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

003725

MAR 21 1984

MEMORANDUM

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

TO: Diane Beavers PM # 21
Fungicide/Herbicide Branch
Registration Division TS-767C

THRU: R. Bruce Jaeger, Section Head
Rev. Sec. # 1/Toxicology Branch
Hazard Evaluation Division TS-769C

RBJ 3/15/84

Subject: PP # 3F2875: Chlorothalonil (CTN) and its 4-OH metabolite in almonds, rice, wheat and meat, milk, poultry and eggs. Petition for tolerances.

Petitioner: SDS Biotech Corp., Painesville OH.(formerly Diamond Shamrock).

Caswell #: 215B.

Tolerances Proposed

Almonds	0.05 ppm
Rice	4.0
Wheat	0.1
Almond Hulls	0.1
Meat	0.1
Milk	0.1
Poultry	0.1
Eggs	0.1

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Recommendation:

1. Although data submitted in support of these tolerances contains presumptive evidence that CTN is an animal carcinogen, Toxicology Branch has no objection to establishing the proposed tolerance of 0.05 ppm combined residues in almonds and of 0.1 ppm in the animal feed item, almond hulls. Basis: The Incremental Exposure for almonds is < 1 % of the TMRC.

Tolerance x Food Factor x 1.5 kg/day = Incremental Exposure

0.05 mg/kg x 0.03 x 1.5 kg/day = 0.0000225 mg/day.

TMRC = 0.01305 mg/day/1.5 kg.

Incr. Exp./TMRC x 100 = 0.2 %

This is in accordance with our recommendations in the "Weight of Evidence Review", 1/31/84, D. Ritter.

Almond hulls is not a human food item. Tolerances are co-pending for residues in meat, eggs, poultry and milk. See (2.) below.

2. Those tolerances that are proposed for rice, wheat, meat, eggs, poultry and milk may not be established.

Basis: Unlike almonds, these racs represent major dietary components, with milk comprising more than 50% of the diet of infants and some elderly individuals who are on "bland" diets. Rice also figures prominently in the diet of these persons.

Thus, since toxicity data submitted in support of this petition contained presumptive evidence that CTN is an animal carcinogen, additional significant new tolerances are not appropriate, pending resolution of the oncogenicity question (Ritter, ibid.).

Review of Data

A number of new toxicity studies were submitted in this petition and were reviewed by either myself or Mr. Bruce Jaeger. The reviews are attached.

David L. Ritter, Toxicologist
Rev. Sec. # 1, Toxicology Branch
Hazard Evaluation Division TS-769C

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DATA EVALUATION REPORT CHLOROTHALONIL

STUDY: 3 Generation Rat Reproduction Study

LABORATORY: Biodynamics Inc., East Millstone, NJ.

STUDY NUMBER & DATE: 78-2278

ACCESSION NUMBER: 071524

MRID:

MATERIAL TESTED: DAC-3701 (major metabolite).

ANIMALS: Sprague Dawley CD rats 30 females and 15 males per group.

Method:

ENVIRONMENTAL PARAMETERS: Standard GLP

HUSBANDRY: STANDARD GLP

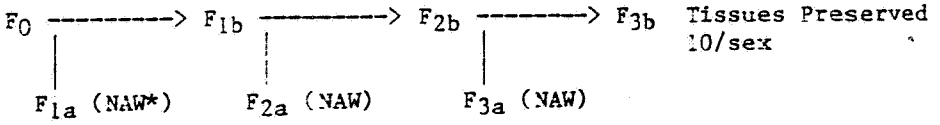
ROUTE OF ADMINSTRATION: Dietary

LEVELS OFFERED: 0, 10, 60 and 125 ppm

SCHEME OF ADMINSTRATION:

F_0 generation: 2 weeks pre-mating, then continuous thereafter for each generation:

Breeding:



- Day 0 - day of vaginal sperm or copulatory plug.
- 14 day rest period between weaning and re-mating.
- Random cull to 10/litter on day four post-partum.

*Necropsied at weaning

OBSERVATIONS:

Daily for mortality and thrift.

Counts on days 0, 4, 14 and 21.

Sexed on days 0 and 4.

Litter weights obtained on days 0, 4 and 14.

Individual pup weights on day 21.

BIOLIGICAL MEASUREMENTS:

Body weights, food consumption and feed content of Test Material obtained weekly.

POST-MORTEM EXAMINATION:

F_0, F_1 and F_2 parents killed and examined grossly after final weaning. Lesions preserved in 10% buffered formalin.

All pups dead or stillborn days on 0 - 4 received gross PM and preservation in 70% EtOH; those perishing 5 days or later post-partum received gross PM only.

Tissues and organs (listed in Appendix A) were preserved in 10% neutral formalin from randomly selected pups of the F_{3b} generation.

MICROSCOPIC EXAMINATION:

Tissues from one F_0 female parent in Group I;

Tissues from two F_{2b} male parents in Group III;

Tissues from one F_{2b} male parent in Group II;

Tissues from one F_{2b} male parent in Group IV.

SEROLOGY:

Performed on selected F_0 and F_{1b} animals for suspected Rat Corona Virus.

STATISTICAL EVALUATION:

Student's "t'" test (Snedecor, et all, 1967)

Dunnet's test (Dunnett, 1964)

Fisher Exact test (Bradley, 1968)

Armitage's test (Armitage, 1955)

Bonferroni Inequality (Miller, 1966)

RESULTS:

OBSERVATIONS:

Mortality, parents:

1 F_{1b} male each in the 10 ppm and 125 pmm groups.

1 F_{2b} male in each treatment group.

1 F_0 female each in the control and 10 ppm group.

Mortality, offspring:

Table I summarizes the Indices of Reproduction.

Signs of non-treatment related disease included randomly occuring:

- sialodacryoadenitis
- purulent bronchopneumonia

Post-mortem examination: Non-remarkable for treatment-related effects. No gross evidence of teratogenic effect reported.

Microscopic examination: Not performed except on the following animals:

One F_0 control female died from lymphosarcoma.

One F_{1a} male pup on 60 ppm had no right eye or optic nerve.

Two F_{2b} parents had purulent bronchopneumonia.

One F_{2b} parent had purulent bronchpneumonia and hepatic, splenic and nephric congestion.

DISCUSSION AND CONCLUSIONS:

1. Quality of Data

Summary sheets of raw data were well presented.

The failure to perform microscopic examinations on tissues and organs, at least from the control and high-dose animals, constitutes a serious deficiency in this study and must be rectified. This is because of the low NOEL discussed below. For this reason this study is graded CORE Supplemental. It is repairable to CORE Guideline with the submission of the missing data.

Our evaluation of the statistical analyses verified sponsor's values for mean and standard deviations.

2. Evaluation of Data

From the limited data presented in TABLE I we conclude that the tentative overall NOEL in this study is 10 ppm over the three generation period. The finding is based on significantly reduced weaning weights in pups on the 60 and 125 ppm dose levels in each of the initial and succeeding generations. The final weaning weight of pups in the 10 ppm F_{1b} litter was significantly reduced below that of the between-treatment control group; however, the mean value for the within-treatment control group was considerably higher than those for all other control groups (55.4 Gm vs 44.2 - 49.1 Gm.), and the 10 ppm value was not materially different from the other 10 ppm groups (ca.49 Gm.). Therefore, we do not consider this deviation to be treatment-related.

CORE RATING:

Supplemental - repairable to Guideline by submission of microscopic exam data.

TABLE I

INDICES OF REPRODUCTION IN THREE GENERATIONS OF RATS FED DAC-3701 IN THE DIET

Litter #	Diet level PPM	Fertility Index (a)	Gestation Index (b)	Viability Index (c)	Lactation Index (d)	Birth Wt. Grams	Weaning Wt. 21 da. Gm.
F_{1a}	0.0	86.7 %	98.6 %	97.2 %	100.0 %	6.2	44.2
	10	73.9	96.8	98.6	98.1	6.3	42.3
	60	85.2	97.6	97.8	100.0	6.5	36.9*
	125	76.9	97.5	97.8	95.5	6.1	31.9*
F_{1b}	0.0	83.3	95.8	98.4	100.0	6.5	55.4
	10	69.2	97.9	95.7	100.0	6.6	49.6*
	60	81.5	97.7	98.0	99.5	6.2	45.2*
	125	65.4	97.7	97.6	98.6	6.2	36.6*
F_{2a}	0.0	96.2	95.1	96.6	99.6	6.3	49.1
	10	95.8	97.9	97.3	99.5	6.4	48.4
	60	96.6	97.4	94.3	98.8	6.3	44.2*
	125	72.7	100.0	98.1	99.3	6.0	33.8*
F_{2b}	0.0	88.9	98.6	97.2	99.5	6.2	48.6
	10	78.3	99.6	96.5	99.4	6.4	48.5
	60	89.7	99.4	99.1	100.0	6.2	43.1*
	125	72.2	100.0	94.4	100.0	6.0	33.6*
F_{3a}	0.0	88.0	97.2	98.0	99.5	6.2	46.6
	10	86.4	99.1	94.9	99.4	6.5	45.8
	60	89.3	95.3	92.0	100.0	6.3	42.9*
	125	90.9	94.2	96.9	99.3	6.0	37.3*
F_{3b}	0.0	92.0	96.1	98.6	100.0	6.1	48.5
	10	66.7	95.0	97.4	99.1	6.4	46.6
	60	84.6	92.1	96.2	100.0	6.0	43.6*
	125	77.8	95.2	98.7	100.0	5.9	35.9*

(a) - Number pregnant/number mated X 100 (b) - Number alive/number born X 100
(c) - Number alive @ 4 days/# born alive (pre-cull) (d) - % weaned (post-cull)

* Statistically significant difference from controls

DATA EVALUATION REPORT

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CHLOROTHALONIL

STUDY: One Generation Rat Reproduction Study

LABORATORY: TR Evans

STUDY NUMBER: 81-0193

ACCESSION NUMBER: 071525

MRID:

MATERIAL TESTED: DAC-3701 (100%)

ANIMALS: Spraque-Dawley CD rats (12 males and 24 females per dose level).

DOSE LEVELS: 0, 10, 20, 30, 60 and 120 ppm in diet.

METHODS: F_0 --------> F1A ------> necropsy at weaning

2 week rest period

F1B --------> necropsy at weaning

Day 0 = Sperm or Plug. Dams to littering cages on day 18.

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Observations:

For mortality and gross effects: twice daily.

Complete physical Examination: weekly.

Body weights & Food consumption (F_0's) Initially and weekly thereafter.

Body weights (F_0's) Day 0, 6, 15 and 20 pre-partum and on days 0, 4, 14 and 21 during lactation.

Pups: culled to 10 pups/litter on day 4; litter weights on days 0, 4, 7, 10, 14, and 21.

Post Mortem:

All animals except those stillborn and those dead before day 4.

Adult F_0 males - testes and epididymes preserved in 10% buffered formalin.

Pups - all abnomalities recorded and preserved in 10% buffered formalin.

$F1_b$ pup - 5/sex/group necropsied and tissues preserved in 10% buffered formalin.

No histopathological examinations done.

Dietary Analysis:

For content on 5 samples obtained at random from 10 ppm and 120 ppm diets.

Stability tested at 7 and 14 days.

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RESULTS:

Mortality and Gross effects - Adults

No treatment related effects in either males or females.

Food consumption & Body weights - No treatment related effects in adults.

Post Mortem:

Adult Males - No treatment related effects.

Adult Females - No treatment related effects.

F_{1a} Pups - No treatment related effects.

F_{1b} Pups - No treatment related effects.

Dietary Analysis

Within acceptable limits.

Discussion and Conclusions:

The only evidence of toxicity reported in this study consists of significantly reduced Viability Indices in the 120 ppm F_{1a} and F_{1b} litters; reduced weaning body weights in the 120 ppm F_{1a} and F_{1b} litters, and in the 60 ppm F_{1b} litters.

The Gestation Indices for the 10 ppm and 30 ppm F_{1a} litters are not considered to be treatment-related since there is no dose-response pattern evident in this parameter. Overall, we conclude that the NOEL for this study is 30 ppm based on reduce weanling weights at the 60 and 120 ppm levels in the F_{1b} litters.

CORE RATING:

Overall, the test procedure follows that of the OECD for a single generation study and therefore we conclude that that it is a scientifically valid One-Generation study. However, our regulations require a two generation study in support of tolerances. Therefore the study does not satisfy our regulatory requirement for a two generation study.

We rate this study as SUPPLEMENTAL. There is no such CORE grade as Supplemental

Should be minimum or better. The CORE grade cannot be based on the EPA regulatory requirements but rather on the scientific quality.
3/26/84 Rbolick

TABLE I

DAC 3701 - Single Generation Rat Dietary Reproduction Study

Litter	Diet Level (ppm)	Fertility Index (a)	Gestations Index (b)	Viability Index (c)	Lactation Index (d)	Birth wt. gm	Weaning wt. gm
F_{1a}	0	73.3	99.1	100.0	98.9	6.6	52.6
	10	81.0	90.7*	98.4	99.4	6.6	51.1
	20	87.5	96.2	96.8	100.0	6.6	49.7
	30	83.3	93.6*	98.6	100.0	6.7	51.5
	60	76.2	97.5	99.5	99.4	6.7	47.1
	120	88.2	95.8	86.9*	97.3	6.6	42.0**
F_{1b}	0	69.2	91.3	100.0	100.0	6.7	55.3
	10	85.7	92.5	100.0	100.0	6.7	52.0
	20	70.8	96.9*	98.9	100.0	6.8	54.6
	30	82.4	96.6	98.9	99.2	6.5	52.2
	60	76.5	97.0*	100.0	100.0	6.5	48.1*
	120	76.5	97.7*	98.0	98.0	6.6	44.3*

a - # pregnant/# mated

b - # alive/# born

c - #alive @ 4 days/# born alive (precall)

d - % weaned (post call)

* Significant difference

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DATA EVALUATION REPORT

STUDY: Chronic Mouse Dietary Study

LABORATORY: T.R. Evans Research Center. Painesville, OH

STUDY NUMBER & DATE: 098-5TX-78-0024-001 2/17/82

ACCESSION NUMBER: 071531

MRID:

MATERIAL TESTED: DS-3701 (4-hydroxy metabolite) 99.6% pure.

ANIMALS: CD-1 mice

METHODS:

ENVIRONMENTAL PARAMETERS: Standard GLP

HUSBANDRY: Standard GLP

ROUTE OF ADMINSTRATION: Dietary. Prepared fresh weekly. Analyzed for DS-3701.

LEVELS OFFERED: 0, 375, 750 and 1500 ppm.

SCHEME OF ADMINSTRATION:

Group	Dose (ppm)	No. Males	No. Females
I	0	60	60
II	375	60	60
III	750	60	60
IV	1500	60	60

OBSERVATIONS:

- 2X daily for mortality and signs of toxicity. Detailed physical examination weekly.
- Food consumption and body weights obtained weekly through week 14; then biweekly through week 26. Monthly thereafter.
- Diets were prepared fresh weekly. Samples were then obtained for subsequent analysis for test material.

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BIOLIGICAL MEASUREMENTS:

Hematology at 12 and 18 months and at termination:

- Hemoglobin
- Hematocrit
- Total RBCs and WBCs
- Differentials

Added at termination: Bone marrow differentials and reticulocytes.

POST MORTEM EXAMINATION:

- All animals dead or moribund during the study were necropsied. All surviving males were terminated at 24 months. All surviving females in the low and middle doses and 10 control females were necropsied at 20 months. Surviving females in the high dose and all remaining control females were necropsied at 22 months.

- The following organs and tissues were preserved, prepared and examined histologically:

Brain *	Thyroid Gl.	Parathyroid Gl.
Trachea	Esophagus	Lungs
Heart *	Pancreas	Salivary Gl.
Stomach	Duodenum	Ileum
Colon	Liver *	Gallbladder
Spleen *	Kidney *	Gonads *
Epididymus	Prostate	Seminal Vesicle
Uterus	Vagina	Eye
Pituitary	Ovary	Lymph nodess
Spinal Cord	Adrenal Gl.	Ureter
Urethra	Aorta	Muscle
Thymus	Peripheral Nerve	Skin
Mammary Gl.	Bone & Marrow	

* organ weights obtained

NOTE:

We combined all reported neoplasms, both malignant and benign, to construct incidence tables for the lungs, the liver, the kidneys and miscellaneous organs and tissues. Every animal bearing one or more neoplasms was counted as one "hit"; thus, an animal with more than one neoplasm counted only once. Table I shows the distribution of neoplastic response in this assay.

RESULTS:

OBSERVATIONS:

- Body weights were significantly lower overall for the high dose males and females.
- Food consumption in the low and middles dose groups was about the same as that for the controls. Food consumption in the high dose male and female groups was increased significantly.
- Actual Dietary Assay

Group	Nominal Concentration (ppm)	Mean (ppm for all analyses)
I	0	0
II	375	384
III	750	780
IV	1500	1552

BIOLOGICAL MEASUREMENTS:

Hematology

Hematological evaluation showed reduced RBCs at twelve months in low and high dose males and in all female treatment groups. At eighteen months RBCs were significantly reduced in the middle dose males and in the middle and high dose females. WBCs were significantly increased in the high dose terminal (20 months) females.

Hemoglobin and hematocrit were not remarkable for toxic effect of DS-3701.

There was a reduction in RBCs at termination (24 months) in the high dose males. WBCs were significantly increased in the middle dose terminal males.

Bone and bone marrow values were not conclusively affected, nor were the total and differential leukocyte counts at the 12 and 18 month intervals or at termination.

POST MORTEM EXAMINATION:

Body Weights and Organ Weights

Liver-to-body weight ratios were significantly decreased in the 750 ppm females sacrificed at 20 months and were significantly increased in the 1500 ppm females scarificed at 22 months. Liver-to-body weight ratios were significantly increased in all treated group males sacrificed at 24 months.

Spleen-to-body weight ratios were significantly increased in the 750 ppm females sacrificed at 20 months.

Brain-to-body weight ratios were slightly, but significantly, increased in the 500 ppm females sacrificed at 22 months.

DISCUSSION:

Hematology

Overall, apart from the finding that there were increased RBC values in treated female mice at all dose levels at the 12 month test period, we consider that variations noted in the hematological parameters studies were within normal limits. For the twelve month female RBCs, this was confirmed as a treatment-related effect in the 18 month female which showed significant reductions in the middle and high dose treatment groups. Therefore, the observed NOEL for this parameter is less than 375 ppm in the diet.

Organ and Body Weights

We consider the finding of reduced liver-to-body weight ratios in all treatment group males to be compound related; therefore, the NOEL for this parameter is less than 375 ppm in the diet.

Histopathology

Table I summarizes Toxicology Branch's evaluation of the neoplasms reported in the individual animal necropsy reports. The great majority of neoplasms were confined to the lungs, liver and kidney and were composed of benign (adenoma and hepatoma) and carcinogenic (lymphosarcoma; hepatocellular carcinomas, etc.) tumors. All benign and malignant neoplasms were considered to be tumors within the definition of the Science Advisory Panel (SAP): "...With regard to lung tumors in CD-1 mice, the Panel agrees that the data for adenomas and carcinomas should be combined..." (Gray, 1983).

With the exception of the low dose males, dietary challenge with DS-3701 in CD-1 mice resulted in decreasing overall tumor incidence with increasing dose. The difference in tumor incidence between the control males and the low dose males is not considered to be significant when taking into acount the lower tumor incidences in the middle and high dose groups.

Examination of the tumor response of individual animals failed to reveal a dose-related effect of DS-3701 on specific carcinogenic lesions. In males the incidence of benign vs. malignant lesions was roughly 1:1 in control and treatment group males in the lungs and liver. In the females the benign lesions occured more frequently in those dose groups where malignant lesions were noted.

There was no dose-related occurrence of animals bearing more than two specific lesions in either males or females at any dose tested.

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CONCLUSIONS:

The systemic NOEL for this study is less than 375 ppm based on reduced liver-to-body weight ratios in males.

Overall, we conclude that DS-3701 does not induce tumors in CD-1 male or female mice when offered in the diet for the lifetime of the animal at levels up to 1500 ppm.

CORE RATING:

1. For systemic effect: Supplemental - no NOEL demonstrated.

2. For tumorigen.. response: Guideline.

must be some other reason than the one given to grade this study Supplementary I say it is minimum 3/26/84 Kokely

no such CORE grade is in existence

TABLE I

Combined Neoplasms per Sex per Dose Level

FEMALES

Site of Neoplasm	0 ppm	Incidence	375 ppm	Incidence	750 ppm	Incidence	1500 ppm	Incidence
Liver	2(44)*	4.5 %	2(57)	3.5 %	0(48)	0.0 %	0(56)	0.0 %
Lungs	9(59)	15.3 %	7(58)	12.1 %	2(57)	3.5 %	2(59)	3.4 %
Kidney	1(44)	2.3 %	0(59)	0.0 %	0(52)	0.0 %	0(58)	0.0 %
Other	1(43)	2.3 &	2(59)	3.3 %	5(57)	8.8 %	1(58)	1.7 %
Total Lesions	13(59**	22.0 %	11(59)	18.6 %	7(57)	13.3 %	3(59)	5.1 %

MALES

Liver	19(58)	32.8 %	15(57)	26.3 %	10(52)	19.2 %	4(54)	7.4 %
Lung	4(58)	6.9 %	11(58)	19.0 %	6(57)	10.5 %	9(57)	15.9 %
Kidney	1(58)	1.7 %	1(57)	1.8 %	2(54)	3.7 %	0(57)	0.0 %
Other	1(58)	1.7 %	1(57)	1.8 %	3(57)	5.3 %	2(57)	3.5 %
Total Lesions	25(58)	43.1 %	28(58)	48.3 %	21(57)	36.8 %	15(57)	26.3 %

* Figures in parentheses represent that number of organs actually examined and reported on the individual necropsy reports.

** Figures in parentheses in Total Lesions line are the maximum number of animals examined.

DATA EVALUATION REPORT

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STUDY: 90 Day Rat Feeding Study

LABORATORY: TR Evans Res Centre

STUDY NUMBER: 5TX-80-0200 (10-19-81)

ACCESSION NUMBER: 071535

MATERIAL TESTED: Tech CTN (DS-2787; 98% pure with [redacted] HCB)

ANIMALS: Charles River, CD 20/sex/dose level

0, 40, 80, 175, 375, 750, 1500 mg/kg/day, equivalent to 800, 1600, 3500, 7500, 15,000 and 30,000 ppm

METHODS:

Environmental Parameters - Standard GLP

Husbandry - Standard GLP

Feed and Water - ad libitum.

Observations:

For mortality and gross effects: twice daily.

Physical examination, body weights and food consumption were recorded one week prior to initiation of feeding and at weekly intervals thereafter.

Hematology and Clinical Chemistry was performed one week pre-test; at 30 days and at termination. Urinalysis was performed at 30 days and at termination.

Hematology:

Hb, HCT, RBC, MCH, MCV, MCHC, WBC, including platelets and lymph/seg ratios.

Clinical Chemistry:

AlkP, BUN, LDH, SGPT, SGOT, Glucose, Total Protein, Alb, Glob and A/G ratio;

Ca^{++}, Na^{+} K^{+}, Cl - Creatinine, Bilirubin, Total Cholesterol and T_3 and T_4.

INERT INGREDIENT INFORMATION IS NOT INCLUDED

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Urinalysis:

Color, Volume, Appearance and Sp. Gr.; Occult blood, protein, pH, bilirubin, ketones, glucose, urobilinogen, sediment and osmolality.

Termination:

Necropsy was performed on all animals:

1. Moribund

2. All suvivors were killed by ether and exsanguination.

RESULTS:[1]

Survival was comparable among all groups. Indication of cathartic action related to compound ingestion was evident in both sexes at 750 and 1500 mg/kg/day. Evidence of soft stools, reduced fecal output, mucus in stools, swelling and irritation of the anus occurred with greater frequency and severity in the two highest dose groups. There were significant dose related body weight reductions in both sexes at dose levels of 375 mg/kg/day and greater. Food consumption comparisons indicated compound related increases throughout the study. There were significant but spurious increases in hemoglobin, hematocrit and erythrocyte counts in males, which are not considered compound related. Mean corpuscular volume, mean corpuscular hemoglobin and mean corpuscular hemoglobin concentration were unaffected by treatment. RBC morphology was normal in all groups. There were compound related decreases in glucose levels at $\geq$ 375 and $\geq$ 750 mg/kg in males and females, respectively. There were similar reductions in BUN at $\geq$ 80 and $\geq$ 375 for males and females, respectively. All other clinical chemistry determinations were normal except for depressed SGPT activity, present in both sexes in all treatment groups. This effect was generally dose related. A special evaluation of serum thyroxine (T-4) and triiodothyronine (T-3) demonstrated depressed T-4 levels at $\geq$ 175 and 1500 mg/kg in males and females, respectively. This depression correlates with the decreased rate of body weight gain at those levels.

There were dose related increases in specific gravity and decreased urine volume for males given $\geq$ 375 mg/kg of chlorothalonil. There was also an increased incidence of dark urine as dose increased from 375 to 1500 mg/kg. There were no similar findings in females.

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[1] Review by R. B. Jaeger for FAO/WHO Joint Committee of Pesticide Residues, Geneva, December 13 - 18, 1983.

Although several absolute and relative organ weight changes were determined, only the kidney weight changes are considered compound related. Relative kidney weights were increased in both sexes at all treatment levels, but gross and histopathological evaluations revealed no correlative compound related effects. Gross necropsy findings were unremarkable among all groups. The only dose related histologic effect of treatment, which was inversely related to dose, was a finding of acute gastritis in the non-glandular portion of the stomach in all treatment groups. Based upon the relative kidney weight changes at all levels with compound related effects on specific gravity and urine volume at $\geq$ 375 mg/kg a clear no adverse effect level has not been demonstrated. The depressed SGPT activity at all treatment levels in both sexes, considered compound related, is difficult to interpret particularly since relative liver weights were increased at $\geq$ 750 mg/kg for both sexes.

NOEL < 40 mg/kg/day

CORE RATING:

Supplemental. A clearcut no effect level was not demonstrated.
Not repairable.

There must be some other reason that the one given to grade this study supplementary. I say it is minimum. Also, there is no such CORE grade as supplemental

3/26/84 Abbotsky

DATA EVALUATION REPORT

STUDY: Rat Two Year Dietary Exposure[1]

LABORATORY: International Research and Development Corporation, Mattawn, MI.

STUDY NUMBER & DATE: DTX-80-0016

ACCESSION NUMBER: 071527

MRID:

MATERIAL TESTED: DS 3701 (100%)

ANIMALS: Charles River CD Rats

METHODS:

ENVIRONMENTAL PARAMETERS: Standard GLP

HUSBANDRY: Standard GLP

"Groups of Sprague-Dawley CD rats (75 males and 75 females/group) were administered 4-hydroxy-2,5,6-trichloroisophthalonitrile in the diet at dosage levels of 0, 0.5 and 3 mg/kg/day for 104 weeks." [Additional] "dosage levels of 15 and 30 mg/kg/day were reduced at week 30 to 10 and 20 mg/kg/day, respectively, because of poor survival and anemia. Animals were observed daily for mortality and gross signs of toxicity/general appearance. Individual body weights and food consumption were measured regularly during the study. Clinical laboratory studies were performed periodically throughout the study on 10 rats/sex/group at six month intervals. Ophthalmological examinations and urinalyses were performed routinely, and feces were collected and examined to evaluate the observed anemia. Interim sacrifices were performed after 1 year on 10 rats/sex in all groups except for the high dose animals which were all necropsied. Terminal necropsies were performed on all surviving animals after 2 years, selected organs weighed, and complete histopathological examinations conducted.

RESULTS:

Pale skin and eyes were evident for the first 30 weeks in high dose males and females with similar but less marked findings in the 15 mg/kg group. Mortality was significantly increased in the 30 mg/kg group males and females, and in the 15 mg/kg group females. The high dose group was sacrificed at 12 months after the dose level had been reduced to 20 mg/kg at week 30. Decreasing the 15 mg/kg/day dose level at week 30 to 10 mg/kg similarly improved the survivability, which was comparable to controls for the remainder of the study. Body weight was !

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1 From: Jaeger, R.B., et al. WHO/FAO Report, 1983, Geneva.

reduced in the 10/15 and 20/30 mg/kg males and females throughout the study, even after reduction of doses. Food consumption was unremarkable except for decreases in 10/15 and 20/30 mg/kg females and 20/30 mg/kg males, consistent with decreased body weights and increased mortality during the first 30 weeks. There were similar decreases in total serum protein, albumin, globulin, and cholesterol in 20/30 mg/kg males and females and 10/15 mg/kg females after 6 months. These returned to control levels for the remainder of the study, after doses were reduced to 20 and 10 mg/kg, respectively.

There were significant hemopoietic effects in the 10/15 and 20/30 mg/kg animals, particularly females, during the first 6 months. Evidence of microcytic anemia was provided by reduced RBC counts, hematocrit, hemoglobin, MCV, and MCH with accompanying increases in MCHC, reticulocytes and metarubricytes. Segmented neutrophiles were increased with corresponding decrease in percentage of lymphocytes. Specially stained bone marrow presented evidence of hypocellularity. Mallory's stain of liver tissue revealed an increased iron content (hemosiderin). After 18 and 24 months exposure the 10/15 mg/kg group females continued to present evidence of anemia (decreased Hct, Hgb, MCV, MCH and increased MCHC) with a positive bone marrow response (increased cellularity with a shift to increasing number of immature erythyroid cell types and increase number animals with a 1:1 M/E ratio). Prussian Blue staining demonstrated the presence of hemosiderin in the 10 mg/kg males and females, not considered significant at 3 mg/kg. After 24 months exposure there were decreased serum potassium levels in all dosed females. Urinalyses and examination for fecal occult blood were unremarkable, except for increased urine volume at 6 months in the high dose group animals.

Ophthalmological examination at 6 months revealed increased pale ocular structures and spontaneous hemorrhage in high dose male and female animals. At 24 months there were increased numbers of dilated pupils (not responding to light) and increased bilateral cataract disease in high dose males.

Comparison of selected organ weights demonstrated decreased absolute organ weights for kidney, heart and brain in high dose males with no significant relative organ-to-body weight changes. High dose females had decreased absolute kidney and heart weights with no relative weight changes except for spleen and brain. Microscopic examination failed to confirm any compound related effects on these organs. There were no significant compound related non-neoplastic organ changes except for hemosiderin in the liver of high dose females and hemorrhage in CNS tissues, hypocellular bone marrow and post-mortem congestion of lymph in high dose male and female rats.

Examination of tissues/organs for neoplastic changes did not indicate any compound related effects at any level tested.

CONCLUSIONS:

Data presented in this study demonstrate that the metabolite, 4-hydroxy-2,5,6-trichloroisophthalonitrile, is without adverse effects on male and female rats at levels up to and including 3 mg/kg/day for 2 years (McGee et al., 1983)."

CORE RATING:

Guideline.

DATA EVALUATION REPORT

STUDY: 13 Week Rat Feeding Study

LABORATORY: Huntington Research Centre

STUDY NUMBER & DATE: 5 TX-81-0213

ACCESSION NUMBER: 071537

MRID:

MATERIAL TESTED: T-117-11 (Technical Chlorothalonil)

ANIMALS: Charles River (CD) Rats

METHODS:

ROUTE OF ADMINSTRATION: In the diet.

SCHEME OF ADMINSTRATION AND LEVELS OFFERED:

Groups of 25 male and 25 females were offered diets containing 0, 1.5, 3.0, 10.0 or 40 mg/kg/day for 13 weeks following a two week acclimatization period. Following termination 10 animals per group were killed and subject to complete necropsy. Remaining animals were placed an untreated rations for an additional thirteen weeks, then killed and subjected to complete necropsy.

ENVIRONMENTAL PARAMETERS:

HUSBANDRY:

Feed and tap water ad lib; animals housed individually. Feed containing test material was analyzed periodically to insure homogeneity.

OBSERVATIONS:

Animals were checked daily for signs of toxication and ill health.
Animals were checked twice daily for mortality and morbidity.
Animals were palpated weekly for masses.
Animals found in extremis were killed and autopsied.

Food and water consumption and body weights were recorded one week prior to initiation of feeding and weekly thereafter.

BIOLIGICAL MEASUREMENTS:

10 rats of each sex were selected prior to intitiation of feeding for hematology and clinical chemistry evaluation and urinalysis in order to establish base-line values. 10 rats per sex per group were selected at weeks 6, 13, 19 and 26 for hematology and clinical chemistry evaluation during the study. The following parameters were measured:

Hematology

PCV, Hb.,RBCs, MCHC, MCV, MCH, reticulocytes, White cells and differentials, platelets and prothrombin times.

Clinical Chemistry

Glucose, Alk.P.,SGPT, SGOT, LDH, BUN, Total Protein, Albumin, Globulin, A/G ratios, Na^+, K^+, Cl^- Ca^{++}, P^{---}, Cholesterol, Bilirubin

Urinalysis

Color, Appearance, Volume, pH, Sp. Gr., Protein, Reducing agents, Glucose, Ketones, Bilirubin, Urobilinogen, Blood, Nitrites, Urine Creatinime and formed elements.

Urine concentrating test

Five of the ten rats used in the laboratory studies above were placed in urine cages and deprived of water for 8 hours. The other five were water loaded by oral intubation of 20 ml/kg and placed in the urine cages with ad lib access to water. The osmolality and volume of urine excreted were measured at 1, 2, 4 and 8 hours. At weeks 12, 18 and 25 the interval at 1.5 hours was also evaluated.

Analysis of Gut Flora

Fecal samples were collected once during the acclimatization period and at 6, 13, 19 and 26 weeks on five animals per sex per group and analyzed for E. coli, C. welchii, S. feacalis, Lactobacillus sp., Bacteriodes sp.an yeasts and molds.

POST MORTEM EXAMINATION:

All animals dead and moribund were necropsied. Of the surviving animals 5 rats per sex per group were killed and necropsied at 7 weeks and subjected to whole body perfusion fixation. Tissues and organ weights were reserved as noted below.

Ten animals per sex per group were killed and necropsied at 13 weeks. They were prepared as above for tissue examination. Organ weights were obtained.

The remaining ten animals per sex per group were killed at 26 weeks, following a 13 week recovery period, and were treated similarly.

The following tissues were prepared for microscopic examination from all animals:

Adrenals	Aorta	Bone Marrow
Bone	Brain *	Epididymes
Eyes	Gonads *	Stomach and Gut
Heart *	Kidneys *	Liver *
Lungs	Lymph Nodes	Esophagus
Pancreas	Peripheral Nerv	Pituitary
Prostate	Salivary Gland	Seminal Vesicles
Muscle	Skin	Skull
Spinal Cord	Spleen	Thymus
Thyroid & Parathyroid	Tongue	Trachea
Bladder	Uterus	Gross Lesions

(*) organs weighed

All tissues were prepared for routine light microscopic examination. In addition, the kidneys and any stomach lesions were prepared for electron microscopy.

Standard Statistical analyses were used to collate the data.

RESULTS:

HUSBANDRY AND MORTALITY

The investigators reported no adverse overt signs of reaction to treatment.

Mortality was confined to three animals that died of causes unrelated to compound ingestion.

OBSERVATIONS:

Statistically significant variations in food and water consumption were of a random nature. No significant effect on body weights were reported at any test level. Actual intake of test material approximated the intended intake for all test levels.

Hematology - normal throughout in all treatment and control groups.

Serum Chemistry - Reduced alkaline phosphatase and GPT values in both sexes in the 10 and 40 mg/kg/day groups.

Urinalysis - negative for effect.

POST MORTEM EXAMINATION

Gross necropsy - increased kidney-to-body weight ratios in the males and females on 3, 10 and 40 mg/kg/day and increased liver ratios in males on 40 mg/kg/day.

Microscopic examination - increased incidence of epitheliar hyperplasia and hyperkeratosis in the non-glandular layers of the stomach in both sexes offered 10 and 40 mg/kg/day CTN.

Increased incidence of dilated renal (medullary) tubules at 10 and 40 mg/kg/day in both sexes.

After a 13 week recovery period these effects were not seen except for persistent cortical mononuclear cells in the treated males.

No histopathological lesions were reported that could explain the increased organ weight ratios noted above.

CONCLUSIONS:

We conclude that CTN had no adverse effects on Sprague-Dawley rats at dietary exposure levels of up to and including 3 mg/kg/day for 13 weeks, based on histopathological effects on the kidneys and stomach.

CORE RATING:

Guideline.

DATA EVALUATION REPORT

STUDY: Micronucleus Test in the Rat

LABORATORY: Laboratoire d'Histopathologique et de Cytopharmacologie, Paris

STUDY NUMBER & DATE: 000-5TX-81-0024-000 (#576) 11-3-81

ACCESSION NUMBER: 071539

MRID:

MATERIAL TESTED: DS-2787 (technical chlorothalonil)

ANIMALS: Wistar Rat, 10 males per test dose.

METHODS:

Unfasted animals were dosed once by gavage with test material or positive control agen suspended in 0.5% methocel (carboxymethyl cellulose) suspending medium. 24 hours later the dose was repeated. Six hours later the animals were killed and the femur bone marrow was removed and processed for examination of "polychromatophilic erythrocytes" (PEs) bearing micronuclei.

LEVELS ADMINSTERED:

0, 8, 40, 200, 1000, 5000 mg/kg.

Positive control material was methyl methanesulfonate (MMS) at 65 mg/kg.

OBSERVATIONS:

Microscopic examination of the prepared smears for the presence of erythrocytes containing micronulei was done. The percentage of cells bearing such nuclei was determined.

RESULTS:

All test dose animals showed no statistically significant differences ($p < 0.05$ from the vehicle controls in the percent of PE cells bearing micronuclei. Positive control animals showed a significant increase in the percentage of PEs bearing micronucei over those of the vehicle control and treated animals.

CONCLUSIONS:

DS-2787 does not induce significantly increased incidences of micronuclei in rat bone marrow erythrocytes at levels up to and including 5000 mg/kg given twice at 24 hour intervals.

DATA EVALUATION REPORT

STUDY: Micronucleus Test in the Mouse

LABORATORY: Laboratoire d'Histopathologique et de Cytopharmacologie, Paris

STUDY NUMBER & DATE: 000-5TX-81-0024-000 (#505) 5-12-81

ACCESSION NUMBER: 071539

MRID:

MATERIAL TESTED: DS-2787 (technical chlorothalonil)

ANIMALS: Swiss CFLP Mouse, 10 males per test dose.

METHODS:

Unfasted animals were dosed once by gavage with test material or positive control agent suspended in 0.5% methocel (carboxymethyl cellulose) suspending medium. 24 hours later the dose was repeated. Six hours later the animals were killed and the femur bone marrow was removed and processed for examination of "polychromatophilic erythrocytes" (PEs) bearing micronuclei.

LEVELS ADMINSTERED:

0, 4, 20, 100, 500, 2500 mg/kg.

Positive control material was methyl methanesulfonate (MMS) at 65 mg/kg.

OBSERVATIONS:

Microscopic examination of the prepared smears for the presence of erythrocytes containing micronulei was done. The percentage of cells bearing such nuclei was determined (see Table I attached).

RESULTS:

All test dose animals showed no statistically significant differences ($p < 0.05$) from the vehicle controls in the percent of PE cells bearing micronuclei. Positive control animals showed a significant increase in the percentage of PEs bearing micronucei over those of the vehicle control and treated animals.

NCLUSIONS:

-2787 does not induce significantly increased incidences of micronuclei in mouse ne marrow erythrocytes at levels up to and including 5000 mg/kg given twice at hour intervals.

TABLE I

fects of Oral Adminstration of DS-2787 on Mouse Polychromatophilic Erythrocytes

DOSE mg/kg	NUMBER OF RATS	PERCENT OF PEs WITH MICRONUCLEI
Vehicle Control	10	0.24 (+/- 0.09)
2 x 4	10	0.25 (+/- 0.06)
2 x 20	10	0.18 (+/- 0.06)
2 x 100	10	0.12 (+/- 0.05)
2 x 500	9	0.21 (+/- 0.05)
2 x 2500	9	0.35 (+/- 0.10)
MS 2 x 65	10	1.36 (+/- 0.24)

DATA EVALUATION REPORT

STUDY: Micronucleus Test in the Chinese Hamster

LABORATORY: Laboratoire d'Histopathologique et de Cytopharmacologie, Paris

STUDY NUMBER & DATE: 000-5TX-81-0024-000 (#591) 12/22/81

ACCESSION NUMBER: 071539

MRID:

MATERIAL TESTED: DS-2787 (technical chlorothalonil)

ANIMALS: Chinese Hamster, 10 males per test dose.

METHODS:

Unfasted animals were dosed once by gavage with test material or positive control agent suspended in 0.5% methocel (carboxymethyl cellulose) suspending medium. 24 hours later the dose was repeated. Six hours later the animals were killed and the femur bone marrow was removed and processed for examination of "polychromatophilic erythrocytes" (PEs) bearing micronuclei.

LEVELS ADMINSTERED:

0, 8, 40, 200, 1000, 5000 mg/kg.

Positive control material was methyl methanesulfonate (MMS) at 65 mg/kg.

OBSERVATIONS:

Microscopic examination of the prepared smears for the presence of erythrocytes containing micronulei was done. The percentage of cells bearing such nuclei was determined (see Table I attached).

RESULTS:

All test dose animals showed no statistically significant differences ($p < 0.05$) from the vehicle controls in the percent of PE cells bearing micronuclei. Positive control animals showed a significant increase in the percentage of PEs bearing micronuclei over those of the vehicle control and treated animals.

CONCLUSIONS:

DS-2787 does not induce significantly increased incidences of micronuclei in Chinese Hamster bone marrow erythrocytes at levels up to and including 5000 mg/kg given twice at 24 hour intervals.

TABLE I

DOSE mg/kg	NUMBER OF RATS	PERCENT OF PEs WITH MICRONUCLEI
Vehicle Control	10	0.19 (+/- 0.05)
2 x 8	10	0.09 (+/- 0.05)
2 x 40	10	0.14 (+/- 0.03)
2 x 200	9	0.13 (+/- 0.05)
2 x 1000	9	0.12 (+/- 0.05)
2 x 5000	10	0.12 (+/- 0.06)
MMS 2 x 65	10	4.08 (+/- 0.80)

003725

DATA EVALUATION REPORT

STUDY: Chromosomal Aberration Assay in the Rat

LABORATORY: Laboratoire d'Histopathologique et de Cytopharmacologie, Paris

STUDY NUMBER & DATE: 000-5TX-81-0025-000 (#590) 5-12-81

ACCESSION NUMBER: 071539

MRID:

MATERIAL TESTED: DS-2787 (technical chlorothalonil)

ANIMALS: Wistar Rat, 10 males per test dose.

METHODS:

Unfasted animals were gavaged twice at 24 hour intervals with vehicle control (0.5% carboxymethyl cellulose), test material suspended in vehicle, or a positive control consisting of methyl methanesulfonate (MMS) suspended in vehicle. Three hours later animals received an IP injection of Colchicine at 7.5 mg/kg body weight. Following an additional period of three hours, animals were killed and the femur marrow was obtained and prepared for chromosomal examination of active mitotic figures for abnormalities.

LEVELS ADMINISTERED:

0, 3, 40, 200, 1000 and 5000 mg/kg.

Positive Control: MMS at 65 mg/kg.

OBSERVATIONS:

100 marrow cells (total per group = 1000) in active mitosis from each animal were examined microscopically for chromatid breakage. These included observations for the occurance of chromatid and isochromatid gaps, single and multiple chromatid breaks, exchanges and pulverizations.

RESULTS:

The sponsor reported that only chromatid and isochromatid gaps were seen in the vehicle control and the DS-2787 treated rats with the exception that one 40 mg/kg rat also showed one chromatid break. The MMS positive control rats demonstrated a significantly increased incidence of anomalies ($p < 0.05$). See Table I.

CONCLUSIONS:

Under the conditions of this assay DS-2787 at oral administration levels up to 5000 mg/kg does not induce a significant increase in the incidence of bone marrow chromosomal anomalies when compared to the incidence of those induced by MMS at 7.5 mg/kg.

TABLE I

Effect of Oral Adminstration of DS-2787 on Chromosomal Anomalies in the Rat

DOSE mg/kg	NUMBER OF MITOSES	PERCENT OF ANOMOLOUS MITOSES
Vehicle Control	1000	0.20
2 x 8	1000	0.10
2 x 40	1000	0.80
2 x 200	1000	0.40
2 x 1000	1000	0.10
2 x 5000	1000	0.10
MMS 2 x 65	1000	3.60

DATA EVALUATION REPORT

STUDY: Chromosomal Aberration Assay in the Mouse

LABORATORY: Laboratoire d'Histopathologique et de Cytopharmacologie, Paris

STUDY NUMBER & DATE: 000-5TX-81-0025-000 (#542) 7-28-81

ACCESSION NUMBER: 071539

MRID:

MATERIAL TESTED: DS-2787 (technical chlorothalonil)

ANIMALS: Swiss CFLP Mice, 10 males per test dose.

METHODS:

Unfasted animals were gavaged twice at 24 hour intervals with vehicle control (0.5% carboxymethyl cellulose), test material suspended in vehicle, or a positive control consisting of Urethan suspended in vehicle. Three hours later animals received an IP injection of Colchicine at 7.5 mg/kg body weight. Following an additional period of three hours, animals were killed and the femur marrow was obtained and prepared for chromosomal examination of active mitotic figures for abnormalities.

LEVELS ADMINISTERED:

0, 4, 20, 100, 500 and 2500 mg/kg.

Positive Control: Urethan at 2000 mg/kg.

OBSERVATIONS:

100 marrow cells (total per group = 1000) in active mitosis from each animal were examined microscopically for chromatid breakage. These included observations for the occurance of chromatid and isochromatid gaps, single and multiple chromatic breaks, exchanges and pulverizations.

RESULTS:

The sponsor reported that only chromatid and isochromatid gaps were seen in the vehicle control and the DS-2787 treated mice. The Urethan positive control mice demonstrated a significantly increased incidence of anomalies ($p < 0.05$). See Table I.

CONCLUSIONS:

Under the conditions of this assay DS-2787 at oral administration levels up to 2500 mg/kg does not induce a significant increase in the incidence of bone marrow chromosomal anomalies in mice when compared to the incidence of those induced by Urethan at 2000 mg/kg.

TABLE I

Effect of Oral Adminstration of DS-2787 on Chromosomal Anomalies in the Mouse

DOSE mg/kg	NUMBER OF MITOSES	PERCENT OF ANOMOLOUS MITOSES
Vehicle Control	1000	0.70
2 x 4	1000	0.50
2 x 20	1000	0.20
2 x 100	1000	0.30
2 x 500	1000	0.20
2 x 2500	900	0.78
Urethan 2 x 2500	1000	23.40

DATA EVALUATION REPORT

STUDY: Chromosomal Aberration Assay in the Chinese Hamster

LABORATORY: Laboratoire d'Histopathologique et de Cytopharmacologie, Paris

STUDY NUMBER & DATE: 000-5TX-81-0025-000 (#525) 7-2-81

ACCESSION NUMBER: 071539

MRID:

MATERIAL TESTED: DS-2787 (technical chlorothalonil)

ANIMALS: Chinese Hamster, 10 males per test dose.

METHODS:

Unfasted animals were gavaged twice at 24 hour intervals with vehicle control (0.5% carboxymethyl cellulose), test material suspended in vehicle, or a positive control consisting of methyl methanesulfonate (MMS) suspended in vehicle. Three hours later animals received an IP injection of Colchicine at 7.5 mg/kg body weight. Following an additional period of three hours, animals were killed and the femur marrow was obtained and prepared for chromosomal examination of active mitotic figures for abnormalities.

LEVELS ADMINISTERED:

0, 8, 40, 200, 1000 and 5000 mg/kg.

Positive Control: MMS at 65 mg/kg.

OBSERVATIONS:

100 marrow cells (total per group = 1000) in active mitosis from each animal were examined microscopically for chromatid breakage. These included observations for the occurance of chromatid and isochromatid gaps, single and multiple chromatid breaks, exchanges and pulverizations.

RESULTS:

The sponsor reported that only chromatid and isochromatid gaps were seen in the vehicle control and the DS-2787 treated hamsters. The 5000 mg/kg animals showed a significant increase in the incidence of mitotic anomilies consisting of chromatid gaps ($p < 0.05$). The MMS positive control hamsters demonstrated a significantly increased incidence of anomalies ($p < 0.05$) over that of the vehicle controls. See Table I.

CONCLUSIONS:

Under the conditions of this assay, DS-2787 at oral administration levels up to 1000 mg/kg does not induce a significant increase in the incidence of bone marrow chromosomal anomalies when compared to the incidence of those induced by MMS at 65 mg/kg. Oral administration of DS-2787 at 5000 mg/kg produced a significant increase in chromosomal anomalies.

TABLE I

Effect of Oral Adminstration of DS-2787 on
Chromosomal Anomalies in the Chinese Hamster

DOSE mg/kg	NUMBER OF MITOSES	PERCENT OF ANOMOLOUS MITOSES
Vehicle Control	1000	0.30
2 x 8	1000	0.20
2 x 40	1000	0.80
2 x 200	1000	0.33
2 x 1000	1000	0.33
2 x 5000	1000	1.11
MMS 2 x 65	1000	4.30

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003725

DATA EVALUATION REPORT

STUDY: Rabbit Teratology Study

LABORATORY: Institute of Environmental Toxicology (Japanese)

STUDY NUMBER & DATE: 000-5TX-75-2077-001 5-30-75

ACCESSION NUMBER: 071539

MRID:

MATERIAL TESTED: Chlorothalonil 99.3% pure

ANIMALS: Japanese White (Funabashi) rabbits: 8 control, 9 low dose, 9 high dose.

METHODS:

3 month old mated does (day of vaginal sperm or plug = day 0 of gestation) were gavaged once daily with 0, 5 or 50 mg/kg of test material in 5% gum arabic suspending medium on days 6 through 18 of gestation.

Test animals were offered basal Rabbit and Guinea Pig diet and tap water ad libitum.

ENVIRONMENTAL PARAMETERS: Not given.

HUSBANDRY: Not given.

OBSERVATIONS:

Body weight, and mortality were recorded initially, daily from days 6 through 18 and on days 24 and 29 of gestation.

Food consumption was recorded initially and on alternate days thereafter.

On day 29 of gestation the uteruses were emptied and the number of viable pups counted. The number of implantation sites and resorption sites were determined.

Pups were weighed and examined grossly for external and visceral anomalies.

Skeleton staining with alizarin was said to have been done; however, no methodology was described for this.

Pups were examined for the occurence of "lumbar ribs" and ossified caudal vertebrae.

No detailed reports for the individual pups were submitted.

RESULTS:

Body weight gain and food consumption did not appear to be affected by treatment with chlorothalonil although the overall rate-of-weight gain was less for the 50 mg/kg does than for the control does.

Four does in the 50 mg/kg group experienced spontaneous abortion in the final five days of the experiment. Examination for malformation or signs of fetal toxicity was not reported on the aborted embryos.

One 5 mg/kg pup had hydrocephalus and one 50 mg/kg pup had cleft palate.

There was no reported effect on the incidence of lumbar ribs or caudal vertebrae.

CONCLUSIONS:

No obvious teratogenic response was reported at oral levels up to 50 mg/kg/day on days 6 - 18 of gestation.

It is not possible to assess the overall fetotoxic effects in this study since detailed analytical data on the offspring was no provided. This should have included individual pup data, such as body weight and individual observations as to their condition, etc.

CORE Rating: Supplementary.

Repairability: Supply individual data on all pups: examination details of aborted embryos in the 50 mg/kg group.

DATA EVALUATION REPORT

STUDY: Chronic Mouse Feeding Study

LABORATORY: Biodynamics Laboratory, East Millstone, NJ

STUDY NUMBER & DATE: DTX-79-0102

ACCESSION NUMBER: 071541

MRID:

MATERIAL TESTED: Technical Chlorothalonil 97.7%

ANIMALS: CD-1 Mice males and females

METHODS:

ENVIRONMENTAL PARAMETERS: Standard GLP

HUSBANDRY: Standard GLP

ROUTE OF ADMINSTRATION: Dietary, prepared fresh weekly with samples of test material taken for analysis.

LEVELS OFFERED: 0, 750, 1500 and 3000 ppm.

SCHEME OF ADMINISTRATION: 60 mice/sex/group. Control and treated diets offered ad libitum.

OBSERVATIONS: Daily for mortality and gross signs of toxicity. Weekly complete physical exam. Body weight and food consumption - pretest, then weekly through week 14; biweekly on weeks 14 through 25, then monthly thereafter until completion of the experiment.

BIOLIGICAL MEASUREMENTS:

Blood samples were obtained by orbital puncture, 10/sex/group, at 12, 18 and 24 months. Parameters measured were:

Hemoglobin	Hemaotcrit	Red cells
Total leukocytes	Differential leukocytes	Red cell morphology

POST-MORTEM EXAMINATION:

Gross examination was made of all animals dead or dying during the study and on all survivors of the 24 month test period. Survivors were killed by exsanguination under ether anesthesia. The following tissues were reserved in 10% neutral buffered formalin for subsequent histopathological examination (*) organ weights obtained.

Adrenal*	Aorta	Bone
Bone marrow	Brain*	Esophagus
Eyes (in Bouin's Solution)	Gallbladder	Gonads* (in Bouin's Solution)
Heart*	Intestine	Kidneys*
Liver*	Lungs	Lymph nodes
Mammary gland	Pancreas	Nerve
Parathyroid	Pituitary	Preputial Gland
Prostate	Salivary	Seminal vesicle
Skeletal muscle	Skin	Spinal Cord
Spleen*	Stomach	Thymus
Thyroid	Tongue	Trachea
Urinary bladder	Uterus	Gross lesions

RESULTS:

OBSERVATIONS:

Mortality - Average survival in all groups was good except for the 3000 ppm males which showed a decreased average survival time when compared to that of the controls:

AVERAGE DAYS SURVIVAL TABLE

	MALES		FEMALES	
DOSE	DAYS*	FULL SURVIVORS†	DAYS	FULL SURVIVORS
Control	660.6	31/60	585.8	18/60
750 ppm	660.3	25/60	609.0	21/60
1500 ppm	675.1	33/60	624.0	23/60
3000 ppm	610.2	21/60	590.6	19/60

* Average number of days on test per group. Does not included animals dying by accident.

† Numerator = total animals alive at 735 days or more. Denominator = total animals begun on test.

Food consumption and weight gain were comparable among the groups.

Decreased hemoglobin, hematocrit and red cell values were reported in high dose males at 24 months and in high dose females at 12 and 24 months. Hyperplastic bone marrow was reported in all treatment group males and females. Hyperplasia of the splenic red pulp was noted in the male treatment groups. Hemosiderosis was not a prominent finding in this study.

[redacted]

POST MORTEM FINDINGS:

GROSS NECROPSY

Relative spleen weights were reported in the high-dose females; no significant pathology was reported, however. Spleen enlargement occured in the mid- and high-dose males. Ovarian weight ratios were decreased in all treatment groups; no histopathological findings were associated with this, however. The same was true for the relative testes weights in the high-dose males. Kidney weights were significantly increased ($p < 0.01$) in all treatment groups. We consider this finding to be dose-related.

Compound related effects in the kidney were described as renal enlargement, discoloration, surface irregularities, pelvic dilation, cysts, nodules and masses in all treatment groups. No other compound-related effects in other organs or tissues were reported.

MICROSCOPIC EXAMINATION

Stomach

The incidence and severity of hyperplasia and hyperkeratosis of the esophageal squamous mucosa in treated males and females was significant and was considered to be dose-related. This was not seen control animals. There was a significant increase in gastric squamous cell tumors in the 1500 ppm females but this was not dose related. Glandular epithelial tumors were present in the treated groups but not in statistically significant numbers. See Table I.

Kidney

Chronic glomerulonephritis was seen in all groups, but the incidence was not significantly different among them, although it was higher in the 3000 ppm males. Increased tubular degeneration was noted in 750 and 1500 ppm males and in the 1500 ppm females. Increased incidences of cortical cysts were seen in all treated males and in the high dose females.

Adenomas and carcinomas of the cortical tubules were increased in all treatment-group males but not in females. See Table I for the incidence of these lesions. The only neoplasm seen in the females was one renal hemangiosarcoma in a low-dose female.

DISCUSSION[1]:

"Chlorothalonil has presented evidence of nephrotoxicity in earlier studies in rats, mice and dogs, predominantly in males. In an NCI rat study [(NCI, etc)] there was presumptive evidence of adenomas and carcinomas of the renal tubular epithelium. Although primary renal tumors are rare in rodents, there was no positive trend for elicitation of adenomas and carcinomas in the renal cortical tubules of male mice in this study, and therefore the evidence for tumorigenicity of chlorothalonil in the kidney remains elusive. The effects on the kidney, in this study, are nonetheless considered compound related ...".

[redacted]

CONCLUSIONS:

CTN produces evidence of hyperplasia and/or tumorigenesis in the squamous cell and epithelial cell layers of the esophagus and stomach in males and females. In addition, renal neoplasms not seen in control animals were reported in males only; their incidence, although statistically significantly increased over that of the controls, did not appear to be dose-related.

A no-effect level for chronic effects has not been demonstrated in this study.

Overall, we conclude that this study presents evidence that CTN can induce gastric and renal neoplasms in CD-1 mice.

CORE RATING:

For Chronic effects: Supplemental/not repairable.

For Oncogenic effects: Guideline.

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[1] From: Jaeger, R.B., et al. WHO/FAO Report, 1983, Geneva.

[redacted]

TABLE I

NEOPLASMS IN MALE CD-1 MICE FED CHLOROTHALONIL IN THE DIET FOR TWO YEARS

STUDY # DTX-79-0102

KIDNEY

	Control	750 ppm	1500 ppm	3000 ppm
Tubular Adenoma	0/60	3/60	4/60	2/60
Tubular Carcinoma	0/60	3/60	0/60	2/60
Total Neoplasms	0/60	6/60	4/60	4/60

The One-Hit Slope Coefficient $B^{(1)}$ =9 .37 x 10^{-4} mg/kg/day^{-1}

Based on the response of the 750 ppm mice Risk = B x $12.6^{(2)}$ Exposure = 0.0118 mg/kg/day^{-1} x 0.0.01305 mg/kg/day (the TMRC) = 1.54 x 10^{-4}.

GASTRIC

	Control	750 ppm	1500 ppm	3000 ppm
Squamous Carcinoma	0/60	1/60	5/60	2/60
Glandular Carcinoma	0/60	1/60	2/60	0/60
Total Neoplasms	0/60	2/60	7/60	2/60

The One-Hit Slope Coefficient B = 4.06 x 10^{-5} mg/kg/day^{-1}

Based on the Squamous Carcinoma response of the 1500 ppm mice, Risk = B x $12.6^{(2)}$ x Exposure = 5.12 mg/kg/day^{-4} x 0.01305 mg/kg/day (the TMRC) = 5.68 x 10^{-6}.

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(1) Slope Coefficient calculated by Roger Gardner, 1/30/84.

(2) Cube root of the ratio of human body weight to mouse body weight.

NEOPLASMS IN FEMALE CD-1 MICE FED CHLOROTHALOPNIL IN THE DIET FOR TWO YEARS

Study # DTX 79-0102

KIDNEY

No lesions were reported in this organ.

GASTRIC

	Control	750 ppm	1500 ppm	3000 ppm
Squamous Carcinoma	0/60	2/60	6/60	5/59
Glandular Carcinoma	0/60	1/60	1/60	2/59

The One Hit Slope Coefficient $B^{(1)} = 7.09 \times 10^{-5}$ mg/kg/day^{-1} is based on the Squamous Carcinoma response of the 1500 ppm females. Risk = B x $12.6^{(2)}$ x Exposure.

Risk = $8.93 \times 10^{-4} \times 0.01305$ mg/kg/day (the TMRC) = 1.75×10^{-5}.

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(1) B value calculated by Roger Gardner, 1/30/84.

(2) Cube root of the ratio of human body weight to mouse body weight.