4-19-91

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

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APR 15 1991

OFFICE OF
PESTICIDES AND TOXIC
SUBSTANCES

MEMORANDUM

SUBJECT: Removal of Rotational Crop Restrictions from the Labels of Chlorothalonil Containing Formulations Bravo 500, W-75, 90DG, S and 720, and Chlorothalonil 75 WP

Caswell No. 215B
HED Project No. 0-1690

FROM: Elizabeth A. Doyle, Ph.D., Section Head
Review Section IV, Tox Branch II (H7509C)
E.A. Doyle 4/19/91

TO: Susan Lewis, PM 21
Registration Division (H7505C)

THRU: Marcia van Gemert, Ph.D., Branch Chief
Tox Branch II
Health Effects Division (H7509C)
M van Gemert 4/9/91

Registrant: Fermenta ASC Corporation

Action Requested: Review of request for removal of the rotational crop restriction on chlorothalonil, including review of toxicology data provided the registrant for soil degradate SDS-46851 (2,5,6-trichloro-3-carboxybenzamide.

Background: Chlorothalonil has been classified as a B2 oncogen based on kidney tumors in rats and mice. The Q_1* for this compound is 1.1×10^{-2} $(mg/kg/day)^{-1}$. In the petition submitted by the registrant, rotational crop studies were cited indicating that residue data have been collected for data are available for plant uptake of chlorothalonil (parent), SDS-3701 (metabolite), HCB and PCBN (formulation contaminants), and SDS-46851 (soil degradate). Structures for these chemicals are attached. These data have been submitted to EFED for evaluation.

Currently, data are available for SDS-3701. Two-year oncogenicity studies in mice and rats (Accession No. 071531, 071527, 072270, 072276) indicated no evidence of oncogenicity. A 3-Generation Reproduction Study in rats (Accession No. 071524) was classified as "Supplementary" data. However, the Reproductive NOEL for SDS-3701 was >125 ppm (HDT). The Developmental NOEL in rabbits was >5 mg/kg/day (HDT) with a Maternal NOEL of 1 mg/kg/day. At 5 mg/kg/day, maternal death and abortion were reported.

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Data for PCBN are limited to a negative Ames assay (Accession No. 0147916). For existing tolerances from directly applied chlorothalonil, PCBN has not been considered due to the determination by HED that the residues of HCB and parent were of overriding significance in estimating cancer risk (cf. Memo from B. Jaeger to E. Saito, 8/5/88). This assumption does not appear pertinent to the current situation.

HCB is a B2 carcinogen with a Q_1^* of 1.7 $(mg/kg/day)^{-1}$.

A substantial body of data has been generated for the SDS-46851 soil degradate. These data are summarized below.

1) Acute Oral Toxicity - Rats (81-1)
MRID No. 415648-01
LD_{50} in male and female Sprague-Dawley rats >5000 mg/kg
Toxicity Category IV
Core - Supplementary (Not enough rats to meet guidelines)

2) 14-Day Feeding Study - Rats
MRID No. 415648-02
Male and female Sprague-Dawley rats were given 0, 125, 250, 500, 1000 or 2000 mg/kg/day in diet for 14 days.
NOEL > 2000 mg/kg/day for male rats
NOEL = 1000 mg/kg/day for female rats
LOEL = 2000 mg/kg/day for female rats based on increased liver weights relative to body weights.
Core - Supplementary (Special study; not guideline)

3) 30-Day Feeding Study - Rats
MRID No. 415648-03
Male and female Sprague-Dawley rats were given 0, 500 or 2000 mg/kg/day in diet for 30 days.
Compound related effects were enlargement of the liver with centrilobular hepatocellular hypertrophy in male and female rats.
NOEL < 500 mg/kg/day in male and female rats.
LOEL = 500 mg/kg/day in male and female rats based on increased liver weights.
Core - Supplementary (Special study; not guideline)

4) 30-Day Feeding Study - Dogs
MRID No. 415648-04
Male and female beagle dogs were given 0, 100, 500 or 1000 mg/kg/day orally. Test material was enclosed in gelatin capsules for administration.
Dose related histopathological and hematological effects were reported for male and female dogs at all treatment levels.
A NOEL was not determined due to a dose related reduction in platelet counts and hepatocytomegaly in females, and delayed sexual maturation in males.

Core - Supplementary (Special study; not guideline)

5) 90-Day Feeding Study - Rats (82-1) and One-Generation Reproduction Study - Rats
MRID No. 415648-06
Male and female Sprague-Dawley rats were given 0, 250, 750 or 2000 mg/kg/day in diet for 90 days. At this time, 10 males and 10 females from each group were sacrificed for histopathological evaluation. The remaining 25 rats from each group were bred. Pups and parental rats were sacrificed on day 21 of lactation.
No significant reproductive effects were reported at any dose tested. Increased adrenal, liver and kidney weights and urinary specific gravity and transient effects on prothrombin time and blood glucose were reported.
Systemic NOEL = not established in females, 250 mg/kg/day in males
Systemic LOEL = 250 mg/kg/day in females based on relative adrenal weights
= 750 mg/kg/day in males based on increased relative kidney and liver weights
Reproductive NOEL = 750 mg/kg/day
Reproductive LOEL = 2000 mg/kg/day based on reduced Day 21 pup weights
Core - Guideline for 90-Day Study
Core - Supplementary for Reproduction Study (Special study; not guideline)

6) 90-Day Oral Study - Dogs (82-1)
MRID No. 415648-05
Male and female beagles were given 0, 5, 15, 50 or 500 mg/kg/day for 90 days. The test material was given orally enclosed in gelatin capsules.
Increased liver weights, decreased urinary pH and increased blood glucose concentrations were reported in male and female dogs.
NOEL = 15 mg/kg/day in male and female dogs
LOEL = 50 mg/kg/day based on effects cited above
Core - Guideline

7) Developmental Toxicity Study - Rats (Range-finding)
MRID No. 415648-07
The test material was given by gavage to pregnant Sprague-Dawley rats on days 6 through 15 of gestation, inclusive at doses of 0, 250, 500, 1000 or 2000 mg/kg/day.
On the basis of this study, dose levels of 0, 500, 1000 and 2000 mg/kg/day were selected for a subsequent Developmental Toxicity Study.
Maternal NOEL = 1000 mg/kg/day
Maternal LOEL = 2000 mg/kg/day based on slight body weight gain and food consumption reductions
Developmental NOEL = not determined

Core - Supplementary (Special study; not guideline)

8) Developmental Toxicity Study - Rats (83-3)
MRID No. 415648-08
Pregnant Sprague-Dawley rats were given the test material by gavage at 0, 500, 1000 or 2000 mg/kg/day on gestational days 6 through 15 inclusive. Neither maternal nor developmental toxicity were reported at 2000 mg/kg/day (HDT). The test material is not a developmental toxicant to rats at doses up to 2000 mg/kg/day.
Core - Guideline

9) Developmental Toxicity - Rabbits (Range-finding)
MRID No. 415648-09
Pregnant New Zealand White rabbits were given test material by gavage on days 6 through 19 inclusive. Treatment levels were 0, 250, 500, 1000 or 2000 mg/kg/day. Based on the findings of this study, doses of 0, 250, 500 and 1000 mg/kg/day were selected for the subsequent Developmental Toxicity Study.
Maternal NOEL = 250 mg/kg/day
Maternal LOEL = 500 mg/kg/day based upon body weight loss during gestation
Developmental NOEL = not determined
Core - Supplementary (Special study; not guideline)

10) Developmental Toxicity - Rabbits (83-3)
MRID No. 415648-10
Pregnant New Zealand White rabbits were given 0, 250, 500 or 1000 mg/kg/day of the test material by gavage on days 7 through 19 of gestation, inclusive.
Maternal NOEL = 250 mg/kg/day
Maternal LOEL = 500 mg/kg/day based on body weight gain decrement and reduced food consumption
Developmental NOEL > 1000 mg/kg/day (HDT)
Core - Guideline

11) Metabolism - Rat (85-1)
MRID No. 415648-18
Male and female Sprague-Dawley rats were given single oral doses of 10 or 1000 mg/kg of ^{14}C-SDS-46851. The major route of excretion was in feces, with a substantial amount of ^{14}C excreted in urine. The test material reached a peak level in the blood rapidly (within 3 hours). The elimination half-lives for the low and high dose were 2.5 and 6.2 hours, respectively.
Core - Supplementary (see attached DER)

12) Mutagenicity - Salmonella/Mammalian Activation Gene Mutation Assay
MRID No. 415648-12
Five dose of 2,4,5-trichloro-3-carboxy-benzamide from 39 to 3900 µg/plate were evaluated by plate incorporation assay.

The 3900 µg/plate level was cytotoxic. S. typhimurium strains TA1535, TA1537, TA1538, TA98 and TA100 were used. No evidence of mutagenicity was reported with or without activation at any concentration tested.
Core - Acceptable

13) Mutagenicity - Mammalian Cells in Culture Gene Mutation Assay in Mouse Lymphoma Cells
MRID No. 415648-13
SDS-46851 was evaluated using L5178Y mouse lymphoma cells with and without activation. Doses tested ranged from 50 to 5000 µg/ml. The 5000 µg/ml dose was excessively cytotoxic. Therefore, doses used for mutant selection were 500, 1000, 2000, 3000 and 4000 µg/ml. No mutagenic effects were reported in the absence of activation. The portion of the study conducted with activation indicated spontaneous mutation frequencies outside the normal range.
Core - Unacceptable

14) Mutagenicity - Mammalian Cells in Culture Gene Mutation Assay in Mouse Lymphoma Cells
MRID No. 415648-14
SDS-46851 was evaluated using L5178Y mouse lymphoma cells with and without activation. Doses tested ranged from 75 to 1000 µg/ml. The 1000 µg/ml dose was adequately cytotoxic. No mutagenic effects were reported with or without activation.
Core - Acceptable

15) Mutagenicity - Unscheduled DNA Synthesis Assay in Primary Rat Hepatocytes
MRID No. 415648-15
Five doses of the test material from 24 to 240 µg/well did not induce unscheduled DNA synthesis in primary rat hepatocytes.
Core - Acceptable

16) Mutagenicity - Mammalian Cells in Culture Sister Chromatid Exchange Assay in Chinese Hamster Ovary (CHO) Cells
MRID No. 415648-16
No evidence of cytotoxicity was reported in the treatment range tested (200 to 2000 µg/ml) with or without activation. Results of the assay were negative. However, the exposure of the nonactivated cells was for only 5 hours and may have been insufficient to have induced sister chromatid exchange.
Core - Unacceptable

17) Mutagenicity - In Vivo Mouse Micronucleus Assay in Mice
MRID No. 415648-17
Male and female Swiss mice were given single oral gavage administrations of the test material equivalent to 0, 500, 2500 or 5000 mg/kg. Bone marrow cells indicated increased frequencies of micronucleated polychromatic erythrocytes in low dose males at 24 and 48 hours after dosing and in high

dose females at 48 hours after dosing. There was no evidence of dose response and the values fell within normal background ranges. Therefore, the results were considered negative.
Core - Unacceptable (Can be upgraded; see DER)

Recommendations: No data are currently available on the oncogenic risk posed by PCBN and SDS-46851. However, the following considerations of chemical structure may be useful in evaluating the behavior of these two materials:

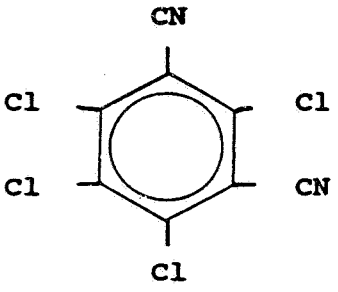
1) As is evident from the structures attached, PCBN is intermediate between the parent and HCB. Therefore, it may be metabolized similarly to these materials, and by inference, have carcinogenic potential intermediate between these two material.

2) The SDS-46851 has two polar groups replacing the nucleophilic groups on the parent. This material is rapidly excreted and may have limited capacity to produce oncogenicity. That is, the SDS-46851 may behave more similarly to the SDS-3701 than to the parent.

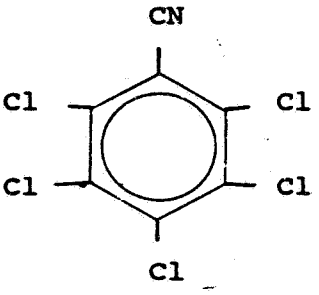
The above two statements can not be definitively defended in the absence of data, but do reflect the current considerations of Tox Branch based upon a structural comparison to the three materials for which data are available.

A new DRES analysis incorporating the rotational crop residue data provided by the registrant should include these considerations in assigning potency factors.

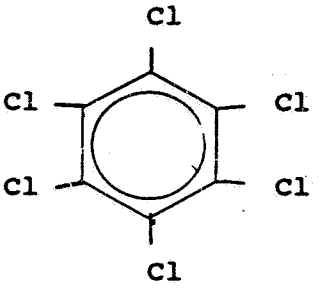
Structures of Chlorothalonil and Related Compounds



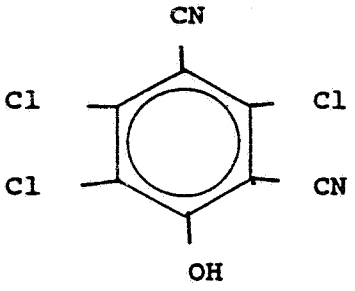
Chlorothalonil
(Parent)



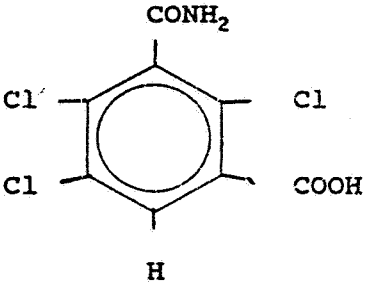
Pentachloro-
benzonitrile



Hexachlorobenzene



SDS-3701



SDS-46851

Reviewed by: Elizabeth A. Doyle, Ph.D. E.A.D. 9/11/90
Section I, Toxicology Branch II (HFAS) (H7509C)
Secondary reviewer: Yiannakis M. Ioannou, Ph.D. 9/11/90
Section I, Toxicology Branch II (HFAS) (H7509C)

008334

DATA EVALUATION REPORT

Study Type: Acute Oral Toxicity - Rats (81-1) Tox. Chem. No.: 215B

MRID Number: 415648-01

Test Material: Chlorothalonil Metabolite

Synonyms: SDS-46851, 2,4,5-Trichloroisophthalamic acid, 2,5,6-trichloro-3-carboxybenzamide, 3-carbamyl-2,4,5-trichlorobenzoic acid

Study Number: 684-5TX-84-0033-001

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

Testing Facility: SDS Biotech Corporation
Department of Safety Assessment
Life Science Toxicology
7528 Auburn Road
Painesville, Ohio 44077

Title of Report: Acute Oral Toxicity Screening in Rats with SDS-46851

Authors: S. K. Shults, M. C. Long and N. H. Wilson

Report Issued: January 2, 1985

Conclusions: Under the conditions of this study, the test material was found to be essentially nontoxic to male and female rats by the oral route.

LD50 >5000 mg/kg in male and female rats

Toxicity Category IV

Classification: core - Supplementary
(Deficient in that the number of rats used in this study does not conform to the guidelines recommendation.)

This study does not satisfy the guideline requirements (81-1) for an "Acute Oral Toxicity Study in Rats".

MATERIALS AND METHODS

The test material used in this study was 2,4,5-trichlorophthalamic acid (purity ≈ 95% a.i.), a white powder. The test material was administered as a single oral dose by gavage at treatment levels of 200, 1000 and 5000 mg/kg. The test material was prepared as 0.5% w/v in methylcellulose.

Two male and two female Charles River CD (Sprague-Dawley) rats were treated with each concentration. Treatment concentrations were 200, 1000 and 5000 mg/kg. Rats were observed once every hour for six hours following dosing and twice daily thereafter through day 10. On days 11 through 14, rats were observed once daily. Body weights were recorded immediately before dosing and on days 3, 7 and 14. All rats were necropsied at termination.

RESULTS AND DISCUSSION

No mortality occurred due to treatment with the test material. No abnormal clinical signs were reported in male or female rats given 200 mg test material/kg. Two rats given 1000 mg/kg developed soft stool containing mucous. At 5000 mg/kg, soft stool containing mucous and tan colored particles was noted in all treated rats. One high dose rat exhibited anogenital staining. These signs developed by about two hours post-treatment and cleared by about six hours post-treatment. Red nasal discharge and chromodacryorrhea occurred in one male and one female high dose rat on days 7 and 10, respectively. These signs persisted until termination. No effect on body weight was reported due to treatment. All groups exhibited similar weight gain patterns during the observation period. No treatment related gross pathology was reported.

The number of rats used in this study was less than that recommended by the guidelines (five per sex/dose). This study was considered to be inadequate on the basis that an insufficient number of rats were used.

CONCLUSIONS

Under the conditions of this study, the test material was found to be essentially nontoxic to male and female rats by the oral route.

LD50 >5000 mg/kg in male and female rats

Toxicity Category IV

Classification: core - Supplementary
(Deficient in that the number of rats used in this study does not conform to the guidelines recommendation.)

This study does not satisfy the guideline requirements (81-1) for an "Acute Oral Toxicity Study in Rats".

E.A.D. 9/12/90

Reviewed by: Elizabeth A. Doyle, Ph.D.
Section I, Tox. Branch II (HFAS) (H7509C)
Secondary Reviewer: Yiannakis M. Ioannou, Ph.D. JMI 9-12-90
Section I, Tox. Branch II (HFAS) (H7509C)

008334

DATA EVALUATION REPORT

STUDY TYPE: 14-Day Feeding - Rats TOX. CHEM. NO.: 215B

MRID NO.: 415648-02

TEST MATERIAL: Chlorothalonil Metabolite

SYNONYMS: SDS-46851, 3-Carboxy-2,5,6-trichlorobenzamide, 2,4,5-Trichloroisophthalamic acid, 3-Carbamyl-2,4,5-trichlorobenzoic acid

STUDY NUMBER: 684-5TX-84-0069-003

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

TESTING FACILITY: SDS Biotech Corporation
Department of Safety Assessment
Life Science Toxicology
Painesville, Ohio 44077

TITLE OF REPORT: A 14-day feeding study in rats with 3-carboxy-2,5,6-trichlorobenzamid (SDS-46851)

AUTHOR(S): A. H. Wilson and E. M. Sadler

REPORT ISSUED: May 15, 1985

CONCLUSIONS: The test material was essentially nontoxic to male and female rats at treatment levels up to 2000 mg/kg/day.

NOEL > 2000 mg/kg/day in male rats
NOEL = 1000 mg/kg/day in female rats
LOEL = 2000 mg/kg/day in female rats based on increased liver weights relative to body weights

Classification: core - Supplementary
(This is not a guideline study.)

A. MATERIALS:

1. Test compound: 3-Carbcxy-2,5,6-trichlorobenzamid, Description: tan microcrystals, Batch #T-165-2, Purity: >94%, contaminants: list in CBI appendix

2. Test animals: Species: rat, Strain: CD (Sprague-Dawley), Age: 40 days, Weight: male - 156-184 g, female - 117-137 g, Source: Charles River Breeding Laboratories, Portage, MI

B. STUDY DESIGN:

1. Animal assignment - Animals were assigned using a weight stratified randomization technique (Concord Woods Animal Facility Standard Operating Procedure) to the following test groups:

Test Group	Dose in diet (mg/kg/day)	Main Study 14 days male	Main Study 14 days female	Interim Sac. - days male	Interim Sac. - days female
I	0	5	5		
II	125	5	5		
III	250	5	5		
IV	500	5	5		
V	1000	5	5		
VI	2000	5	5		

2. Diet preparation - Diet was prepared weekly and stored at room temperature in the dark. Samples of treated food were analyzed for concentration, stability and homogeneity prior to the initiation of the study using test batches of diet containing 1250 ppm and 20,000 ppm (125 and 2000 mg/kg/day) prepared in the same manner as the test diets for use in the study. Two samples of each diet were also taken during the study to confirm concentration.

Results - Analyses conducted to determine stability of the test material in food indicated that it is stable for at least 14 days. Homogeneity was also confirmed.

Weekly diet samples from 97 to 102% of nominal for male rats and 37 to 103% for female rats.

3. Animals received food (Purina Certified Rodent Chow #5002) and water ad libitum.

4. Statistics - Per the report, "For each week, the mean body weights and food consumption (absolute and relative to body weight) of all test groups were compared with the control group mean values using analysis of variance and Dunnett's multiple comparison tables. This method of statistical analysis of test versus control group data also was conducted for the clinical pathology values (where

appropriate), the absolute organ weight values, organ weight to body weight values and organ weight to brain weight values obtained at termination."

5. Quality assurance was documented by signed and dated GLP and quality assurance statements.

C. METHODS AND RESULTS:

1. Observations - Animals were inspected twice daily for signs of toxicity and mortality.

   Results - Toxicity - During week 1 of the study, one male form Group V and one female from Group IV exhibited slight red nasal discharge and chromodacryorrhea. No other apparent treatment related effects were reported.

   Mortality (survival) - All rats survived to terminal sacrifice.

2. Body weight - Rats were weighed weekly beginning one week prior to administration of the test material until termination of the study.

   Results - No treatment related effects were reported.

3. Food consumption and compound intake - Consumption was measured over a six day period weekly and mean daily diet consumption was calculated. Efficiency and compound intake were calculated from the consumption and body weight gain data.

   Results - Food consumption - No treatment related effects were reported. Group V male food consumption during week 1 was significantly greater than the control. However, this difference did not appear to be biologically significant in that no apparent relation to dose was reported and the difference did not persist during week 2.

   Food efficiency - Food intake as a function of body weight was not affected by treatment.

   Compound intake - Compound intake for males was 86-90% and 83-89% of nominal during weeks 1 and 2, respectively. Comparable values for females were 86-90% and 87-90%, respectively.

4. Ophthalmological examinations were not performed.

5. Blood was collected at termination for hematology and clinical analysis from all animals. The CHECKED (X) parameters were examined.

a. Hematology:

X		X	
X	Hematocrit (HCT)		Total plasma protein (TP)
X	Hemoglobin (HGB)		Leukocyte differential count
X	Leukocyte count (WBC)		Mean corpuscular HGB (MCH)
X	Erythrocyte count (RBC)		Mean corpuscular HGB conc. (MCHC)
	Platelet count		Mean corpuscular volume (MCV)

Results - The suggestion of dose related increases in WBC in males and females was apparent in the data. However, no statistically significant differences were reported and the absolute differences in WBC were small. No other treatment related effects were reported.

b. Clinical Chemistry

X		X	
	Electrolytes:		Other:
	Calcium	X	Albumin
X	Chloride		Blood creatinine
	Magnesium	X	Blood urea nitrogen
	Phosphorous		Cholesterol
X	Potassium	X	Globulins
X	Sodium		Glucose
	Enzymes:		Total Bilirubin
X	Alkaline phosphatase	X	Total Protein
	Cholinesterase		Triglycerides
	Creatinine phosphokinase		
	Lactic acid dehydrogenase		
X	Serum alanine aminotransferase (also SGPT)		
	Serum aspartate aminotransferase (also SGOT)		

Results - Females exhibited a slight but nonsignificant dose related decrease in SGPT. In addition, group II, V and VI females had reduced alkaline phosphatase activity. No other treatment related effects were reported.

c. Urinalysis - Urine was collected from fasted animals at termination. The CHECKED (X) parameters were examined.

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

Results - No treatment related effects were reported.

7. Sacrifice and Pathology - All animals that died and that were sacrificed on schedule were subject to gross pathological examination and the CHECKED (X) tissues were collected for histological examination. The (XX) organs were also weighed.

X	X	X
Digestive system	Cardiovas./Hematol.	Neurologic
Tongue	Aorta	XX Brain
Salivary glands	XX Heart	Periph. nerve
Esophagus	Bone marrow	Spinal cord (3 levels)
X Stomach	Lymph nodes	Pituitary
X Duodenum	Spleen	Eyes (optic nerve)
Jejunum	Thymus	Glandular
Ileum	Urogenital	XX Adrenals
Cecum	XX Kidneys	Lacrimal gland
Colon	Urinary bladder	Mammary gland
Rectum	XX Testes	X Parathyroids
XX Liver	Epididymides	X Thyroids
Gall bladder	Prostate	Other
Pancreas	Seminal vesicle	Bone
Respiratory	XX Ovaries	Skeletal muscle
Trachea	Uterus	Skin
Lung		X All gross lesions and masses

Results

a. Organ weight - No treatment related effect on absolute organ weight, organ weight per 100 g of body weight and organ weight per g of brain weight were reported for male rats at any treatment level.

In females, slight, dose related increases in absolute liver, kidney and ovary weights were reported. When normalized to body weight and brain weight, no difference in kidney weights remained. However, dose related trends in liver and ovary weights persisted after normalization. The only statistically significant increase was in the liver weight relative to body weight in females from the 2000 mg/kg/day group.

ORGAN WEIGHTS - FEMALE RATS

Dose Level (mg/kg/day)	Absolute (g)	Relative to Body Weight (g/100 g BW)	Relative to Brain Weight (g/g Brain)
Liver			
0	5.528	3.458	3.256
125	5.922	3.502	3.510
250	6.350	3.624	3.680
500	5.714	3.534	3.352
1000	6.422	3.698	3.676
2000	6.354	3.944**	3.674
Kidneys			
0	1.574	0.980	0.926
125	1.658	0.984	0.984
250	1.772	1.022	1.024
500	1.616	1.002	0.948
1000	1.696	0.980	0.970
2000	1.676	1.038	0.970
Ovaries			
0	0.0656	0.0413	0.0386
125	0.0738	0.0435	0.0436
250	0.0752	0.0430	0.0435
500	0.0726	0.0449	0.0426
1000	0.0802	0.0463	0.0459
2000	0.0766	0.0479	0.0443

**Significantly different from the control, $p<0.01$

b. Gross pathology - No treatment related gross abnormalities were reported.

c. Microscopic pathology - No treatment related effects were reported.

D. DISCUSSION: Treatment levels of up to 2000 mg/kg/day for 14 days appeared to be largely without adverse effect in this study. Although the suggestion of treatment related effects on white blood cell counts and liver enzymes were cited above for high dose rats, the magnitude of the effects reported was so small as to be of questionable biological significance. Similarly, differences in organ weights were small in magnitude and may reflect adaptation and metabolism of the test material.

E. CONCLUSIONS: The test material was essentially nontoxic to male and female rats at treatment levels up to 2000 mg/kg/day.

NOEL > 2000 mg/kg/day in male rats
NOEL = 1000 mg/kg/day in female rats
LOEL = 2000 mg/kg/day in female rats based on increased liver weight relative to body weight

CLASSIFICATION: core - Supplementary
(This is not a guideline study.)

E.A. Doyle 9/30/90

Reviewed by: Elizabeth A. Doyle, Ph.D.
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Secondary Reviewer: Yiannakis M. Ioannou, Ph.D. JMI 9/14/90
Section I, Tox. Branch II (HFAS) (H7509C)

002334

DATA EVALUATION REPORT

STUDY TYPE: 30-Day Feeding Study - Rats TOX. CHEM. NO.: 215B

MRID NO.: 415648-03

TEST MATERIAL: Chlorothalonil Metabolite

SYNONYMS: 3-Carboxy-2,5,6-trichlorobenzamide, 2,4,5-Trichloroisophthalamic acid, 3-Carbamyl-2,4,5-trichlorobenzoic acid, SDS-46851

STUDY NUMBER: 736-5TX-85-0007-003

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

TESTING FACILITY: SDS Biotech Corporation
Department of Safety Assessment
Life Science Toxicology
7528 Auburn Road
Painesville, Ohio 44077

TITLE OF REPORT: A 30-Day Feeding Study in Rats with 3-Carboxy-2,5,6-Trichlorobenzamide

AUTHORS: D. M. Serrone, N. H. Wilson and J. C. Killeen

REPORT ISSUED: February 21, 1986

CONCLUSIONS: Treatment with the test material in diet for 30 days resulted in enlargement of the liver and centrilobular hepatocellular hypertrophy in male and female rats.

NOEL < 500 mg/kg/day in male and female rats
LOEL = 500 mg/kg/day in male and female rats based on increased liver weights

CLASSIFICATION: core - Supplementary
(This is not a guideline study.)

A. MATERIALS:

1. Test compound: 3-Carboxy-2,5,6-trichlorobenzamide Description: tan microcrystals Batch #T-165-2, Purity >94%, contaminants: list in CBI appendix

2. Test animals: Species: rat, Strain: CD (Sprague-Dawley), Age: 41 days, Weight: male - 170-209 g, female - 132-168 g, Source: Charles River Breeding Laboratories, Portage, MI

B. STUDY DESIGN:

1. Animal assignment - Animals were assigned using a weight stratified randomization procedure (Concord Woods Animal Facility Standard Operating Procedure) to the following test groups:

Test Group	Dose Level (mg/kg/day)	Main Study 30 days male	Main Study 30 days female	Interim Sac. - days male	Interim Sac. - days female
1 Cont.	0	5	5	5	5
2 Low (LDT)	500	5	5	5	5
3 High (HDT)	2000	5	5	5	5

2. Diet preparation - Diet was prepared weekly and stored at room temperature in the dark. Samples of treated food were analyzed for concentration, stability and homogeneity prior to the initiation of the study using test batches of diet containing 5000 ppm and 20,000 ppm (500 and 2000 mg/kg/day) prepared in the same manner as the test diets for use in the study. Two samples of each diet were also taken during the study to confirm concentration.

Results - The test material was found to be stable in diet for at least 14 days. Homogeneity analyses indicated that the nominal 5000 ppm diet contained 4880 ppm ± 2% and the 20000 ppm diet contained 20327 ppm ± 2%. Weekly diet samples tested for verification of concentration contained 99-103% and 94-103% of nominal for the 5000 and 20000 ppm diets fed to females and 98-102% and 20-102% of the nominal concentrations in diets fed to males.

3. Animals received food (Purina Certified Rodent Chow #5002) and water ad libitum.

4. Statistics - Per the report, "For each week, the mean body weight and food consumption (absolute and relative to body weight) values of all test groups were compared with the control group mean values using analysis of variance and Dunnett's multiple comparison tables. This method of statistical analysis of test versus control group data also was conducted for the clinical pathology values (where appropriate), the absolute organ weight values, organ weight to body weight values and organ weight to brain weight values

obtained at termination. A value of $p < 0.05$ was accepted as significant."

5. Quality assurance was documented by signed and dated GLP and quality assurance statements.

C. METHODS AND RESULTS:

1. Observations - Animals were inspected twice daily for signs of toxicity and mortality. A complete physical examination was made once per week.

   Results - Toxicity - No treatment related clinical signs of toxicity were reported.

   Mortality (survival) - All rats survived to scheduled termination.

2. Body weight - Rats were weighed weekly for the duration of the study.

   Results - No treatment related effects were reported.

3. Food consumption and compound intake - Consumption was determined and mean daily diet consumption was calculated. Efficiency and compound intake were calculated from the consumption and body weight gain data.

   Results - Food consumption and food efficiency - No treatment related effects were reported.

   Compound intake - Compound intake was within 8% of the target dose for the four weeks of feeding treated diet.

4. Ophthalmological examinations were not performed.

5. Blood was collected at termination of the study (30 days) for hematology and clinical analysis from all animals. The CHECKED (X) parameters were examined.

a. Hematology:

X	Hematocrit (HCT)	X	Total plasma protein (TP)
X	Hemoglobin (HGB)		Leukocyte differential count
X	Leukocyte count (WBC)		Mean corpuscular HGB (MCH)
X	Erythrocyte count (RBC)		Mean corpuscular HGB conc. (MCHC)
	Platelet count		Mean corpuscular volume (MCV)

Results - No treatment related effects were reported.

b. Clinical Chemistry

X	Electrolytes:	X	Other:
	Calcium	X	Albumin
X	Chloride	X	Blood creatinine
	Magnesium	X	Blood urea nitrogen
	Phosphorous		Cholesterol
X	Potassium	X	Globulins
X	Sodium	X	Glucose
	Enzymes:		Total Bilirubin
X	Alkaline phosphatase	X	Total Protein
	Cholinesterase		Triglycerides
	Creatinine phosphokinase		
	Lactic acid dehydrogenase		
X	Serum alanine aminotransferase (also SGPT)		
	Serum aspartate aminotransferase (also SGOT)		

Results - No treatment related effects were reported. Although a statistically significant ($p < 0.05$) increase in total protein was reported in males from the low dose group, this observation was considered anomalous in that the high dose value was similar to the control.

6. Urinalysis - Urine was collected from fasted animals prior to the 30 day sacrifice. The CHECKED (X) parameters were examined.

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

Results - No treatment related effects were reported.

7. Sacrifice and Pathology - All animals that died and that were sacrificed on schedule were subject to gross pathological examination and the CHECKED (X) tissues were collected for histological examination. The (XX) organs were also weighed.

X		X		X	
	Digestive system		Cardiovas./Hematol.		Neurologic
	Tongue		Aorta	XX	Brain
X	Salivary glands	XX	Heart		Periph. nerve
X	Esophagus	X	Bone marrow	X	Spinal cord (3 levels)
X	Stomach	X	Lymph nodes	X	Pituitary
X	Duodenum	X	Spleen	X	Eyes (optic nerve)
X	Jejunum	X	Thymus		Glandular
X	Ileum		Urogenital	XX	Adrenals
X	Cecum	XX	Kidneys		Lacrimal gland
X	Colon	X	Urinary bladder		Mammary gland
X	Rectum	XX	Testes	X	Parathyroids
XX	Liver	X	Epididymides	X	Thyroids
	Gall bladder	X	Prostate		Other
X	Pancreas		Seminal vesicle	X	Bone
	Respiratory	XX	Ovaries	X	Skeletal muscle
X	Trachea	X	Uterus	X	Skin
X	Lung			X	All gross lesions and masses

Results -

a. Organ weight - Males and females exhibited dose related increases in liver weights expressed as absolute weight or relative to body or brain weight. The increases appeared to be biologically and statistically significant, contrary to arguments presented by the registrant.

Kidney weights, expressed in all three forms, were slightly increased in a dose related manner in males only. The magnitude of increase was so small as to be of questionable significance.

No other treatment related effects on organ weights were reported.

ORGAN WEIGHTS

Dose Level (mg/kg/day)	Absolute (g)	Relative to Body Weight (g/100 g BW)	Relative to Brain Weight (g/g Brain)
		Males	
Liver			
0	9.850	2.910	5.086
500	11.308	3.280	5.828
2000	11.798*	3.432*	6.098*
Kidney			
0	2.588	0.764	1.336
500	2.750	0.798	1.416
2000	2.964	0.862	1.530
		Females	
Liver			
0	5.708	2.816	3.118
500	6.130	3.088*	3.450
2000	6.552	3.294**	3.666

*Significantly different from the control, $p < 0.05$.
**Significantly different from the control, $p < 0.01$.

b. Gross pathology - At necropsy, two of five high dose females were reported with dilated renal pelvis, described in the pathology report as "incidental to treatment". No other unusual abnormalities were reported.

c. Microscopic pathology - Centrilobular hepatocellular hypertrophy occurred in all high dose male and female rats. The severity was characterized as slight to very slight. The enlarged hepatocytes were reported to contain an increased amount of eosinophilic cytoplasm. One low dose male also exhibited very slight hepatocellular hypertrophy.

D. DISCUSSION: The effects of the test material appeared to be largely on the liver. Increased size as indicated by liver weight was apparent at both treatment levels. The registrant indicated that the liver weight data was not evidence of treatment effect because weights were not outside the historical control range for rats from this laboratory. However, the data exhibited a clear dose related trend and statistical significance for specific treatment levels (both absolute and normalized values) relative to the concurrent control. In addition, the hepatocellular hypertrophy reported at the high dose confirms the liver as the

target organ for the test material. This is consistent with effects generally associated with chlorinated aromatic compounds.

E. CONCLUSIONS: Treatment with the test material in diet for 30 days resulted in enlargement of the liver and centrilobular hepatocellular hypertrophy in male and female rats.

NOEL < 500 mg/kg/day in male and female rats
LOEL = 500 mg/kg/day in male and female rats based on increased liver weights

CLASSIFICATION: core - Supplementary
(This is not a guideline study.)

Reviewed by: Elizabeth A. Doyle, Ph.D. E.A.D. 9/13/90
Section I, Tox. Branch II (HFAS) (H7509C)
Secondary Reviewer: Yiannakis M. Ioannou, Ph.D. JM.I 9/12/90
Section I, Tox. Branch II (HFAS) (H7509C)

008334

DATA EVALUATION REPORT

STUDY TYPE: 30-Day Feeding - Dog TOX. CHEM. NO.: 215B

MRID NO.: 415648-04

TEST MATERIAL: Chlorothalonil Metabolite

SYNONYMS: 3-Carbamyl-2,4,5-trichlorobenzoic acid, 2,4,5-trichloroisophthalamic acid, 3-carboxy-2,5,6-trichlorobenzamide, 2,5,6-trichloro-3-carboxybenzamide, SDS-46851

STUDY NUMBER: 1644-87-0073-TX-003

SPONSOR: Fermenta Plant Protection Company
5966 Heisley Road
P. O. Box 8000
Mentor, Ohio 44061-8000

TESTING FACILITY: Bio\dynamics Inc.
P. O. Box 2360
East Millstone, New Jersey 08875-2360

and

Experimental Pathology Laboratories
P. O. 474
Herndon, Virginia 22070

TITLE OF REPORT: A 30-Day Oral Toxicity Study in dogs with 3-Carbamyl-2,4,5-Trichlorobenzoic Acid (SDS-46851)

AUTHOR(S): D. M. Serrone and J. C. Killeen, Jr.

REPORT ISSUED: January 12, 1989

CONCLUSIONS: The test material caused dose related histopathological and hematological abnormalities in male and female dogs at all treatment levels tested (100 to 1000 mg/kg/day).

NOEL was not determined due to dose related reduction in platelet counts and hepatocytomegaly in males and females, and delayed sexual maturation in males.

CLASSIFICATION: core - Supplementary
(This is not a guideline study. Deficient in that insufficient numbers of animals were used to permit interpretation of the data.)

A. MATERIALS:

1. Test compound: 3-carbamyl-2,4,5-trichlorobenzoic acid Description: beige powder Batch #T-165-2, Purity 98%, contaminants: not given

2. Test animals: Species: dog, Strain: beagle, Age: 5-6 months, Weight: males - 7.2-8.6 kg, females - 4.7-7.1 kg, Source: Marshall Farms, USA, North Rose, New York 14516

B. STUDY DESIGN:

1. Animal assignment - Animals were assigned by weight stratified randomization to the following test groups:

Test Group	Dose (mg/kg/day)	Main Study 37-38 days male	Main Study 37-38 days female	Interim Sac. - day male	Interim Sac. - day female
1 Cont.	0	2	2		
2 Low (LDT)	100	2	2		
3 Mid (MDT)	500	2	2		
4 High (HDT)	1000	2	2		

2. Treatment administration - The test compound was measured into the gelatin capsules based upon the body weight of the dogs at the beginning of each treatment week. Capsules for each dog were prepared weekly. Dogs were dosed 30 minutes following consumption of half of their daily feed ration. They were then observed for 30 minutes and given the remainder of their ration.

3. Animals received 400 g of Wayne Bite Size laboratory diet daily. Water was available ad libitum.

4. Statistics - No statistical analyses were conducted.

5. Quality assurance was documented by signed and dated GLP and quality assurance statements.

C. METHODS AND RESULTS:

1. Observations - Animals were inspected twice daily for signs of toxicity and mortality.

Results - Toxicity - One male (#4002) and one female (#4502) from the high dose group were thin and lethargic during week 5 and weeks 4 and 5, respectively. The male had tarry stools on day 31 and yellow ocular discharge and/or slight ocular opacity during weeks 4 and 5. The female exhibited red stool, emesis, red emesis and recumbency on one or more occasions between days 31 and 38.

No other abnormal observations were reported.

Mortality (survival) - All dogs survived to scheduled sacrifice.

2. Body weight - Dogs were weighed pretest, weekly for the duration of the study and immediately prior to sacrifice.

Results - No adverse effect on body weight or body weight gain occurred in low dose males or females. In the mid and high dose groups, body weight gains were similar to the control through the week 2 weighing. During the remaining treatment weeks, one male from the mid and high dose groups and one female from the high dose group lost weight to the extent that total body weight change for the treatment period represented a net loss relative to start weight. The remaining animals from the mid and high dose groups had reduced body weight gains relative to the control. The effect on body weight appeared to be dose related, however, the limited number of dogs used in the study reduces the certainty of this observation. No apparent difference between males and females was noted.

BODY WEIGHTS BY WEEK (KG)

Animal #	Sex	Dose	0	1	2	3	4	5
1001	M	0	8.1	8.4	8.5	8.9	9.1	9.2
1002	M	0	7.8	8.6	9.0	9.3	9.5	9.6
2001	M	100	8.0	8.5	8.4	8.6	8.6	9.1
2002	M	100	8.6	8.8	9.4	9.3	9.5	9.5
3001	M	500	8.2	8.5	8.6	8.8	8.8	9.0
3002	M	500	7.2	7.5	7.9	7.8	7.4	6.2
4001	M	1000	7.7	8.2	8.2	8.3	8.4	8.3
4002	M	1000	8.2	8.8	8.8	8.5	7.2	5.9
1501	F	0	4.7	4.9	5.1	5.4	5.7	5.7
1502	F	0	7.1	7.7	8.0	8.4	8.4	8.8
2501	F	100	5.2	5.4	5.7	5.8	6.2	6.1
2502	F	100	6.6	7.1	7.4	7.6	8.0	8.2
3501	F	500	5.5	5.7	5.8	5.9	6.2	6.2
3502	F	500	5.7	6.1	6.2	6.2	6.3	6.1
4501	F	1000	5.8	6.2	6.3	6.3	6.5	6.7
4502	F	1000	5.3	5.6	5.5	5.1	4.5	4.8

BODY WEIGHT CHANGE

Dose (mg/kg/day)	Animal # (males)	Change (kg)	Animal # (females)	Change (kg)
0	1001	1.1	1501	1.0
	1002	1.8	1502	1.7
100	2001	1.1	2501	0.9
	2002	0.9	2502	1.6
500	3001	0.8	3501	0.7
	3002	-1.0	3502	0.4
1000	4001	0.6	4501	0.9
	4002	-2.3	4502	-0.5

3. Food consumption - Consumption was monitored visually during each feeding (daily).

   Results - As indicated above, food consumption measurements were visual estimates of the amount of food consumed daily by each dog. Generally, food consumption data paralleled body weight gain data, with control dogs of both sexes consuming all or almost all of their food consistently throughout the study. Treatment related reductions in food consumption roughly exhibited dose response, with the greatest decrease in the high dose dogs.

4. Ophthalmological examinations were performed pretest and after 30 days on all animals.

   Results - No treatment related effects were reported.

5. Blood was collected before treatment and at 37-38 days for hematology and clinical analysis from all animals. The CHECKED (X) parameters were examined.

a. Hematology:

X	Hematocrit (HCT)	X	Prothrombin Time
X	Hemoglobin (HGB)	X	Leukocyte differential count
X	Leukocyte count (WBC)	X	Mean corpuscular HGB (MCH)
X	Erythrocyte count (RBC)	X	Mean corpuscular HGB conc. (MCHC)
X	Platelet count	X	Mean corpuscular volume (MCV)

Results - Terminal platelet counts exhibited a dose related decrease in male and female dogs. One mid dose male (#3002) and one high dose male (#4002) exhibited increased HGB, HCT and erythrocyte counts. Both of these dogs had net body weight losses. The registrant offered that this effect in #4002 was suggestive of dehydration when coupled with the observation that the dog appeared thin and lethargic at sacrifice. In addition, this may partially account for the higher platelet count observed in this dog (3.44 x $10^5/\mu l$). Similar arguments can be made for #3002 although the observed effects were less pronounced.

PLATELET COUNTS (x $10^5/\mu L$) - TERMINATION

	Dose (mg/kg/day)			
Sex	0	100	500	1000
Male	4.76	3.96	2.77	2.64
Male	4.69	3.13	1.87	3.44
Female	4.41	3.59	4.00	2.92
Female	4.70	3.89	4.25	3.32

No other treatment related effects were reported.

b. Clinical Chemistry

X Electrolytes:
- X Calcium
- X Chloride
- Magnesium
- Phosphorous
- X Potassium
- X Sodium

Enzymes:
- X Alkaline phosphatase
- Cholinesterase
- Creatinine phosphokinase
- Lactic acid dehydrogenase
- X Serum alanine aminotransferase (also SGPT)
- X Serum aspartate aminotransferase (also SGOT)

X Other:
- X Albumin
- X Blood creatinine
- X Blood urea nitrogen
- Cholesterol
- X Globulins
- X Glucose
- X Total Bilirubin
- X Total Protein
- Triglycerides

Results - No consistent treatment related effect on clinical chemistries was reported. However, data presented were highly variable, especially with respect to enzymes. Due to the limited number of animals at each treatment level and the variability associated with the data, no real conclusions concerning the effects of the test material on clinical chemistry data can be drawn.

6. Urinalysis - Urine was collected from fasted animals pretest and after 37-38 days. The CHECKED (X) parameters were examined.

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

Results - Urinary pH was decreased in a dose related manner at terminal sacrifice in males and females. No other treatment related effects were reported.

URINARY pH - TERMINATION

	Dose (mg/kg/day)			
Sex	0	100	500	1000
Male	8.0	8.0	8.0	7.5
Male	8.0	8.0	6.0	6.0
Female	8.5	7.5	8.0	6.0
Female	8.0	8.0	7.0	6.0

7. Sacrifice and Pathology - All animals that died and that were sacrificed on schedule were subject to gross pathological examination and the CHECKED (X) tissues were collected for histological examination. The (XX) organs were also weighed.

X		X		X	
	Digestive system		Cardiovas./Hematol.		Neurologic
X	Tongue	X	Aorta	XX	Brain
X	Salivary glands	XX	Heart	X	Periph. nerve
X	Esophagus	X	Bone marrow	X	Spinal cord (3 levels)
X	Stomach	X	Lymph nodes	X	Pituitary
X	Duodenum	X	Spleen	X	Eyes (optic nerve)
X	Jejunum	X	Thymus		Glandular
X	Ileum		Urogenital	XX	Adrenals
X	Cecum	XX	Kidneys		Lacrimal gland
X	Colon	X	Urinary bladder	X	Mammary gland
X	Rectum	XX	Testes	XX	Parathyroids
XX	Liver	X	Epididymides	XX	Thyroids
X	Gall bladder	X	Prostate		Other
X	Pancreas		Seminal vesicle	X	Bone
	Respiratory	XX	Ovaries	X	Skeletal muscle
X	Trachea	X	Uterus	X	Skin
X	Lung			X	All gross lesions and masses

Results -

a. Organ weight - Male and female dogs from the high dose groups and one male (#3002) and one female (#3502) dog from the mid dose group had increased liver weights relative to body weight. No effect on absolute liver weight or liver weight relative to brain weight was reported. The mid dose male with increased relative liver weight experienced net body weight loss during the treatment period.

Both high dose males and mid dose #3002 exhibited reduced absolute testes weights. However when normalized to body and brain weight, only the high dose dogs remained different from the control.

One high dose female (#4502) had a reduced ovary weight relative to the control. This difference persisted when normalized to brain and body weights.

No other treatment related effects were reported.

ORGAN WEIGHTS

Dose (mg/kg/day)	Animal Number	Liver: Absolute Weight (g)	Liver: Relative Body Weight (x100)	Liver: Relative Brain Weight (x1)	Testes or Ovaries: Absolute Weight (g)	Testes or Ovaries: Relative Body Weight (multiplier below)	Testes or Ovaries: Relative Brain Weight (multiplier below)
Males						(x1000)	(x10)
0	1001	354.7	3.66	4.64	13.7	1.41	1.79
0	1002	287.6	2.93	4.50	11.8	1.20	1.85
100	2001	320.5	3.48	4.22	14.0	1.52	1.84
100	2002	349.0	3.67	4.26	18.0	1.89	2.20
500	3001	334.7	3.76	4.30	13.7	1.54	1.76
500	3002*	323.0	5.77	4.00	9.9	1.77	1.23
1000	4001	439.9	5.30	6.20	4.5	0.54	0.63
1000	4002*	262.1	4.85	3.77	5.9	1.09	0.85
Females						(x100000)	(x1000)
0	1501	214.2	3.69	2.79	0.399	6.88	5.20
0	1502	290.3	3.23	3.85	0.662	7.36	8.77
100	2501	226.3	3.77	3.28	0.607	10.12	8.80
100	2502	264.7	3.27	3.70	0.608	7.51	8.49
500	3501	229.8	3.77	3.51	0.391	6.41	5.97
500	3502	276.9	4.54	4.13	0.461	7.56	6.88
1000	4501	317.0	4.66	4.84	0.447	6.57	6.82
1000	4502*	215.6	5.26	2.77	0.237	5.78	3.05

*Dog lost weight during study.

b. Gross pathology - The only noteworthy gross pathology reported was the occurrence of abnormally small testes in both high dose males.

A number of other abnormalities were reported which occurred only in single mid and high dose animals, but not in the controls or low dose animals. However, they cannot be unequivocally attributed to the test material due to the lack of replication. Of particular interest, high dose male #4002 was found to have reddened mucosa of the jejunum at necropsy and mid dose male #3002 and mid dose female #3502 had red or red streaked mucosa of the cecum.

Confounding factors to the data observed at necropsy included the observation of apparent emphysema in female #3502 and evidence of severe trauma to the chest in female #4502.

c. Microscopic pathology - All treated dogs exhibited a dose related multifocal centrilobular to diffuse hypertrophy of hepatocytes. These changes graded from minimal to slight at 100 mg/kg/day to moderate to moderately severe in dogs given 500 or 1000 mg/kg/day. In dogs with moderately severe hepatocytomegaly, eosinophilic cytoplasmic bodies were also reported. The pathology report indicated that these bodies are consistent with proliferation of smooth endoplasmic reticulum involved in detoxification processes in severely affected livers.

The testes of high dose males had diffuse hypoplasia of the seminiferous tubules and were aspermatogenic. Mid dose males had reduced spermatogenesis. The prostates of high dose males had moderately severe hypoplasia; both mid dose and one low dose male had minimal or slight diffuse hypoplasia of the prostate. The ovaries and uterus of one high dose female exhibited moderately severe diffuse hypoplasia. The pathology report suggests that these changes indicate delayed sexual maturation and may reflect the poor weight gain reported.

No other treatment related microscopic lesions were reported.

D. DISCUSSION: Few conclusions can be drawn from this study due to the limited amount of data provided. The interpretation is further clouded by the necessity of eliminating data from female #4502 due to the appearance of apparent severe trauma to the chest. Within the confines of these statements, the following conclusions were made.

No NOEL was established during this study. An apparent dose related reduction in platelet count and a dose related increase in severity of centrilobular to diffuse hepatocytomegaly were seen at all treatment levels in male and female dogs. In addition, males exhibited a treatment related delay in sexual maturation as indicated by hypoplasia of the testes and prostate. This effect is probably not a direct toxic effect but rather a reflection of the poor weight gain observed.

E. CONCLUSIONS: The test material caused dose related histopathological and hematological abnormalities in male and female dogs at all treatment levels tested (100 to 1000 mg/kg/day).

NOEL was not determined due to dose related reduction in platelet counts and hepatocytomegaly in males and females, and delayed sexual maturation in males.

CLASSIFICATION: core - Supplementary
(This is not a guideline study. Deficient in that insufficient numbers of animals were used to permit interpretation of the data.)

Reviewed by: Elizabeth A. Doyle, Ph.D. E.A. Doyle 9/30/90
Section I, Tox. Branch II (HFAS) (H7509C)
Secondary Reviewer: Yiannakis M. Ioannou, Ph.D. YMI 9/14/90
Section I, Tox. Branch II (HFAS) (H7509C)

008334

DATA EVALUATION REPORT

STUDY TYPE: 30-Day Feeding Study - Rats TOX. CHEM. NO.: 215B

MRID NO.: 415648-03

TEST MATERIAL: Chlorothalonil Metabolite

SYNONYMS: 3-Carboxy-2,5,6-trichlorobenzamide, 2,4,5-Trichloroisophthalamic acid, 3-Carbamyl-2,4,5-trichlorobenzoic acid, SDS-46851

STUDY NUMBER: 736-5TX-85-0007-003

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

TESTING FACILITY: SDS Biotech Corporation
Department of Safety Assessment
Life Science Toxicology
7528 Auburn Road
Painesville, Ohio 44077

TITLE OF REPORT: A 30-Day Feeding Study in Rats with 3-Carboxy-2,5,6-Trichlorobenzamide

AUTHORS: D. M. Serrone, N. H. Wilson and J. C. Killeen

REPORT ISSUED: February 21, 1986

CONCLUSIONS: Treatment with the test material in diet for 30 days resulted in enlargement of the liver and centrilobular hepatocellular hypertrophy in male and female rats.

NOEL < 500 mg/kg/day in male and female rats
LOEL = 500 mg/kg/day in male and female rats based on increased liver weights

CLASSIFICATION: core - Supplementary
(This is not a guideline study.)

A. MATERIALS:

1. Test compound: 3-Carboxy-2,5,6-trichlorobenzamide Description: tan microcrystals Batch #T-165-2, Purity >94%, contaminants: list in CBI appendix

2. Test animals: Species: rat, Strain: CD (Sprague-Dawley), Age: 41 days, Weight: male - 170-209 g, female - 132-168 g, Source: Charles River Breeding Laboratories, Portage, MI

B. STUDY DESIGN:

1. Animal assignment - Animals were assigned using a weight stratified randomization procedure (Concord Woods Animal Facility Standard Operating Procedure) to the following test groups:

Test Group	Dose Level (mg/kg/day)	Main Study 30 days male	Main Study 30 days female	Interim Sac. - days male	Interim Sac. - days female
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2 Low (LDT)	500	5	5	5	5
3 High (HDT)	2000	5	5	5	5

2. Diet preparation - Diet was prepared weekly and stored at room temperature in the dark. Samples of treated food were analyzed for concentration, stability and homogeneity prior to the initiation of the study using test batches of diet containing 5000 ppm and 20,000 ppm (500 and 2000 mg/kg/day) prepared in the same manner as the test diets for use in the study. Two samples of each diet were also taken during the study to confirm concentration.

Results - The test material was found to be stable in diet for at least 14 days. Homogeneity analyses indicated that the nominal 5000 ppm diet contained 4880 ppm ± 2% and the 20000 ppm diet contained 20327 ppm ± 2%. Weekly diet samples tested for verification of concentration contained 99-103% and 94-103% of nominal for the 5000 and 20000 ppm diets fed to females and 98-102% and 20-102% of the nominal concentrations in diets fed to males.

3. Animals received food (Purina Certified Rodent Chow #5002) and water ad libitum.

4. Statistics - Per the report, "For each week, the mean body weight and food consumption (absolute and relative to body weight) values of all test groups were compared with the control group mean values using analysis of variance and Dunnett's multiple comparison tables. This method of statistical analysis of test versus control group data also was conducted for the clinical pathology values (where appropriate), the absolute organ weight values, organ weight to body weight values and organ weight to brain weight values

obtained at termination. A value of $p < 0.05$ was accepted as significant."

5. Quality assurance was documented by signed and dated GLP and quality assurance statements.

C. METHODS AND RESULTS:

1. Observations - Animals were inspected twice daily for signs of toxicity and mortality. A complete physical examination was made once per week.

   Results - Toxicity - No treatment related clinical signs of toxicity were reported.

   Mortality (survival) - All rats survived to scheduled termination.

2. Body weight - Rats were weighed weekly for the duration of the study.

   Results - No treatment related effects were reported.

3. Food consumption and compound intake - Consumption was determined and mean daily diet consumption was calculated. Efficiency and compound intake were calculated from the consumption and body weight gain data.

   Results - Food consumption and food efficiency - No treatment related effects were reported.

   Compound intake - Compound intake was within 8% of the target dose for the four weeks of feeding treated diet.

4. Ophthalmological examinations were not performed.

5. Blood was collected at termination of the study (30 days) for hematology and clinical analysis from all animals. The CHECKED (X) parameters were examined.

a. Hematology:

X		X	
X	Hematocrit (HCT)		Total plasma protein (TP)
X	Hemoglobin (HGB)		Leukocyte differential count
X	Leukocyte count (WBC)		Mean corpuscular HGB (MCH)
X	Erythrocyte count (RBC)		Mean corpuscular HGB conc. (MCHC)
	Platelet count		Mean corpuscular volume (MCV)

Results - No treatment related effects were reported.

b. Clinical Chemistry

X	Electrolytes:	X	Other:
	Calcium	X	Albumin
X	Chloride	X	Blood creatinine
	Magnesium	X	Blood urea nitrogen
	Phosphorous		Cholesterol
X	Potassium	X	Globulins
X	Sodium	X	Glucose
	Enzymes:		Total Bilirubin
X	Alkaline phosphatase	X	Total Protein
	Cholinesterase		Triglycerides
	Creatinine phosphokinase		
	Lactic acid dehydrogenase		
X	Serum alanine aminotransferase (also SGPT)		
	Serum aspartate aminotransferase (also SGOT)		

Results - No treatment related effects were reported. Although a statistically significant ($p < 0.05$) increase in total protein was reported in males from the low dose group, this observation was considered anomalous in that the high dose value was similar to the control.

6. Urinalysis - Urine was collected from fasted animals prior to the 30 day sacrifice. The CHECKED (X) parameters were examined.

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

Results - No treatment related effects were reported.

7. Sacrifice and Pathology - All animals that died and that were sacrificed on schedule were subject to gross pathological examination and the CHECKED (X) tissues were collected for histological examination. The (XX) organs were also weighed.

X	X	X
Digestive system	Cardiovas./Hematol.	Neurologic
Tongue	Aorta	XX Brain
X Salivary glands	XX Heart	Periph. nerve
X Esophagus	X Bone marrow	X Spinal cord (3 levels)
X Stomach	X Lymph nodes	X Pituitary
X Duodenum	X Spleen	X Eyes (optic nerve)
X Jejunum	X Thymus	Glandular
X Ileum	Urogenital	XX Adrenals
X Cecum	XX Kidneys	Lacrimal gland
X Colon	X Urinary bladder	Mammary gland
X Rectum	XX Testes	X Parathyroids
XX Liver	X Epididymides	X Thyroids
Gall bladder	X Prostate	Other
X Pancreas	Seminal vesicle	X Bone
Respiratory	XX Ovaries	X Skeletal muscle
X Trachea	X Uterus	X Skin
X Lung		X All gross lesions and masses

Results -

a. Organ weight - Males and females exhibited dose related increases in liver weights expressed as absolute weight or relative to body or brain weight. The increases appeared to be biologically and statistically significant, contrary to arguments presented by the registrant.

Kidney weights, expressed in all three forms, were slightly increased in a dose related manner in males only. The magnitude of increase was so small as to be of questionable significance.

No other treatment related effects on organ weights were reported.

ORGAN WEIGHTS

Dose Level (mg/kg/day)	Absolute (g)	Relative to Body Weight (g/100 g BW)	Relative to Brain Weight (g/g Brain)
		Males	
Liver			
0	9.850	2.910	5.086
500	11.308	3.280	5.828
2000	11.798*	3.432*	6.098*
Kidney			
o	2.588	0.764	1.336
500	2.750	0.798	1.416
2000	2.964	0.862	1.530
		Females	
Liver			
0	5.708	2.816	3.118
500	6.130	3.088*	3.450
2000	6.552	3.294**	3.666

*Significantly different from the control, $p < 0.05$.
**Significantly different from the control, $p < 0.01$.

b. Gross pathology - At necropsy, two of five high dose females were reported with dilated renal pelvis, described in the pathology report as "incidental to treatment". No other unusual abnormalities were reported.

c. Microscopic pathology - Centrilobular hepatocellular hypertrophy occurred in all high dose male and female rats. The severity was characterized as slight to very slight. The enlarged hepatocytes were reported to contain an increased amount of eosinophilic cytoplasm. One low dose male also exhibited very slight hepatocellular hypertrophy.

D. DISCUSSION: The effects of the test material appeared to be largely on the liver. Increased size as indicated by liver weight was apparent at both treatment levels. The registrant indicated that the liver weight data was not evidence of treatment effect because weights were not outside the historical control range for rats from this laboratory. However, the data exhibited a clear dose related trend and statistical significance for specific treatment levels (both absolute and normalized values) relative to the concurrent control. In addition, the hepatocellular hypertrophy reported at the high dose confirms the liver as the

target organ for the test material. This is consistent with effects generally associated with chlorinated aromatic compounds.

E. CONCLUSIONS: Treatment with the test material in diet for 30 days resulted in enlargement of the liver and centrilobular hepatocellular hypertrophy in male and female rats.

NOEL < 500 mg/kg/day in male and female rats
LOEL = 500 mg/kg/day in male and female rats based on increased liver weights

CLASSIFICATION: core - Supplementary
(This is not a guideline study.)

Reviewed by: Elizabeth A. Doyle, Ph.D. E.A. Doyle 10/22/90
Section IV, Tox. Branch II (HFAS) (H7509C)
Secondary Reviewer: Marcia van Gemert, Ph.D. mvanGemert 10/26/90
Tox. Branch II (HFAS) (H7509C)

008334

DATA EVALUATION REPORT

STUDY TYPE: 90-Day Feeding (82-1) and One-Generation Reproduction - Rat

TOX. CHEM. NO.: 215B

MRID NO.: 415648-06

TEST MATERIAL: Chlorothalonil Metabolite

SYNONYMS: 3-Carbamyl-2,4,5-trichlorobenzoic acid, 2,4,5-trichloroisophthalamic acid, 3-carboxy-2,5,6-trichlorobenzamide, 2,5,6-trichloro-3-carboxybenzamide, SDS-46851

STUDY NUMBER: 1111-86-0055-TX-003

SPONSOR: Fermenta Plant Protection Company
5966 Heisley Road
P. O. Box 8000
Mentor, Ohio 44061-8000

TESTING FACILITY: Ricerca, Inc.
Department of Toxicology and Animal Metabolism
7528 Auburn Road
Painesville, Ohio 44077

and

Experimental Pathology Laboratories, Inc.
P. O. Box 474
Ross, Ohio 45061

TITLE OF REPORT: Combined 90-Day Feeding Study and One-Generation Reproduction Study in Rats with 3-Carboxy-2,5,6-Trichlorobenzamide

AUTHOR(S): D. M. Serrone and J. C. Killeen, Jr.

REPORT ISSUED: August 25, 1988

CONCLUSIONS: This chlorothalonil metabolite has essentially no reproductive effects in rats. Increased adrenal, liver and kidney weights and urinary specific gravity and transient effects on prothrombin time and blood glucose were reported.

Systemic NOEL = not established in females, 250 mg/kg/day in males
Systemic LOEL = 250 mg/kg/day in females based on relative adrenal weights
= 750 mg/kg/day in males based on increased relative kidney and liver weights
Reproductive NOEL = 750 mg/kg/day
Reproductive LOEL = 2000 mg/kg/day based on reduced Day 21 pup weight

CLASSIFICATION: Core - Guideline for 90-Day Study
Core - Supplementary for Reproduction Study
(Not a guideline study)

This study satisfies the guideline requirements (82-1) for a "90-Day Feeding Study in Rats".

A. MATERIALS:

1. Test compound: 3-carboxy-2,5,6-trichlorobenzamide Description: light brown powder Batch #T-165-3, Purity >99%, contaminants: list in CBI appendix

2. Test animals: Species: rat, Strain: CD (Sprague-Dawley), Age: 40 days, Weight: males - 140-202 g, females - 113-161 g, Source: Charles River Breeding Laboratory, Inc., Portage, MI 49081

B. STUDY DESIGN:

1. Animal assignment - Animals were assigned according to AF SOP 05-0006 to the following test groups:

Test Group	Dose (mg/kg/day)	Main Study 3 months male	Main Study 3 months female	Repro. Study Thru lactation male	Repro. Study Thru lactation female
1 Cont.	0	10	10	25	25
2 Low (LDT)	250	10	10	25	25
3 Mid (MDT)	750	10	10	25	25
4 High (HDT)	2000	10	10	25	25

2. Diet preparation - Diet was prepared weekly and stored at room temperature in the dark. The concentration of test material in the diet was adjusted based upon the preceding week's body weight and food consumption data to achieve the target treatment rate. Samples of treated food were analyzed for stability, homogeneity and concentration prior to the initiation of the study using test batches of diet containing 2500 ppm and 40,000 ppm of the test material prepared in the same manner as the test diets for use in the study. Samples of each diet were placed in feed containers under actual study conditions for 7 and 14 days to confirm stability. Two samples of each diet were also taken during the study to confirm concentration.

Results - Feed samples analyzed for homogeneity prior to initiation produced concentrations of 2478 ± 64 ppm for the 2500 ppm diet and 42,260 ± 367 ppm for the 40,000 ppm diet. These values corresponded to concentrations that were 99% and 106% of nominal, respectively. Similar analyses were performed at week 6 of the study when new batches of feed were prepared. These diets contained 101 to 105% of nominal concentration.

Stability of the test material in diet was verified to be greater than seven days, with 2500 and 40,000 diets assayed as containing 102 and 97% of the assay concentration at preparation on day 7. When assayed again at 14 days following preparation, the diets were found to contain 103 and 93% of the originally measured concentrations, respectively.

Diet samples collected during the study were found to contain between 99 and 102% of the nominal concentration except on two occasions. During week 4 of the study, the diet prepared for females in Group III was found to contain 110.2% of the target concentration. During week 12, the diet prepared for Group IV males contained 89.3% of the target concentration.

3. Animals received food (Purina Certified Rodent Chow #5002) and water ad libitum.

4. Statistics - The following are excerpts from the study report.

   90-Day Feeding Phase - "For each week, body weight and food consumption (absolute and relative to body weight) means will be compared with control group means using analysis of variance and Dunnett's multiple comparison tables to judge significance of differences. These statistical methods also will be used to analyze clinical pathology values, where appropriate, and absolute organ weights, organ weight relative to body weight and organ weight relative to brain weight. The survival of groups will be compared using the appropriate chi-square test."

   Reproduction Phase - "Statistical analysis of body weight values will be done on males, dams that mated, completed gestation, delivered a live litter and completed 21 days of lactation. Statistical analysis will be done on number of pups and pup litter weights at day 0, 4, 7, 14 and 21 of lactation. Only those pups and litters that complete day 21 of lactation will be included."

5. Quality assurance was documented by signed and dated GLP and quality assurance statements.

C. METHODS AND RESULTS - 90-Day Study:

1. Observations - Animals were inspected twice daily for signs of toxicity and mortality.

   Results - Toxicity - Signs of toxicity were limited to the occurrence of soft stool in high dose males and females. The effect was more pronounced in males than in females and occurred earlier (seven weeks vs. nine weeks).

   No other signs of toxicity were reported.

   Mortality (survival) - All rats survived the 90-day study phase.

2. Body weight - Rats were weighed weekly until termination.

   Results - No treatment related effects were reported.

3. Food consumption and compound intake - Consumption was determined and mean daily diet consumption was calculated. Efficiency and

compound intake were calculated from the consumption and body weight gain data.

Results - Food consumption and food efficiency - No treatment related effects were reported. Although occasional indications of statistical significance occurred throughout the data, the magnitude of difference from the control was too small to be considered biologically significant.

Compound intake - The calculated compound intake was between 87 and 104% of target during weeks 1 through 5. During weeks 6 to 13, compound intake varied between 93 and 102% of target.

4. Ophthalmological examinations were performed on Day 75 on all animals.

Results - No treatment related effects were reported.

5. Blood was collected during week 6 and 13 for hematology and clinical analysis from all animals. The CHECKED (X) parameters were examined.

a. Hematology:

X	Hematocrit (HCT)	X	Prothrombin time
X	Hemoglobin (HGB)	X	Leukocyte differential count
X	Leukocyte count (WBC)		Mean corpuscular HGB (MCH)
X	Erythrocyte count (RBC)		Mean corpuscular HGB conc. (MCHC)
	Platelet count		Mean corpuscular volume (MCV)

Results - Prothrombin time was increased significantly ($p<0.01$) in high dose males at the week 6 sampling time. At week 13, prothrombin time in high dose males was still slightly higher than the control, although not to a statistically significant extent.

No other treatment related effects were reported.

PROTHROMBIN TIME - MALES (sec)

Time (weeks)	Treatment Level (mg/kg/day) 0	250	750	2000
6	14.95	14.79	14.20	17.78**
12	14.22	14.06	13.90	15.13

**Significantly different from the control ($p<0.01$)

b. Clinical Chemistry

X		X	
	Electrolytes:		Other:
	Calcium	X	Albumin
X	Chloride	X	Blood creatinine
	Magnesium	X	Blood urea nitrogen
	Phosphorous		Cholesterol
X	Potassium	X	Globulins
X	Sodium	X	Glucose
	Enzymes:		Total Bilirubin
X	Alkaline phosphatase	X	Total Protein
	Cholinesterase		Triglycerides
	Creatinine phosphokinase		
	Lactic acid dehydrogenase		
X	Serum alanine aminotransferase (also SGPT)		
	Serum aspartate aminotransferase (also SGOT)		

Results - Blood glucose levels were increased and alkaline phosphatase levels decreased in high dose males at both sampling time. High dose females had decreased ($p<0.05$) SGPT levels at the week 6 sampling time only.

No other treatment related effects were reported.

CLINICAL CHEMISTRY DATA - MALES

Time (weeks)	Treatment Level (mg/kg/day) 0	250	750	2000
Glucose (mg/100 ml)				
6	92.5	102.7	98.7	119.3*
12	114.9	122.0	122.2	129.5
Alkaline phosphatase (U/l)				
6	105.2	113.2	113.7	92.8
12	66.0	65.7	68.7	53.0

*Significantly different from the control ($p<0.05$)

6. Urinalysis - Urine was collected from fasted animals at 5 and 12 weeks. The CHECKED (X) parameters were examined.

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

Results - The specific gravity of urine from high dose males was significantly increased relative to the control at both sampling times.

No other treatment related effects were reported.

SPECIFIC GRAVITY - MALES

Time (weeks)	Treatment Level (mg/kg/day) 0	250	750	2000
5	1.026	1.019	1.020	1.044*
12	1.025	1.024	1.030	1.049**

*Significantly different from the control ($p<0.05$)
**Significantly different from the control ($p<0.01$)

7. Sacrifice and Pathology - The first ten males and ten females as indicated by the sequential numbering system were sacrificed on days 92 and 93 of the study. All animals that were sacrificed on schedule were subject to gross pathological examination and the CHECKED (X) tissues were collected for histological examination. The (XX) organs were also weighed.

X		X		X	
	Digestive system		Cardiovas./Hematol.		Neurologic
	Tongue	X	Aorta	XX	Brain
X	Salivary glands	X	Heart		Periph. nerve
X	Esophagus	X	Bone marrow	X	Spinal cord (3 levels)
X	Stomach	X	Lymph nodes	X	Pituitary
X	Duodenum	X	Spleen	X	Eyes (optic nerve)
X	Jejunum	X	Thymus		Glandular
X	Ileum		Urogenital	X	Adrenals
X	Cecum	XX	Kidneys		Lacrimal gland
X	Colon	X	Urinary bladder	X	Mammary gland
X	Rectum	XX	Testes	X	Parathyroids
XX	Liver	X	Epididymides	X	Thyroids
	Gall bladder	X	Prostate		Other
X	Pancreas		Seminal vesicle	X	Bone
	Respiratory	XX	Ovaries	X	Skeletal muscle
X	Trachea	X	Uterus	X	Skin
X	Lung			X	All gross lesions and masses

Results -

a. Organ weight - No clear dose response was apparent in absolute organ weights except for adrenals. In all treated groups from both sexes, adrenal weights were increased relative to the control. In female rats, adrenals exhibited a statistically significant, dose related increase. The livers of mid and high dose males and females were slightly heavier than the control, but did not exhibit dose response. Low dose females had reduced liver weights relative to the control.

When organ weights were normalized to body weights, liver weights of mid and high dose males and high dose females were increased to a statistically significant extent. Relative liver weights of mid dose females were also increased, but not significantly. Relative kidney weights of males from all treatment groups were increased relative to the control. In females all groups were similar to the control. Relative testes weights were increased in mid and high dose males, but did not exhibit dose response. Relative adrenal weights were increased in all treated females, and in high dose males only.

When normalized to brain weight, the only difference in organ weights that was apparent was increased adrenal weights. Treated females exhibited a dose related trend for increased adrenal weight

relative to brain weight, with the mid and high dose groups increased to a statistically significant extent.

ABSOLUTE ORGAN WEIGHTS (g)

Dose Level (mg/kg/day)	Liver	Kidneys	Testes	Adrenals*
Males				
0	12.707	2.922	3.373	49.7
250	12.843	2.968	3.304	51.6
750	13.883	3.308*	3.607	50.5
2000	12.919	2.971	3.285	52.4
Females				
0	6.609	1.718	---	52.3
250	6.395	1.585	---	59.7
750	6.938	1.720	---	62.1*
2000	7.204	1.737	---	66.1**

*Significantly different from the control, $p<0.05$
**Significantly different from the control, $p<0.01$
*Weight expressed in mg

ORGAN WEIGHTS RELATIVE TO BODY WEIGHTS
(g/100 g BW)

Dose Level (mg/kg/day)	Liver	Kidneys	Testes	Adrenals*
Males				
0	2.540	0.586	0.686	10.1
250	2.579	0.601	0.671	10.5
750	2.761*	0.659**	0.720	10.1
2000	2.802**	0.646*	0.717	11.4
Females				
0	2.518	0.651	---	20.0
250	2.566	0.638	---	24.1*
750	2.670	0.644	---	24.0*
2000	2.761*	0.666	---	25.3**

*Significantly different from the control, $p<0.05$
**Significantly different from the control, $p<0.01$
*Units = mg/100 g BW

ORGAN WEIGHTS RELATIVE TO BRAIN WEIGHTS
(g/g Brain)

Dose Level (mg/kg/day)	Liver	Kidneys	Testes	Adrenals*
Males				
0	6.181	1.422	1.646	24.2
250	6.245	1.442	1.664	25.1
750	6.588	1.569	1.710	24.0
2000	6.400	1.469	1.628	26.0
Females				
0	3.556	0.921	---	28.1
250	3.473	0.860	---	32.5
750	3.733	0.925	---	33.4*
2000	3.937	0.947	---	36.1**

*Significantly different from the control, $p<0.05$
**Significantly different from the control, $p<0.01$
*Units = mg/g brain

b. Gross pathology - No treatment related effects were reported.

c. Microscopic pathology - No treatment related effects were reported.

D. DISCUSSION - 90-Day Study: Only minor effects due to the test material were reported. Adrenal weights both as absolute weight or relative to brain or body weight were increased in a dose related manner in females. In males, kidney weights relative to body weight were increased in a dose related manner.

Prothrombin time and blood glucose were significantly increased in high dose males at the week 6 sampling. However, by week 12, values, while still slightly greater than the control were no longer significantly different. Urinary specific gravity was increased significantly in high dose males at both sampling times.

With the exception of a slight increase in relative adrenal weights in females, no treatment related effects were seen in rats given 250 mg test material/kg/day.

E. METHODS - ONE-GENERATION REPRODUCTION STUDY - This study was designed to assess the developmental toxicity potential of 3-carboxy-2,5,6-trichlorobenzene when administered in diet to male and female rats as a continuation of the study above until day 21 of lactation.

1. Mating - Females were individually housed with males from the same treatment level daily from 4 PM until 8 AM the following morning. Females were examined for evidence of mating. The presence of a sperm plug or sperm in the vaginal smear was the criterion for confirmation of mating. If mating was not confirmed, this procedure was repeated for up to ten days. If mating still had not occurred, the female was housed for up to five days with a male from the same treatment level that had successfully mated. After 15 days, no further matings were attempted.

2. Group Arrangement - See Section B above.

3. Dosing - See section B above.

4. Observations

a. Parental animals: Observations and the schedule for those observations is summarized from the report as follows:

Type of observation	Number of animals per sex per group	Frequency
Mortality and signs of toxicity	All	Twice a day during premating and growth periods.
Body weight	All	At beginning of study and weekly through growth and mating periods.
	Maternal animals	Days 0, 6, 15, and 20 of gestation; days 0, 4, 14, and 21 post partum.
	Paternal animals	Weekly through mating period

b. Reproductive performance: The following indices were calculated:

$$\text{Mating Index (Male)} = \frac{\text{No. males with confirmed matings}}{\text{Total no. males mated}} \times 100$$

$$\text{Male fertility index} = \frac{\text{No. females with implantations}}{\text{No. males mated}} \times 100$$

$$\text{Mating Index (Female)} = \frac{\text{No. of females confirmed matings}}{\text{Total no. females mated}} \times 100$$

$$\text{Female fertility index} = \frac{\text{No. females with implantations}}{\text{Total no. females mated}} \times 100$$

$$\text{Gestation index} = \frac{\text{No. live litters born}}{\text{No. females with implantations}} \times 100$$

c. Litter observations: The following litter observations were made:

Observation	Time of observation (lactation day) Birth	Day 4	Day 14	Day 21
Number of live pups	X	X	X	X
Pup weight (stillborn)	X			
Litter weight	X	X	X	X
External alterations	X			
Number of dead pups	X	X	X	X
Sex of each pup	X	X	X	X

Dead pups were examined grossly for external and internal abnormalities, and a possible cause of death was determined for pups born or found dead.

The following indices were calculated:

$$\text{Live Born index} = \frac{\text{No. live pups born}}{\text{No. live + dead pups born}} \times 100$$

$$\text{Stillborn index} = \frac{\text{No. of pups stillborn}}{\text{Total no. pups born}} \times 100$$

$$\text{Day 4 pup viability index} = \frac{\text{No. pups alive day 4 (precull)}}{\text{Total no. pups born alive}} \times 100$$

$$\text{Lactation index} = \frac{\text{No. pups alive day 21}}{\text{No. pups alive day 4 (postcull)}} \times 100$$

$$\text{Viability index} = \frac{\text{No. litters with live pups at day 21}}{\text{No. litters with pups born alive}} \times 100$$

5. Necropsy

a. Parental animals: All surviving parental males were sacrificed as soon as possible after the litters were produced. Maternal animals were sacrificed after the litters of each generation were weaned. These animals were subjected to post mortem examinations.

b. Offspring: The F1 offspring were sacrificed at 21 days of age. These animals and any found dead or moribund were subjected to gross post mortem examinations.

c. Necropsy observations: Gross necropsy consisted of external and internal examinations including the cervical, thoracic, and abdominal viscera.

Tissues were collected as described in section C.7 above, but were not processed or evaluated microscopically.

Results for the parental animals are summarized from the report as follows:

Observation	Dose group Control	Low	Mid	High
Males				
Cohabited	24	25	25	25
Mated	19	23	21	23
Fertile	17	18	19	22
Intercurrent deaths	0	0	0	0
Females				
Cohabited	24	25	25	25
Mated	22	25	24	24
With implantations	18	20	20+	23
Delivering pups	18	20	19	23
Intercurrent deaths	0	0	0	0
Median gestation interval (days)	22.1	22.0	22.0	22.1
Number of litters	18	20	19	23
Total litter losses	0	0	0	0
Mean litter size (Day 1)	11.8	12.5	11.8	12.3
Mean litter size (Day 21)	8.9	9.1	9.6	9.3
Number of live pups (Day 1)	212	249	224	283
Number of dead pups (Day 1)	9	5	3	5
Pup viability (Day 4, precull)	207	232	218	270
Number of pups (Day 4, postcull)	161	184	183	215
Number of pups (Day 21)	160	182	183	214
Pup deaths (Days 1-21)	6	19	6	14
Mean pup weight (g) (Day 1)	6.3	6.2	6.3	6.2
Mean pup weight (g) (Day 21)	45.4	43.4	43.4	41.3*

*Scatistically significantly different from control, $p<0.05$.
+One female was omitted during counts of implantation sites. Data from this female was not included in further analyses.

d. Necropsy results - Gross pathological examination revealed no treatment related abnormalities.

2. Offspring

a. Viability and clinical signs: No treatment related effects were reported.

b. Body weight: Selected group mean body weights are summarized from the report as follows:

Body Weights (g)	Dose group			
	Control	Low	Mid	High
	F1 Generation			
Day 0	6.3	6.2	6.3	6.2
Day 4 - Pre-cull	9.6	9.6	9.9	9.9
Day 4 - Post-cull	9.6	9.5	9.9	9.9
Day 7	14.8	14.2	14.8	14.7
Day 14	28.3	27.4	27.4	26.6
Day 21	45.4	43.4	43.4	41.3*

* Statistically significantly different from control, $p<0.05$.

c. Necropsy results - Gross pathological examination indicated no treatment related effects.

G. DISCUSSION - ONE-GENERATION REPRODUCTION: The only apparent treatment related effect on reproduction reported in this study was the reduction in mean pup weight in the high dose group at day 21 of lactation. No other treatment related effects occurred in this study.

H. CONCLUSIONS: This chlorothalonil metabolite has essentially no reproductive effects in rats. Increased adrenal, liver and kidney weights and urinary specific gravity and transient effects on prothrombin time and blood glucose were reported.

Systemic NOEL = not established in females, 250 mg/kg/day in males
Systemic LOEL = 250 mg/kg/day in females based on relative adrenal weights
= 750 mg/kg/day in males based on increased relative kidney and liver weights
Reproductive NOEL = 750 mg/kg/day
Reproductive LOEL = 2000 mg/kg/day based on reduced Day 21 pup weight

CLASSIFICATION: Core - Guideline for 90-Day Study
Core - Supplementary for Reproduction Study
(Not a guideline study)

This study satisfies the guideline requirements (82-1) for a "90-Day Feeding Study in Rats".

Reviewed by: Elizabeth A. Doyle, Ph.D. E.A. Doyle 9/24/90
Section I, Tox. Branch II (HFAS) (H7509C)
Secondary Reviewer: Yiannakis M. Ioannou, Ph.D. J.M.I 9/24/90
Section I, Tox. Branch II (HFAS) (H7509C)

DATA EVALUATION REPORT 002334

STUDY TYPE: 90-Day Oral - Dog (82-1) TOX. CHEM. NO.: 215B

MRID NO.: 415648-05

TEST MATERIAL: Chlorothalonil Metabolite

SYNONYMS: 3-Carbamyl-2,4,5-trichlorobenzoic acid, 2,4,5-trichloro-isophthalamic acid, 3-carboxy-2,5,6-trichlorobenzamide, 2,5,6-trichloro-3-carboxybenzamide, SDS-46851

STUDY NUMBER: 3080-88-0082-TX-003

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

TESTING FACILITY: Bio\dynamics Inc.
Mettlers Road
P. O. Box 2360
East Millstone, New Jersey 08875-2360

and

Experimental Pathology Laboratories
P. O. Box 474
Herndon, Virginia 22070

TITLE OF REPORT: A 90-Day Oral Toxicity Study in Dogs with 3-Carbamyl-2,4,5-trichlorobenzoic Acid (SDS-46851)

AUTHOR(S): D. M. Serrone and J. C. Killeen, Jr.

REPORT ISSUED: February 5, 1990

CONCLUSIONS: SDS-46851 caused increased liver weights, decreased urinary pH and increased blood glucose concentrations in male and female dogs.

NOEL = 15 mg/kg/day in male and female dogs
LOEL = 50 mg/kg/day in male and female dogs based on increased liver weights, decreased urinary pH and increased blood glucose levels.

Classification: core - Guideline

This study satisfies the guideline requirements (82-1) for a "90-Day Oral Toxicity Study in Dogs".

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A. MATERIALS:

1. Test compound: 3-carbamyl-2,4,5-trichlorobenzoic acid Description: off-white microcrystals Batch #T-165-5, Purity >99%, contaminants: not given

2. Test animals: Species: dog, Strain: Beagle, Age: 6 months, Weight: males - 8.0-10.6 kg, females - 7.5-9.4 kg, Source: Marshall Farms, U.S.A., Inc., North Rose, New York

B. STUDY DESIGN:

1. Animal assignment - Animals were assigned by weight stratified randomization to the following test groups:

Test Group	Dose (mg/kg/day)	Main Study 99-102 days male	Main Study 99-102 days female	Interim Sac. - months male	Interim Sac. - months female
I	0	4	4		
II	5	4	4		
III	15	4	4		
IV	50	4	4		
V	500	4	4		

2. Treatment administration - The test compound was measured into the gelatin capsules based upon the body weight of the dogs at the beginning of each treatment week. Capsules for each dog were prepared weekly. Dogs were dosed 30 minutes following consumption of half of their daily feed ration. They were then observed for 30 minutes and given the remainder of their ration.

3. Animals received 200 g of Wayne Bite Size laboratory diet twice daily. Water was available ad libitum.

4. Statistics - Per the study report, "Statistical evaluation of equality of means was made by the appropriate one way analysis technique, followed by a multiple comparison procedure if needed. First, Bartlett's test was performed to determine if groups had equal variance. If the variances were equal, parametric procedures were used; if not, nonparametric procedures were used. The parametric procedures were the standard one way ANOVA using the F distribution to assess significance. If significant differences among the means were indicated, Dunnett's test was used to determine which means were significantly different from the control. If a nonparametric procedure for testing equality of means was needed, the Kruskal-Wallis test was used, and if differences were indicated a summed rank test (Dunn) was used to determine which treatments differed from control.

"A statistical test for trend in the dose levels was also per-

formed. In the parametric case (i.e., equal variance) standard regression techniques with Jonckheere's test for monotonic trend was used.

"The test for equal variance (Bartlett's) was conducted at the 1%, two-sided risk level. All other statistical tests were conducted at the 5% and 1%, two-sided risk level."

5. Quality assurance was documented by signed and dated GLP and quality assurance statements.

C. METHODS AND RESULTS:

1. Observations - Animals were inspected twice daily for signs of toxicity and mortality. A detailed physical examination was made weekly.

Results - Toxicity - No apparent treatment related adverse effects were reported.

Watery stool and emesis occurred with similar frequency in all groups. The only difference reported was the occurrence of watery stool in all eight dogs on the 500 mg/kg/day treatment regimen compared to six dogs from all other groups.

Mortality (survival) - All dogs survived to scheduled sacrifice.

2. Body weight - Dogs were weighed pretest, weekly during treatment, and at termination.

Results - Body weights and body weight gains in female dogs from all treatment groups were similar to the control.

Males from groups given 5, 15 or 50 mg/kg/day had body weights and body weight gains that were similar to the control for the entire treatment period. High dose males had body weights that diverged from the control by the second week of treatment. Body weight gain for the high dose group was also notably lower than the control. However, neither body weight nor body weight gain differed from the control to a statistically significant extent at any time during the study.

MEAN BODY WEIGHTS (KG) - MALES

Time (weeks)	Treatment Level (mg/kg/day 0	5	15	50	500
0	9.7 (0.7)*	9.4 (0.9)	9.5 (1.1)	10.1 (0.5)	9.6 (0.7)
3	10.8 (1.0)	10.5 (1.5)	10.3 (1.1)	10.6 (0.9)	9.9 (1.0)
6	12.2 (1.1)	11.5 (1.9)	11.6 (1.0)	11.9 (1.5)	10.9 (1.2)
9	12.4 (1.2)	11.8 (2.0)	12.0 (1.5)	12.8 (1.2)	11.1 (1.4)
12	13.0 (1.3)	12.6 (2.3)	12.7 (1.2)	13.1 (1.8)	11.4 (1.4)
14	12.9 (1.3)	12.4 (2.3)	12.7 (1.2)	13.2 (1.9)	11.2 (1.4)

*Numbers in parentheses are standard deviations.

BODY WEIGHT GAINS (KG)
Weeks 0 to 14 - Males

Group I 0 mg/kg/day		Group II 5 mg/kg/day		Group III 15 mg/kg/day		Group IV 50 mg/kg/day		Group V 500 mg/kg/day	
An.No.	Gain	An.No.	Gain	An.No.	Gain	An.No.	Gain	An.No.	Gain
1001	3.2	2001	3.4	3001	3.6	4001	1.1	5001	0.5
1002	3.9	2002	4.4	3002	3.6	4002	3.4	5002	3.2
1003	3.3	2003	0.9	3003	2.5	4003	4.2	5003	1.4
1004	2.2	2004	3.3	3004	3.4	4004	3.7	5004	1.1
Mean	3.2		3.0		3.3		3.1		1.6
S.D.	0.7		1.5		0.5		1.4		1.2
N	4		4		4		4		4

3. Food consumption - Consumption was monitored visually during each feeding (daily).

Results - No treatment related effects were reported.

4. Ophthalmological examinations were performed pretest and at termination on all animals.

Results - No treatment related effects were reported.

5. Blood was collected before treatment, at week 8 and at termination for hematology and clinical analysis from all animals. The CHECKED (X) parameters were examined.

a. Hematology:

X	Hematocrit (HCT)	X	Prothrombin time
X	Hemoglobin (HGB)	X	Leukocyte differential count
X	Leukocyte count (WBC)	X	Mean corpuscular HGB (MCH)
X	Erythrocyte count (RBC)	X	Mean corpuscular HGB conc. (MCHC)
X	Platelet count	X	Mean corpuscular volume (MCV)

Results - No biologically or statistically significant effects on hematological parameters were reported in male or female dogs from any treatment group at the eight week sampling time or in treated females at termination. Males given 50 or 500 mg/kg/day had reduced HCT and RBC at the terminal sacrifice. Although specific data means were not significantly different from the control, a statistically significant dose related trend ($p<0.05$) was reported for these two parameters. The magnitude of difference was small in each case and of questionable biological significance. HCT values 53, 57, 52, 49 and 49% for increasing doses. Erythrocyte counts were 7.08, 7.52, 6.77, 6.31 and 6.47 million per μl. HGB was also indicated to exhibit a significant dose related trend, however, this appears to have been due to abnormally high values for the 5 mg/kg/day group.

b. Clinical Chemistry

	Electrolytes:		Other:
X	Calcium	X	Albumin
X	Chloride	X	Blood creatinine
	Magnesium	X	Blood urea nitrogen
X	Phosphorous		Cholesterol
X	Potassium	X	Globulins
X	Sodium	X	Glucose
	Enzymes:	X	Total Bilirubin
X	Alkaline phosphatase	X	Total Protein
	Cholinesterase		Triglycerides
	Creatinine phosphokinase		

	Lactic acid dehydrogenase
X	Serum alanine aminotransferase (also SGPT)
X	Serum aspartate aminotransferase (also SGOT)

Results - No differences from the control were reported for any parameter except for blood glucose concentration. A statistically significant increase occurred in glucose concentration at the week 8 sampling in 15 and 500 mg/kg/day males and 50 and 500 mg/kg/day females. Although not statistically significant at termination, 50 and 500 mg/kg/day females continued to have elevated glucose levels relative to the control. High dose males exhibited a slight increase in blood glucose at termination. Due to the lack of an effect in 50 mg/kg/day males and the lack of an effect at termination, the elevation in 15 mg/kg/day males at week 8 is of questionable biological significance.

BLOOD GLUCOSE CONCENTRATIONS (mg/dl)

		Treatment Level (mg/kg/day)				
Sex	Week	0	5	15	50	500
Male	8	83	86	101*	93	109**
		(6)[a]	(5)	(9)	(5)	(10)
	16	103	113	108	110	119
		(9)	(6)	(11)	(14)	(6)
Female	8	79	83	86	95*	104**
		(4)	(6)	(5)	(15)	(2)
	16	89	96	99	104	115
		(8)	(15)	(20)	(12)	(13)

*Significantly different from the control ($p<0.05$)
**Significantly different from the control ($p<0.01$)
[a]Standard deviation

6. Urinalysis - Urine was collected from fasted animals pretest, at 8 weeks and at termination months. The CHECKED (X) parameters were examined.

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

Results - Urinary pH was reduced in male and female dogs receiving 50 and 500 mg/kg/day at both time periods. However, the effect was less pronounced at termination than at week 8.

No other treatment related effects were reported.

7. Sacrifice and Pathology - All animals that died and that were sacrificed on schedule were subject to gross pathological examination and the CHECKED (X) tissues were collected for histological examination. The (XX) organs were also weighed.

X	X	X
Digestive system	Cardiovas./Hematol.	Neurologic
X Tongue	X Aorta	XX Brain
X Salivary glands	XX Heart	X Periph. nerve
X Esophagus	X Bone marrow	X Spinal cord (3 levels)
X Stomach	X Lymph nodes	X Pituitary
X Duodenum	X Spleen	X Eyes (optic nerve)
X Jejunum	X Thymus	Glandular
X Ileum	Urogenital	XX Adrenals
X Cecum	XX Kidneys	Lacrimal gland
X Colon	X Urinary bladder	X Mammary gland
X Rectum	XX Testes	XX Parathyroids
XX Liver	X Epididymides	XX Thyroids
X Gall bladder	X Prostate	Other
X Pancreas	Seminal vesicle	X Bone
Respiratory	XX Ovaries	X Skeletal muscle
X Trachea	X Uterus	X Skin
X Lung		X All gross lesions and masses

Results -

a. Organ weight - The only organ that exhibited a treatment related effect on weight was the liver. Males given 500 mg/kg/day had a 10% increase in liver weight. When normalized to body weight, this constituted a 28% increase, which was statistically significant relative to the control ($p<0.05$). Females from the 50 and 500 mg/kg/day treatment groups had increases in liver weight of 14 and 23% relative to the control. When normalized to body weight, these increases represented changes of 2 and 23%, respectively.

No other treatment related effects on organ weight were reported.

(Comparisons to brain weight were discounted due to the indication in the study report that brain weights were highly variable, in part due to inconsistencies in trimming procedures used prior to weighing when tissues were collected.)

MEAN LIVER WEIGHT AND LIVER/BODY WEIGHT ± STD. DEV.

	Male		Female	
mg/kg/day	Liver Weight (g)	Liver/Body Weight (x100)	Liver Weight (g)	Liver/Body Weight (x100)
0	308.2 ± 25.0	2.46 ± 0.21	263.2 ± 49.1	2.61 ± 0.54
5	312.6 ± 70.7 (+1%)	2.52 ± 0.14 (+2%)	260.8 ± 32.1 (-1%)	2.55 ± 0.31 (-2%)
15	325.9 ± 17.3 (+6%)	2.59 ± 0.18 (+5%)	266.9 ± 30.2 (+1%)	2.63 ± 0.28 (+1%)
50	325.6 ± 20.5 (+6%)	2.55 ± 0.21 (+4%)	299.4 ± 40.6 (+14%)	2.67 ± 0.31 (+2%)
500	340.5 ± 39.1 (+10%)	3.16* ± 0.29 (+28%)	324.8 ± 39.7 (+23%)	3.21 ± 0.35 (+23%)

*Significantly different from the control ($p<0.05$)
(%) Percent change from control

b. Gross pathology - No treatment related gross pathology was reported.

c. Microscopic pathology - No apparent treatment related microscopic abnormalities were apparent in the accompanying pathology report. The pathologist's summary confirmed this observation.

D. DISCUSSION: The major effects of the test material were reduced urinary pH in males and females given 50 or 500 mg/kg/day, and increased liver weights and decreased body weights and body weight gains in high dose males and females. An apparent effect on blood glucose levels occurred at both sampling times in males and females given 500 mg/kg/day. Increased blood glucose also occurred in 15 mg/kg/day males at week 8 only and 50 mg/kg/day females at both sampling times. Increased liver weights were not accompanied by treatment related pathology and probably represent adaptive change.

E. CONCLUSIONS: The test material caused increased liver weights, decreased urinary pH and increased blood glucose concentrations in male and female dogs.

NOEL = 15 mg/kg/day in male and female dogs
LOEL = 50 mg/kg/day in male and female dogs based on increased liver weights, decreased urinary pH and increased blood glucose levels.

CLASSIFICATION: core - Guideline

This study satisfies the guideline requirements (82-1) for a "90-Day Oral Toxicity Study in Dogs".

Primary Review by: Elizabeth A. Doyle, Ph.D. E.A. Doyle 10/15/90
Review Section IV, Toxicology Branch II (HFAS)(H7509C)
Secondary Review by: Marcia van Gemert, Ph.D. M van Gemert 10/29/90
Toxicology Branch II (HFAS)(H7509C)

008334

DATA EVALUATION RECORD

Study Type: Teratology - Developmental Toxicity (Range-finding)
Species: Rat

MRID No.: 415648-07 Caswell No.: 215B

Test Material: Chlorothalonil Metabolite

Synonyms: 3-Carbamyl-2,4,5-trichlorobenzoic acid, 2,4,5-Trichloroisophthalamic acid, 2,5,6-trichloro-3-carboxybenzamide, 3-carboxy-2,5,6-trichlorobenzamide

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

Study Number: 1554-87-0094-TX-001

Testing Facility: Ricerca, Inc.
Department of Toxicology and Animal Metabolism
7528 Auburn Road
Painesville, Ohio 44077

Title of Report: A Teratology Dose Range-Finding Study in Rats with 3-Carbamyl-2,4,5-trichlorobenzoic Acid

Authors: J. S. Chun, N. H. Wilson and J. C. Killeen, Jr.

Report Issued: June 28, 1989

Conclusions: Proposed treatment levels for a teratology study in rats of 500, 1000 and 2000 mg/kg/day are appropriate.

Maternal NOEL = 1000 mg/kg/day
Maternal LOEL = 2000 mg/kg/day based on slight body weight gain and food consumption reductions

Classification: Supplementary
(This study was not intended to fulfil regulatory requirements, but rather to set doses for an upcoming guideline study.)

I. Materials and Methods

A. Test Compound - Purity: 99% Description: white powder Lot No.: T-165-4 Contaminant: not given

B. Vehicle - 0.5% (w/v) aqueous methyl cellulose Lot No.: 104F-0601 Source: Sigma Chemical Company

C. Test Animal - Species: Rat Strain: CD (Sprague-Dawley) Source: Charles River Breeding Laboratory, Inc., Portage, MI Age: 75 days at initiation of mating Weight: 182-259 g

D. Study Design - This study was designed to establish doses for an impending teratology study of the subject compound in rats. The test material was administered by gavage to 40 female rats on gestation days 6 through 15, inclusive.

1. Mating - Sixty male and 60 female rats were mated naturally. Females were randomly assigned to males and pairs were cohoused overnight. The following morning, females were examined for the presence of a sperm plug and/or the presence of sperm in the vaginal smear. A positive finding for either observation was taken as confirmation of mating. If mating was not confirmed, females were cohoused with the same male for each evening until 40 mated females were obtained.

2. Group Arrangement:

Test Group	Dose Level (mg/kg)	Number Assigned
I	0	8
II	250	8
III	500	8
IV	1000	8
V	2000	8

3. Dosing: All doses were in a volume of 10 ml/kg of body weight/day prepared immediately prior to the dosing period. The dosing solutions were analyzed for concentration and stability. Dosing was based on the most recent body weight taken.

E. Observations

1. Maternal Observations and Evaluations - The animals were checked twice daily for mortality or abnormal condition for the term of the study. Dams were sacrificed on day 20 of gestation. At sacrifice, the abdominal cavity was opened and the uterus tied off at the cervix and removed without ovaries. The uterus was weighed and examined externally for the presence of implantation sites. A gross examination of all females was performed at necropsy.

2. Fetal Evaluations - No fetal examinations were performed.

3. Historical control data were not provided to allow comparison with concurrent controls.

F. Statistical analysis - Body weights, body weight gains and food consumption were analyzed as interval data. Below are the study report entries describing the statistical analyses employed.

"Bartlett's test was performed to test for normality/homogeneity of variance. If the test indicated significance, nonparametric procedures were used; if not, parametric procedures were used.

"The parametric procedures were the standard one-way ANOVA using the F distribution to assess significance. Dunnett's test was used to determine which treatment means were significantly different from the control group mean.

"If a nonparametric procedure for testing equality of means was needed, the Kruskal-Wallis test was used, and Dunn's summed rank test for comparing treatments to the control group was used.

"A statistical test for trend in the dose levels was performed. In the parametric case standard regression techniques with a test for trend and lack of fit were used. In the nonparametric case, Jonckheere's test for monotonic trend was used.

"Bartlett's test was conducted at the 1% level of significance. All other statistical tests were conducted at the two-sided 5% and 1% levels of significance."

G. Compliance - Signed and dated GLP and Quality Assurance statements were provided.

II. Results

A. Maternal Toxicity

1. Mortality - All females survived to scheduled sacrifice.

2. Clinical Observations - Six of eight females in high dose group had soft stool. This observation first occurred on day 7 or 8 of gestation and lasted from one to six days. No other treatment related effects were reported.

3. Body Weight - Slight body weight and body weight gain decrements were reported by the registrant for the period covering gestational days 6-9. However, no clear dose response was apparent from the data provided. Although treatment groups II, IV and V had lower mean body weight gains during the day 6-9 interval, treatment group III gained more weight than the control. In the absence of clinical signs of toxicity, the

biological significance of the reduced body weight gain in groups II and IV is questionable.

The individual animal data for groups II and IV are similar to the control for the 6-9 day interval except for one animal from each group which exhibited substantial weight loss (II (#105408) = -25 g, IV (#105425) = -11 g). The registrant attributed this observation in group IV to possible gavage injury. No comment was made concerning the group II animal. Recalculated without these two rats, average weight gains for this period were 7 and 5.7 g for group II and IV, respectively.

In the high dose group (group V), this reduction in body weight gain coincided with reports of loose stool.

MEAN BODY WEIGHTS (g)

Gestation Interval	Dose Level (mg/kg/day) 0	250	500	1000	2000
Day 0	228.9	222.5	223.0	213.6	226.5
Day 6	259.4	252.8	252.4	242.8	257.4
Day 9	266.8	255.8	260.4	246.4	258.5
Day 12	281.1	273.3	277.1	261.5	275.0
Day 16	299.6	293.8	298.1	284.9	297.3
Day 20	363.0	352.3	361.5	341.4	358.4
Day 20*	291.9	286.5	295.3	276.1	286.9

*Body weight corrected for uterine weight.

MEAN BODY WEIGHT GAINS (g)

Gestation Interval	Dose Level (mg/kg/day) 0	250	500	1000	2000
Days 0-6	30.5	30.3	29.4	29.1	30.9
Days 6-9	7.4	3.0	8.0	3.6	1.1
Days 9-12	14.4	17.5	16.8	15.1	16.5
Days 12-16	18.5	20.5	21.0	23.4	22.3
Days 16-20	63.4	58.5	63.4	56.5	61.1

4. Food Consumption - Rats given 1000 and 2000 mg/kg/day exhibited reduced food consumption during the gestational period including days 6-9. This reduction was evident both as absolute food consumption and when normalized to body weight and occurred only during the 6-9 day time period following initiation of treatment.

As indicated above (II,a,3), two animals exhibited unusual weight gain patterns and may have sustained injury during gavage. If data for these animals are excluded, mean absolute food consumption for groups II and IV during days 6-9 was 20.6 and 17.6 g/day, respectively, and relative food consumption was 79.1 and 70.4 g/kg/day, respectively. These values are more similar to the control, indicating that the 1000 mg/kg/day group was probably not different from the control group.

No other treatment related effects were reported.

MEAN ABSOLUTE FOOD CONSUMPTION (g/day)

Gestation Interval	Dose Level (mg/kg/day) 0	250	500	1000	2000
Days 0-6	19.5	18.3	19.6	19.3	19.6
Days 6-9	20.1	18.9	19.8	16.0	15.5
Days 9-12	20.9	21.6	21.9	19.6	20.1
Days 12-16	21.4	22.8	22.1	21.4	22.4
Days 16-20	25.1	25.0	26.8	24.6	25.4

MEAN RELATIVE FOOD CONSUMPTION (g/kg/day)

Gestation Interval	Dose Level (mg/kg/day) 0	250	500	1000	2000
Days 0-6	74.9	72.4	77.5	79.4	76.3
Days 6-9	75.5	73.0	75.9	64.3	60.0*
Days 9-12	74.5	78.9	78.8	75.1	73.5
Days 12-16	71.5	77.4	74.1	74.8	75.3
Days 16-20	69.4	70.9	73.9	72.4	70.6

*Significantly different from the control ($p<0.05$)

5. Gross Pathology - No treatment related gross pathological lesions were reported. Uterine weights from all groups were similar.

All females were pregnant at sacrifice.

III. Discussion: The test material was not found to be maternally toxic at levels up to 1000 mg/kg/day. At the highest dose tested, only transient signs of toxicity were reported. These consisted of reduced body weight gain, reduced food consumption and soft stool in females following the initiation of treatment during days 6-9 of gestation.

No spontaneous abortions occurred. Uterine weights were similar in all treatment groups, suggesting that litter weights were not affected by the test material.

IV. Conclusions: Proposed treatment levels for a teratology study in rats of 500, 1000 and 2000 mg/kg/day are appropriate.

Maternal NOEL = 1000 mg/kg/day
Maternal LOEL = 2000 mg/kg/day based on slight body weight gain and food consumption reductions

Classification: Supplementary
(This study was not intended to fulfil regulatory requirements, but rather to set doses for an upcoming guideline study.)

Primary Review by: Elizabeth A. Doyle, Ph.D. E. A. Doyle 10/22/90
Review Section IV, Toxicology Branch II (HFAS)(H7509C)
Secondary Review by: Marcia van Gemert, Ph.D. M van Gemert 10/25/90
Toxicology Branch II (HFAS)(H7509C)

008334

DATA EVALUATION RECORD

Study Type: Teratology - Developmental Toxicity (Range-finding)
Species: Rabbit

MRID No.: 415648-09 Caswell No.: 215B

Test Material: Chlorothalonil Metabolite

Synonyms: 3-Carbamyl-2,4,5-trichlorobenzoic acid, 2,4,5-Trichloroisophthalamic acid, 2,5,6-trichloro-3-carboxybenzamide, 3-carboxy-2,5,6-trichlorobenzamide

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

Study Number: 1112-86-0056-TX-002

Testing Facility: Argus Research Laboratories, Inc.
935 Horsham Road
Horsham, Pennsylvania 19044

and

Ricerca, Inc.
Department of Toxicology and Animal Metabolism
7528 Auburn Road
Painesville, Ohio 44077

Title of Report: A Teratology Dose Range-Finding Study in Rabbits with 3-Carbamyl-2,4,5-trichlorobenzoic Acid (SDS-46851)

Authors: D. M. Serrone and J. C. Killeen, Jr.

Report Issued: February 8, 1989

Conclusions: Based upon the results of this study, the dose levels of 250, 500 and 1000 mg/kg/day of this chlorothalonil metabolite selected for a teratology study in rabbits appear appropriate.

Maternal NOEL = 250 mg/kg/day in rabbits
Maternal LOEL = 500 mg/kg/day in rabbits based on body weight loss during gestation
Developmental NOEL = Not determined

Classification: Core - Supplementary
(This study was not designed to fulfil regulatory requirements but only to set dose levels for an upcoming study.)

I. Materials and Methods

A. Test Compound - Purity: >99% Description: Light brown powder Lot No.: 46851-0202 Contaminant: not given

B. Vehicle - 0.5% (w/v) methyl cellulose in water

C. Test Animal - Species: Rabbit Strain: New Zealand White [Hra:(NZW)SPF] Source: Hazleton Research Animals, Denver, Pa Age: 5 months Weight: 2.5-4.5 kg

D. Study Design - This study was designed to assess the developmental toxicity potential of the test material when administered by gavage to pregnant rabbits on gestation days 6 through 19, inclusive.

1. Mating - Females were given human chorionic gonadotropin (20 USP Units/kg) intravenously and artificially inseminated.

2. Group Arrangement:

Test Group	Dose Level (mg/kg)	Number Assigned
I	0	7
II	250	7
III	500	7
IV	1000	7
V	2000	7

3. Dosing: All doses were in a volume of 10 ml/kg of body weight/day prepared weekly during the dosing period. The dosing solutions were analyzed for concentration and stability. Dosing was based on the most recent body weight taken.

E. Observations

1. Maternal Observations and Evaluations - The animals were checked twice daily for mortality or abnormal condition throughout the study period. Dams were sacrificed on day 20 of gestation. Data collected at sacrifice included uterine weights, number and placement of implantations, early and late resorptions and live and dead fetuses and number of corpora lutea. Maternal tissues were saved in neutral buffered formalin only if gross abnormalities were evident. Females found dead were necropsied and the cause of death determined where possible.

2. Fetal Evaluations - No fetal evaluations were performed during this study.

3. Historical control data were not provided to allow comparison with concurrent controls.

F. Statistical Analysis - The description below is taken from the study report.

"Physical sign data for inseminated female rabbits will be analyzed using the variance test for homogeneity of the binomial distribution.

"Mean maternal body weight data will be based on surviving pregnant does and will be analyzed using Bartlett's test of homogeneity of variances and the Analysis of variance. If the Analysis of Variance is significant and appropriate, i.e., it passed Bartlett's test ($P>0.05$), then Dunnett's test will be used to identify the statistical significance of individual groups. If the Analysis of Variance is not appropriate ($P<0.05$), the Kruskal-Wallis test will be used; in cases where statistical significance occurred, Dunn's method of multiple comparisons will be used to identify statistical significance of individual groups.

"The Analysis of Covariance will be used to evaluate average maternal body weight change from day 0 to day 6 of gestation and from day 0 to day 20 of gestation. This test will also be used to evaluate changes in average maternal body weight from day 6 to days 9, 12, 15, 19 and 20 of gestation.

"Data obtained at Caesarean-sectioning of does will be evaluated using the Kruskal-Wallis test; in cases where statistical significance occurred ($P<0.05$), Dunn's method of multiple comparisons will be used to identify the statistical significance of individual groups.

"Uterine weights will be analyzed using Bartlett's test of homogeneity of variances and the Analysis of Variance. If the Analysis of Variance is significant and appropriate, i.e., it passed Bartlett's test, then Dunnett's test will be used to identify the statistical significance of individual groups. If the Analysis of Variance is not appropriate, the Kruskal-Wallis test will be used; in cases where statistical significance occurs, Dunn's method of multiple comparisons will be used to identify the statistical significance of individual groups.

"Observations for delivered and dead conceptuses will be excluded from fetal body weight, summaries and statistical analyses. Additional statistical tests may be conducted if necessary or deemed appropriate."

G. Compliance - Signed and dated GLP and Quality Assurance statements were provided.

II. Results

A. Maternal Toxicity

1. Mortality - Two does given 2000 mg/kg/day and one doe given 1000 mg/kg/day died before the scheduled termination of the study. The high dose does were found dead on gestation days 10 and 17. Both rabbits had exhibited reduce food consumption and body weight loss following initiation of dosing. The doe found dead on day 17 had

exhibited altered fecal consistency beginning on day 9 and gestation. At necropsy this doe was found to have stomach ulcerations and a fur ball.

The doe found dead from the 1000 mg/kg/day group died due to perforation of the right diaphragmatic lobe of the lungs during intubation. This death was not test material related.

Fetuses in each of these does were apparently alive and normal for their respective developmental ages at the time that the dams died.

2. Clinical Observations - The fecal consistency of six does from the 2000 mg/kg/day group and three does from the 1000 mg/kg/day group changed, apparently in concert with a decrease in food consumption. Stool during this period was described as soft or liquid or dried. The changes occurred beginning on day 7 for the 2000 mg/kg/day group and day 11 for the 1000 mg/kg/day group. One doe each from the 500 mg/kg/day and control groups also had transient occurrences of abnormal feces.

Decreased motor activity was reported in one high dose doe on day 20 of gestation. No definitive comment can be made concerning the causal relationship between treatment and this observation.

3. Body Weight - Body weights were reduced beginning on day 9 in females given 1000 or 2000 mg/kg/day this was the first weighing following initiation of dosing. Body weight decrement in these two groups persisted until sacrifice. Gravid uterine weights from these two treatment groups were also reduced relative to the control, but the difference was insignificant in the overall weight reduction observed. Terminal body weights corrected for gravid uterine weight reflected the reduction in groups given 1000 and 2000 mg/kg/day.

Body weight losses due to treatment with the test material at levels of 500, 1000 and 2000 mg/kg/day were evident beginning immediately after initiation of treatment. Body weight change demonstrated a dose response for the three highest treatment groups beginning with the 6-9 day gestation interval and continuing until sacrifice. When corrected for gravid uterine weight and presented as cumulative body weight change for either the entire gestation period or the dosing period only, the differences due to treatment exhibit a clear dose response.

MATERNAL BODY WEIGHTS (kg)

Gestation Day	Treatment Level (mg/kg/day) 0	250	500	1000	2000
0	3.68	3.73	3.77	3.76	3.81
6	3.83	3.89	3.94	3.92	4.00
9	3.83	3.89	3.92	3.80	3.84
12	3.85	3.91	3.93	3.74	3.79
15	3.91	3.95	3.92	3.71	3.68
19	3.93	3.96	3.85	3.53	3.55
20	3.92	3.97	3.87	3.51	3.52
Gravid Uterine Weight (g)	138.11	136.99	140.66	86.52	118.51
20C[a]	3.78	3.83	3.73	3.42	3.39

[a]Body weight corrected for gravid uterine weight

MATERNAL BODY WEIGHT CHANGES (kg)

Gestation Interval (days)	Treatment Level (mg/kg/day) 0	250	500	1000	2000
0 - 6	+0.15	+0.16	+0.17	+0.15	+0.19
6 - 9	0.00	0.00	-0.01	-0.02	-0.16**
9 - 12	+0.02	+0.02	+0.08	-0.07	-0.12
12 - 15	+0.07	+0.03	-0.01	-0.03	-0.12**
15 - 20	+0.00	+0.02	-0.05	-0.20**	-0.16**
0 - 20C[a]	+0.10	+0.10	-0.02	-0.24*	-0.44**
6 - 20C[a]	-0.05	-0.06	-0.21	-0.40**	-0.63**

*Significantly different from the control ($p \leq 0.05$)
**Significantly different from the control ($p \leq 0.01$)
[a]Body weight gains corrected for gravid uterine weights

4. Food Consumption - Absolute food consumption was reduced in females in the two highest treatment groups beginning with the first measurement period following initiation of treatment (days 6-9). In the 500 mg/kg/day group, absolute food consumption was similar to the control during the days 6-9 interval, began to diverge from the control by the days 9-12 interval. A dose response was apparent with respect to food consumption reduction at all time intervals.

These observations were confirmed when the food consumption was normalized to body weight. As for absolute food consumption above, the relative food consumption in the two high dose groups diverged from the control beginning with the initiation of dosing. During the second gestational interval in which food consumption was measured (days 9-12), the 500 mg/kg/day group exhibited lower food consumption than the control. At all time points following initiation of treatment, dose response was evident in the data.

MATERNAL FOOD CONSUMPTION (g/day)

Gestation Interval (days)	Treatment Level (mg/kg/day) 0	250	500	1000	2000
0 - 6	149.0	167.0	168.3	160.2	170.9
6 - 9	154.7	163.2	161.6	141.6	100.9**
9 - 12	144.5	152.2	133.5	90.3*	14.7**
12 - 15	136.2	144.9	91.4	60.4	39.4*
15 - 20	124.5	134.3	78.6	29.6*	10.2**

*Significantly different from the control ($p \leq 0.05$)
**Significantly different from the control ($p \leq 0.01$)

MATERNAL FOOD CONSUMPTION RELATIVE TO BODY WEIGHT (g/kg/day)

Gestation Interval (days)	Treatment Level (mg/kg/day) 0	250	500	1000	2000
0 - 6	40.7	43.9	43.2	41.8	44.8
6 - 9	40.6	41.2	41.2	37.2	25.4
9 - 12	37.6	38.8	33.6	23.8	4.2*
12 - 15	35.1	35.2	22.8	15.8*	9.9*
15 - 20	31.2	33.0	19.9	8.0*	2.7**

*Significantly different from the control ($p \leq 0.05$)
**Significantly different from the control ($p \leq 0.01$)

5. Gross Pathology - Five rabbits from the 2000 mg/kg/day group and

two from the group given 1000 mg/kg/day were found to have fur balls in their stomachs (gastric trichobezoar) at necropsy. Rabbits from the lower dose groups and the control group did not have gastric trichobezoar. Gastric ulceration occurred in three high dose rabbits only.

6. Litter Data - Data from litters taken at terminal sacrifice indicated no apparent effect on caesarean section parameters at scheduled sacrifice. The registrant indicated that inclusion of implantation data from the does that died during the study provided possible indication that the test material may have interfered with implantation in the high dose group; however, this comment is highly speculative in natural.

Cesarean Section Observations[a]

Dose (mg/kg/day):	0	250	500	1000	2000
Animals Assigned	7	7	7	7	7
#Animals Inseminated	7	7	7	7	7
Pregnancy Rate (%)	100	100	85.7	71.4	85.7
Maternal Wastage					
#Died	0	0	0	1	2
#Died/pregnant	0	0	0	1	2
#Nonpregnant	0	0	1	2	1
#Aborted	0	0	0	0	0
#Premature Delivery	0	0	0	0	0
Corpora Lutea/Dam	10.3	10.3	10.8	10.0	10.5
Implantations/Dam	8.0	8.6	8.7	7.2	8.2
Live Fetuses	56	55	50	20	32
Live Fetuses/Dam	8.0	7.6	8.3	5.0	8.0
Dams with Resorptions	0	4	2	2	1
Resorptions/Dam	0.0	0.7	0.3	2.2**	0.2
Early	0.0	0.6	0.2	1.8	0.2
Late	0.0	0.1	0.2	0.5	0.0
Total Dead Fetuses	0	0	0	0	0
Resorbed/Dam (%)	0.0	8.2*	4.8	25.6	2.8

* Significantly different from the control ($p<0.05$)
**Significantly different from the control ($p<0.01$)

B. Developmental Toxicity - Fetuses not evaluated for variations.

III. Discussion: The test material caused a reduction in body weight and an actual body weight loss almost immediately following initiation of treatment in the 1000 and 2000 mg/kg/day treatment groups (gestational day 6). This observation was coupled with simultaneous reduction in food consumption, and change in fecal consistency. Rabbits from the 500 mg/kg/day group had minor body weight reduction and reduced food consumption beginning after gestational day 9. No adverse clinical signs were reported for the 500 mg/kg/day group. Body weight loss and reduced food consumption exhibited dose response.

Two does from the high dose group died following reported thin appearance and development of abnormal fecal consistency. These deaths appeared to result from treatment with the test material.

Adverse effects on implantation and developing fetuses were questionable if present at all.

IV. Conclusions: Based upon the results of this study, the dose levels of 250, 500 and 1000 mg/kg/day of this chlorothalonil metabolite selected for a teratology study in rabbits appear appropriate.

Maternal NOEL = 250 mg/kg/day in rabbits
Maternal LOEL = 500 mg/kg/day in rabbits based on body weight loss during gestation
Developmental NOEL = Not determined

Classification: Core - Supplementary
(This study was not designed to fulfil regulatory requirements but only to set dose levels for an upcoming study.)

Primary Review by: Elizabeth A. Doyle, Ph.D. E.A. Doyle 3/27/91
Review Section IV, Toxicology Branch II (HFAS)(H7509C)
Secondary Review by: Marcia van Gemert, Ph.D. Marcia van Gemert 3/27/91
Toxicology Branch II (HFAS)(H7509C)

008334

DATA EVALUATION RECORD

Study Type: Developmental Toxicity - Rabbit (83-3) Tox. Chem. No.: 215B

MRID No.: 415648-10

Test Material: Chlorothalonil Metabolite

Synonyms: 3-Carbamyl-2,4,5-trichlorobenzoic acid, 2,4,5-Trichloroisophthalamic acid, 2,5,6-trichloro-3-carboxybenzamide, 3-carboxy-2,5,6-trichlorobenzamide

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

Study Number: 1112-88-0013-TX-002

Testing Facility: Hazleton Laboratories America, Inc.
3301 Kinsman Boulevard
Madison, WI 53704

Title of Report: A Teratology Study in Rabbits with 3-Carbamyl-2,4,5-trichlorobenzoic Acid (SDS-46851)

Authors: D. M. Serrone and J. C. Killeen, Jr.

Report Issued: June 14, 1989

Conclusions - The test material is not a developmental toxicant to rabbits under the conditions of this study.

Maternal NOEL = 250 mg/kg/day
Maternal LOEL = 500 mg/kg/day based upon body weight gain decrement and reduced food consumption
Developmental NOEL = 1000 mg/kg/day (highest dose tested)

Classification: Core - Guideline

This study satisfies the guideline requirements (83-3) for a "Developmental Toxicity Study in Rabbits".

I. Materials and Methods

A. Test Compound - Purity: >99% Description: light brown powder Lot No.: T-165-2 Contaminant: not given

B. Vehicle - 0.5% w/v aqueous methyl cellulose

C. Test Animal - Species: rabbit Strain: New Zealand White [Hra:(NZW)SPF] Source: Hazleton Research Products, Inc., Denver, PA Age: 5 months Weight: 3.00-4.18 kg

D. Study Design - This study was designed to assess the developmental toxicity potential of the test material when administered by gavage to pregnant rabbits on gestation days 7 through 19, inclusive.

1. Mating - Ovulation was induced by injection with 20 IU of human chorionic gonadotropin/kg. Females were artificially inseminated with semen from male New Zealand White rabbits.

2. Group Arrangement:

Test Group	Dose Level (mg/kg)	Number Assigned
Control	0	20
Low Dose	250	20
Mid Dose	500	20
High Dose	1000	20

3. Dosing: All doses were in a volume of 4 ml/kg of body weight/day prepared weekly during the dosing period. The dosing solutions were analyzed for concentration and stability. Dosing was based on the most recent body weight.

E. Observations

1. Maternal Observations and Evaluations - The animals were checked twice daily for mortality or abnormal condition for the duration of the study.

All surviving dams were sacrificed on day 29 of gestation. These and females found moribund or dead were necropsied. In females dying before scheduled sacrifice, an attempt was made to determine the cause of death. Tissues with gross lesions were fixed in 10% neutral buffered saline. The number of corpora lutea and number and location of implantations was recorded.

2. Fetal Evaluations - Live and dead fetuses were weighed and examined for gross malformations. Only dead fetuses with obvious external malformations were processed for skeletal examination. Late resorptions were discarded.

Fetuses were examined by microdissection for soft tissue malformations. Carcasses were then eviscerated. The eyes were examined for gross abnormalities during skinning. The skull was opened by making transverse cuts with a razor blade parallel and posterior to the frontal-parietal suture and through the cerebral hemispheres for examination of the brain.

3. Historical control data were provided to allow comparison with concurrent controls.

F. Statistical Analysis - For the purposes of analysis of the data from this study, the dam or litter were considered the experimental units. The statistical methods used in evaluation of this study are presented below as described in the study report.

"One-way analysis of variance (ANOVA) with transformations (square root, log, reciprocal, arc sine, and rank) will be done on the following data: maternal body weights and body weight changes (uncorrected and corrected) for pregnant animals; gravid uterine weight; the number of corpora lutea and implantations; implantation efficiency; fetal viability; the number of live, dead and resorbed fetuses; and sex ratio. Dunnett's t-test will be done on the transformed data when analysis of variance is significant. If none of the transformations produce homogeneous variance, ANOVA and Dunnett's t-test will be done on the ranked data, as well as Kruskal-Wallis ad Terpstra-Jonckheere tests for monotonic trend.

"Fetal weights will be analyzed by one-way analysis of covariance (ANCOVA) using the number of live fetuses as the covariate. When the test is significant, Dunnett's t-test will be used to determine significant differences between the control and treated groups.

"The proportion of litters and fetuses with visceral and skeletal abnormalities in the treated groups will be compared with the control by the Cochran-Armitage test for trend and departure and by a Fisher-Irwin exact test."

G. Compliance - Signed and dated GLP and Quality Assurance statements were provided.

II. Results

A. Maternal Toxicity

1. Mortality - Three control, one 250 mg/kg and two 1000 mg/kg females were found dead during the study. Based upon subsequent necropsy of the carcasses, the deaths were attributed to gavage error with the exception of the high dose female found dead on day 12 of gestation.

Two, one and seven rats from the low, mid and high dose groups, respectively, were sacrificed after abortion or premature delivery.

2. Clinical Observations - Rabbits given the test material exhibited a dose related change in quantity of feces produced and in consistency. These observations coincided with development of anorexia and, in six of the high dose females, an appearance of thinness.

CLINICAL OBSERVATIONS

Observation	Treatment Level (mg/kg/day)			
	0	250	500	1000
Few or no feces	10	17	19	20
Soft feces	2	6	6	16
Black/tan/green mucus-like feces	0	0	3	14
Anorexia	0	6	10	19
Thin	0	1	1	6

3. Body Weight - Body weights for the low dose group were similar to the control for the entire study period. In the mid dose group, body weights diverged from the control beginning on day 13 of gestation (the first weighing after initiation of treatment) and continuing for the remainder of the study. High dose rabbits similarly had lower body weights than the control beginning on day 13 of gestation, although the magnitude of difference was greater in the high dose females. Gravid uterine weights exhibited a dose related decrease in the mid and high dose females. Correction for gravid uterine weights accounted for about half of the difference in the terminal body weights.

Body weight gains demonstrated a similar time related pattern of difference as the absolute body weights. Reduced body weight gain was evident beginning with the first measurement period following initiation of treatment. As with the body weight data, a dose related reduction was reported beginning with the days 7-13 gestation interval and continuing through day 19-24. During the final period of days 24-29 following cessation of dosing, the mid and low dose groups reversed this trend and gained with while the control and low dose groups recorded actual weight losses. This may reflect recovery upon removal of the test material. When evaluated over the entire treatment period, all treated groups were similar but had lower weight gains than the control group.

BODY WEIGHTS (kg) AND GRAVID UTERINE WEIGHTS (g)

Gestation Day	Treatment Level (mg/kg/day) 0	250	500	1000
0	3.64	3.69	3.56	3.53
7	3.78	3.85	3.70	3.72
13	3.80	3.86	3.73	3.62
16	3.85	3.87	3.75	3.55*
19	3.87	3.88	3.72	3.46*
24	3.92	3.91	3.69	3.39*
29	3.89	3.87	3.72	3.57
29[c]	3.43	3.46	3.32	3.20
Gravid Uterine Weight	465.5	456.8	393.4	365.0

*Significantly different from the control ($p<0.05$)
[c]Body weight minus gravid uterine weight.

BODY WEIGHT GAIN (kg)

Gestation Day	Treatment Level (mg/kg/day) 0	250	500	1000
0 - 7	0.14	0.16	0.14	0.18
7 - 13	0.02	0.01	0.03	-0.11*
13 - 16	0.05	0.01	0.02	-0.07*
16 - 19	0.04	0.01	-0.03	-0.09*
19 - 24	0.05	0.03	-0.02	-0.07
24 - 29	-0.03	-0.04	0.01	0.03
0 - 29	0.28	0.16	0.18	0.13

*Significantly different from the control ($p<0.05$)

4. Food Consumption - Food consumption directly mirrored the treatment related pattern of differences in body weight gain reported above. Food consumption declined in the mid and high dose group in a dose related manner immediately following initial treatment. Food consumption remained depressed in the higher dose groups during the treatment period (days 7-19) and into the following period (days 20-25). However, during the final period (days 25-29), an apparent rebound occurred with increased food consumption compared to the control and low dose groups.

FOOD CONSUMPTION (g)

Gestation Day	Treatment Level (mg/kg/day) 0	250	500	1000
0 - 7	1,777.6	1,223.2	1,247.4	1,195.0
7 - 14	1,044.5	1,076.6	1,165.9	799.9*
14 - 17	428.0	417.7	388.7	152.8*
17 - 20	446.5	461.5	271.1*	108.9*
2 - 25	548.5	601.7	460.0	273.3
25 - 29	269.8	268.2	299.7	334.4

*Significantly different from the control ($p<0.05$)

5. Gross Pathology - Apparent treatment related effects were limited to hard, raised, black areas on the gallbladder (2), enlarged gallbladder (3) and focal erosion of the stomach (4) in the high dose females.

6. Litter Data - High dose females had increased premature deliveries and abortions relative to the control group. In addition, The number of live fetuses/dam and percent live fetuses were reduced in the high dose group relative to the control group. However, neither observation achieved statistical significance.

No other treatment related effects were reported.

Cesarean Section Observations

Dose (mg/kg/day):	0	250	500	1000
#Animals Assigned	20	20	20	20
#Animals Mated/Inseminated	20	20	20	20
Pregnancy Rate (%)	85	85	85	90
Maternal Wastage				
#Died	3	1	0	3
#Died/pregnant	3	1	0	3
#Non pregnant	3	3	3	2
#Aborted	0	1	1	2
#Premature Delivery	0	1	0	5
Corpora Lutea/Doe	11.0	11.6	11.6	11.3
Implantations/Doe	8.2	9.2	8.5	7.9
Implantation Efficiency	74.5	82.4	72.4	70.7
Total Litters Delivered	14	15	16	9
Live Fetuses/Doe	8.5	8.3	7.5	6.6
Percent Live Fetuses	98.0	88.6	90.1	81.6
Total Resorpcions/Doe	0.2	0.5	0.9	0.9
Early	0.2	0.4	0.4	0.5
Late	0.0	0.1	0.5	0.4
Dead Fetuses/Doe	0.0	0.7	0.0	0.6
Mean Fetal Weight (gm)	39.2	36.6	36.8	34.7
Sex Ratio (% Male)	55.3	42.7	49.7	48.9

B. Developmental Toxicity

1. External Examination - No apparent treatment related effects were reported.

2. Viscera. Examination - No treatment related soft tissue abnormalities were reported. However, the report noted the absence of the azygous lobe of the lung and/or the arising of the left carotid artery from the innominate in a number of pups from control and treated groups.

3. Skeletal Examination - No apparent treatment related effects were reported.

III. Discussion: Only slight maternal toxicity was reported for pregnant rabbits given up to 1000 mg/kg/day of the test material on gestational days 7 to 19 inclusive. Signs of toxicity were limited to slight reductions in body weight gain and food consumption and abnormal feces.

No treatment related developmental abnormalities were reported. However, the number of live fetuses per doe and percent live fetuses were slightly but nonsignificantly decreased at the high dose level.

IV. Conclusions: The test material is not a developmental toxicant to rabbits under the conditions of this study.

Maternal NOEL = 250 mg/kg/day
Maternal LOEL = 500 mg/kg/day based upon body weight gain decrement and reduced food consumption
Developmental NOEL = 1000 mg/kg/day (highest dose tested)

V. Classification: Core - Guideline

This study satisfies the guideline requirements (83-3) for a "Developmental Toxicity Study in Rabbits".

Primary Review by: Elizabeth A. Doyle, Ph.D. E.A. Doyle 10/2/90
Review Section IV, Toxicology Branch II (HFAS)(H7509C)
Secondary Review by: Marcia van Gemert, Ph.D.
Toxicology Branch II (HFAS)(H7509C) MvanGemert 10/25/90

DATA EVALUATION RECORD 002334

Study Type: Developmental Toxicity - Rat (83-3) Tox. Chem. No.: 215B

MRID No.: 415648-08

Test Material: Chlorothalonil Metabolite

Synonyms: 3-Carbamyl-2,4,5-trichlorobenzoic acid, 2,4,5-Trichloroisophthalamic acid, 2,5,6-trichloro-3-carboxybenzamide, 3-carboxy-2,5,6-trichlorobenzamide

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

Study Number: 1554-87-0095-TX-002

Testing Facility: Ricerca, Inc.
Department of Toxicology and Animal Metabolism
7528 Auburn Road
Painesville, Ohio 44077

Title of Report: A Teratology Study in Rats with 3-Carbamyl-2,4,5-trichlorobenzoic Acid

Authors: J. S. Chun, N. H. Wilson and J. C. Killeen, Jr.

Report Issued: August 15, 1989

Conclusions - The test material is not a developmental toxicant at doses up to or exceeding 2000 mg/kg/day in rats.

Maternal NOEL = 2000 mg/kg/day
Maternal LOEL > 2000 mg/kg/day
Developmental Toxicity NOEL = 2000 mg/kg/day
Developmental Toxicity LOEL > 2000 mg/kg/day

Classification: Core - Guideline

This study satisfies the guideline requirements (83-3) for a "Developmental Toxicity Study in Rats".

SU

F. RESULTS - ONE-GENERATION REPRODUCTION STUDY

1. Parental animals

a. Mortality and clinical signs: One female was found dead on the morning prior to mating. She had been noted to have broken teeth when examined two days earlier. This rat had not been eating well and was reported to have appeared thin with a rough coat. Gross necropsy revealed no remarkable lesions.

b. Body weight and food consumption: Food consumption was not measured during this phase of the study.

Selected group mean body weights values for pregnant or nursing dams were summarized in the report as follows:

Observation and study time	Dose group: Control	Low	Mid	High
	F_0 Generation			
Mean body weight (g)				
Day 0 of gestation	291.0	288.0	285.1	290.2
Day 20 of gestation	404.3	403.6	383.5	400.5
Day 0 of lactation	324.2	324.4	307.9	321.5
Day 21 of lactation	303.2	308.2	299.0	315.4
Mean body weight gain (g)				
Days 0-20 of gestation	113.3	115.6	98.4	110.3
Day 0-21 of lactation	-21.0	-15.7	-8.9	-6.1

*Statistically significantly different from control, $p<0.05$.
**Statistically significantly different from control, $p<0.01$.

c. Reproductive performance: No effects on reproductive performance noted by the investigators except for a slight but statistically significant decrement in Day 21 pup weight in the high dose litters.

I. Materials and Methods

A. Test Compound - Purity: 99%, Description: beige powder, Lot No.: T-165-4, Contaminant: not given

B. Vehicle - 1.0% (w/v) methyl cellulose in water, Source: Sigma Chemical Company, Lot No.: 57F-0199 and 77F-0601

C. Test Animal - Species: Rat, Strain: CD (Sprague-Dawley), Source: Charles River Breeding Laboratories, Inc., Age: 69 days, Weight: mated females - 214 g (186-261 g)

D. Study Design - This study was designed to assess the developmental toxicity potential of the test material when administered by gavage to females rats on gestation days 6 through 15, inclusive.

1. Mating - One hundred sixty-four male and 164 female rats were mated naturally. Females were randomly assigned to males and pairs were cohoused overnight. The following morning, females were examined for the presence of a sperm plug and/or the presence of sperm in the vaginal smear. A positive finding for either observation was taken as confirmation of mating. If mating was not confirmed, females were cohoused with the same male for each evening until 100 mated females were obtained.

2. Group Arrangement:

Test Group	Dose Level (mg/kg/day)	Number Assigned	Proportion of Fetuses Examined/Litter		
			External	Soft Tissue	Skeletal
I	0	25	All	1/2	1/2
II	500	25	All	1/2	1/2
III	1000	25	All	1/2	1/2
IV	2000	25	All	1/2	1/2

3. Dosing: All doses were in a volume of 10 ml/kg of body weight/day prepared immediately prior to the dosing period. The dosing solutions were analyzed for concentration and stability. Dosing was based on the most recent body weight taken.

E. Observations

1. Maternal Observations and Evaluations - The animals were checked twice daily for mortality or abnormal condition. Rats were given a thorough physical examination on days 0, 6, 9, 12, 16 and 20 of gestation. All mated females were sacrificed on day 20 of gestation and subjected to a gross post mortem. This examination included external surfaces, all orifices, the cranial cavity, carcass, the external surface of the spinal cord and sectioned surfaces of the brain, nasal cavity and paranasal sinuses, the

thoracic, abdominal and pelvic cavities and their viscera and the cervical tissues and organs.

Only tissues in which lesions were observed during necropsy were saved.

Intact uteri with ovaries attached were removed and weighed and the number and location of live fetuses, dead fetuses, late and early resorptions and implantation sites were recorded. The number of corpora lutea were determined for each ovary.

2. Fetal Evaluations - All fetuses were subjected to gross examination for external malformations or variations including palatal defects. Individual fetuses were weighed and sexed.

   One half of the fetuses were evaluated for visceral abnormalities. Fetuses were decapitated and the heads fixed in Bouin's solution. The carcasses were dissected for evaluation of thoracic and abdominal tissues. Fetuses were then eviscerated and placed in 70% ethanol. Following a period of fixation, the heads were sectioned with a razor blade into serial, transverse sections. The sections were evaluated for malformations of the eyes palate and brain under a dissecting microscope.

   The remaining fetuses were eviscerated and processed for staining of the ossified structures using Alizarin Red.

   Late resorptions were examined grossly for external malformations and discarded.

3. Historical control data were not provided to allow comparison with concurrent controls.

F. Statistical analysis - The following description of the statistical analyses employed is taken from the study report.

"Bartlett's test will be performed to test for normality/homogeneity of variance. If the test indicates significance, nonparametric procedures will be used; if not, parametric procedures will be used.

"The parametric procedures will be the standard one-way ANOVA using the F distribution to assess significance. In addition, an analysis of covariance, with litter size as the covariant will be performed on the fetal weight. Dunnett's test will be used to determine which treatment means are significantly different from the control group mean.

"If a nonparametric procedure for testing equality of means is needed, the Kruskal-Wallis test will be used, and Dunn's summed rank test for comparing treatments to the control group will be used.

"A statistical test for trend in the dose levels will also be performed. In the parametric case standard regression techniques with a test for

trend and lack of fit will be used. In the nonparametric case, Jonckheere's test for monotonic trend will be used.

"Bartlett's test will be conducted at the 1% level of significance. All other statistical tests will be conducted at the two-sided 5% and 1% levels of significance.

"All ratios will be transformed via the arc sine transformation prior to analysis. However, the data will be presented untransformed."

"Statistical analysis of incidence data will be performed using contingency tables. Fetal incidence data will be presented on both a per fetus and a per litter basis. However, to preclude any erroneous results due to "litter effects", the data will be analyzed only on a per litter basis. Each treatment group will be compared to the control group using Fisher's Exact Test for a 2x2 table; the significance level will be corrected via Bonferroni inequality to assure an overall test of the stated significance level. In addition, Armitage's test for linear trend in the dosage groups will be performed.

"The results of all tests will be reported at the two-sided 5% and 1% levels of significance."

G. Compliance - Signed and dated GLP and Quality Assurance statements were provided.

II. Results

A. Maternal Toxicity

1. Mortality - All rats survived scheduled sacrifice.

2. Clinical Observations - No treatment related adverse clinical signs were reported.

3. Body Weight - No treatment related effect on body weight was reported.

Body weight change was not affected by treatment with the test material during any monitoring period or when evaluated as change for the entire dosing period (Days 6-16 inclusive). Terminal body weights corrected for gravid uterine weight also were similar to the control in all treatment groups.

MATERNAL BODY WEIGHT CHANGE - GESTATION (g)

Gestation Interval (days)	Dose Level (mg/kg/day) 0	500	1000	2000
0 - 6	31	31	32	29
6 - 9	7	8	10	10
9 - 12	15	14	15	14
12 - 16	27	26	28	28
16 - 20	59	56	59	56
6 - 16*	49	48	52	51
6 - 20[c]	32.4	30.7	34.3	32.9

*Body weight change during dosing period
[c]Body weight change corrected for gravid uterine weight

4. Food Consumption - No treatment related effect were reported.

5. Gross Pathology - No adverse effects due to treatment were reported at the post mortem.

6. Litter Data - The test material caused no apparent effect on litter production at the treatment levels tested in this study.

Cesarean Section Observations[a]

Dose:	Control	LDT	MDT	HDT
#Animals Assigned	25	25	25	25
#Animals Mated	25	25	25	25
Pregnancy Rate (%)	88.0	100.0	92.0	100.0
Maternal Wastage				
#Died	0	0	0	0
#Nonpregnant	3	0	2	0
#Aborted	0	0	0	0
#Premature Delivery	0	0	0	0
Total Corpora Lutea	355	381	375	400
Corpora Lutea/Dam	16.1	15.2	16.3	16.0
Total Implantation	309	359	340	360
Implantations/Dam	14.0	14.4	14.8	14.4
Total Live Fetuses	296	331	324	345
Live Fetuses/Dam	13.5	13.2	14.1	13.8
Total Resorptions	12	28	16	15
Early				
Late				
Resorptions/Dam	0.5	1.1	0.7	0.6
Total Dead Fetuses	1	0	0	0
Mean Fetal Weight (gm)	3.45	3.43	3.45	3.39
Preimplantation Loss(%)	12.5	5.4	8.3	9.6
Postimplantation Loss(%)	3.8	8.7	4.6	4.1
Sex Ratio (# Male/# Female)	1.0	0.9	1.0	0.8

B. Developmental Toxicity

1. External Examination - One dead edematous fetus was observed in a female from the 1000 mg/kg/day group. One fetus with exencephaly, protruding tongue and an open eye occurred in the high dose group. No other external fetal abnormalities were reported.

2. Visceral Examination - No apparent treatment related effects were reported. Individual fetal and litter rates of variations were similar for all groups.

3. Skeletal Examination - Rates of skeletal variations were comparable between all groups examined.

III. Discussion: No treatment related effects on any of the parameters evaluated were reported in this study.

IV. Conclusions: The test material is not a developmental toxicant at doses up to or exceeding 2000 mg/kg/day in rats.

Maternal NOEL = 2000 mg/kg/day
Maternal LOEL > 2000 mg/kg/day
Developmental Toxicity NOEL = 2000 mg/kg/day
Developmental Toxicity LOEL > 2000 mg/kg/day

Classification: Core - Guideline

This study satisfies the guideline requirements (83-3) for a "Developmental Toxicity Study in Rats".

EPA No.: 68D80056
DYNAMAC No.: 333-F
TASK No.: 3-33F
January 11, 1991

008334

DATA EVALUATION RECORD

3-CARBOXY-2,5,6-TRICHLOROBENZAMIDE

Mutagenicity--*In vivo* Micronucleus Assay in Mice

APPROVED BY:

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

Signature: Robert J. Weir

Date: 1/10/91

Guideline Series 84: **MUTAGENICITY**

EPA No.: 68D80056
DYNAMAC No.: 333-F
TASK No.: 3-33F
January 1[illegible], 1991

008334

DATA EVALUATION RECORD

3-CARBOXY-2,5,6-TRICHLOROBENZAMIDE

Mutagenicity--In vivo Micronucleus Assay in Mice

REVIEWED BY:

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

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Date: 1-11-91

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Date: 1-10-91

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Yiannakis M. Ioannou, Ph.D.
EPA Section Head, Section I
Toxicology Branch II
(H-7509C)

Signature: E. A. Doyle for
Date: 3/18/91

Micronucleus

DATA EVALUATION RECORD

Tox. Chem. No.:

EPA File Symbol:

CHEMICAL: 2,5,6-Trichloro-3-carboxybenzamide.

STUDY TYPE: Mutagenicity--in vivo micronucleus assay in mice.

ACCESSION OR MRID NUMBER: 415648-17.

SYNONYMS/CAS Number: T-165-2; SDS-46851; 3-carboxy-2,5,6,-trichlorobenzamide.

SPONSOR: Fermenta ASC Corp., Mentor, OH.

TESTING FACILITY: C.E.R.T.I. Laboratoire d'Histopathologie, Versailles, France.

TITLE OF REPORT: The Micronucleus Test in Mice with 2,5,6-Trichloro-3-carboxybenzamide (SDS 46851).

AUTHORS: Siou, G. and Lerond-Conan, L. (C.E.R.T.I.); Mizens, M. and Killeen, J.C. (SDS Biotech Corp.).

STUDY NUMBER: 5TX-84-0096.

REPORT ISSUED: February 13, 1985 (C.E.R.T.I); November 21, 1985 (SDS Biotech Corp.).

NOTE: Report prepared by the sponsor's representative, SDS Biotech Corp., is a summary of the laboratory report prepared by C.E.R.T.I. The following review focuses on the reported data furnished by C.E.R.T.I.

CONCLUSIONS - Executive Summary: Groups of seven male and seven female Swiss mice received single oral gavage administrations of 2,5,6-trichloro-3-carboxybenzamide suspensions to yield doses of 500, 2500, and 5000 mg/kg. Bone marrow cells were harvested 24, 48, and 72 hours postexposure. Owing to poor staining of cells collected at the 24-hour harvest interval, this phase of the study was repeated with the high dose. Results from the first trial indicated that the test material was not toxic to the animals and failed to induce a cytotoxic response in the target organ (i.e., bone marrow cells). Significant increases in the frequency of micronucleated polychromatic erythrocytes (MPEs) were seen in the low-dose males 24 and 48 hours postexposure and in high-dose females 48 hours postexposure. These increases were, however, not dose-related and fell within the normal spontaneous MPE frequency for mice. In the repeat trial with 5000 mg/kg, no cytotoxic or clastogenic effects were observed in either sex at the 24-hour cell harvest. Although the findings were negative, the missing information listed below renders the study unacceptable:

1. Since dosage of all animals was based on individual body weights, such data should have been included in the study report.

2. The information provided on animal maintenance and environmental conditions was insufficient.

3. No analytical data were provided to verify actual concentrations used in the assay.

The study, therefore, does not fully satisfy Guideline requirements for genotoxic effects Category II, Structural Chromosomal Aberrations.

Study Classification: The study is currently unacceptable but can be upgraded if the missing information, listed above, is furnished by the study authors.

A. MATERIALS:

1. Test Material:

Name:	2,5,6-Trichloro-2-carboxybenzamide.
Description:	Beige, crystalline powder (aggregates).
Identification Number:	T-165-2.
Purity:	≥92.3%.
Contaminants:	Not listed.
Solvent used:	0.5% Aqueous methocel.

Other comments: The test material was stored at room temperature, protected from light. No information was provided on test material stability. Analytical determinations of the target concentrations used in the study were not performed. The test material was crushed with a mortar and suspended in 0.5% aqueous methocel at the time of use.

2. Control Materials:

Negative/Route of administration: None.

Vehicle/Final concentration/Route of administration: 0.5% aqueous methocel at a dosing volume of 0.25 mL/10 g body weight was administered by oral gavage.

Positive/Final concentration/Route of administration: Urethane was dissolved in distilled water and administered by oral gavage at 1000 mg/kg.

3. Test Compound:

Route of administration: Oral gavage.

Dose levels used:

a. Preliminary toxicity study: 20,000 mg/kg (four males and three females; dosing volume = 0.5 mL/10 g body weight) and 10,000 mg/kg (five males and five females; dosing volume = 0.25 mL/10 g body weight).

b. Micronucleus assay:

1) Trial 1: 500, 2500, and 5000 mg/kg.

2) Trial 2: 5000 mg/kg.

Note: The report did not indicate whether animals were weighed prior to dosing; individual body weight data were not provided.

4. Test Animals:

a. Species: Mouse; Strain: Swiss; Age: Not reported; Weight Range 25 to 30 g (not specified by sex). Source: Centre d' Elevage R. Janvier, Le Genest, France.

b. No. animals used per dose: Seven males and seven females/group/sacrifice time.

c. Properly maintained? Insufficient information provided. The study authors did not describe the environmental conditions of animal housing or indicate that animals were randomized.

B. TEST PERFORMANCE:

1. Treatment and Sampling Times:

a. Test compound:
Dosing: __x__ once ____ twice (24 hr apart
__N/A__ other describe: _____
Sampling (after last dose): _____ 6 hr _____ 12 hr
__x__ 24 hr __x__ 48 hr __x__ 72 hr.

b. Vehicle control:
Dosing: __x__ once _____ (24 hr apart)
__N/A__ other describe: _____
Sampling (after last dose): _x_ 24 hr _x_ 48 hr _x_ 72 hr

c. Positive control:
Dosing: __x__ once _____ twice (24 hr apart)
__N/A__ other describe: _____
Sampling (after last dose): __x__ 24 hr

2. Tissues and Cells Examined:

__x__ bone marrow __N/A__ other list:
No. of polychromatic erythrocytes (PCEs) examined per animal: __2000__.
No. of normochromatic erythrocytes (PCEs, more mature RBCs): Determined while scoring 1000 erythrocytes per animal.

3. Details of Slide Preparation: At 24, 48, and 72 hours after administration of the test material or negative control and 24 hours following administration of the positive control, the appropriate groups of animals were sacrificed by cervical dislocation. Bone marrow cells were removed from one femur/animal and suspended in fetal calf serum. Cell suspensions were centrifuged, spread onto slides, and air dried. Slides were stained in May-Gruenwald and Giemsa solutions and coded.

4. Statistical Methods: The data were evaluated for statistical significance by the Student's T-test and Mann-Whitney U-test.

5. Evaluation Criteria: No criteria to evaluate assay validity, a positive response, or the biological significance of the findings were presented.

C. Reported Results:

1. Preliminary Toxicity Study: The report stated that two groups of mice received single oral doses of 10,000 mg/kg (five males and five females) and 20,000 mg/kg of the test material (four males and three females). Animals were observed for 72 hours. No deaths or other signs of compound toxicity occurred in either group; however, diarrhea, which our reviewers assume was caused by the increased dosing volume (0.5 mL/10 g body weight), was reported in the high-dose animals. No primary data were provided to support the animal observation information. The study authors further stated that because of the inability to maintain a homogeneous suspension at 400 mg/mL, a suspension containing 200 mg/mL of the test material was prepared, constantly mixed, and used to achieve the high dose (5000 mg/kg) selected for the micronucleus assay.

2. Micronucleus Assay:

a. Animal observations: One female administered the high dose (5000 mg/kg) died approximately 7 hours posttreatment. Two males in the mid-dose group died within 48 hours of dosing, and one female in the mid-dose group was found dead after 2 days. Necropsies were performed on all animals except one of the mid-dose males; this animal was cannibalized. The macroscopic evaluation of dead animals revealed no compound-related effects.

b. Micronucleus assay:

1) Trial 1: Groups of seven male and seven female mice were administered single oral gavage doses of 500, 2500, and 5000 mg/kg of the test material and were sacrificed at 24, 48, and 72 hours posttreatment. The study authors stated that cells harvested at the 24-hour sacrifice interval stained poorly because of technical problems. Slides from the 24-hour harvest were scored, nevertheless, and results were presented. As shown in Table 1, significantly

increased frequencies of MPEs were seen in the low-dose males at 24 hours ($p < 0.02$) and 48 hours ($p < 0.01$) postexposure. MPEs were also significantly increased in the high-dose females ($p < 0.01$) at the 48-hour cell harvest. The study authors disputed the biological significance of these increases because they were observed only in the low-dose males and only at one harvest interval in the females. Our reviewers tend to agree with this assessment, and further note that no dose-related effects were seen in either sex. Additionally, all significant increases were well within the reported spontaneous MPE frequency for mice (1 to 3%)[1]. However, using this background range, the significant increases seen with the positive control would, therefore, be considered unacceptable evidence of assay sensitivity.

The test material was also not cytotoxic to the target organ. In general, the ratio of PCEs to NCEs did not suggest adverse effects on hematopoiesis; however, there was a significant decrease ($p < 0.05$) in PCEs:NCEs in the high-dose females at the 72-hour harvest.

2) Trial 2: As previously stated, the assay was repeated because the cells harvested 24 hours postexposure were poorly stained. Only the high-dose of the test material was assayed in parallel with the vehicle and positive controls. All groups, consisting of seven males and seven females, were sacrificed 24 hours posttreatment. The report stated that two of the seven females receiving 5000 mg/kg of the test material died at ~2 and 7 hours postdosing; no compound-related toxic effects were uncovered at necropsy. Results from the second trial, presented in Table 2, indicate that the high dose did not induce significant increases in the frequency of MPEs in cells harvested from either

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[1]Heddle, J.A.; Hite, M.; Kirkhart, B.; Mavournin, K.; MacGregor, J.T.; Newell, G.W.; and Salamone, M.F. The induction of micronuclei as a measure of genotoxicity. A report of the U.S. Environmental Protection Agency Gene-Tox Program. Mutat. Res. 123(1983): 61-118.

TABLE 1. Results of the Micronucleus Assay in Mice with 2,5,6-Trichloro-3-Carboxybenzamide: Trial 1

Substance	Dose (mg/kg)	Exposure Time[a] (hours)	Sex	No. of Animals Analyzed per Group	No. of PCEs Analyzed per Group	Percent MPEs per Group ± S.D.	Group PCE:NCE ± S.D.
Vehicle Control							
0.5% Methocel	--	24	M	7	14,000	0.16 ± 0.06	2.04 ± 0.09
	--		F	7	14,000	0.23 ± 0.05	2.13 ± 0.29
	--	48	M	F	14,000	0.16 ± 0.04	2.02 ± 0.21
	--		F	7	14,000	0.14 ± 0.03	1.63 ± 0.32
	--	72	M	7	14,000	0.15 ± 0.07	2.22 ± 0.13
	--		F	7	14,000	0.20 ± 0.06	2.14 ± 0.20
Positive Control							
Urethane	1000	24	M	7	14,000	1.24 ± 0.42***	1.86 ± 0.34
			F	7	14,000	0.86 ± 0.20***	1.72 ± 0.25
Test Material							
2,5,6-Trichloro-3-carboxy-benzamide	500	24	M	7	14,000	0.29 ± 0.07**	2.00 ± 0.22
			F	7	14,000	0.17 ± 0.07	2.01 ± 0.28
		48	M	7	14,000	0.27 ± 0.05***	2.10 ± 0.18
			F	7	14,000	0.17 ± 0.06	1.99 ± 0.17
		72	M	7	14,000	0.16 ± 0.04	2.17 ± 0.24
			F	7	14,000	0.24 ± 0.07	1.89 ± 0.54
	2500	24	M	7	14,000	0.24 ± 0.06	1.95 ± 0.28
			F	7	14,000	0.20 ± 0.09	1.89 ± 0.23
		48	M	7	14,000	0.16 ± 0.03	2.02 ± 0.39
			F	7	14,000	0.14 ± 0.04	1.80 ± 0.30
		72	M	5[b]	10,000	0.14 ± 0.04	2.14 ± 0.22
			F	6[b]	12,000	0.23 ± 0.05	1.94 ± 0.23
	5000	24	M	F	14,000	0.22 ± 0.05	2.15 ± 0.20
			F	7	14,000	0.15 ± 0.07	1.92 ± 0.38
		48	M	7	14,000	0.16 ± 0.05	1.75 ± 0.34
			F	7	14,000	0.24 ± 0.05***	1.49 ± 0.43
		72	M	7	14,000	0.21 ± 0.06	1.99 ± 0.21
			F	6[b]	12,000	0.16 ± 0.06	1.72 ± 0.26*

[a]Time after compound administration.
[b]Deaths not related to compound exposure occurred in these groups.
Abbreviations used:
PCE--Polychromatic erythrocyte
MPE--Micronucleated polychromatic erythrocyte
NCE--Normochromatic erythrocyte
*Significantly lower than the corresponding vehicle control ($p < 0.05$) by Student's T or Mann-Whitney U test.
**Significantly higher than the corresponding vehicle control ($p < 0.02$) by Student's T or Mann-Whitney U test.
***Significantly higher than the corresponding vehicle control ($p < 0.01$) by Student's T or Mann-Whitney U test.

TABLE 2. Results of the Micronucleus Assay in Mice with 2,5,6-Trichloro-3-Carboxybenzamide: Trial 2

Substance	Dose (mg/kg)	Exposure Time[a] (hours)	Sex	No. of Animals Analyzed per Group	No. of PCEs Analyzed per Group	Percent MPEs per Group ± S.D.	Group PCE:NCE ± S.D.
Vehicle Control							
0.5% Methocel	--	24	M	7	14,000	0.16 ± 0.03	1.88 ± 0.28
	--	24	F	7	14,000	0.14 ± 0.06	2.20 ± 0.15
Positive Control							
Urethane	1000	24	M	7	14,000	6.96 ± 0.87*	1.69 ± 0.22
			F	7	14,000	6.95 ± 0.92*	1.47 ± 0.26**
Test Material							
2,5,6-Trichloro-3-carboxybenzamide	5000	24	M	7	14,000	0.14 ± 0.07	2.00 ± 0.12
			F	5[b]	10,000	0.21 ± 0.08	2.06 ± 0.29

[a]Time after compound administration.
[b]Deaths not related to compound exposure occurred in this group.
Abbreviations used:
PCE--Polychromatic erythrocytes
MPE--Micronucleated polychromatic erythrocytes
NCE--Normochromatic erythrocytes
*Significantly higher than the corresponding vehicle control ($p < 0.01$) by Student's T or Mann-Whitney U test.
**Significantly lower than the corresponding vehicle control ($p < 0.001$) by Student's T or Mann-Whitney U test.

male or female mice 24 hours postexposure to 5000 mg/kg of the test material. Similarly, 2,5,6-trichloro-3-carboxybenzamide had no adverse effects on hematopoiesis. By contrast, MPEs were markedly increased at 24 hours in male and female mice receiving the positive control (1000 mg/kg urethane); the increases for both sexes were significant ($p < 0.01$). Based on the overall findings, the study author concluded that the test material was negative in the mouse micronucleus assay.

D. REVIEWERS' COMMENTS AND INTERPRETATION OF STUDY RESULTS:

We assess that 2,5,6-trichloro-3-carboxybenzamide, assayed to 5000 µg/kg, was not toxic to the test animals, and failed to induce a cytotoxic or clastogenic response in the target organ (i.e., bone marrow cells). We further assess that the significantly increased MPE frequencies seen in the first trial were probably artifactual and resulted from the low values scored for the vehicle control animals. Although there was no evidence of clastogenesis, the study does not fully comply with Guideline requirements for the following reasons:

1. Since animals were dosed on individual body weights, these data should have been included in the study report.

2. Insufficient information was reported on animal maintenance and environmental conditions.

3. Analytical determinations of the target concentrations were not reported. Since the test material was administered as a suspension, this information is necessary to determine the accuracy of dose preparation.

E. QUALITY ASSURANCE MEASURES: A quality assurance statement from the performing laboratory was signed but not dated.

F. CBI APPENDIX: Appendix A, Materials and Methods (SDS Biotech Corp.) CBI pp. 13-15; Appendix 3, Protocol (C.E.R.T.I.) CBI pp. 27-38; Appendix C, Materials and Methods (C.E.R.T.I., English Translation) CBI pp. 47-53.

APPENDIX A

Materials and Methods
(SDS Biotech Corp.) Methods
CBI pp. 13-15

Chlorothalonil

Page_____ is not included in this copy.

Pages 108 through 132 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.

Guideline Series 84: Mutagenicity
EPA No.: 68D80056
DYNAMAC No.: 333-B
TASK No.: 3-33B
December 18, 1990

008334

DATA EVALUATION RECORD

3-CARBOXY-2,5,6-TRICHLOROBENZAMIDE

Mutagenicity--Unscheduled DNA Synthesis Assay in Primary Rat Hepatocytes

REVIEWED BY:

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Date: 1/7/91

UDS

DATA EVALUATION RECORD

Tox. Chem. No.:
EPA File Symbol:

CHEMICAL: 3-Carboxy-2,5,6-trichlorobenzamide.

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

MRID NUMBER: 415648-15.

SYNONYM/CAS NUMBER: T-165-2; SDS-46851.

SPONSOR: Fermenta ASC Corp., Mentor, OH.

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

TITLE OF REPORT: Rat Hepatocyte Primary Culture/DNA Repair Test PH 311-SDS-002-84.

AUTHORS: Barfknecht, T. R., Naismith, R. W., and Matthews, R. J. (Pharmakon Research International, Inc.); Jones, R. E. and Killeen, J. C. (SDS Biotech Corp.).

STUDY NUMBER: PH 311-SDS-002-84.

REPORT ISSUED: December 6, 1984 (Pharmakon Research International, Inc.); July 8, 1985 (SDS Biotech Corp.).

NOTE: Report prepared by the sponsor's representative, SDS Biotech Corp., is a summary of the laboratory report prepared by Pharmakon Research International, Inc. The following review focuses on the reported data furnished by Pharmakon Research International, Inc.

CONCLUSION(S) - Executive Summary: Under the conditions of the primary rat hepatocyte culture unscheduled DNA synthesis (UDS) assay, five doses of 3-carboxy-2,5,6-trichlorobenzamide (2.4 to 240 µg/well) did not induce a cytotoxic or genotoxic effect. The highest assay level was reported to be the limit of test material solubility. We assess that an adequate range of test material concentrations was evaluated and there was no indication of a positive response in a well-conducted study. We conclude, therefore, that the study fulfills Guideline requirements for Category III, Other Mutagenic Mechanisms.

Study Classification: The study is acceptable.

A. MATERIALS:

1. Test Material:

Name:	3-Carboxy-2,5,6-trichlorobenzamide
Description:	White powder
Identification No.:	T-165-2
Purity:	99.4%
Contaminants:	Not listed
Solvent used:	95% ethanol (ETOH)
Other comments:	The test material was stored at room temperature protected from light; dosing solutions were prepared on the day of use. The report indicated that the limit of solubility of the test material in culture medium (Williams Medium E containing 10% calf serum) was 240 µg/well.

2. Indicator Cells: Primary rat hepatocytes were obtained by the in situ perfusion of the liver of an adult male Fischer 344 rat weighing 207 g and purchased from Charles River Breeding Laboratories.

3. Control Substances: ETOH at a final concentration of 1% was used as the solvent control, and 2-acetylaminofluorene (2-AAF) at a final concentration of 1 x 10^{-4} M was used as the positive control.

4. Medium: WME: Williams' Medium E; WMES: WME with 10% fetal calf serum.

B. STUDY DESIGN:

1. Cell Preparation:

a. Perfusion technique: The liver was perfused with Hank's balanced salts containing 0.5 mM EGTA and HEPES buffer, pH 7.35, for 4 minutes and with WME containing

100 units/mL collagenase for 10 minutes. The liver was excised, trimmed of excess tissue, placed in a culture dish containing WME and collagenase, and combed with a camelhair brush to release the hepatocytes.

b. Hepatocyte harvest/culture preparation: Recovered cells were allowed to settle for 10 minutes, and cell viability was determined by trypan blue exclusion. Approximately 1 x 10^5 viable cells were inoculated onto plastic coverslips in microwell culture dishes containing WMES and allowed to attach for ≈2 hours in a humidified, 37°C, 5% CO_2 incubator; cultures were rinsed and refed with WME containing 10 µCi/mL [^{3}H]thymidine.

2. UDS Assay:

a. Treatment/slide preparation: Triplicate monolayer cultures were exposed to the selected doses of the test material, negative control (medium) solvent control (ETOH), or positive control (2-AAF) for 18 to 20 hours. Treated monolayers were washed three times and cells, attached to coverslips, were exposed to 1% sodium citrate for 10 to 15 minutes, fixed in acetic acid:ethanol (1:3), dried, and mounted.

b. Preparation of autoradiographs/grain development: Slides were coated with Kodak NTB-2 emulsion, dried for 7 days at 4°C in light-tight desiccated boxes, developed in Kodak D-19, fixed, stained with Harris alum hematoxylin and eosin, coded, and counted.

c. Grain counting: The nuclear grains of 60 cells (20/coverslip) for each test dose and negative, solvent, and positive controls were counted microscopically. Net nuclear grain counts were determined by subtracting the average cytoplasmic grain count of three nuclear-sized areas adjacent to each nucleus from the nuclear grain count of each cell.

3. Evaluation Criteria:

a. Assay validity: For the assay to be considered valid, the following criteria must be satisfied:

(1) the negative or solvent control should have net nuclear grain counts of ≤1 and fall within the 95% confidence limits of the presented mean historical data, and

(2) the positive control should yield a mean net nuclear count that is within one standard deviation of the mean historical value.

b. Positive response: The assay was considered positive if a minimum net grain count of 5 grains/nucleus was consistently observed in triplicate cultures and the increase was accompanied by a dose response.

NOTE: A complete copy of all primary data generated in this study was provided.

C. REPORTED RESULTS:

Five doses of the test material (2.4, 8.0, 24.0, 80.0, and 240.0 µg/well) were evaluated in the UDS assay. The study authors stated that the high dose was the limit of test material solubility. None of the treatment levels produced a cytotoxic effect or increased the frequency of UDS in the treated hepatocytes. By contrast, the positive control (1×10^{-7} M 2AAF) induced a powerful UDS response. Representative results are presented in Table 1.

Based on these results, the study author concluded that 3-carboxy-2,5,6-trichlorobenzamide was negative in the rat hepatocyte UDS assay.

D. REVIEWER'S DISCUSSION/INTERPRETATION OF STUDY RESULTS:

We assess that the study was properly conducted and that the study authors interpreted the data correctly. 3-Carboxy-2,5,6-trichlorobenzamide was assayed to the limit of solubility and failed to induce either a cytotoxic or genotoxic response in a well-controlled study. In addition, the findings with the positive control (1×10^{-7} M 2AAF) demonstrated that the test system was adequately sensitive to detect UDS.

E. QUALITY ASSURANCE MEASURES: A quality assurance statement from the performing laboratory was signed and dated November 8, 1989.

F. CBI APPENDIX: Appendix A, Materials and Methods (SDS Biotech Corp.) CBI pp. 13-14; Appendix B, Protocol (Pharmakon Research International, Inc.) CBI pp. 17-40; Appendix C, Materials and Methods (Pharmakon Research International, Inc.) CBI pp. 47-50.

TABLE 1. Representative Results of the Unscheduled DNA Synthesis Rat Hepatocyte Assay with 3-Carboxy-2,5,6-Trichlorobenzamide

Treatment	Dose/Well	Cells Scored	Mean Net Nuclear Grain Count ± SD[a]
Negative Control			
(Culture medium)	--	60	0.2 ± 0.4
Solvent Control			
95% Ethanol	1%	60	0.3 ± 0.3
Positive Control			
2-Acetylamino-fluorene	1×10^{-7} M	60	29.9 ± 2.1[b]
Test Material			
3-Carboxy-2,5,6-trichlorobenzamide	240.0 µg[c]	60	0.4 ± 0.4

[a]Mean value for triplicate coverslips ± standard deviations.

[b]Fulfills reporting laboratory's criteria for a positive effect (i.e., ≥5 mean net nuclear grains over the solvent control and ≥1 standard deviation of the historical positive control value).

[c]Highest assayed dose; reported to be the limit of test material solubility. Results for lower concentrations (2.4, 8.0, 24.0, and 80.0 µg/well) did not suggest a genotoxic effect.

APPENDIX A

Materials and Methods
(SDS Biotech Corp.)
CBI pp. 13-14

Chlorothalonil

Page_____ is not included in this copy.

Pages 140 through 171 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.

EPA No.: 68D80056
DYNAMAC No.: 333-C
TASK No.: 3-33C
December 18, 1990

008334

DATA EVALUATION RECORD

3-CARBOXY-2,5,6-TRICHLOROBENZAMIDE

Mutagenicity--Mammalian Cells in Culture Gene Mutation Assay in Mouse Lymphoma Cells

APPROVED BY:

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

Signature: Tom McLellan for

Date: 12-18-90

Guideline Series 84: **MUTAGENICITY**

EPA No.: 68D80056
DYNAMAC No.: 333-C
TASK No.: 3-33C
December 18, 1990

908334

DATA EVALUATION RECORD

3-CARBOXY-2,5,6-TRICHLOROBENZAMIDE

Mutagenicity--Mammalian Cells in Culture Gene Mutation Assay in Mouse Lymphoma Cells

REVIEWED BY:

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

Signature: Nancy E. McCarroll
Date: 12-18-90

I. Cecil Felkner, Ph.D.
Independent Reviewer
Dynamac Corporation

Signature: W. McLellan
Date: 12-18-90

APPROVED BY:

Nicolas P. Hajjar, Ph.D.
Department Manager
Dynamac Corporation

Signature: W. McLellan for
Date: 12-18-90

Elizabeth Doyle, Ph.D.
EPA Reviewer, Section IV
Toxicology Branch II
(H-7509C)

Signature: E. A. Doyle
Date: 1/2/91

Marcia Van Gemert, Ph.D.
Branch Chief
Toxicology Branch II
(H-7509C)

Signature: Marcia Van Gemert
Date: 1/4/91

MAMMALIAN CELLS IN CULTURE GENE MUTATION

008334

DATA EVALUATION RECORD

Tox. Chem. No.:

EPA File Symbol:

CHEMICAL: 3-Carboxy-2,5,6-trichlorobenzamide.

STUDY TYPE: Mutagenicity--Mammalian cells in culture gene mutation assay in mouse lymphoma cells.

MRID NUMBER: 415648-14.

SYNONYM/CAS NUMBER: T-165-2; SDS-46851.

SPONSOR: Fermenta Plant Protection Co., Mentor, OH.

TESTING FACILITY: Microbiological Associates, Inc., Bethesda, MD.

TITLE OF REPORT: L5178Y $TK^{+/-}$ Mouse Lymphoma Forward Mutation Assay with 3-Carboxy-2,5,6-Trichlorobenzamide (SDS-46851).

AUTHORS: Mizens, M. and Killeen, J. C. (Ricerca, Inc.); Rodgers-Back, A. (Microbiological Associates, Inc.).

STUDY NUMBER: T5288.701.

REPORT ISSUED: March 10, 1987 Microbiological Associates, Inc.); February 4, 1988 (Ricerca, Inc.).

NOTE: Report prepared by the sponsor's representative, Ricerca, Inc., is a summary of the laboratory report prepared by Microbiological Associates, Inc. The following review focuses on the reported data furnished by Microbiological Associates, Inc.

CONCLUSIONS - Executive Summary: 3-Carboxy-2,5,6-trichlorobenzamide was evaluated in two independently performed mouse lymphoma forward mutation assays. Over a nonactivated and S9-activated concentration range of 75 to 1000 µg/mL, the test material did not induce a significant increase in the mutation frequency (MF) in either the initial or confirmatory assay. Although the high dose both with and without S9 activation was only marginally cytotoxic (≈63% relative survival), higher concentrations could not be evaluated because of the excessive osmotic pressure of stock solutions ≥1.5 mg/mL.

Based on the overall results, we conclude that 3-carboxy-2,5,6-trichlorobenzamide was adequately tested over an appropriate concentration range and did not induce a mutagenic response in this test system. The study, therefore, fulfills Guideline requirements for Category I, Gene Mutations.

Study Classification: This study is acceptable.

MAMMALIAN CELLS IN CULTURE GENE MUTATION

A. MATERIALS:

1. Test Material:

Name:	3-Carboxy-2,5,6-trichlorobenzamide
Description:	White powder
Identification No.:	T-165-2
Purity:	≥98%
Contaminants:	Not listed
Solvent used:	Dimethylsulfoxide (DMSO)
Other comments:	The test material was stored at room temperature protected from light. Stability studies conducted by Ricerca, Inc., indicated that the test material was stable in DMSO at 1000 µg/mL for at least 24 hours.

2. Control Materials:
Negative: None.

Solvent/final concentration: DMSO/not listed but reported to be a concentration that was not cytotoxic to the cells.

Positive: Nonactivation: (concentrations, solvent): Ethyl methanesulfonate (EMS) was prepared in DMSO to yield final concentrations of 0.5 and 1.0 µL/mL.

Activation (concentrations, solvent): 7,12-dimethylbenz(a)anthracene (DMBA) was prepared in acetone to yield final concentrations of 5.0 and 7.5 µg/mL.

3. Activation: S9 derived from

x Aroclor 1254	x induced	x rat	x liver
_____ phenobarbital	_____ noninduced	_____ mouse	_____ lung
_____ none		_____ hamster	_____ other
_____ other		_____ other	

If other, describe below. Describe S9 composition (if purchased, give details).

The S9 fraction was prepared by the performing laboratory and characterized for its ability to metabolize selected promutagens to mutagenic forms prior to use. The S9 mix contained the following components/mL:

NADP	6.0 mg
Isocitric acid	11.25 mg
Culture medium (F_0P)	0.75 mL
S9 homogenate	0.25 mL

MAMMALIAN CELLS IN CULTURE GENE MUTATION

4. Test Cells: mammalian cells in culture

__x__ mouse lymphoma L5178Y cells
____ Chinese hamster ovary (CHO) cells
____ V79 cells (Chinese hamster lung fibroblasts)
____ other (list):

Properly maintained? Yes.
Periodically checked for mycoplasma contamination? Not reported.
Periodically checked for karyotype stability? Not reported.
Periodically "cleansed" against high spontaneous background? Yes.

5. Locus Examined:

__x__ thymidine kinase (TK)
Selection agent: __3 µg/mL__ trifluorothymidine (TFT)
(give concentration) _____ fluorodeoxyuridine (FdU)

____ hypoxanthine-guanine-phosphoribosyl transferase (HPRT)
Selection agent: _____ 8-azaguanine (8-AG)
(give concentration) _____ 6-thioguanine (6-TG)

____ Na^+/K^+ATPase
Selection agent: _____ ouabain
(give concentration)

____ other (locus and/or selection agent; give details)

6. Test Compound Concentrations Used:

a. Preliminary cytotoxicity assay: Seven concentrations (0.01, 0.1, 1, 10, 100, 1,000, 10,000 µg/mL) were assayed with and without S9 activation.

b. Mutation assay:

1) Initial assay: Sixteen doses (13 to 1000 µg/mL) were tested with and without S9 activation. Ten doses (75, 100, 133, 178, 237, 316, 422, 563, 750, and 1000 µg/mL +/-S9) were selected for cloning in the initial mutation assay.

2) Confirmatory assay: As above for the initial mutation assay.

B. TEST PERFORMANCE:

1. Cell Treatments:

a. Cells exposed to test compound for:
4 hours (nonactivated) 4 hours (activated)

b. Cells exposed to positive controls for:
4 hours (nonactivated) 4 hours (activated)

c. Cells exposed to negative and/or solvent controls for:
4 hours (nonactivated) 4 hours (activated)

d. After washing, cells cultured for 2 days (expression period) before cell selection

e. After expression, cells cultured for 10-12 days in selection medium to determine numbers of mutants and for 10-12 days without selection medium to determine cloning efficiency

2. Preliminary Cytotoxicity Assay: Cells (1×10^6 cells/mL) were exposed to seven test material doses (0.01 to 1000 µg/mL) and to the solvent (DMSO) for 4 hours both in the presence and absence of S9 activation. Following exposure, cells were washed, refed fresh medium, and reincubated. Cell viability was determined 24 and 48 hours posttreatment. The percent relative suspension growth (RSG) was determined and used to establish a dose range for the mutation assay.

3. Mutation Assay: Cells seeded at 1×10^5 cells/mL were exposed to the 16 selected test material doses, solvent, or positive controls with or without S9 activation for 4 hours. Cells were washed, resuspended in growth medium, and reincubated.

Daily cell counts were performed at 24 and 48 hours, and cells were diluted when appropriate to maintain an optimal growth rate. At the end of the expression period, 10 doses were chosen for mutant selection.

For mutant selection, 1×10^6 cells were seeded into triplicate selection medium plates. The cloning efficiency (CE) was determined by plating 200 cells/plate (in triplicate) in cloning medium. After 10 to 12 days of incubation, TFT-resistant colonies and the total number of viable cells were counted; cloning efficiencies (CE),

relative percent total growth (TG), and mutation frequencies (MFs) were calculated.

4. Statistical Analysis: The data were evaluated for statistical significance at $p \leq 0.05$ using the Kastenbaum and Bowman tables.

5. Evaluation Criteria:

   a. Assay acceptability: For the assay to be considered acceptable, the following criteria must be satisfied: (1) the CE of the solvent control should be >50%; (2) the background MF of the solvent control should range from 0.2 to 1.0 mutants/10^4 survivors; and the MF for the positive control must be at least 2-fold higher than the appropriate solvent control cultures.

   b. Positive response: The test material was considered positive if there was a dose-response and one or more of the doses in the ≥10% TG range exhibited a ≥2-fold increase in the MF compared to the solvent control.

C. REPORTED RESULTS:

1. Preliminary Cytotoxicity Assay: The preliminary cytotoxicity assay was conducted with seven doses ranging from 0.01 to 10,000 µg/mL in the absence and presence of S9 activation. The study authors stated that the pH of the highest test solution was acidic and was, therefore, neutralized with 1N NaOH.

   No cells survived exposure to 10,000 µg/mL in the nonactivated assay. RSG at all lower nonactivated levels was ≥99%. In the presence of S9 activation, RSG at 10,000 µg/mL was 33% and for the remaining doses was ≥92%. The investigators also performed osmolality tests and found that the osmolality of 1 mg/mL of the test material was 452 mOsm/kg; at ≥1.5 mg/mL, the osmolality was ≥610 mOsm/kg. Based on the high osmotic pressure at concentrations ≥1.5 mg/mL, the dose range selected for further investigation ranged from 13 to 1000 µg/mL.

2. Mutation Assay: Two independent mutation assays were performed; the results were as follows:

   a. Initial mutation assay: The RSG for cells exposed to the high dose (1000 µg/mL) both with and without S9 activation was 63%. Accordingly, cells treated with

concentrations ranging from 75 to 1000 µg/mL +/-S9 were chosen for mutant selection. Under nonactivated and S9-activated conditions, the total mutant colonies and the MFs for test groups were not significantly higher than the solvent control values.

b. Confirmatory mutation assay: Results from t. confirmatory assay with comparable doses of 3-carboxy-2,5,6-trichlorobenzamide indicated that the high dose (1000 µg/mL +/-S9) was less cytotoxic; however, there was no evidence of a mutagenic response at any nonactivated or S9-activated level of the test material.

In both assays, the nonactivated (0.5 and 1.0 µg/mL EMS) and the S9-activated (5 and 7.5 µg/mL DMBA) positive controls induced dose-related mutagenic effects. Representative results from the two assays are presented in Table 1.

Based on the results, the study authors concluded that 3-carboxy-2,5,6-trichlorobenzamide was not mutagenic in this mammalian cell assay.

D. REVIEWERS' DISCUSSION AND INTERPRETATION OF RESULTS:

We assess that the study was properly conducted and that the study authors' interpretation of the data was correct. In both the absence and presence of S9 activation, 3-carboxy-2,5,6-trichlorobenzamide was assayed over an appropriate concentration range and failed to induce a mutagenic response in two independently performed assays. We further assess, in agreement with the study authors, that the osmotic pressure at stock concentrations ≥1.5 mg/mL precluded the testing of higher levels.

In contrast to the uniformly negative results with the test material, findings with the nonactivated (EMS at 0.5 and 1.0 µg/mL) and the S9-activated (DMBA at 5 and 7.5 µg/mL) positive controls demonstrated that the test system was adequately sensitive for the detection of mutagenesis.

We conclude, therefore, that 3-carboxy-2,5,6-trichlorobenzamide was found to be nonmutagenic in a well-controlled mammalian cell gene mutation assay.

MAMMALIAN CELLS IN CULTURE GENE MUTATION

TABLE 1. Representative Results from the Mouse Lymphoma Forward Mutation Assays with 3-Carboxy-2,5,6-Trichlorobenzamide

Substance	Dose/mL	S9 Activation	Relative Percent Suspension Growth[a]	Average Mutant Colonies[b]	Average Viable Colonies[b]	Relative Percent Total Growth	Mutation Frequency x 10^{-6} Survivors[c]
Solvent Control							
Dimethylsulfoxide	-	-[d]	100	31	112	--	0.60
	-	-[e]	100	51	175	--	0.60
	-	+[d]	100	33	102	--	0.65
	-	+[e]	100	61	152	--	0.80
Positive Control[f]							
Ethylmethane-sulfonate	0.5 µg	-[d]	60	139	29	17	13.0
	0.5 µg	-[e]	57	280	70	20	8.0
7,12-Dimethyl-benz(a)anthracene	5.0 µg	+[d]	71	112	64	51	3.5
	5.0 µg	+[e]	56	231	125	43	3.7
Test Material							
3-Carboxy-2,5,6-trichlorobenzamide	1000 µg[g]	-[d]	63	33	122	65	0.53
	1000 µg[g]	-[e]	83	55	186	89	0.60
	1000 µg[g]	+[d]	63	32	105	65	0.60
	1000 µg[g]	+[e]	121	65	189	151	0.70

[a]Relative Percent Suspension Growth = $\frac{\text{Suspension Growth (test group)}}{\text{Suspension Growth (solvent control)}} \times 100$.

[b]Average count of at least three replicates per group.

[c]Mutation Frequency (MF) = $\frac{\text{Average Mutant Colonies}}{\text{Average Viable Colonies}} \times 2$.

[d]Results from the first assay.

[e]Results from the confirmatory assay.

[f]Two levels of each positive control were assayed; the lowest dose for each positive control was selected as representative.

[g]Results for lower concentrations (75, 100, 133, 178, 237, 316, 422, 563, and 750 µg/mL +/-S9) in both trials did not indicate a mutagenic effect.

NOTE: 7,12-Dimethylbenz(a)anthracene was dissolved in acetone; the MFs for acetone were 0.75 and 0.8 x 10^{-6} survivors in the first and confirmatory assays, respectively.

E. QUALITY ASSURANCE MEASURES: A quality assurance statement from the performing laboratory was signed and dated March 10, 1987.

F. CBI APPENDIX: Appendix A, Materials and Methods (Ricerca, Inc.) CBI pp. 14-16; Appendix B, Protocol (Microbiological Associates, Inc.) CBI pp. 27-53; Appendix C, Materials and Methods (Microbiological Associates, Inc.) CBI pp. 61-70.

APPENDIX A

Materials and Methods
(Ricerca, Inc.)
CBI pp. 14-16

Chlorothalonil

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Pages 184 through 225 are not included.

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____ 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) _____.

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EPA No.: 68D80056
DYNAMAC No.: 333-D
TASK No.: 3-33D
December 18, 1990

008334

DATA EVALUATION RECORD

3-CARBOXY-2,5,6-TRICHLOROBENZAMIDE

Mutagenicity--Mammalian Cells in Culture Gene Mutation Assay in Mouse Lymphoma Cells

APPROVED BY:

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

Date: 12-18-90

226

Guideline Series 84: **MUTAGENICITY**

EPA No.: 68D80056
DYNAMAC No.: 333-D
TASK No.: 3-33D
December 18, 1990

008334

DATA EVALUATION RECORD

3-CARBOXY-2,5,6-TRICHLOROBENZAMIDE

Mutagenicity--Mammalian Cells in Culture Gene Mutation Assay in Mouse Lymphoma Cells

REVIEWED BY:

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

Signature: Nancy E. McCarroll
Date: 12-18-90

I. Cecil Felkner, Ph.D.
Independent Reviewer
Dynamac Corporation

Signature: W. McLellan for
Date: 12-18-90

APPROVED BY:

Nicolas P. Hajjar, Ph.D.
Department Manager
Dynamac Corporation

Signature: William L. McLellan for
Date: 12-18-90

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Signature: E. A. Doyle
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Signature: Marcia Van Gemert
Date: 1/7/91

227

MAMMALIAN CELLS IN CULTURE GENE MUTATION

008334

DATA EVALUATION RECORD

Tox. Chem. No.:

EPA File Symbol:

CHEMICAL: 3-Carboxy-2,5,6-trichlorobenzamide.

STUDY TYPE: Mutagenicity--Mammalian cells in culture gene mutation assay in mouse lymphoma cells.

MRID NUMBER: 415648-13.

SYNONYM/CAS NUMBER: T-165-2; SDS-46851.

SPONSOR: Fermenta ASC Corp., Mentor, OH.

TESTING FACILITY: Hazleton Biotechnologies Corp., Vienna, VA.

TITLE OF REPORT: L5178Y TK+/- Mouse Lymphoma Forward Mutation Assay with and without Activation with 3-Carboxy-2,5,6-Trichlorobenzamide.

AUTHOR: Jones, R. E. and Killeen, J. C. (SDS Biotech Corp.); Sernau, R. C. and Cavagnaro, J. (Hazleton Biotechnologies Corp.).

STUDY NUMBER: 2312-100.

REPORT ISSUED: June 26, 1985 (Hazleton Biotechnologies Corp.); July 25, 1985 (SDS Biotech Corp.).

NOTE: Report prepared by the sponsor's representative, SDS Biotech Corp., is a summary of the laboratory report prepared by Hazleton Biotechnologies Corp. The following review focuses on the reported data furnished by Hazleton Biotechnologies Corp.

MAMMALIAN CELLS IN CULTURE GENE MUTATION

CONCLUSIONS - Executive Summary: 3-Carboxy-2,5,6-trichlorobenzamide was evaluated for the potential to induce forward mutations in the L5178Y mouse lymphoma assay. The dose range assayed with and without S9 activation was 50 to 5000 µg/mL. In both the presence and absence of S9 activation, ≈ ≤10% of the cells survived exposure to 5000 µg/mL; this concentration caused an acidic shift in the pH of the culture medium. Based on the severe cytotoxic response induced at 5000 µg/mL +/-S9, the doses chosen for mutant selection were 500, 1000, 2000, 3000, and 4000 µg/mL. No mutagenic effect was observed under nonactivated conditions. In the presence of S9 activation, increased mutant colony counts and mutation frequencies (MF), compared to the concurrent dimethylsulfoxide-treated cells, were noted at all dose levels. Although the results suggested a dose-related effect, we assess that the finding should be interpreted with caution for the following reasons:

1. The background MF for the DMSO +S9 solvent control group (12 mutants/10^5 survivors) fell outside the accepted spontaneous MF for mouse lymphoma cells (15 to 110 mutants/10^6 survivors).

2. The increased MFs, compared to the concurrent solvent control, for all S9-activated doses of the test material were well within the above-cited normal background frequency. For example, the greatest increase in the MF (55 mutants/10^5 survivors) was calculated for the 1000-µg/mL dose group; this value would, therefore, fall within the midrange of the accepted background frequency.

Although the study authors should have rejected the S9-activated assay because of the low spontaneous MF of the solvent-treated cells, it is likely that repeating the assay with comparable doses and test conditions would have produced similar findings. It is, therefore, reasonable to assume that these investigators would have concluded that 3-carboxy-2,5,6-trichlorobenzamide was mutagenic in this test system. However, the subsequent mouse lymphoma assays conducted by an independent laboratory (see Data Evaluation Record 333C) provided sufficient evidence to support the conclusion that the increased MFs seen in the currently reviewed study presumably were caused by pH and osmolality effects on the culture medium rather than by a true mutagenic response.

Caspary, W. J., Lee, Y. J., Poulton, S., Myhr, B. C., Mitchell, A. D., and Rudd, C. J. Evaluation of the L5178Y mouse lymphoma cell mutagenesis assay: Quality-control guidelines and response categories. Environ. Molec. Mutagen. (1988) 12:19.

MAMMALIAN CELLS IN CULTURE GENE MUTATION

Although this study does not fulfill Guideline requirements for Category I, Gene Mutations, the subsequent study was fully acceptable and negative for gene mutation in this mammalian cell system.

Study Classification: This study is unacceptable; however, see Data Evaluation Record 333C for the acceptable results.

MAMMALIAN CELLS IN CULTURE GENE MUTATION

A. MATERIALS:

1. Test Material:
Name: T-165-2(3-carboxy-2,5,6-trichlorobenzamide)
Description: White powder
Reference No.: 5TX-84-0083
Purity: 99.4%
Contaminants: Not listed
Solvent used: Dimethylsulfoxide (DMSO)
Other comments: The test material was stored at room temperature protected from light. No information on test material stability was presented, and neither the summarized nor the original laboratory reports indicated that analytical determinations of test solutions had been performed. The test material was soluble in DMSO at 500 mg/mL.

2. Control Materials:
Negative: Fischer's medium containing sodium pyruvate, pluronics, and antibiotics.

Solvent/final concentration: DMSO/1%.

Positive: Nonactivation: (concentrations, solvent): Ethylmethanesulfonate (EMS) was prepared in Fischer's medium to yield a final concentration of 300 µg/mL.

Activation (concentrations, solvent): 3-Methylcholanthrene (MCA) was prepared in DMSO to yield a final concentration of 4 µg/mL.

3. Activation: S9 derived from

x Aroclor 1254	x induced	x rat	x liver
_____ phenobarbital	_____ noninduced	_____ mouse	_____ lung
_____ none		_____ hamster	_____ other
_____ other		_____ other	

If other, describe below. Describe S9 composition (if purchased, give details).

The S9 fraction was prepared by the performing laboratory and characterized for its ability to metabolize MCA to a mutagenic form in the mouse lymphoma assay prior to use. The S9 mix contained the following components/mL:

NADP	6.0 mg
Isocitric acid	11.25 mg
Fischer's medium (F_0P)	0.75 mL
S9 homogenate	0.25 mL

MAMMALIAN CELLS IN CULTURE GENE MUTATION

4. Test Cells: mammalian cells in culture

__x__ mouse lymphoma L5178Y cells
____ Chinese hamster ovary (CHO) cells
____ V79 cells (Chinese hamster lung fibroblasts)
____ other (list):

Properly maintained? Yes.
Periodically checked for mycoplasma contamination? Not reported.
Periodically checked for karyotype stability? Not reported.
Periodically "cleansed" against high spontaneous background? Yes.

5. Locus Examined:

__x__ thymidine kinase (TK)
Selection agent: __5 µg/mL__ trifluorothymidine (TFT)
(give concentration) _____ fluorodeoxyuridine (FdU)

____ hypoxanthine-guanine-phosphoribosyl transferase (HPRT)
Selection agent: _____ 8-azaguanine (8-AG)
(give concentration) _____ 6-thioguanine (6-TG)

____ Na^+/K^+ATPase
Selection agent: _____ ouabain
(give concentration)

____ other (locus and/or selection agent; give details)

6. Test Compound Concentrations Used.

a. Preliminary cytotoxicity assay: An unspecified number of doses ranging from 5 to 7500 µg/mL were assayed with and without S9 activation.

b. Mutation assay: Eight concentrations (50 to 5000 µg/mL +/-S9) were initially tested; five dose levels (500, 1000, 2000, 3000, and 4000 µg/mL -/-S9) were selected for cloning.

B. TEST PERFORMANCE:

1. Cell Treatments:

a. Cells exposed to test compound for:
__4__ hours (nonactivated) __4__ hours (activated)

b. Cells exposed to positive controls for:
4 hours (nonactivated) 4 hours (activated)

c. Cells exposed to negative and/or solvent controls for:
4 hours (nonactivated) 4 hours (activated)

d. After washing, cells cultured for 2 days (expression period) before cell selection

e. After expression, cells cultured for ≈10 days in selection medium to determine numbers of mutants and for ≈10 days without selection medium to determine cloning efficiency

2. Preliminary Cytotoxicity Assay: Cells (≈0.6 x 10^6 cells/mL) were exposed to test material doses ranging from 5 to 7500 µg/mL, the solvent (DMSO), or the medium control for 4 hours in both the presence and absence of S9 activation. Following exposure, cells were washed, refed fresh medium, and reincubated. Cell viability was determined 24 and 48 hours posttreatment. The percent relative suspension growth (RSG) was determined and used to establish a dose range for the mutation assay.

3. Mutagenicity Assay: Duplicate cultures of prepared cells were exposed to the eight selected test material doses, negative, solvent, or positive controls with or without S9 activation for 4 hours. Cells were washed, resuspended in growth medium, and reincubated.

Daily cell counts were performed at 24 and 48 hours, and cells were diluted when appropriate to maintain an optimal growth rate. At the end of the 2-day expression period, six doses were chosen for mutant selection.

For mutant selection, 1 x 10^5 cells were seeded into triplicate selection medium plates. The cloning efficiency (CE) was determined by plating 200 cells/plate in triplicate) in cloning medium. After ≈10 days of incubation, TFT-resistant colonies and the total number of viable cells were counted; relative percent total growth (TG), and mutation frequencies (MFs) were calculated.

4. Evaluation Criteria:

a. Assay validity: The assay was considered valid if the MFs of the solvent and positive controls were within the acceptable ranges of the performing laboratory's historical control data.

b. Positive response: The test material was considered positive if there was a dose-response and at least two dose levels had MFs that were ≥2-fold higher than the solvent control. MFs were not calculated for dose levels with ≤10% RSG.

C. REPORTED RESULTS:

1. Preliminary Cytotoxicity Assay: The report stated that at the conclusion of the 4-hour exposure of mouse lymphoma cells to 3-carboxy-2,5,6-trichlorobenzamide, an acidic change in the pH of the culture medium containing 5000 and 7500 µg/mL of the test material was observed. The pH of the 5000-µg/mL culture supernatant was found to be 5.9 (-S9) and 6.4 (+S9); for the 7500-µg/mL culture supernatant, the pH was 3.6 (-S9) and 6.0 (+S9). Doses <5000 µg/mL did not appear to alter in the pH of the culture medium. No information on RSG was provided in the laboratory report. The summarized report prepared by the sponsor's representative stated, however, that <10% of the cells survived exposure to 7500 µg/mL (+/-S9) and to 5000 µg/mL (-S9); survival at 5000 µg/mL (+S9) was reported to be 30%. Based on these findings, the high dose selected for the nonactivated and S9-activated assay was 5000 µg/mL.

2. Mutation Assay: There was no indication that the pH of the high-dose solution or the culture medium containing 5000 µg/mL was adjusted to neutrality. RSGs for the 5000-µg/mL dose group were 1.6% (-S9) and 10.8% (+S9). Below this level, the average RSG for the nonactivated doses ranged from ≈53% at 4000 µg/mL to ≈99% at 50 µg/mL. In the presence of S9 activation, the average RSG ranged from ≈38% at 4000 µg/mL to ≈91% at 50 µg/mL. Based on these findings, cultures treated with nonactivated and S9-activated doses of 500, 1000, 2000, 3000, and 4000 µg/mL were cloned. As shown in Table 1, the MF for the highest nonactivated dose was less than the solvent control; the remaining nonactivated doses did not induce a mutagenic response. In the presence of S9 activation, average mutant colony counts and the MFs for all assayed levels were higher

TABLE 1. Representative Results from the Mouse Lymphoma Forward Mutation Assays with T-165-2 (3-Carboxy-2,5,6-Trichlorobenzamide)

Substance	Dose/mL	S9 Activation	Average Relative Percent Suspension Growth[a]	Average Mutant Colonies[a]	Average Viable Colonies[a]	Average Relative Percent Total Growth[a]	Average Mutation Frequency $x10^{-6}$[a,b]	Fold Increase[c]
Negative Control								
Culture medium	--	-	100	18	218	100.0	1.7	--
	--	+	100	28	238	100.0	2.4	--
Solvent Control								
Dimethylsulfoxide	1%	-	100	21	277	100.0	1.5	--
	1%	+	100	25	425	100.0	1.2	--
Positive Control								
Ethylmethane sulfonate	300 µg	-	77.5	198	188	66.8	21.1	12.4[d]
3 Methylcholanthrene	4 µg	+	40.9	173	194	18.6	17.9	14.9
Test Material								
T-165-2	4000 µg[e,f]	-	52.6	16	284	50.3	1.2	0.9
	500 µg	+	87.8	43	306	62.1	2.9	2.4
	1000 µg	+	87.8	44	333	67.0	2.6	2.2
	2000 µg	+	88.1	53	246	51.0	4.4	3.6
	3000 µg	+	61.1	66	242	34.6	5.5	4.6
	4000 µg[e]	+	37.8	66	288	25.7	4.8	4.0

[a]Average count of three cultures for the medium, solvent, and positive controls and duplicate cultures for the test material dose groups; average values were calculated by our reviewers.

[b]Mutation Frequency (MF) = $\frac{\text{Average Mutant Colonies}}{\text{Average Viable Colonies } (0.3 \times 10^{-6})}$.

[c]Fold Increase = $\frac{\text{MF of Test Group}}{\text{MF of Solvent Control}}$.

[d]MF for ethylmethane sulfonate was compared to the nonactivated medium control.

[e]Highest assayed level, 5000 µg/mL, was too cytotoxic to be carried through the mutant selection phase of the study.

[f]Results for lower nonactivated doses (500, 1000, 2000, and 3000 µg/mL) did not suggest a mutagenic effect.

than the solvent control. The study authors calculated increases in the MF over the solvent control that ranged from 2.4-fold at the low dose (500 μg/mL) to 4.0-fold at the high dose (4000 μg/mL). However, our reviewers noted the MFs for the S9-activated solvent control (1.2×10^{-5} or 12 mutants/10^6 survivors) was below the acceptable spontaneous range for mouse lymphoma cells (15 to 110 mutants/10^6 survivors). According to quality control guidelines established by Caspary et al. (1988)[2] for evaluation of the mouse lymphoma assay, studies in which the solvent control MF falls below 15 mutants/10^6 survivors are usually rejected. Similarly, the increased test material MFs compared to the concurrent solvent control, which ranged from a low of 26 mutants/10^6 survivors at 1000 μg/mL to a high of 55 mutants/10^6 survivors at 3000 μg/mL, were within the accepted spontaneous background frequency.

The study authors stated that the MF for the S9-activated medium control was elevated (≈2-fold) compared to the S9-activated solvent control and concluded that the results of the S9-activated assay with the test material were equivocal. They recommend that S9-activated assay be repeated to confirm that the results were compound related.

D. REVIEWERS' DISCUSSION AND INTERPRETATION OF RESULTS:

We agree with the study authors that the S9-activated assay performed with the test material should be repeated. However, our reasons for considering the S9-activated results to be equivocal differ from those presented by the study authors. Based on our evaluation of the data, the extremely low MF for the S9-activated DMSO control group may have resulted from solvent interference. Nevertheless, the sensitivity of the test system to detect a mutagenic effect was adequately demonstrated by the findings with the positive controls, particularly the S9-activated control (4 μg MCA), which was dissolved in DMSO.

There appeared to be a dose-related increase in mutant colonies and the MFs over the S9-activated range of test material doses. However, none of the test material MFs approached the higher

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[2]Caspary, W. J., et al. Environ. Molec. Mutagen. (1988) 12:19-36.

end of the accepted spontaneous background frequency range for mouse lymphoma cells (110 mutants/10^6 survivors), nor did the response appear to be related to compound toxicity. It further appears that at the time this study was performed, the laboratory investigators were unaware of the high osmotic pressure of test material concentrations >1.5 mg/mL (see Data Evaluation Record 333C). It is likely, therefore, that had the study been repeated with comparable test doses and test conditions, similar results would have been produced, leading the investigators to conclude that 3-carboxy-2,5,6-trichlorobenzamide was mutagenic in this test system. However, the subsequent mouse lymphoma assays performed by independent investigators with 3-carboxy-2,5,6-trichlorobenzamide (see Data Evaluation Record 333C) were negative, and they provided adequate evidence to support the conclusion that the increased MFs seen in the currently reviewed study probably resulted from compound effects on pH and osmolality of the culture medium rather than a true mutagenic response.

E. QUALITY ASSURANCE MEASURES: A quality assurance statement from the performing laboratory was signed and dated June 26, 1985.

F. CBI APPENDIX: Appendix A, Materials and Methods (SDS Biotech Corp.), CBI pp. 13-15; Appendix B, Protocol (Hazleton Biotechnologies, Inc.) CBI pp. 21-37; Appendix C, Materials and Methods (Hazleton Biotechnologies, Inc.) CBI pp. 45-49.

APPENDIX A

Materials and Methods
(SDS Biotech Corp.)
CBI pp. 13-15

Chlorothalonil

Page_____ is not included in this copy.

Pages 239 through 265 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.

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____ The product confidential statement of formula.

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EPA No.: 68D80056
DYNAMAC No.: 333-E
TASK No.: 3-33E
December 18, 1990

008334

DATA EVALUATION RECORD

3-CARBOXY-2,5,6-TRICHLOROBENZAMIDE

Mutagenicity--Salmonella typhimurium/Mammalian Microsome Mutagenicity Assay

APPROVED BY:

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

Signature: [signature]

Date: 12-18-90

008334

Guideline Series 84: Mutagenicity
EPA No.: 68D80056
DYNAMAC No.: 333-E
TASK No.: 3-33E
December 18, 1990

DATA EVALUATION RECORD

3-CARBOXY-2,5,6-TRICHLOROBENZAMIDE

Mutagenicity--Salmonella typhimurium/Mammalian Microsome Mutagenicity Assay

REVIEWED BY:

Nancy E. McCarroll
Principal Reviewer
Dynamac Corporation

Signature: Nancy E. McCarroll
Date: 12-18-90

I. Cecil Felkner, Ph.D.
Independent Reviewer
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Date: 12-18-90

APPROVED BY:

Nicolas P. Hajjar, Ph.D.
Department Manager
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Date: 12-18-90

Elizabeth Doyle, Ph.D.
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(H-7509C)

Signature: E. A. Doyle
Date: 1/2/91

Marcia Van Gemert, Ph.D.
Branch Chief
Toxicology Branch II
(H-7509C)

Signature: Marcia Van Gemert
Date: 1/7/91

DATA EVALUATION RECORD

CHEMICAL: 3-Carboxy-2,5,6-Trichlorobenzamide.

TEST MATERIAL: 2,4,5-Trichloro-3-carboxybenzamide. [Previously reviewed studies (see Data Evaluation Records 333A through D) listed the test material with one of the three chlorine ions at the meta-position on the benzene ring and none at the para-position].

STUDY TYPE: Mutagenicity--Salmonella/mammalian activation gene mutation assay.

MRID NUMBER: 415648-12.

SYNONYMS/CAS NUMBER: T-165-2; SDS-46851.

SPONSOR: Fermenta ASC Corp., Mentor, OH.

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

TITLE OF REPORT: Salmonella/Mammalian-Microsome Plate Incorporation Assay (Ames Test) With and Without Activation with 2,4,5-Trichloro-3-Carboxy-Benzamide.

AUTHORS: Godek, E. G., Naismith, R. W., and Matthews, R. J. (Pharmakon Research International, Inc.); Jones, R. E. and Killeen, J. C. (SDS Biotech Corp.).

STUDY NUMBER: PH 301-SDS-002-84.

REPORT ISSUED: December 6, 1984, (Pharmakon Research International, Inc.); April 16, 1985 (SDS Biotech Corp.).

NOTE: Report prepared by the sponsor's representative, SDS Biotech Corp., is a summary of the laboratory report prepared by Pharmakon Research International, Inc. The following review focuses on the reported data furnished by Pharmakon Research International, Inc.

SALMONELLA

CONCLUSION(S) - Executive Summary: Five doses of 2,4,5-trichloro-3-carboxy-benzamide ranging from 39 to 3900 µg/plate were evaluated for the potential to cause gene mutations in the Salmonella typhimurium/mammalian microsome plate incorporation mutagenicity assay. The highest nonactivated dose (3900 µg/plate) was cytotoxic in all tester strains (S. typhimurium TA1535, TA1537, TA1538, TA98, and TA100). In the presence of S9 activation, reduced colony counts were observed for the majority of the strains at 3900 µg/plate. There was, however, no indication of a mutagenic response in any strain at any dose level with or without S9 activation. We assess that 2,4,5-trichloro-3-carboxybenzamide was adequately tested and found to be not mutagenic in a well-controlled study. It was concluded, therefore, that the study satisfies Guideline requirements for genotoxic effects Category I, Gene Mutations.

Study Classification: The study is acceptable; however the sponsor should clarify the discrepancy in chemical nomenclature. Either the name of the test material is incorrect or the test material is different than the compound evaluated in the other reviewed genetic toxicology studies.

Note: Previously reviewed studies (see Data Evaluation Records 333A through D) listed the test material with one of the three chlorine ions at the meta-position on the benzene ring and none at the para-position).

A. MATERIALS:

1. Test Material:

Name:	2,4,5-Trichloro-3-carboxy-benzamide
Description:	White powder
Identification No.:	T-165-2
Purity:	99.4%
Contaminants:	Not listed
Solvent used:	Dimethylsulfoxide (DMSO)
Other comments:	The test material was stored at room temperature protected from light; dosing solutions were prepared on the day of use. The report indicated that the initial cytotoxicity assessment was conducted with solutions of the test material dissolved in 95% ethanol (high concentration = 15 mg/mL). However, since there was no cytotoxicity, further solubility studies were conducted with DMSO. In this solvent, a stock solution containing 50 mg/mL of the test material was achieved; therefore, DMSO was selected as the solvent of choice.

2. Control Materials:
Negative: None
Solvent/final concentration: DMSO/100 µL/plate
Positive: Nonactivation:
Sodium azide 10.0 µg/plate TA100, TA1535
2-Nitrofluorene 5.0 µg/plate TA98, TA1538
9-Aminoacridine 150 µg/plate TA1537
Other:

Activation:
2-Aminoanthracene 5.0 µg/plate all strains.

3. Activation: S9 derived from

x Aroclor 1254	x induced	x rat	x liver
_____ phenobarbital	_____ noninduced	_____ mouse	_____ lung
_____ none		_____ hamster	_____ other
_____ other		_____ other	

If other, describe below. Describe S9 composition (if purchased, give details). The S9 liver homogenate was prepared by the performing laboratory and contained 38.3 mg protein/mL.

The S9 mix contained the following ingredients/mL:

0.2 M	Phosphate buffer, pH 7.4	500 µL
1.0 M	Glucose 6-phosphate	5 µL
0.1 M	NADP	40 µL
0.4 M	$MgCl_2$	20 µL
1.65 M	KCl	20 µL
	S9	80 µL
	H_2O	335 µL

4. Test Organism Used: S. typhimurium strains

_____	TA97	__x__	TA98	__x__	TA100	_____	TA102	_____	TA104
__x__	TA1535	__x__	TA1537	__x__	TA1538; list any others:				

Test organisms were properly maintained? Yes.
Checked for appropriate genetic markers (rfa mutation, R factor)? Yes.

5. Test Compound Concentrations Used:

a. Preliminary cytotoxicity assays:

1) Initial test: Five nonactivated doses prepared in 95% ethanol (15, 50, 150, 500, and 1500 µg/plate) were evaluated in strains TA1538 and TA100.

2) Repeat test: Five nonactivated doses prepared in DMSO (50, 166, 500, 1666, and 5000 µg/plate) were assayed with strains 1538 and TA100.

b. Mutation assay: Five doses (39, 130, 390, 1300, and 3900 µg/plate) were evaluated in both the presence and absence of S9 activation with all tester strains.

B. TEST PERFORMANCE:

1. Type of Salmonella Assay: __x__ Standard plate test
_____ Pre-incubation (___) minutes
_____ "Prival" modification
_____ Spot test
_____ Other (describe).

2. Protocol:

a. Preliminary assay: In general, similar procedures were used for the preliminary cytotoxicity and the mutation assays.

To tubes containing 2-mL volumes of molten top agar, 0.1 mL of an overnight broth culture of the appropriate tester strain and 0.1 mL of the appropriate test material dose, solvent, or positive controls were added. For the S9-activated test, 0.5 mL of the S9-cofactor mix was added. The contents of the tubes were mixed, poured over Vogel-Bonner minimal medium E, and incubated at 37°C for 48 to 72 hours. At the end of incubation, plates were scored for

revertant colonies using an automatic colony counter. Means and standard deviations were determined for the mutation assay.

Bacterial titers were determined by plating serial dilutions of each strain on nutrient agar. Sterility tests were performed on the top agar, S9 mix, solvent, and the highest prepared concentration of the test material.

b. Evaluation criteria:

1) Assay validity: The assay was considered valid if the solvent control values for each strain were within the 95% confidence limits of the performing laboratory's historical mean data.

2) Positive response: The test material was considered positive if it induced a reproducible, dose-related increase in the number of mutant colonies of any strain and the highest increase was ≥3-fold higher than the corresponding solvent control.

NOTE: A complete copy of all primary data for this study was included with the final report.

C. REPORTED RESULTS:

1. Preliminary Assay: Two preliminary assays were performed with the test material. In the first study, solutions of the test material were prepared in 95% ethanol to yield nonactivated doses ranging from 15 to 1500 µg/plate. Owing to the lack of cytotoxicity, the test was repeated and DMSO was selected as the solvent. For this test, the dose range evaluated was 50 to 5000 µg/plate (-S9). A review of the primary data indicated that 5000 µg/plate induced a slight cytotoxic response (i.e., thinning of the background lawn of growth) in both strains TA1538 and TA100; revertant colonies were not counted. Based on these findings, the dose range selected for the nonactivated and S9-activated mutation assay was 39 to 3900 µg/plate.

2. Mutation Assay: Representative results from the mutation assay conducted with the test material are presented in Table 1. As shown, the highest nonactivated dose (3900 µg/plate) was cytotoxic in all strains as indicated by the ≈≥50% decrease in mutant colonies compared to the respective solvent control. Although reduced colony counts were also seen for several strains at 1300 µg/plate -S9, the reductions were not clearly indicative of cytotoxicity. Results for the remaining nonactivated doses showed no cytotoxic or mutagenic effect.

   In the presence of S9 activation, lower than solvent control mutant colony counts were seen for the majority of strains at the high dose (3900 µg/plate). The remaining S9-activated doses were neither cytotoxic nor mutagenic.

   By contrast, all nonactivated and S9-activated positive controls induced a mutagenic response in the appropriate tester strains. Based on these finding, the study authors concluded that 2,4,5-trichloro-3-carboxy-benzamide was not mutagenic in this microbial test system.

D. REVIEWER'S DISCUSSION/INTERPRETATION OF STUDY RESULTS:

We assess that the study was properly conducted and that the study authors interpreted the data correctly. 2,4,5-Trichloro-3-carboxy-benzamide was assayed to a cytotoxic level and failed to induce a mutagenic response in a well-controlled study. In addition, the findings with the positive controls demonstrated that the test system was adequately sensitive to detect mutagenesis.

E. QUALITY ASSURANCE MEASURES: A quality assurance statement from the performing laboratory was signed and dated November 28, 1994.

F. CBI APPENDIX: Appendix A, Materials and Methods (SDS Biotech Corp.) CBI pp. 13-14; Appendix B, Protocol (Pharmakon Research International, Inc.) CBI pp. 18-45; Appendix C, Materials and Methods (Pharmakon Research International, Inc.) CBI pp. 52-56.

TABLE 1. Representative Results of the Salmonella typhimurium Mutagenicity Assay with 2,4,5-Trichloro-3-Carboxy-Benzamide

Substance	S9 Activation	Dose/plate	Revertants per Plate of Bacterial Tester Strain[a] TA1535	TA1537	TA1538	TA98	TA100
Solvent Control							
Dimethylsulfoxide	-	100 µL	15 ± 5	10 ± 4	12 ± 2	19 ± 7	225 ± 19
	+	100 µL	10 ± 4	14 ± 5	18 ± 4	29 ± 1	213 ± 18
Positive Control							
Sodium azide	-	10 µg	536 ± 31	--	--	--	359 ± 33
2-Nitrofluorene	-	5 µg	--	--	389 ± 64	350 ± 18	--
9-Aminoacridine	-	150 µg	--	927 ± 36	--	--	--
2-Aminoanthracene	+	5 µg	379 ± 33	682 ± 138	727 ± 392	2121 ± 114	2079 ± 76
Test Material							
2,4,5-Trichloro-3-carboxy-benzamide		1300 µg[b]	10 ± 3	6 ± 3	12 ± 4	20 ± 8	133 ± 15
		3900 µg	7 ± 5	2 ± 3	0 ± 1	9 ± 4	104 ± 69
	+	3900 µg[b]	10 ± 4	8 ± 3	15 ± 4	25 ± 11	133 ± 26

[a]Means and standard deviations of counts from triplicate plates.

[b]Results for lower concentrations (39, 130 and 390 µg/plate -S9 and 39, 130, 390, and 1300 µg/plate +S9) did not indicate a mutagenic response.

APPENDIX A

Materials and Methods
(SDS Biotech Corp.)
CBI pp. 13-14

Chlorothalonil

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Pages 276 through 312 are not included.

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____ Description of the product manufacturing process.

____ Description of quality control procedures.

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X FIFRA registration data.

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008334

EPA No.: 68D80056
DYNAMAC No.: 333-G
TASK No.: 3-33G
February 15, 1991

DATA EVALUATION RECORD

3-CARBOXY-2,5,6-TRICHLOROBENZAMIDE

Pharmacokinetics/Metabolism in Rats

STUDY IDENTIFICATION: Savides, M.C., Marciniszyn, J.P., and Killeen Jr., J.C. Pharmacokinetics study to determine the effects of dose level on the metabolism of ^{14}C-SDS-46851 by rats. (Unpublished study No. 3043-88-0056-AM-001 performed by Ricerca, Inc., Painesville, OH; dated July 11, 1990.) MRID No. 415648-18.

APPROVED BY:

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

Signature: William L. McLellan for

Date: Feb 15, 1991

1. CHEMICAL: 3-Carboxy-2,5,6-trichlorobenzamide; SDS-4681.

2. TEST MATERIAL: 3-Carbamyl-2,4,5-trichlorobenzoic acid-ring-UL-^{14}C (^{14}C-SDS-46851; synthesized by Ricerca, Inc., Painesville, OH; Animal Metabolism Radiation Inventory No. 14C-063) with a specific activity of 3.51 mCi/mmol (13.1 µCi/mg). A radiochemical purity of 95.2 percent was used.

3. STUDY/ACTION TYPE: Pharmacokinetics and metabolism in rats.

4. STUDY IDENTIFICATION: Savides, M.C., Marciniszyn, J.P., and Killeen Jr., J.C. Pharmacokinetics study to determine the effects of dose level on the metabolism of ^{14}C-SDS-46851 by rats. (Unpublished study No. 3043-88-0056-AM-001 performed by Ricerca, Inc., Painesville, OH; dated July 11, 1990.) MRID No. 415648-18.

5. REVIEWED BY:

Peter W. Woodman, Ph.D.
Principal Reviewer
Dynamac Corporation

Signature: Sharon M Ambrose for
Date: 2-15-91

William L. McLellan, Ph.D.
Independent Reviewer
Dynamac Corporation

Signature: William L. McLellan
Date: Feb 15, 1991

6. APPROVED BY:

Nicolas P. Hajjar, Ph.D.
Department Manager
Dynamac Corporation

Signature: William L. McLellan for
Date: Feb 15, 1991

Elizabeth Doyle, Ph.D.
Acting Section Head
Review Section IV
Toxicology Branch II
(H-7509C)

Signature: Elizabeth C. Doyle
Date: 2/18/91

7. CONCLUSIONS:

A. A single oral dose of 10 mg/kg (no-effect-level) or 1000 mg/kg (effect level) of a suspension of ^{14}C-SDS-46851 was administered to groups of male and female Sprague-Dawley rats (three subgroups/dose level; minimum of five animals/sex/subgroup). Controls (seven males; six females) received a single oral dose of the vehicle. Following dosing, animals were placed in metabolism cages, and blood, urine, and feces were collected at various intervals until study termination. At 36, 42, or 48 hours after dosing, animals in each subgroup were sacrificed, and selected tissues were collected for radioanalysis. The average percentage of the administered dose recovered ranged from 77 percent to 100 percent in animals that received the low dose, and from 61 percent to 95 percent in the high-dose group. In all treated groups, cage washes contained an average of ≤2.3 percent of the administered dose. No metabolites were identified. Since the carcass and the majority of the tissues from each animal were not analyzed, and since urine and feces losses were reported, and radioactivity levels in the blood were not considered in the recovery calculations, the materials balance data underestimate total recoveries of the administered dose.

In the low-dose subgroups, the minimum average values for the excretion of the radiolabel in urine ranged from 10.3 percent to 16.8 percent in males, and from 9.5 percent to 19.5 percent of the administered dose in females. In contrast, urinary excretion of the ^{14}C label in the high-dose subgroups ranged from an average of 3.6 percent to 6.7 percent of the administered dose for males, and from 3.2 percent to 6.2 percent of the dose for females. The low-dose animals excreted an average of 87 percent of the total urinary radiolabel during the first 12 hours following dosing. In the high-dose group, an average of only 42 percent of the total ^{14}C label excreted in the urine was eliminated during the same period. This decrease in the percentage of the administered dose excreted in the urine and the ^{14}C label by the high-dose group animals was thought to reflect either prolonged or decreased absorption of the test material; saturation of an elimination pathway involving active secretion of SDS-46851 and/or its metabolites is another possible explanation for the observed effects.

The feces represented the major route of excretion of the ^{14}C label in both sexes and at both dose levels. Low-dose males excreted an average of 73.7 percent to 87.4 percent of the administered dose, and females an average of 61.2 percent to 80.5 percent of the dose. In the high-dose subgroups, males excreted an average of 54.3 percent to

64.4 percent, and females excreted an average of 44.0 percent to 79.5 percent of the administered dose. As was the case for urinary excretion, the percentage of the ^{14}C label eliminated in the feces decreased in the high-dose subgroups. During the first 12 hours following dosing, an average of 49 percent of the ^{14}C label was excreted via the feces by the low-dose groups versus 18 percent by the high dose groups during the same period.

Peak concentrations of the radiolabel were rapidly achieved in the blood of all animals that received ^{14}C-SDS-46851. The peak concentrations were essentially the same for both male and female rats in each dose group, and occurred in the low-dose group at the 0.5-hour collection point (males 903 ± 157 nanogram equivalents per gram (ng eq/g); females 861 ± 174 ng eq/g). Radiolabel blood concentrations in the high-dose males appeared to peak between 0.5 hours (26,119 ± 10,257 ng eq/g) and 3.0 hours (27,762 ± 4,298 ng eq/g), and in high-dose females between 0.5 hours (28,787 ± 9,346 ng eq/g) and 2 hours (32,084 ± 2,929 ng eq/g). The concentration of the radiolabel in the blood declined rapidly in the low-dose animals, and more slowly in the high-dose group. The average elimination half-life calculated was 2.5 hours for the low-dose group and 6.2 hours for the high-dose group. The corresponding elimination rate constants (k_{el}) were 0.111 and 0.277 per hour. No significant differences were found between these values or the total area under the blood concentration (AUC) versus time curve between sexes within either dose group. However, AUC values for the high (277,000 hr*ng-eq/mL) and low (4,050 hr*ng-eq/mL) doses were significantly ($p < 0.0001$) different, and the ratio of the AUCs was 68 (range 43 to 109; 95 percent confidence interval), which encompassed the dose ratio of 100 (i.e., 1000 mg/kg/10 mg/kg). These findings suggest that the bioavailability of SDS-46851 and/or its metabolites was proportional to the dose administered. Assumptions used to calculate the elimination half-life and rate constants are considered acceptable for the low-dose group, but not for the high-dose group owing to the apparent continued absorption of radiolabel during the elimination phase. A possible secondary peak in radiolabel blood concentrations occurred in the low-dose groups (males 213 ± 15 ng eq/g; females 270 ± 35 ng eq/g) at 14 hours postdosing, and in the high-dose groups at 8 hours in the females (17,824 ± 3,395 ng eq/g) and at 9 hours in the males (24,724 ± 11,922 ng eq/g).

Radioactivity levels were analyzed in selected tissues. Thirty-six hours after dosing, the total level of radioactivity in the liver, kidneys, and gastrointestinal (G.I.) tract in each subgroup ranged from 1.14 ± 0.44 to

7.32 ± 6.06 percent of the administered dose. The highest levels were found in the G.I. tract in the high-dose males (4.67 ± 1.74%) and females (7.23 ± 6.05%). At 48 hours postdosing, ^{14}C levels had fallen, and values for all dose groups ranged from 0.39 ± 0.05 percent to 1.62 ± 1.94 percent of the administered dose. Only trace amounts remained in the liver and kidneys, two potential target organs.

B. This study provides preliminary data on metabolism. The study does not, however, meet current § 85-1 guidelines (Pesticide Assessment Guidelines, Subdivision F, Hazard Evaluation: Human and Domestic Animals, Office of Pesticides and Toxic Substances, U.S. Environmental Protection Agency, Washington, DC, p. 156). No repeat doses of ^{14}C-SDS-46851 were administered. No data were provided to support the choice of the no-effect (10 mg/kg) and effect (1000 mg/kg) dose levels utilized. Expired air was not monitored for excretion of ^{14}C volatiles, and no justification for this omission was provided. No attention was given to the effect of the methylcellulose vehicle used and its possible interference with the kinetics of absorption of the test substance. No metabolites were identified, and no measurements were made on the levels of residual radioactivity in all tissues required by EPA Guideline 85-1. Finally, the 48-hour observation period used to monitor 90%+ excretion of the administered dose was too short for two of the high-dose animal subgroups. In addition, radiochemical analytical data on the purity of some of the ^{14}C-SDS-46851 dose preparations appear to be missing.

Items 8 through 10--see footnote 1.

11. MATERIALS AND METHODS (PROTOCOLS):

A. Materials and Methods: (See Appendix A for details.)

1. 3-Carbamyl-2,4,5-trichlorobenzoic acid-ring-UL-^{14}C (^{14}C-SDS-46851; Animal Metabolism Radiation Inventory No. 14C-063; specific activity 3.51 mCi/mmol [13.1 μCi/mg]) was synthesized by Ricerca, Inc., Painesville, OH. No details were provided on its

[1]Only items appropriate for this study are included in this DER.

synthesis or purification. Radiochemical purity (95.2 percent) was determined by high-performance liquid chromatography (HPLC) on a Whatman RAC semi-preparatory column and by subsequent liquid scintillation counting (LSC). ^{14}C-SDS-46851 was stored in the dark at -70°C prior to dose preparation. No other details were provided. Non-^{14}C-labeled 3-carbamyl-2,4,5-trichlorobenzoic acid (SDS-46851; Lot No. 0203) was used as the analytical standard and in the preparation of the 50-µCi dose for the 1000-mg/kg body weight dose. The source of SDS-46851 was not identified. The chemical purity (99.7 percent) was determined by HPLC. The sample was prepared in methanol/5 mg/mL Trizma® Base, pH adjusted to 8.0 by H_3PO_4 (1:4), and chromatographed in a mobile phase of methanol/0.05 M ammonium dihydrogen phosphate (10:90). SDS-46851 was stored at 4°C until used.

2. Sprague-Dawley rats Crl:CD® BR VAF/Plus® purchased from Charles River Breeding Laboratories, Inc. (Portage, MI) were used. Animals were received in three separate shipments and quarantined for 7 days before exposure to the test material. At the time of dosing, males (no age stated) weighed between 243 and 329 g (low dose) and between 252 and 295 g (high dose), and female weights ranged from 200 to 232 g in both the low- and high-dose groups. It should be noted that the protocol specified a range of 250 to 300 g for male rats at the time of dosing. Several of the randomly numbered males were within 10 percent above or below this specified weight range, and the protocol was amended since the slight difference was not considered to be important.

3. The specific activity of the ^{14}C-SDS-46851, 3.51 mCi/mmol, was too low to administer 50 µCi/animal for the 10-mg/kg dose level as originally proposed. Through an amendment to the protocol to allow administration of 30 µCi/animal, the low dose was prepared by the addition of a small volume of the 0.75 percent methylcellulose/water (w/v) vehicle to 100.1 mg of ^{14}C-SDS-46851. The moistened ^{14}C test material was ground using a mortar and pestle and placed in a 100-mL volumetric flask; the volume was adjusted to 100 mL with the vehicle. The 1000-mg/kg body weight, (50 µCi) dose was prepared by dissolving 186 mg of ^{14}C-SDS-46851 and 10.73 g of unlabeled SDS-46851 in a mixture of dimethyl formamide (DMF)/acetone (v:v 4:3). The solubilized material was recrystallized by dropwise addition of water and vacuum dried; 10.9 g was suspended in 0.75 percent methylcellulose as described for the low dose. The chemical concentration for the low dose was 1.00 mg/mL and for the high dose was 109.1

mg/mL. The specific activities of the compound in the low- and high-dose suspensions were 2.89 and 0.033 mCi/mmol, respectively, and the average radiochemical purities, as determined by high-performance liquid chromatography and subsequent liquid scintillation counting, were 93.5 percent and 95.5 percent. Data in Appendix C were not clearly marked and some appear to be missing; thus, these values could not be validated.

4. Initially, groups of 15 animals/sex/dose were assigned to receive a single oral dose of the suspension by gavage (10 mg/kg or 1000 mg/kg of ^{14}C-SDS-46851; low or high dose, respectively). The volume of the dosing suspension used was approximately 2.5 mL for males and 2.0 mL for females. The following average doses were actually received: male and female rats in the low-dose group 10.1 ± 0.2 mg SDS-46851/kg; high-dose group males 1044 ± 24 mg SDS-46851/kg; and high-dose females 1038 ± 031 mg SDS-46851/kg. After dosing, each dose group was divided into three subgroups of a minimum of five rats each for the collection of blood, urine, and feces samples. Controls (7 males and 6 females) received a single oral dose of the vehicle, 0.75 percent methylcellulose (w/v). The breakdown of the test groups, numbers of males and females actually used, and blood collection times are shown in Table 1. No additional information was provided on the controls. Animals were fasted for approximately 16 hours before dosing and then allowed food 4 hours after administration of the dose. Water was allowed at all times.

Following dosing, animals were placed in metabolism cages. Blood samples, with the exception of terminal bloods, were obtained by orbital sinus puncture at the times shown in Table 1. Animals were lightly anesthetized, and 200-uL samples were collected in heparinized tubes. Duplicate blood samples were combusted and the radiolabel content analyzed by LSC of the trapped $^{14}CO_2$. Urine and feces excreted during the time animals were removed from the cages for blood collection were not recovered. During the times the animals were in the metabolism cages, urine and feces were collected over dry ice. Collections were made at 0-12, 12-24, and 24-36 hours for all of the subgroups and from 36 hours until study termination for subgroups 2 and 3. Urine samples were assayed for radioactivity directly by LSC. Fecal pellets were weighed prior to homogenization, and then ground with dry ice and combusted; the trapped $^{14}CO_2$ was counted by LSC.

TABLE 1. Study Design for Administration of ^{14}C-SDS-46851 Low and High Dose, and Blood Collection Times

Group	No. & Sex	Dose Level	Blood Collection Times
Low Dose			
Subgroup 1[a]	5 males	10 mg/10 mL/kg	0.5, 3, 6, 9, 14, 24[d], 36 hrs
	5 females	10 mg/10 mL/kg	0.5, 3, 6, 9, 14, 24[d], 36 hrs
Subgroup 2[b]	5 males	10 mg/10 mL/kg	1, 4, 7, 10, 16, 26, 42 hrs
	5 females	10 mg/10 mL/kg	1, 4, 7, 10, 16, 26, 42 hrs
Subgroup 3[c]	6 males[e]	10 mg/10 mL/kg	2, 5, 8, 12, 18, 28, 48 hrs
	5 females	10 mg/10 mL/kg	2, 5, 8, 12, 18, 28, 48 hrs
High Dose			
Subgroup 1[a]	9 males[f]	1000 mg/ 10 mL/kg	0.5, 3, 6, 9, 14, 24, 36 hrs
	5 females	1000 mg/10 mL/kg	0.5, 3, 6, 9, 14, 24, 36 hrs
Subgroup 2[b]	5 males	1000 mg/10 mL/kg	1, 4, 7, 10, 16, 26, 42 hrs
	5 females	1000 mg/10 mL/kg	1, 4, 7, 10, 16, 26, 42 hrs
Subgroup 3[c]	5 males	1000 mg/10 mL/kg	2, 5, 8, 12, 18, 28, 48 hrs
	5 females	1000 mg/10 mL/kg	2, 5, 8, 12, 18, 28, 48 hrs

[a]Animals were terminated 36 hours after dose administration.

[b]Animals were terminated 42 hours after dose administration.

[c]Animals were terminated 48 hours after dose administration.

[d]Collections were not made at this time.

[e]An additional animal was dosed to replace an animal that died while blood was being collected.

[f]Four additional animals were unintentionally dosed.

SOURCE: CBI Text Table, CBI pp.16.

Expired air was not monitored for the excretion of ^{14}C volatiles.

5. At the end of the assigned exposure periods, 36, 42 and 48 hours after dosing, animals were terminated under anesthesia by exsanguination via the dorsal aorta, and the blood was collected and analyzed for the ^{14}C radiolabel as described previously. Details on when the controls were terminated were not provided. It is assumed they were sacrificed 48 hours after dosing to provide the necessary background samples for LSC. Selected tissues (identified in the protocol only as all major tissues and organs such as liver, kidney, gonads, adrenals, prostate, thyroid, muscle, etc.) were removed from all animals and frozen at <-10°C, along with the residual carcass in labeled bags. The liver, kidneys and G.I. tract of each animal were analyzed for the ^{14}C radiolabel. Tissues were thawed, weighed, and minced; the G.I. tract was also homogenized. Duplicate samples were combusted and the trapped $^{14}CO_2$ was counted by LSC. Cages were washed with HPLC-grade water and methanol to collect remaining radiolabeled material. Appropriate measures were taken to correct for combustion efficiency, and all LSC analytical data were corrected for background and quenching.

6. To determine if the absorption and/or elimination of ^{14}C-SDS-46851 from the blood was proportional to the dose following oral administration, the concentrations of radioactivity in the blood determined in each test animal, expressed as ng-eq/g, were analyzed statistically to determine the time to peak radiolabel concentration, the elimination half-life, the elimination rate constant (k_{el}), and the AUC.

B. Protocol: A protocol for this study and its amendments are presented in the Appendix to this report.

12. REPORTED RESULTS:

A. Tables 2 and 3 summarize data on the excretion and distribution of the administered radiolabel in the low- and high-dose groups, respectively. Approximately 49 to 100 percent of the administered dose was recovered from the urine, feces, tissues, and cage washes following oral dosing with a suspension of ^{14}C-SDS-46851, with the percent recoveries (77 to 100 percent) higher in the low-dose animals. The percent recovery of the administered dose from the blood was not computed. The average

TABLE 2. Summary of the Excretion and Distribution of Administered Radiolabel (Low Dose)

Sample	Time (hours)	Mean Percentage of Administered Dose					
		Males/Subgroup			Females/Subgroup		
		1	2	3	1	2	3
Urine							
	12	16.23	8.71	13.95	11.45	7.20	16.19
	24	16.39	9.65	15.04	11.70	8.62	18.09
	36	16.75	--	15.36	12.16	--	18.69
	42	--	10.32	--	--	9.50	--
	48	--	--	15.65	--	--	19.52
	Total	16.75	10.32	15.65	12.16	9.50	19.52
Feces							
	12	19.61	53.43	38.34	25.32	45.80	38.32
	24	70.92	83.03	75.44	53.45	74.90	60.74
	36	78.69	--	78.67	61.24	--	63.86
	42	--	87.41	--	--	80.51	--
	48	--	--	81.57	--	--	65.24
	Total	78.69	87.41	81.57	61.24	80.51	65.24
Summary							
Urine & Feces		95.44	97.73	97.22	73.40	90.01	84.76
Tissues[a]		1.14	0.65	0.39	1.50	2.89	0.52
Cagewash		0.91	1.25	0.57	1.88	1.22	2.12
	Total	97.49	99.62	98.17	76.78	94.13	87.40

[a]Includes only liver, kidney and gastrointestinal tract.

SOURCE: CBI Table 14, CBI pp. 83-86

TABLE 3. Summary of the Excretion and Distribution of Administered Radiolabel (High Dose)

Sample	Time (hours)	Mean Percentage of Administered Dose					
		Males/Subgroup			Females/Subgroup		
		1	2	3	1	2	3
Urine							
	12	2.15	1.40	2.86	1.05	1.79	3.02
	24	4.12	3.20	5.21	3.96	2.79	5.02
	36	4.50	3.40	5.78	6.17	3.01	5.70
	42	--	3.59	--	--	--	--
	48	--	--	6.68	--	3.20	6.20
	Total	4.50	3.59	6.68	6.17	3.20	6.20
Feces							
	12	1.94	6.86	21.21	9.90	13.41	13.72
	24	51.52	39.79	43.76	66.16	31.94	42.79
	36	62.95	49.05	53.03	79.46	40.73	52.76
	42	--	54.28	--	--	43.96	--
	48	--	--	64.40	--	--	61.41
	Total	62.95	54.28	64.40	79.46	43.96	61.41
Summary							
Urine and feces		67.45	57.87	71.08	85.63	47.16	67.61
Tissues[a]		4.77	1.10	1.62	7.32	0.50	1.03
Cagewash		1.64	2.23	2.29	2.25	1.14	1.62
	Total	82.96	61.21	74.99	95.19	48.81	70.26

[a]Includes only liver, kidney, and gastrointestinal tract.

excretion of the ^{14}C label in the urine by male rats that received the low dose ranged from 10.3 percent to 16.8 percent of the administered dose. The female rats excreted an average of 9.5 percent to 19.5 percent of the administered dose. Urinary excretion of the ^{14}C label by rats that received the high dose ranged from an average of 3.6 percent to 6.7 percent of the administered dose in male rats, and from 3.2 percent to 6.2 percent for the females. A larger percentage of the administered dose was excreted in the urine at the low dose than was excreted by the animals that received the high dose. Over the first 12 hours following dosing, low-dose animals eliminated an average of 87 percent of the total radiolabel excreted in the urine. By contrast, animals in the high dose group excreted only an average of 42 percent of the total radiolabel during the same time period. The average level of excretion of ^{14}C radiolabel in the feces of the low-dose male rats ranged from 78.7 percent to 87 percent of the administered dose in subgroups 1 to 3. The average percent of the administered dose found in the feces for subgroups 1 to 3 of the females ranged from 61.2 percent to 80.5 percent. Males that received the high dose excreted an average of 54.3 percent to 64.4 percent of the administered dose. Female rats excreted an average of 44.0 percent to 79.5 percent of the administered dose. The rate of elimination of the ^{14}C radiolabel in the feces paralleled that in the urine. During the first 12 hours following dosing, an average of 49 percent of the ^{14}C radiolabel excreted via the feces was eliminated by the animals that received the low dose. In contrast, animals that received the high dose eliminated only 18 percent of the total ^{14}C radiolabel in the feces during the same observation period.

The mean distribution of the ^{14}C radiolabel in the kidneys, liver and G.I. tract, presented as a percent of the administered dose, are summarized in Table 4. Only trace amounts of the ^{14}C-radiolabel were present in the liver or kidneys at either the 36-, 42-, or 48-hour sacrifice. Thirty-six hours after dosing, the total level of radioactivity in the liver, kidneys, and G.I. tract in each subgroup ranged from 1.14 ± 0.44 to 7.32 ± 6.06 percent of the administered dose. The highest levels were found in the G.I. tract in the high-dose males (4.67 ± 1.74%) and females (7.23 ± 6.05%) at 3 hours postdosing. At 48 hours postdosing, however, tissue levels had fallen, and values for all dose groups ranged from 0.39 ± 0.05 percent to 1.62 ± 1.94 percent of the administered dose. In all treated groups, cage washes contained an average of ≤2.3

TABLE 4

SUMMARY OF THE DISTRIBUTION OF RADIOLABEL IN TISSUES[†]

Mean ± Standard Deviation

Dose Level and Sex	Sub-group	% AD Liver	% AD Kidney	% AD G.I. Tract	Total % AD Tissues
Low Male	1[a]	0.30 ± 0.04	0.03 ± 0.01	0.80 ± 0.41	1.14 ± 0.44
Low Males	2[b]	0.26 ± 0.02	0.03 ± 0.00	0.36 ± 0.32	0.65 ± 0.32
Low Males	3[c]	0.21 ± 0.04	0.03 ± 0.01	0.16 ± 0.05	0.39 ± 0.05
Low Female	1[a]	0.21 ± 0.03	0.03 ± 0.01	1.27 ± 1.88	1.50 ± 1.88
Low Female	2[b]	0.17 ± 0.04	0.03 ± 0.01	2.69 ± 1.21	2.89 ± 1.23
Low Female	3[c]	0.16 ± 0.03	0.02 ± 0.01	0.33 ± 0.33	0.52 ± 0.34
High Male	1[a]	0.09 ± 0.01	0.01 ± 0.00	4.67 ± 1.74	4.77 ± 1.74
High Male	2[b]	0.07 ± 0.01	0.00 ± 0.01	1.02 ± 0.55	1.10 ± 0.56
High Male	3[c]	0.06 ± 0.01	0.00 ± 0.01	1.56 ± 1.92	1.62 ± 1.94
High Female	1[a]	0.08 ± 0.01	0.01 ± 0.01	7.23 ± 6.05	7.32 ± 6.06
High Female	2[b]	0.04 ± 0.02	0.00 ± 0.00	0.46 ± 0.29	0.50 ± 0.43
High Female	3[c]	0.05 ± 0.01	0.00 ± 0.00	0.98 ± 0.64	1.03 ± 0.63

[†]See TABLE 11 for individual animal data.
[a]Animals were terminated 36 hours after dose administration.
[b]Animals were terminated 42 hours after dose administration.
[c]Animals were terminated 48 hours after dose administration.

dose. Carcasses and other body tissues from the animals were not analyzed for ^{14}C radioactivity. No metabolites were identified.

B. Levels of ^{14}C radioactivity in the blood rapidly peaked in all animals that received ^{14}C-SDS-46851 (Figures 1 and 2). Peak concentrations were essentially the same for both male and female rats in each dose group and occurred in the low dose group at the 0.5-hour collection point (males 903 ± 157 ng eq/g; females 861 ± 174 ng eq/g). Blood concentrations of the radiolabel in the high-dose males appeared to peak between 0.5 hours (26,119 ± 10,257 ng eq/g) and 3.0 hours (27,762 ±4,298 ng eq/g), and in high-dose females between 0.5 hours (28,787 ± 9,346 ng eq/g) and 2 hours (32,084 ± 2,929 ng eq/g).

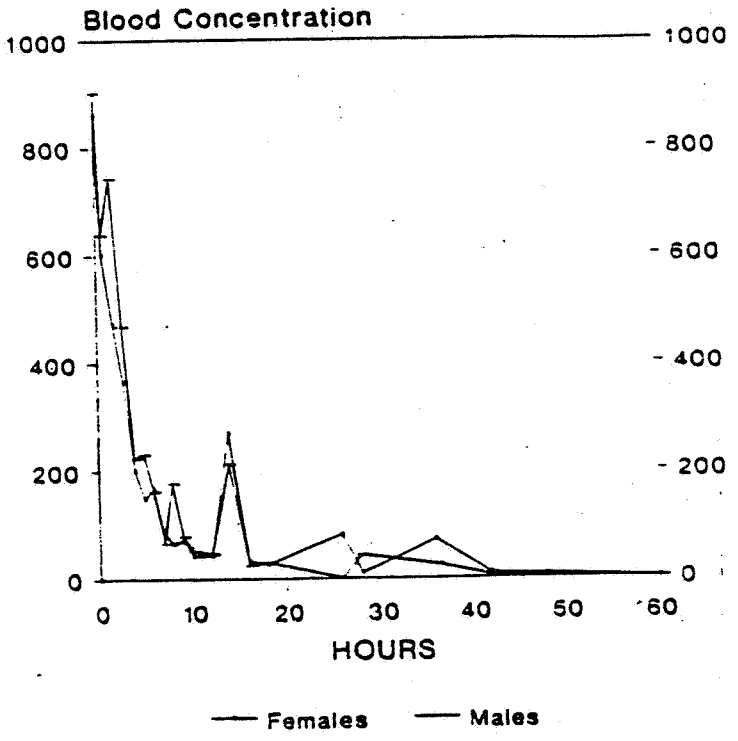
Table 5 presents data on blood levels at selected intervals. The concentration of the radiolabel in the blood declined rapidly in the low-dose animals (Figure 1), and more slowly in the high-dose groups (Figure 2). The average elimination half-life calculated (assuming first-order kinetics) was 2.5 hours for the low-dose group and 6.2 hours for the high-dose group. The corresponding elimination rate constants were 0.111 and 0.277 per hour. No significant differences were found in these values or the total AUC between sexes within either dose group. However, AUC values for the high (277,000 hr*ng-eq/mL; range 160,000 to 478,000, 95 percent confidence interval) and low (4,050 hr*ng-eq/mL; range 2,280 to 7,210, 95 percent confidence interval) doses were significantly ($p < 0.0001$) different, and the ratio of the AUCs (277,000/4,505) was 68 (range 43 to 109, 95 percent confidence interval), which encompassed the dose ratio of 100 (i.e., 1000 mg/kg/10 mg/kg). This suggested that the bioavailability of SDS-46851 and/or its metabolites was proportional to the dose administered. Assumptions used to calculate the elimination half-life and rate constants were considered acceptable for the low-dose group, but not for the high-dose group owing to the apparent continued absorption of radiolabel during the elimination phase. A possible secondary peak in blood radiolabel concentrations occurred in the low-dose groups (males 213 ± 15 ng eq/g; females 270 ± 35 ng eq/g) at 14 hours postdosing, and in the high-dose groups at 8 hours in the females (17,824 ± 3,395 ng eq/g) and at 9 hours in the males (24,724 ± 11,922 ng eq/g).

Figure 1. Low-Dose Group: Blood Concentrations (ng/mL) of ^{14}C Radioactivity versus Time

Source: CBI Figure 2d, CBI pp. 262.

FIGURE 1

BLOOD CONCENTRATION (ng/ml) BY HOUR
Low Dose Group



All Subgroups

Figure 2. High-Dose Group: Blood Concentrations (ng/mL) of ^{14}C Radioactivity versus Time

Source: CBI Figure 3d, CBI pp. 266.

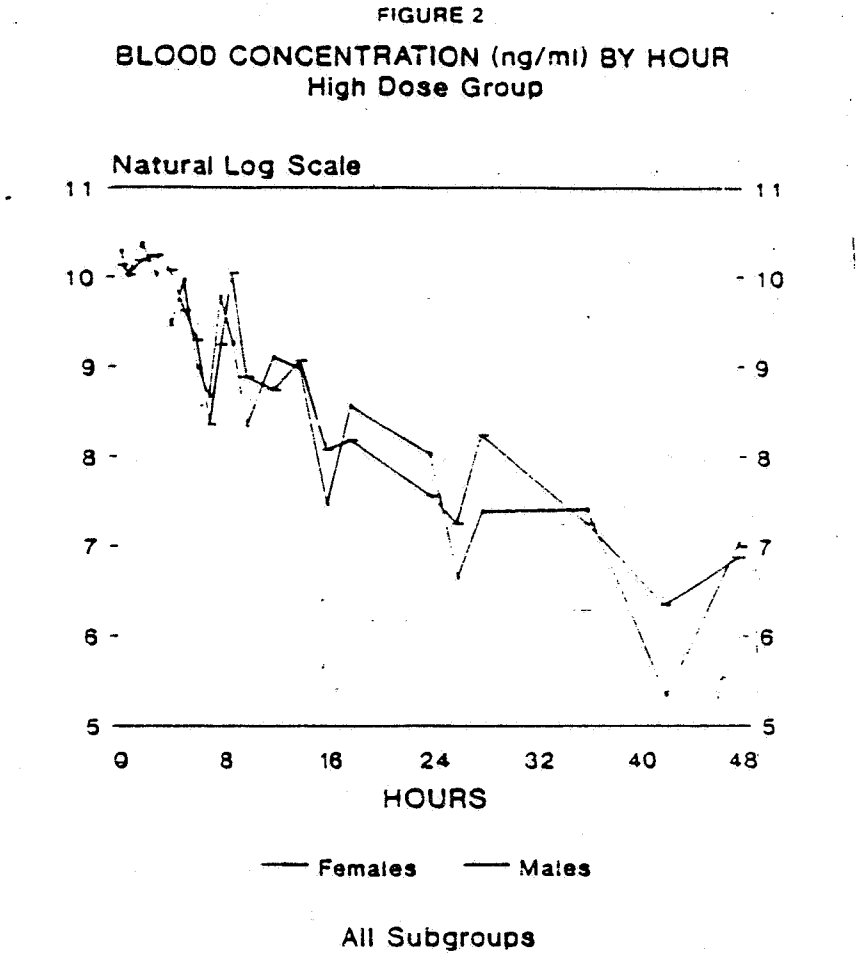


TABLE 5. Summary of Mean Blood Concentrations (nanogram equivalents/gram)

	ng Equivalent/Gram[a]			
	Low Dose		High Dose	
Hours	Males	Females	Males	Females
1	638	602	22799	22873
4	224	201	23679	13724
7	65	35	4849	6662
10	51	42	7609	4692

[a]Data for animals terminated at 42 hours (Group b); data for all subgroups combined are shown in Figures 1 and 2.

SOURCE: CBI Table 4, CBI pp. 49-50.

13. STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES:

A. The study authors concluded that the bioavailability of ^{14}C-SDS-46851 and/or its metabolites was proportional to the dose administered. They determined that there were no significant differences between the pharmacokinetic parameters calculated for the male and female rats at either dose level. The half-life for the elimination of the ^{14}C radiolabel was 2.5 hours following a 10-mg/kg dose of ^{14}C-SDS-46851. However, the elimination of the ^{14}C radiolabel following the 1000-mg/kg dose of ^{14}C-SDS-46851 occurred during a prolonged phase of absorption; thus, the true elimination half-life could not be meaningfully determined. The feces were considered the primary route of elimination of ^{14}C-SDS-46851 following oral administration. The ^{14}C radiolabel was excreted at a decreased rate via the urine and feces following administration of the high dose of ^{14}C-SDS-46851 compared with the rate following administration of the low dose. Only traces of the ^{14}C radiolabel remained in the liver and kidneys, considered two potential target organs, at 36, 42, or 48 hours following a single dose of ^{14}C-SDS-46851 at either the no-effect-level (10 mg/kg) or the effect level (1000 mg/kg). Analysis of the G.I. tract and contents indicated that more than 48 hours were required for complete elimination of the radiolabel.

B. A quality assurance statement signed and dated July 9, 1990, and a statement of compliance with Good Laboratory Practices Regulations (as set forth in Title 40 CFR 160), signed and dated July 11, 1990, were included. However, the study authors noted exceptions for deviations in the maintenance of temperature (below 70°F on three different days; protocol-specified range was 70-76°F) and humidity (low-dose animals were exposed to daily fluctuations in humidity between 55 and 73%; protocol-specified range was 40-60%) in the animal rooms. In addition, it was acknowledged that blood samples were inadvertently not collected at 24 hours in one subgroup of animals following administration of the low dose of ^{14}C-SDS-46851. The study authors did not consider this time point critical.

14. REVIEWER'S DISCUSSION AND INTERPRETATION OF STUDY RESULTS:

This study provides preliminary data on the pharmacokinetics and metabolism of SDS-46851 and the effect of dose on the elimination of the compound from rats following oral administration. There is a significant ($p < 0.0001$) difference between the area under the blood concentration versus time curve (AUC) for the high- and low-dose groups, and the apparent effect of the 1000-mg/kg dose on prolonging the absorption phase of

^{14}C-SDS-46851, support the contention that the bioavailability of SDS-46851 and/or its metabolites was proportional to the dose administered.

The average percent of the administered dose recovered ranged from 77 percent to 100 percent in animals that received the low dose, and from 61 percent to 95 percent in the high-dose group. However, recoveries would actually have been higher if radioactivity from the blood (data available but not included by study authors), expired air, carcass, and other tissues (samples taken but not analyzed) had been included.

Despite several deficiencies in the study, the conclusions reached by the authors are generally supported by the data. However, based on § 85-1 guidelines (Pesticide Assessment Guidelines, Subdivision F, Hazard Evaluation: Human and Domestic Animals, Office of Pesticides and Toxic Substances, U.S. Environmental Protection Agency, Washington, DC, p. 156), major problems include a failure to study the effect(s) of a series of single oral daily doses of SDS-46851 administered over a period of at least 14 days, followed at 24 hours after the last dose by a single oral dose of ^{14}C-SDS-46851 on the metabolism and pharmacokinetics of the compound; a failure to monitor for the excretion of ^{14}C volatiles in the expired air (or justification for this omission); failure to consider in discussion of the data on the possible effect of the 0.75 percent methylcellulose vehicle on the kinetics of absorption, of SDS-46851; and a failure to identify metabolites or to measure residual levels of radioactivity in several of the tissues required by EPA Guideline 85-1. The measurement of radioactivity in all the tissues required by guidelines, however, may not have been feasible because of low levels of radioactivity particularly at the high dose. Other deficiencies include a failure to use a sufficiently long observation period for two of the high-dose subgroups to monitor >90% excretion of the dose -- a shortcoming acknowledged by the study authors. In addition the study authors failed to document the rationale or present previous study findings in support of the 10-mg/kg no-effect-level and the 1000-mg/kg effect level, and there was an apparent lack of HPLC/LSC analytical data on the purity of the various ^{14}C-SDS-46851 preparations.

Items 15 and 16--see footnote 1.

17. CBI APPENDIX: Appendix A, Protocol and Amendments, CBI pp. 88-117.

APPENDIX A

Protocol and Amendments
(CBI pp. 88-117)

Chlorothalonil

Page_____ is not included in this copy.

Pages 333 through 361 are not included.

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EPA No.: 68D80056
DYNAMAC No.: 333-A
TASK No.: 3-33A
December 18, 1990

008334

DATA EVALUATION RECORD

3-CARBOXY-2,5,6-TRICHLOROBENZAMIDE

Mutagenicity--Mammalian Cells in Culture Sister Chromatid Exchange Assay in Chinese Hamster Ovary (CHO) Cells

APPROVED BY:

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

Signature: William L McLellan for

Date: Dec 18, 1990

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Guideline Series 84: **MUTAGENICITY**

EPA No.: 68D80056
DYNAMAC No.: 333-A
TASK No.: 3-33A
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008334

DATA EVALUATION RECORD

3-CARBOXY-2,5,6-TRICHLOROBENZAMIDE

Mutagenicity--Mammalian Cells in Culture Sister Chromatid Exchange Assay in Chinese Hamster Ovary (CHO) Cells

REVIEWED BY:

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Toxicology Branch II
(H-7509C)

Signature: Marcia Van Gemert
Date: 1/7/91

DATA EVALUATION RECORD

Tox. Chem. No.:

EPA File Symbol:

008334

CHEMICAL: 3-Carboxy-2,5,6-Trichlorobenzamide.

STUDY TYPE: Mutagenicity--Mammalian cells in culture sister chromatid exchange assay in Chinese hamster ovary (CHO) cells.

MRID NUMBER: 415648-16.

SYNONYM/CAS NUMBER: T-165-2; SDS-46851.

SPONSOR: Fermenta ASC Corp., Mentor, OH.

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

TITLE OF REPORT: In Vitro Sister Chromatid Exchange Assay in Chinese Hamster Ovary (CHO) Cells with 3-Carboxy-2,5,6-Trichlorobenzamide.

AUTHORS: San Sebastian, J. R., Naismith, R. W., and Matthews, R. J. (Pharmakon Research International, Inc.); Jones, R. E. and Killeen, J. C. (SDS Biotech Corp.).

STUDY NUMBER: PH 319-SDS-002-84.

REPORT ISSUED: April 17, 1985 (Pharmakon Research International, Inc.); July 8, 1985 (SDS Biotech Corp.).

NOTE: Report prepared by the sponsor's representative, SDS Biotech Corp., is a summary of the laboratory report prepared by Pharmakon Research International, Inc. The following review focuses on the reported data furnished by Pharmakon Research International, Inc.

CONCLUSIONS - Executive Summary: No evidence of cytotoxicity or an increase in the frequency of sister chromatid exchange (SCE) was observed in Chinese hamster ovary (CHO) cells exposed for 5 hours to nonactivated and S9-activated doses of 3-carboxy-2,5,6-trichlorobenzamide ranging from 200 to 2000 µg/mL. The limit of solubility was reported to be 300 mg/mL, and an acidic change in the culture medium occurred at doses ≥1000 µg/mL. Although the results were negative, the 5-hour treatment time in the nonactivated assay may have been too short to allow detection of an SCE response. The currently accepted approach is to continuously expose CHO cells to the nonactivated test material throughout the period of incubation.[1] We assess, therefore, that the nonactivated assay should be repeated using a continuous cell exposure to assure that optimal conditions are available to detect genotoxicity. In addition, there were no analytical data to support the actual concentrations of 3-carboxy-2,5,6-trichlorobenzamide used in the study.

It was, therefore, concluded that the study does not satisfy Guideline requirements for Category III, Other Mutagenic Mechanisms.

Study Classification: The nonactivated phase of this study is unacceptable.

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[1] Galloway, S. M., Bloom, A. D., Resnick, M., Margolin, B. H., Nakamura, F., Archer, P., and Zeiger, E. Development of a standard protocol for in vitro cytogenetic testing with Chinese hamster ovary cells: Comparison of results for 22 compounds in two laboratories. Environ. Mutagen. (1985) 7:1-51.

A. MATERIALS:

1. Test Material:

Name:	3-Carboxy-2,5,6-trichlorobenzamide.
Description:	Beige powder; Note: Raw data submitted with the report listed the test material as a white powder.
Identification No.:	T-165-2.
Purity:	99.4%.
Contaminants:	Not listed.
Solvent used:	Dimethylsulfoxide (DMSO)
Other comments:	The test material was stored at room temperature; dosing solutions were used within 2 hours of preparation. The report stated that an acidic change in the pH of the culture medium [F12FCM (5%)] was observed at dose levels ≥1000 µg/mL; the pH of the culture medium was not adjusted to neutrality.

2. Control Materials:

Negative: F12 medium supplemented with 5% fetal calf serum.

Solvent/final concentration: DMSO/1%.

Positive: Nonactivation (concentrations, solvent): Ethylmethane sulfonate (EMS) was prepared in distilled water to yield a final concentration of 124 µg/mL.

Activation (concentrations, solvent): Dimethylnitrosamine (DMN) was prepared in distilled water to yield a final concentration of 25 µg/mL.

3. Activation: S9 derived from

X Aroclor 1254	X induced	X rat	X liver
____ phenobarbital	____ noninduced	____ mouse	____ lung
____ none		____ hamster	____ other
____ other		____ other	

If other, describe below. Describe S9 composition (if purchased, give details). The S9 fraction was prepared by the performing laboratory and contained 33.6 mg protein/mL.

The composition of the S9 mix per mL was as follows:

$MgCL_2.6H_2O$	8	µm
$CaCL_2.2H_2O$	8	µm
KCL	33	µm
Glucose-6-phosphate	5	µm
NADP	4	µm
Sodium phosphate buffer (pH 7.4)	50	µm
S-9	0.1	mL

4. Test Compound Concentrations Used:

   a. Preliminary cytotoxicity assay: Ten doses (1, 3, 10, 33, 100, 200, 333, 500, 750, and 1000 µg/mL) were evaluated with or without S9 activation.

   b. SCE assay: Six doses (200, 500, 750, 1000, 1500, and 2000 µg/mL) were tested with and without S9 activation.

5. Test Cells: CHO-K_1-BH4 were obtained from Dr. A. W. Hsie, Oak Ridge National Laboratories. Prior to use, exponential CHO cells were grown for 16 to 24 hours in F12FCM (5%).

   Properly maintained? Yes.

   Cell line or strain periodically checked for mycoplasma contamination? Not reported.

   Cell line or strain periodically checked for karyotype stability? Not reported.

B. TEST PERFORMANCE:

1. Cell Treatments:

   a. Cells exposed to test compound for:
   5 hours (nonactivated) 5 hours (activated.

   b. Cells exposed to positive controls for:
   5 hours (nonactivated) 5 hours (activated).

   c. Cells exposed to negative and/or solvent controls for:
   5 hours (nonactivated) 5 hours (activated)

2. Protocol:

a. Preliminary assay: Prepared cultures, seeded at 8×10^5 cells/15 mL of medium, were exposed with or without S9 activation to 10 doses of the test material (1 to 1000 µg/mL) or the solvent control (DMSO) for 5 hours. At the conclusion of treatment, cells were washed, refed fresh medium containing 100 µL BrdU, and reincubated for 27 hours. Colcemid (final concentration, 2×10^{-7} M) was added to each culture 2 hours prior to cell harvest. Cells were collected by mitotic shake-off; metaphases were harvested, fixed, and stained using the modified fluorescent-plus-Giemsa technique of Perry and Wolff.[2] One hundred cells from each dose group were examined for the percentage of first division (M_1), second division (M_2), and third division (M_3) metaphases. Mean cell cycle and average proliferation times were calculated. Based on these results, dose selection and harvest times were established for the SCE assay.

b. SCE assay:

1) Treatment: Prepared cultures (in duplicate), seeded at 8×10^5 cells/15 mL of medium, were exposed to the selected test material doses, the negative control (culture medium), the solvent control (DMSO), or the positive controls (EMS -S9 or DMN +S9) in a manner similar to that described for the preliminary cytotoxicity assay, with the exception that the incubation time in BrdU was 28 hours.

2) Slide analysis: Slides were stained as described and coded prior to scoring. Two hundred metaphase cells per group were scored to determine the percentage of first (M_1), second (M_2), or third (M_3) division metaphases. Fifty M_2 cells per group were scored for the frequency of SCEs.

3) Statistical methods: SCE/cell data were transformed by a standard square root transformation and evaluated for statistical significance at $p \leq 0.05$ by a "t" test.

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[2]Perry, P., and Wolff, S. New Giemsa method for the differential staining of sister chromatids. Nature (1974) 251:156-158.

4) Evaluation criteria:

a. Assay validity: The assay was considered valid if the SCEs/cell in the positive control group was significantly higher ($p \leq 0.05$) than the solvent control group.

b. Positive response: The test material was considered positive if it caused a dose-related and significant increase in the mean number of SCEs/cell or a ≥2-fold increase in the SCE frequency over the solvent control in at least one dose.

II. REPORTED RESULTS:

1. Preliminary Cytotoxicity Assay: The report indicated that an acidic change in the pH of the culture medium was observed at 1000 µg/mL. There were, however, no adverse effects on cell cycling or the proliferation time at this or lower doses ranging from 1 to 750 µg/mL either in the presence or absence of S9 activation. The study authors further stated that 300 mg/mL was the limit of test material solubility. However, owing to the acidic pH and in agreement with the study sponsor, the nonactivated and S9-activated SCE assay was performed with a dose range of [illegible] to [illegible] µg/mL.

2. SCE Assay: Six doses were initially assayed (210, 500, 750, [illegible], and 2000 µg/mL); the 750-µg/mL dose group was not scored. Results presented in Table 1 indicate that the evaluated doses of the test material both with and without S9 activation were neither cytotoxic nor caused an appreciable increase in the SCE frequency of the treated CHO cells. By contrast, the SCE cell frequencies induced by the nonactivated (124 µg/mL EMS) and the S9-activated (25 µg/mL DMN) positive controls were significantly ($p \leq 0.05$) higher than the corresponding solvent control value.

Based on the findings, the study authors concluded that [illegible]-carboxy-3,5,6-trichlorobenzamide was negative in this test system.

TABLE 1. Representative Results of the Sister Chromatid Exchange Assay in Chinese Hamster Ovary Cells Treated with 3-Carboxy-2,5,6-trichlorobenzamide

Substance	Dose/mL	S9 Activation	No. of Metaphases Scored for SCEs	% Cells[a] M_1	% Cells[a] M_1^+	% Cells[a] M_2	% Cells[a] M_2^+	SCEs/Chromosome	Mean SCEs/Cell
Negative Control									
Culture medium	--	-	50	6.0	56.0	38.0	-	0.56	11.28 ± 3.33
	--	+	50	3.0	15.5	81.0	0.5	0.79	15.84 ± 3.83
Solvent Control									
Dimethylsulfoxide	1%	-	50	12.0	56.5	31.5	-	0.57	11.36 ± 3.83
	1%	+	50	7.5	28.0	64.5	-	0.78	15.56 ± 3.5-
Positive Control									
Ethylmethane sulfonate	124 µg	-	50	6.5	26.0	58.0	9.5	1.78	35.58 ± 7.3e*
Dimethylnitrosamine	25 µg	+	50	9.5	30.0	60.5	-	1.24	24.82 ± 16.-3*
Test Material									
3-Carboxy-2,5,6-trichlorobenzamide	2000 µg[b]	-	50	11.5	47.0	39.0	2.5	0.63	12.56 ± 3.7[illegible]
	2000 µg	+	50	5.5	37.5	56.5	0.5	0.76	15.02 ± 3.[illegible]

[a]Percent cell in first (M_1), between first and second (M_1^+), in second (M_2), or between second and third (M_2^+) division/200 cells scored; no third-division metaphases was seen.

Results for lower doses (200, 500, 1000, and 1500 µg/mL +/-S9) did not suggest a positive response.

*Significantly higher ($p \leq 0.05$) than the solvent control (culture medium for both positive controls) by t test.

D. REVIEWERS' COMMENTS AND INTERPRETATION OF STUDY RESULTS:

We assess, in agreement with the study authors, that 3-carboxy-2,5,6-trichlorobenzamide assayed to a level that approached the limit of solubility failed to induce a cytotoxic or a genotoxic response. However, the currently accepted protocol (Galloway et al., 1985)[3] requires the continuous exposure of cells to the nonactivated test material throughout the period of incubation. We conclude, therefore, that the 5-hour treatment time used in the nonactivated phase of this study may have been too short to allow detection of SCE induction. The nonactivated portion of this study should be repeated to assure that optimum conditions are available for the detection of genotoxicity.

E. QUALITY ASSURANCE MEASURES: A quality assurance statement from the performing laboratory was signed and dated January 28, 1985.

F. CBI APPENDIX: Appendix A, Materials and Methods (SDS Biotech Corp.), CBI pp. 13-14; Appendix B, Protocol (Pharmakon Research International, Inc.), CBI pp. 20-35; Appendix C, Materials and Methods (Pharmakon Research International, Inc.), CBI pp. 43-46.

[3]Galloway, S.M. et al. Environ. Mutagen. (1985) 7:1-51.

APPENDIX A

Materials and Methods
(SDS Biotech Corp.)
CBI pp. 13-14

Chlorothalonil

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