 and 91% of the radiolabel in the feces was excreted by both sexes in the low-dose group within 48 hours. It required 72 hours for fecal excretion to be greater than 90% complete for the animals in the high-dose group.

2. Tissue Distribution

Mean recoveries of radioactivity after oral dosing in the tissues were less than 0.2% of the administered dose. The radiolabel found in the individual blood and organ samples was negligible and contained ≤0.05% of the administered dose (Table 2).

No major sex-related differences were seen in residue levels. Mean concentrations in organs were highest in the liver of the animals at both dose levels. The average nanogram equivalents (ng-eq/g) per gram in the liver of animals at the low-dose level

were 95 ng-eq/g for males and 67 ng-eq/g for females. For the animals in the high-dose group, the liver contained an average of 5917 ng-eq/g for the males and 4687 ng-eq/g for the females. The study authors pointed out that these total nanogram equivalent values appear to be considerably greater than zero, but the values were calculated from disintegrations per minute (DPM) which approximated background. The study authors stated that because of the insignificance of the DPM values, care should be taken in assessing the importance of the nanogram equivalent values.

D. STUDY AUTHORS' CONCLUSIONS/QUALITY ASSURANCE MEASURES

The predominant route of excretion following oral administration was in the feces. The urine was the secondary route of elimination. Essentially no radiolabel was excreted in the expired air for either sex in both dose groups. Although there were some sex related differences in the elimination of SDS-46851 following oral dosing, these differences were not statistically significant. At the end of 7 days, approximately three-quarters of the orally-dosed radioactivity in both male and female rats was excreted in the feces (68.48-76.40%), while the other one-quarter of dosed radioactivity was excreted in the urine (16.68-26.37%) (Table 1). There were, however, dose-related differences in the rate of both urinary and fecal excretion. During the first 24 hours following dose administration, the animals in the low-dose group excreted a greater cumulative percentage of radiolabel in the urine (71-85% complete) and feces (72-83% complete) than did the animals in the high-dose group (42-51% complete for urine; 26-35% complete for feces). About 90% of the total radiolabel in both urine and feces was excreted by both sexes in the low-dose group within 48 hours. However, for animals in the high-dose group, 90% completion of urinary excretion required 96 hours; 90% completion of fecal excretion required 72 hours. The study authors indicated that the slower urinary excretion of radiolabel by the animals in the high-dose group indicated prolonged absorption of the test material. Tissue content of ^{14}C-SDS-46851 was mostly insignificant. Overall, most tissues showed low activities (<0.2% of administered dose) (Table 2).

The quality assurance statement was signed on 10/25/90 and the statement of compliance with Good Laboratory Practices for the study was signed on 10/25/90 and 10/26/90.

E. CONCLUSIONS BASED ON REVIEWERS' DISCUSSION AND INTERPRETATION OF DATA

The study authors did not determine the amounts and rates of absorption of SDS-46851 at different dose levels. Also, no attempt was made to identify the metabolites of SDS-46851 or the metabolic pathways. Repetitive dosing and I.V. dosing were not performed. This study described the distribution and excretion of ^{14}C-SDS-46851 following a single oral exposure. The data indicated that the major route of elimination was the feces and that the urine was the secondary route of elimination. Essentially no radiolabel was excreted in the expired air for either sex in both dose groups. The appearance of radioactivity in

the feces could be due to biliary excretion and poor absorption from the gastrointestinal tract; however, since I.V. dosing was not performed, it is difficult to determine the origin of the radioactivity in the feces. Although there were some sex related differences in the elimination of SDS-46851 following oral dosing, these differences were not statistically significant. Although absorption was not determined, it occurred readily as evidenced by the appearance of radioactivity in the urine within the first 24 hours for both sexes in both dose groups. There were dose-related differences in the rate of both urinary and fecal excretion, with the animals in the low-dose group excreting radioactivity in the urine and feces at a faster rate than the animals in the high-dose group. The reviewers note that the total amounts eliminated over the 7-day period were not affected. Although the study authors stated that the slower urinary excretion of radiolabel by the animals in the high-dose group indicated prolonged absorption of the test material, the reviewers feel that an additional explanation for the dose-dependant pattern could be saturation of the metabolic pathway. The low tissue levels of radioactivity demonstrated that bioaccumulation and retention of SDS-46851 were low. Recovery of the radioactivity was nearly complete (90.68-98.69%) (Table 1). Statistical methods were used to analyze the data; however, the statistically significant results were not indicated in the tables--they were only reported to be statistically significant in the text.

This study was unacceptable. The study did not meet the minimum requirements set forth under Guideline 85-1 (and Addendum 7) for a metabolism study in rats. It was judged to be unacceptable for the following reasons: (1) although the test material was rapidly and extensively absorbed, the authors failed to determine the amounts and rates of absorption at different dose levels; (2) no attempt was made to identify the metabolic pathways or the metabolites of SDS-46851 in the urine and fecal samples; (3) repetitive dosing and I.V. dosing were not used, only a single oral dose was used; (4) the results of the control animals dosed with the vehicle were not presented, thus the potential interference of the dosing vehicle to the kinetics of SDS-46851 is unknown; (5) the study authors' rationale for choosing the high-dose level, 1000 mg/kg, was that it was an effect level; however, according the Combined Chronic Toxicity/Oncogenicity Study in Rats (One Year Interim Report) (MRID# 420901-04), no apparent toxic effects were seen at the 1000-mg/kg dose level.

TABLE 1. Mean Percent Recovery of Radioactivity 7 Days After Oral Administration of [^{14}C]-SDS-46851 to Rats

Dose Group	Sex	Percent of Administered Dose[a]					
		Urine	Feces	Expired Air	Tissues	Cage Wash	Total Recovery
10 mg/kg	Male	21.77	68.48	0.00	0.10	0.34	90.68
	Female	24.00	73.70	0.00	0.20	0.79	98.69
1000 mg/kg	Male	26.37	69.38	0.02	0.13	0.70	96.60
	Female	16.68	76.40	0.00	0.05	0.89	94.02

[a]Based on individual means.

Source: CBI Table 2, pp. 41-42

TABLE 2. Distribution of Radioactivity in Tissues of Rats 7 Days After Oral Administration of SDS-46851

	ng equivalents ^{14}C-SDS-46851/g[a] of tissue in rats dosed at:			
	10 mg/kg		1000 mg/kg	
Tissue/Organ	Males	Females	Males	Females
Whole Blood	2 (0.00)	1 (0.00)	80 (0.00)	109 (0.00)
Heart	7 (0.00)	5 (0.00)	310 (0.00)	187 (0.00)
Lung	2 (0.00)	1 (0.00)	33 (0.00)	113 (0.00)
Spleen	0 (0.00)	2 (0.00)	375 (0.00)	0 (0.00)
Adrenal	77 (0.00)	1 (0.00)	1116 (0.00)	489 (0.00)
Kidney	39 (0.00)	51 (0.00)	1823 (0.00)	722 (0.00)
Liver	95 (0.05)	67 (0.03)	5917 (0.03)	4687 (0.02)
Gonads	5 (0.00)	5 (0.00)	80 (0.00)	146 (0.00)
Brain	2 (0.00)	0 (0.00)	121 (0.00)	42 (0.00)
Carcass	4 (0.05)	16 (0.17)	1004 (0.10)	2[illegible] (0.03)
Fat	7 (0.01)	7 (0.01)	689 (0.01)	266 (0.00)
Muscle	2 (0.01)	7 (0.04)	0 (0.00)	23 (0.00)
Bone	6 (0.00)	0 (0.00)	243 (0.00)	479 (0.00)
Tissues[b]	- (0.09)	- (0.20)	- (0.13)	- (0.04)

[a]Each value represents the mean of five rats; values in parentheses represent mean percent of administered dose.

[b]Does not include mesenteric fat, muscle, or bone. These tissues are included in the carcass.

Source: CBI Tables 5-6, pp. 47-50

CBI APPENDIX

Study Protocol
(CBI pp. 20-31)

Chlorothalonil

Page_____ is not included in this copy.

Pages 36 through 47 are not included.

The material not included contains the following type of information:

____ Identity of product inert ingredients.

____ Identity of product impurities.

____ Description of the product manufacturing process.

____ Description of quality control procedures.

____ Identity of the source of product ingredients.

____ Sales or other commercial/financial information.

____ A draft product label.

____ The product confidential statement of formula.

____ Information about a pending registration action.

X FIFRA registration data.

____ The document is a duplicate of page(s) _____.

____ The document is not responsive to the request.

The information not included is generally considered confidential by product registrants. If you have any questions, please contact the individual who prepared the response to your request.

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DOC 920189

FINAL

DATA EVAL .TION REPORT

SDS-46851

Study Title:
A Combined Chronic Toxicity/Oncogenicity Study in Rats with 3-Carbamyl-2,4,5-Trichlorobenzoic Acid (SDS-46851) One Year Interim Report

Prepared for:

Office of Pesticide Programs
U.S. Environmental Protection Agency
1921 Jefferson Davis Highway
Arlington, VA 22202

Prepared by:

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

February 14, 1992

Principal Author: _____ Date 2/14/92
John Liccione

Reviewer : _____ Date 2/14/92
Wayne Reichardt

QA/QC Manager: _____ Date 2/13/92
Sharon Segal

Contract Number: 68D10075
Work Assignment Number: 1-70
Clement Number: 91-215
Project Officer: James E. Scott

EPA Reviewer and Section Head:
Dr. Elizabeth Doyle
Review Section IV, Toxicology Branch II,
Health Effects Division

Signature: E.A. Doyle
Date: 2/20/92

DATA EVALUATION REPORT

STUDY TYPE: Combined chronic toxicity/oncogenicity study in rats

TEST MATERIAL: SDS-46851

MRID Number: 420901-04

SYNONYMS: 3-Carbamyl-2,4,5-trichlorobenzoic acid

STUDY NUMBER: 3533-90-0030-TX-001

SPONSOR: ISK Biotech Corporation, 5966 Heisley Road, P.O. Box 8000, Mentor, Ohio, 44061-8000

TESTING FACILITY: Ricera, Inc., Department of Toxicology and Animal Metabolism, 7528 Auburn Road, P.O. Box 1000, Painesville, Ohio, 44077-1000

TITLE OF REPORT: A Combined Chronic Toxicity/Oncogenicity Study in Rats with 3-Carbamyl-2,4,5-Trichlorobenzoic Acid (SDS-46851): One-Year Interim Report

AUTHORS: Killeen J.C. Jr., Serrone D.M., Gelin B.S., and Benz G.

REPORT ISSUED: August 9, 1991

CONCLUSIONS: This study provides supplementary information regarding the potential chronic toxicity and carcinogenicity of SDS-46851 in rats since it is an interim report (first 65 weeks) of an ongoing 2-year chronic toxicity/oncogenicity study. A maximum tolerated dose (MTD) has not been achieved thus far. The no-observable-effect-level (NOEL) is 1000 mg/kg.

Core classification: Core Supplementary, since this is an interim report.

A. MATERIALS, METHODS, AND RESULTS

1. Test Article Description

Name: SDS-46851

Formula: 3-Carbamyl-2,4,5-trichlorobenzoic acid

Batch description: SDS-46851-0207

Purity: 99.3% (Sponsor analysis)

Physical Property: Off-white crystals

Stability: 14 days (determined in the interim report)

2. Test Article Analyses for Purity and Stability

The test article was administered in the diet. Diets were prepared fresh weekly, and the amount of test article was adjusted according to the mean body weight and mean food consumption data for the first 13 weeks, and adjusted monthly according to mean body weight and mean food consumption for the remainder of the study. A premix containing test substance and feed was ground in a mortar, transferred to a 1 cubic foot Patterson-Kelly V-Shell blender, and mixed with a portion of the remaining feed. Additional feed was added to the blender after it was used to remove any remains of the test substance from the mortar and pestle. The balance of the feed for each particular dose level was added and the mixture was blended for 10 minutes.

Homogeneity and stability of the test material in the diet were evaluated prior to the initiation of the study. Two test diets containing 800 and 20000 µg/g were prepared according to the experimental procedure, and five random samples were taken from each of the test diets for homogeneity analysis. The authors reported that the feed prepared according to the protocol was homogeneously dispersed, although the homogeneity analysis data were not included in the study report. Additional samples were taken from the test diets prior to study initiation and maintained at room temperature to determine the stability of the test substance in the diet for 7 and 14 days after preparation. The authors reported that the test material was stable for at least 14 days under study conditions although actual stability data were not provided.

Additional diet samples taken in duplicate were collected and frozen during the study. One of each of the control pair and test samples were analyzed for concentration of test material. The study authors did not indicate in the interim report the actual intended nominal concentration (in ppm) of the test material in the diets. All diets were reported by the study authors to be within ±10% of nominal concentrations with the exception of the low-dose female diet at week 22, which was at 74% of the nominal concentration; however, the results of dietary analysis at week 22 were not provided for evaluation. The study authors also indicated that the diets for the low-dose females were within 10% of the nominal concentrations from weeks 21 to 23; however, dietary data for weeks 22 and 23 were not

provided for verification of results.

3. Animals

Rats (CD VAF/Plus®, 800 animals) were received from Charles River Breeding Laboratories, Portage, Michigan. Of the 800 animals received, 600 (300 males, 300 females) were selected for study after a physical examination, and the remaining rats were terminated or used for the development of the experimental method. Rats were 28 days old upon arrival and were 48 days old at the initiation of the study. In order to facilitate acclimation to the laboratory environment, rats were housed 2-3 animals/cage for three days, and thereafter were housed individually in stainless steel cages. Temperature and relative humidity controls were set within the range of 67-75°F and 40-70%, respectively, with greater than 12 air changes/hour. A 12-hour light/dark cycle was maintained. Food (Purina® Certified Rodent Chow® #5002) and water (municipal) were provided ad libitum throughout the acclimation and study periods.

Animals were assigned by sex to the following dose groups using a computer-generated randomization procedure:

Dose Levels (mg/kg/day)	65-Week Study	
	Males	Females
0 (control)	60	60
80 (low)	60	60
200 (mid-1)	60	60
500 (mid-2)	60	60
1000 (high)	60	60

The rationale for dose selection in the chronic toxicity/oncogenicity rats study was not discussed in the report; however, a 28-day range-finding study (ISK Biotech Corporation, Study Number 90-0155, April 17, 1991) in mice and a 90-day oral study (ISK Biotech Corporation, Study Number 90-0317, July 12, 1991) in mice were available for review. As reported in the subchronic study in mice, SDS-46851 was administered via diet to 4 groups of 10 male and 10 female mice at dietary levels of 250, 750, 2200, and 7500 ppm. A control group of 10 male and 10 female mice received basal diet. There were no treatment-related effects on body weight, body weight gains, food consumption, hematology parameters, organ weights, and mortality. Renal tubular epithelial hyperplasia was observed in male mice fed

250, 750, and 7500 ppm; however, the renal lesion was not found in males receiving 2200 ppm. Also, there was no dose-response relationship and the severity of the renal lesion did not increase with dose. Renal tubular epithelial hyperplasia was not found in the females. As reported in the 28-day range-finding study, SDS-46851 was administerd via diet to mice at dietary levels of 0, 250, 500, 1000, 5000, or 10000 ppm. There were no treatment-related effects on body weight, body weight gain, food consumption, mortality, and hematology parameters. Renal tubular epithelial hyperplasia was noted in males receiving 250, 1000, or 10000 ppm, but not in males receiving 500 or 5000 ppm. A dose-response relationship was not established and the severity of the lesion did not increase with the dose. Renal tubular epithelial hyperplasia was not present in the females.

4. Statistics

No statistical information was provided in the study report with the exception of group means and standard deviation for body weight and compound consumption.

5. Quality Assurance

A quality assurance statement was signed and dated August 2, 1991.

6. General Observations

(a) Mortality/moribundity/survival

The animals were observed twice daily for mortality and/or moribundity.

Treatment had no effect on mortality. Mortality did not exceed 5% in any of the groups at 1 year.

(b) Clinical observations

Animals were observed twice daily for general health. Complete physical examinations were conducted on all animals weekly from one week prior to initiation of treatment and throughout the duration of the study.

There were no treatment-related effects on clinical observations.

(c) Body weights/food consumption/test material intake

Body weights. Body weights were recorded one week prior to initiation of treatment, weekly from week 1 to week 13 of

treatment, and monthly thereafter.

Tables 1 and 2 summarize mean body weights and mean body weight gain, respectively, at selected intervals. Mean body weights and body weight gains in all treated males were comparable to those of controls throughout the study. Mean body weights in all treated females were similar to those of controls throughout the study. Mean body weight gain in females receiving 80 mg/kg/day was slightly but statistically significantly increased at weekly intervals of 0-4, 0-5, 0-8, 0-9, 0-12, and 0-13 when compared to that of controls. Mean body weight gain in females receiving 500 mg/kg/day was slightly but statistically significantly increased at weeks 0-8 when compared to that of controls. Mean body weight gain in the high-dose females was slightly but significantly increased at weeks 0-8, 0-10, 0-11, and 0-12 when compared to that of controls. The mean body weight gain in females receiving 200 mg/kg/day were similar to that of controls throughout the study. The increases in mean body weight gains in females receiving 80, 500, or 1000 mg/kg/day were not considered by the study authors to be treatment-related.

Food consumption. Food consumption was recorded one week prior to dosing, weekly for the first 13 weeks of treatment, and monthly thereafter.

Table 3 summarizes mean relative food consumption data. Slight but statistically significant increases in relative (to body weight) food consumption were noted in treated males and females at occasional intervals of the study. Slight increases in absolute food consumption were also noted in treated males and females at occasional intervals of the study. The study authors attributed the increase in food intake in the treated males and females to the increase in dietary intake to compensate for the amount of test material in the food.

Test article intake. Compound intake was recorded weekly for the first 13 weeks of treatment and monthly thereafter. Compound intake was calculated from body weight, food consumption, and the nominal dietary concentration of the test substance.

Actual compound intakes for males receiving 80, 200, 500, or 1000 mg/kg/day at the 0-65-week interval ranged from 67.3-84.2, 162.9-210.0, 414.1-519.0, and 805.7-1048.0 mg/kg/day, respectively. In females receiving the same doses at this time interval, the actual compound intakes ranged from 63.2-84.7, 161.3-208.3, 405.9-522.4, and 816.6-1045.0 mg/kg/day, respectively.

TABLE 1. Mean Body Weights at Selected Intervals for Rats Fed SDS-46851 for 65 Weeks[a]

Week(s)	Dose (mg/kg/day) 0	80	200	500	1000
			Males		
0	224.0±14.5	221.3±19.5	217.6±16.0	223.8±20.7	218.4±19.5
7	452.7±37.9	454.9±46.0	448.9±36.3	446.9±38.2	451.0±40.6
13	525.5±52.0	524.6±55.5	524.1±46.7	519.6±48.7	525.8±50.3
25	598.3±68.0	600.7±64.7	603.0±64.4	601.0±58.2	610.3±63.5
49	713.7±100.6	720.7±103.4	702.4±88.4	698.3±78.9	705.9±77.1
65	752.0±118.6	765.4±117.3	747.5±92.2	730.2±95.3	738.8±86.1
			Females		
0	161.1±11.8	161.1±12.7	157.4±12.0	159.8±10.9	158.7±11.2
7	249.4±21.5	256.3±21.4	250.5±21.4	252.4±22.1	253.3±21.4
13	277.8±28.0	289.4±24.6	281.8±26.8	283.3±27.5	286.4±24.9
25	312.7±36.9	323.3±33.5	321.3±37.6	317.4±38.3	319.8±32.0
49	388.5±68.6	405.3±66.0	404.6±6u.5	398.8±66.4	394.3±57.2
65	434.0±78.4	456.5±82.3	454.9±79.2	436.5±79.7	439.7±66.0

[a]Data extracted from Tables 7 and 9 of the study report.

TABLE 2. Mean Body Weight Gain at Selected Intervals in Rats Fed SDS-46851 for 65 Weeks[a]

	Dose (mg/kg/day)				
Week(s)	0	80	200	500	1000
			Males		
0-7	228.8±30.9	233.6±38.1	231.3±27.6	223.1±28.5	232.6±28.2
0-13	301.1±44.9	302.8±48.2	307.1±40.5	295.6±43.9	307.6±38.6
0-25	373.8±62.3	378.9±57.3	386.0±57.8	377.0±53.3	392.0±52.7
0-53	502.3±99.5	510.7±102.3	498.6±83.2	489.5±78.6	501.0±68.4
0-65	528.3±116.1	544.1±113.2	530.5±89.3	506.6±91.3	521.6±77.0
			Females		
0-7	88.2±15.1	94.6±14.0	93.2±14.5	92.7±14.8	94.6±14.0
0-13	116.9±21.9	128.0±16.6*	126.1±21.7	124.1±21.0	127.1±19.6
0-25	151.8±31.4	161.6±27.2	165.6±32.9	158.4±33.3	160.4±27.7
0-53	236.5±67.1	254.4±61.0	255.8±66.6	247.1±63.7	246.8±51.7
0-65	272.7±72.3	294.9±75.9	297.7±74.8	277.5±76.9	281.2±63.5

[a]Data extracted from Tables 8 and 10 of the study report.

*Significantly different from control values, $p<0.05$.

TABLE 3. Mean Relative Food Consumption at Selected Intervals for Rats Fed SDS-46851 for 65 Weeks[a]

Week(s)	Dose (mg/kg/day) 0	80	200	500	1000
			Males		
0	107.5±5.5	108.2±5.2	109.4±5.6	107.7±5.9	109.3±5.2
7	58.2±3.9	59.2±5.6	60.7±3.9*	60.4±4.0*	60.6±3.0*
13	49.8±2.5	50.4±3.4	50.4±2.7	50.7±2.9	50.8±2.7
25	43.7±2.9	43.8±2.7	43.2±5.3	43.2±3.2	43.7±3.1
49	38.0±4.6	38.6±2.2	41.3±7.0*	40.0±3.3*	40.3±3.1*
65	38.4±4.7	38.0±3.5	39.3±2.9	41.1±3.7	41.6±4.1
			Females		
0	110.7±5.0	109.5±6.4	111.4±5.4	111.7±5.4	112.1±5.5
7	72.1±4.0	71.9±3.9	74.8±4.2*	73.5±4.0	76.6±4.1*
13	64.2±4.4	63.5±5.0	62.4±5.0	63.6±5.2	63.9±3.8
25	58.2±5.8	58.6±5.0	59.3±4.6	57.3±4.9	56.7±3.3
49	52.0±4.9	53.8±5.5	53.2±5.6	54.7±5.3	53.9±5.8
65	50.0±7.2	50.2±5.9	50.6±7.6	52.0±6.8	52.0±6.2

[a]Data extracted from Tables 12 and 14 of the study report.

*Significantly different from control values, $p<0.01$.

(d) Ophthalmoscopic examination

An ophthalmologic examination of both eyes was conducted on all animals prior to dosing, and at the end of one year.

There were no compound-related ophthalmological findings.

7. Clinical Pathology

Blood samples were taken from the orbital sinus of 10 rats/sex/group (randomly selected at first collection) for hematology and clinical chemistry evaluations at weeks 13, 26 and 52. Rats were fasted approximately 16 hours prior to sample collection. For blood sample collection, rats were anesthetized with ether. Those parameters indicated by an X were examined:

(a) Hematology

X Hematocrit (HCT)
X Hemoglobin (HGB)*
X Leukocyte count (WBC)*
X Erythrocyte count (RBC)*
X Platelet count*
Reticulocyte count (RETIC)
Red cell morphology
X Leukocyte differential count*
X Mean corpuscular HGB (MCH)
X Mean corpuscular HGB concentration (MCHC)
X Mean corpuscular volume (MCV)
Coagulation: thromboplastin time (PT)

* = Recommended by Subdivision F (November 1984) Guidelines

No effects of treatment with SDS-46851 on hematology parameters were observed.

(b) Blood (clinical) chemistry

Electrolytes

X Calcium*
X Chloride*
Magnesium
X Phosphorus
X Potassium*
X Sodium*

Enzymes

Alkaline phosphatase (ALP)
Cholinesterase
X Creatinine phosphokinase*
Lactic acid dehydrogenase
X Serum alanine aminotransferase (SGPT)*

Other

X Albumin*
X Albumin/globulin ratio
X Blood creatinine
Blood urea nitrogen*
Cholesterol
X Globulins
X Glucose*
X Total bilirubin*
Direct bilirubin
X Total protein*
Triglycerides

X Serum aspartate aminotransferase (SGOT)*
Gamma glutamyltransferase (GGT)

* = Recommended by Subdivision F (November 1984) Guidelines

No effects of treatment with SDS-46851 on clinical chemistry parameters were observed.

(c) Urinalysis

Urinalysis was conducted on 10 rats/sex/group (random selection). Rats were fasted approximately 16 hours prior to urine collection. Urine was collected at weeks 12, 25, and 52. The following parameters (X) were examined:

X Appearance*	X Sediment (microscopic)*	X Bilirubin*
X Volume*	X Protein*	X Blood*
X Specific gravity*	X Glucose*	X Nitrate
X pH*	X Ketones*	X Urobilinogen

* = Recommended by Subdivision F (November 1984) Guidelines

No effects of treatment with SDS-46851 were noted on urinalysis parameters. Although a statistically significant trend towards an increased urinary specific gravity for treated males occurred at week 52, the increases in specific gravity values were slight and not signficantly different compared to those of controls.

8. Sacrifice and Pathology

An interim sacrifice was not performed.

B. DISCUSSION

This 1-year interim study reports the results of the first 65 weeks of an ongoing 2-year combined chronic toxicity/oncogenicity study. Therefore, this 1-year interim study provides supplementary information regarding the potential toxicity and oncogenicity of SDS-46851 in rats.

Based on the data presented thus far, an MTD has not been achieved. The highest dietary level (1000 mg/kg/day - the limit dose) did not induce a toxic effect from which an MTD can be defined, for example, a biologically significant decrease in body weight gain. Although a rationale for dose selection was not discussed in the interim report, the results of a previous subchronic oral study in mice indicated that the high dose (approximately 2000 mg/kg/day) did not result in any signs of overt toxicity. While these results pertain to mice rather than rats, they suggest the possibility that rats may have tolerated a higher dose level

limit dose). Thus, it is possible that the dose levels chosen for the chronic study of SDS-46851 in rats may not be adequate to determine the carcinogenic potential of the compound.

The interim report did not indicate if an interim sacrice of 10 rats/sex for pathological examinations was performed. Such an interim sacrifice might identify the possible development of renal tubular epithelial hyperplasia, a finding noted in both the range-finding and subchronic oral studies in mice. As mentioned before, the toxicological significance of the renal tubular epithelial hyperplasia in mice has not been ascertained.

The cover page of the study report lists Guidelines 83-1 and 83-2 as the guidelines for this study. Guideline 83-5 is the pertinent guideline for a combined chronic toxicity/oncogenicity study. Stability and homogeneity data on the test material were not presented. Data on the analysis of the concentration of the test material in the diets at weeks 22 and 23 were not provided.

In summary, results of the interim study provide supplementary information regarding the potential toxicity and carcinogenicity of SDS-46851. Thus far, a NOEL of 1000 mg/kg/day (the limit dose) has been determined; however, an MTD has not been achieved.