

2-19-86



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

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OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

TO: H. Jacoby, PM # 21
Herbicides - Fungicide Branch
Registration Division TS-767C

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

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

PRX 2/19/86

*SDR 2-10-86
H/WB 2/17/86*

Subject: EPA Reg. No. 50534-7 Data Call In Submission. Chlorothalonil Registration Standard; review of data.

Petitioner: SDS Biotech Corp., Painesville, OH.

Caswell #: 215B

Action Requested:

Review of a rat oncogenicity study and other toxicity data on Chlorothalonil.

Recommendation:

The studies have been reviewed (DERs attached). All Registration Standard requirements have been submitted and reviewed with the exception of a 21 dayermal toxicity study final report.

Additional dietary oncogenicity studies in rats and mice are in progress and are due for submission in 1987 - 88.

Additional metabolism data to further define metabolic pathways are in progress.

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Detailed Considerations:

Oncogenicity

Our evaluation of the Rat Oncogenicity Study (# 099-5TX-80-234-008; 5/28/85) has revealed that Chlorothalonil (CTN) produces a dose-dependent increase in renal tubular adenomas and carcinomas in male and female rats, and a statistically significant increase in the incidence of gastric mucosal tumors in the high dose females. Together with evidence of renal tubular tumorigenicity, oncogenicity from the earlier mouse study (108-5TX-79-0192-004; 2/8/79) reviewed under PP # 3F 2875, D. Ritter, 3/21/84, and with a calculated Q_1^* value of 2.4×10^{-2} (Toxicology Branch Risk Assessment, H. Lacayo, 5/17/85), we tentatively conclude that Chlorothalonil is a Class B2 oncogen.

Current HED procedure for evaluating these oncogens requires Peer Review, and a panel has been established for that purpose within Toxicology Branch. Peer Review requires that tumor occurrence data from historical control animals be provided; accordingly, the Petitioner should be asked to submit such information if it is available. In the present instance we need historical control data on renal tubular hyperplasia, adenomas and carcinomas, and on gastric mucosal tumors in Fischer 344 male and female rats from the laboratory which conducted the studies during the same time period.

Mutagenicity

Sixteen new mutagenicity studies using CTN and several of its related congeners were submitted and reviewed by Dr. Chen, who concluded that fifteen studies showed negative response, while one Chinese Hamster bone marrow study showed a weakly positive clastogenic response. These studies confirm our previous findings that CTN is not mutagenic (e.g., see the review of PP # 3F 2875, D. Ritter, 3/21/84).

Metabolism

Additional biochemical investigations were performed to elucidate further the role of rate-limiting renal excretion mechanisms involving glutathione interactions. These effects are fully discussed in the WHO/JMPR 1985 Monograph, R. Jaeger, 11/20/85. It was stated that "... the data demonstrate preferential excretion via the bile and feces, with secondary excretion in the urine." There is also evidence that the kidney, liver or gut forms nephrotoxic thiol metabolites which are excreted in the urine. There appears to be a rate-limiting step in the renal excretion of CTN and/or its metabolites that reaches saturation at doses between 50 - 160 mg/kg bw. Several studies were performed which indicated that CTN and/or its metabolites conjugate with reduced glutathione in the liver followed by renal excretion.

Tolerance Considerations

No additional tolerances are proposed under this submission. We concluded in the Monograph (Jaeger, ibid.) that the temporary ADI for man is 5.0×10^{-4} mg/kg bw.

Additional dietary oncogenicity studies in rats and mice are currently ongoing, with completion scheduled for mid- 1987 or late 1988. The JMPR is awaiting results of these studies.

At present, we are recommending no change in tolerances in accordance with the "Weight of Evidence Review" of 1/31/84, D. Ritter.

Further tolerance considerations await receipt of the historical control data on Fischer 344 rats regarding renal neoplasms (adenomas, carcinomas, etc.) and HED Peer Review, and additional data on the metabolism of CTM and/or its metabolites, as well as the 21 day dermal toxicity study cited above.

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DATA EVALUATION REPORT

CHLOROTHALONIL

STUDY: Tumorigenicity Study in Rats.

LABORATORY: IRDC.

STUDY NUMBER & DATE: 099-5TX-80-234-008 5/28/85

W..D. Busey.

ACCESSION NUMBER: 258759.

MATERIAL TESTED: Chlorothalonil 98.1 % (containing [REDACTED] % ECB or less).

ANIMALS: Fischer 344 rats, 60 per sex per group.

METHODS:

Dosing:

Rats were offered diets containing 0, 800, 1600 or 3500 ppm for 116 weeks (males) and for 129 weeks (females). These levels are equivalent to 40, 80 and 175 mg/kg/day, respectively.

Husbandry, Food Consumption, Body Weights and Observations for Effects:

Standard GLP.

Necropsy:

Standard GLP.

RESULTS: (Jaeger, R.B., 1985 WHO/JMPR monograph, 11/20/85)

"Survival was comparable in all groups, both sexes, for the first 24 months. Continuation on study decreased survival in high dose males resulting in all males sacrificed at 27 months. Females were terminated on schedule at 30 months. The major cageside clinical observation included dark yellow urine in high dose males and females from weeks 27-91. An increased brown, red staining around the anogenital region of mid- and high dose females was also observed. There was a significant body weight decrease (10-29%) in high dose males and females throughout study, as well as a 5-12% body weight decrease in both sexes at the mid dose. There was no body weight reduction in low dose animals. Food consumption was unaffected, except for an increase in high dose animals, generally towards the last half of the study.

"Mononuclear cell leukemia is a common finding (approx. 20%) in Fischer 344 rats at an average age of 2 years (so-called "Fischer rat leukemia"). In this particular study there was an inverse relationship with dose in that this finding was most pronounced in controls. This was supported by numerous hematological, clinical chemistry and micropathological findings. These effects were most noticeable in controls males. They included: decreased RBC, Hgb, Hct, and platelet counts, with increased MCV, MCH, reticulocytes, nucleated RBC and segmented

INERT INGREDIENT INFORMATION IS NOT INCLUDED

neutrophils. These changes were accompanied by enlarged spleen at 0 and 40 mg/kg, and are suggestive of a macrocytic normochromic regenerative anemia. Also, in control males, there were increases in total bilirubin, aspartate amino transferase (AST), alanine amino transferase (ALT) and alkaline phosphatase levels; findings which are common in the Fischer rats in later stages of this disease.

"Parameters measured which were compound related and associated with the effects on the kidneys included increased BUN and serum creatinine in high dose males and females, decreased serum albumin and serum glucose in high dose males and females, increased urine volume and decreased specific gravity in all treated males throughout the study, and in all treated females initially (first year), but in high dose females only, after the first year. The relative kidney weights were significantly increased in all treated males and in mid- dose and high dose females only. Relative liver weight was effected in the same groups, being significantly increased in all dosed males, and mid- and high-dose females only. Gross necropsy of all animals demonstrated a compound related effect on the kidneys and stomach. In all dosed male groups and the high dose female group there were kidney masses and/or nodules as well as increased granularity of the surface of the kidneys (the latter observed in all dose groups). There were increased incidences of erosions and ulcerations in the non-glandular stomach of all dosed rats as well as a significant increase in discoloration of the mucosa in high dose males.

"Histologically there was evidence of compound related effects on the kidneys, esophagus, stomach and duodenum. Non-neoplastic changes in the kidney included: chronic glomerulonephritis which increased in severity in a dose-related manner in all groups; dose related increase in cortical tubular hyperlasia in all dosed rats; increased incidence of tubular cysts in all dosed rats; and increased incidence in dosed males only of hyperplasia of the papillary/pelvic epithelium. Other changes included increased hyperplasia/hyperkeratosis of the squamous mucosa of the esophagus (all dose groups); increased mucosal hypertrophy of the duodenum (all dose groups); hyperplasia/ hyperkeratosis of the parathyroid (all dosed male and high dose female groups, considered a secondary lesion as a result of severe chronic renal disease); increased hyperplasia/hyperkeratosis of the squamous mucosa of all dose groups; increased incidences of foci of necrosis or ulcers in the glandular stomach of all dose groups; increased incidence of suppurative prostatitis in all male dose groups (considered associated with treatment related renal lesions); complete involution of the thymus was increased in high dose males and all female dose groups. Interesting inverse dose related changes included: chronic interstitial prostatitis (increased in control and low dose male groups); increased incidence of medullary tumors of the adrenal (Control and low dose female groups); increased incidences of osteosclerosis of the femur and sternum (control females); and an increased incidence of basophilic cell focus/foci of the liver (control females - a common finding in aging Fischer 344 rats).

"Neoplastic changes associated with treatment were observed in kidneys and stomach (forestomach). Tubular adenomas and carcinomas, anaplastic renal carcinomas and transitional cell carcinomas were observed in the kidney of treated rats only, being statistically significant in all dosed rats except low dose females. There was also a possible decrease in time to tumor in high dose rats for renal adenomas and carcinomas. There was no evidence however, that the occurrence of

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cortical tubular hyperplasia or tubular cysts predisposed animals to such tumors since only 1/11 tumor bearing low dose rats also had a tubular cyst. This was also true at the higher doses as well.

"Papillomas and carcinomas of the squamous mucosa of the stomach were present in treated rats only, but statistically significant in high dose females only. Again, there was no evidence that hyperplasia or hyperkeratosis predisposed the animals to such tumors as the degree or severity of hyperplasia/hyperkeratosis varied from slight to marked in these animals, and was equally prevalent in non-tumor bearing rats, although the severity of response increased in a dose-related manner."

CONCLUSIONS:

Results of this study demonstrate that chlorothalonil produced renal adenomas and carcinomas in Fischer 344 rats (both sexes) at ≥ 40 mg/kg b.wt. (see Tables 1 - 5, appended). Secondary to this response was a dose-related increase in papillomas of the stomach (0/60, 1/60, 1/60, 2/60 for 0, 40, 80 and 175 mg/kg males; and 0/60, 1/60, 2/60 and 6/60 for 0, 40, 80 and 175 mg/kg females; see Table 6, appended).

CORE RATING:

Guideline.

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TABLE 1

Incidence^a of Renal Tumors of Epithelial Origin, Original Report

<u>Tumor Type</u>	<u>Control</u>		<u>40 mg/kg/day</u>		<u>80 mg/kg/day</u>		<u>175 mg/kg/day</u>	
	<u>M</u>	<u>F</u>	<u>M</u>	<u>F</u>	<u>M</u>	<u>F</u>	<u>M</u>	<u>F</u>
Tubular Adenoma	0	0	2	2	4	4	**11 ^b	**9
Tubular Carcinoma	0	0	5	1	2	2	*7	**11
Transitional-cell Carcinoma	0	0	0	0	0	0	2 ^b	0
Anaplastic Renal Carcinoma	0	0	0	0	1	0	0	3
Total Animals with These Tumors	0	0	*7	3	*7	*6	**19	**23

^aKidneys from 60 animals of each sex were examined for all groups.

^bIncludes one male with tubular adenoma and transitional cell carcinoma.

*Statistically different from control - $p < 0.05$ (Fisher's exact test).

**Statistically different from control - $p < 0.01$ (Fisher's exact test).

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TABLE 2

Incidence of Renal Tumors of Epithelial Origin, Independent Evaluation

Tumor Type	Control		40 mg/kg/day		80 mg/kg/day		175 mg/kg/day	
	M	F	M	F	M	F	M	F
Tubular Adenoma	0	0	3	3	5	10	7 ^a	15 ^b
Tubular Carcinoma	0	0	4	1	2	0	14 ^a	12 ^b
Total Animals with tumors	0	0	7	4 ^d	7	10 ^e	19	24 ^c

a. Includes 2 males with combined incidence of tubular adenoma and tubular carcinoma.

b. Includes 3 females with combined incidence of tubular adenoma and tubular carcinoma.

c. Includes one female with a tubular carcinoma, originally diagnosed as invasive lipomatous tumor.

d. Includes one female with a tubular adenoma, originally diagnosed as negative.

e. Includes 4 females with a tubular adenoma, originally diagnosed as negative.

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TABLE 3

Correlation of Renal Hyperplasia with Tubular Adenoma
and Carcinoma, Original Report (Males)

<u>Pathological Finding</u>	<u>Control</u>	<u>40 mg/kg/day</u>	<u>80 mg/kg/day</u>	<u>175 mg/kg/day</u>
Glomerulo - nephritis	39/60	56/60	56/60	60/60
Cortical Tubular hyperplasia	0/60	7/60	9/60	22/60
Kidney Adenoma or Carcinoma	0/60	7/60	7/60	19/60
Number of Tumor bearing rats with renal hyperplasia	0/0	0/7	0/7	3/19

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TABLE 4

Correlation of Renal Hyperplasia with Tubular Adenoma
and Carcinoma, Independent Evaluation (Males)

<u>Pathological Finding</u>	<u>Control</u>	<u>40 mg/kg/day</u>	<u>80 mg/kg/day</u>	<u>175 mg/kg/day</u>
Chronic progressive nephropathy	47/60	52/60	54/60	57/60
Focal Epithelial Hyper- plasia (Prox. Conv. Tub.)	0/60	6/60	20/60	6/60
Epithelial Hyperplasia (Prox. Conv. Tub.)	0/60	32/60	30/60	36/60
Kidney Adenoma or carcinoma	0/60	7/60	7/60	19/60
Number of Tumor bearing rats with renal hyperplasia.	0/0	6/7	7/7	19/19

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TABLE 5

Correlation of Renal Hyperplasia with Tubular Adenoma
and Carcinoma, Independent Evaluation (Females)

<u>Pathological Finding</u>	<u>Control</u>	<u>40 mg/kg/day</u>	<u>80 mg/kg/day</u>	<u>175 mg/kg/day</u>
Chronic Progressive Nephropathy	45/60	49/60	47/60	51/60
Focal Epithelial Hyperplasia (Prox. Conv. Tub.)	6/60	22/60	34/60	42/60
Epithelial Hyperplasia (Prox. Conv. Tub.)	5/60	35/60	39/60	48/60
Kidney Adenoma or Carcinoma	0/60	4/60	10/60	24/60
Number of Tumor bearing rats with renal hyperplasia	0/0	4/4	10/10	21/24

The incidence of papillomas and carcinomas of the stomach were dose-related, but statistically significant only in high dose females (0/60, 1/60, 1/60, 2/60 for males and 0/60, 1/60, 2/60, and 6/60 for females at 0, 40, 80 and 175 mg/kg dose levels, respectively) [See Table 6]. Although there was no apparent correlation between forestomach tumors and the incidence of hyperplasia or hyperkeratosis, the non-neoplastic changes may have been obscured by the progression to tumor. Nonetheless, there was a dose-related increase in the severity of hyperplasia/hyperkeratosis in the forestomach.

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TABLE 6

Incidence^a of Tumors in the Gastric Mucosa

Site/ Tumor Type	Control		40 mg/kg/day		80 mg/kg/day		175 mg/kg/day	
	<u>M</u>	<u>F</u>	<u>M</u>	<u>F</u>	<u>M</u>	<u>F</u>	<u>M</u>	<u>F</u>
Forestomach/ Papilloma:	0	0	1	1	1	2	2	6*
Squamous Carcinoma:	0	0	0	0	0	0	1	1
Total number of animals with fore- stomach tumors:	0	0	1	1	1	2	3	7*
Fundal stomach/ Mucosal polyp:	1	0	0	0	0	0	0	0
Adenocarcinoma:	0	0	0	0	0	1	0	0

^aStomachs from 60 animals of each sex were examined.*Statistically different from control - $p < 0.05$ (Fisher's exact test)

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DATA EVALUATION REPORT

CHLOROTHALONIL

STUDY: Ninety Day Mouse Feeding Study - Histopathologic Re-evaluation.

LABORATORY: Experimental Pathology Labs. (Concorde Woods Animal Facility, SDS Biotech).

STUDY NUMBER & DATE: 5TX-79-0102 (Ref. # 618-5TX-83-0007-C04; 9/2/83)

ACCESSION NUMBER: 258769 (Ref. # 072269).

MATERIAL TESTED: Technical Chlorothalonil.

ANIMALS: CD-1 Mice, 41 days old.

METHODS:

See the Review of D. Ritter, PP # 4F 3025, 5/7/84.

RESULTS: (Jaeger, R.B., 1985 WHO/JMPR monograph, 11/20/85)

"Histopathological re-evaluation of the kidneys in the Shults, 1983 study, identified microscopic kidney changes in males at 750 ppm. These changes which consisted of hyperplasia of the epithelium of the proximal convoluted tubules, were minimal to slight (severity), involved only 3/15 males, and were not considered to be clearly treatment-related effects."

CONCLUSIONS:

The NOEL for this study remains at 15 ppm.

CORE RATING:

Acceptable.

DATA EVALUATION REPORT

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CHLOROTHALONIL

STUDY: 13 Week Rat Feeding Study - Histopathologic Re-evaluation.

LABORATORY: Huntington Research Centre.

STUDY NUMBER & DATE: 562-5TX-81-0213-004-001 28/6/83 J. Colley.
(Ref. # 5TX-81-0213. Wilson, 1981)

ACCESSION NUMBER: 258768.

MRID:

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

ANIMALS: Charles River CD Rats.

METHODS:

Groups of 25 males and 25 females were offered diets containing 0, 1.5, 3.0, 10 or 40 mg/kg/day for thirteen weeks. Animals were then allowed a thirteen week rest period. They were sacrificed and Electron micrography was performed on the renal tissues. See the review of D. Ritter, PP # 3F 287, 3/21/84.

RESULTS: (Jaeger, R.B., 1985 WHO/JMPR monograph, 11/20/85).

"EM and light microscopy of renal tissue from the Wilson et al 1983 a., b. study confirmed the absence of a demonstrated microscopic change in female kidneys. In males there was evidence of an increased incidence of hyperplasia of the proximal convoluted tubules at 40 mg/kg b. weight. Electron and light microscopy identified a compound related increased number of irregular intracytoplasmic inclusion bodies in the proximal convoluted tubules of all males, including controls. The number of such inclusions increased with dose at > 1.5 mg/kg b. weight but showed a tendency to reversal at the low dose (e.g., 105 mg/kg) only following a 13 week recovery period."

CONCLUSIONS:

"The exact toxicological significance of these inclusion bodies is unknown since there were no associated degenerative renal changes, a spontaneous occurrence in controls was also observed and there was a tendency to reversal after a 13 week recovery period. The NOEL is ≥ 1.5 mg/kg b.weight. The previous NOEL was 3 mg/kg b.wt."

CORE RATING:

~~Acceptable.~~ The previous NOEL of 3 mg/kg BW is ~~downgraded~~ to 1.5 mg/kg BW.

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DATA EVALUATION REPORTCHLOROTHALONIL

STUDY: Identification of Metabolites in Urine and Blood Following Oral Administration of 14 -C-labeled Chlorothalonil to Male Rats: The Thiol metabolites in Urine (Interim Report).

LABORATORY: Concord Woods Laboratories, Painesville, OH.

STUDY NUMBER & DATE: # 621-4AM-83-0061-001; J.P. Marciniszyn, 6/28/85.

ACCESSION NUMBER: # 258776

MATERIAL TESTED: 99.7 % pure 14 -C-DS-2787 with specific activity of 124.7 mCi/ μ mol.

ANIMALS: CD Sprague-Dawley male rats.

METHODS:

One group of 8 males (group I) and one group of 5 males (group II) each received 200 mg test material/ kg body weight on different days. Three males were undosed and served as controls.

Group I rats had urine collected at 24 and 48 hours. Group II had samples collected at 17 hours (termination).

Blood samples were taken just prior to necropsy for group I at 48 hours and for group II at 17 hours (to be analyzed later).

Only four group I urine samples could be used because of fecal contamination. These were pooled and subjected to extraction and LSC and MS analyses for urinary metabolites (procedures attached).

No group II urines were used for analysis because of the time difference.

RESULTS:

The authors calculated that each of the four remaining animals received 55.1 mg of radio-labeled DS-2787 in 0.75 % methylcellulose. The combined urines contained 2.4 % of the total administered dose. Ethyl acetate extraction removed 15.4 % of this or 0.35 % of the administered dose. 54.5 % of the labeled DS-2787 or 1.3 % of the administered dose was removed by subsequent acidification/ethyl acetate extraction. The remainder was deemed to be unextractable (30 % of label or 0.73 % of administered dose).

Two metabolites were subsequently identified by GC/MS analyses: dithiodichloroisophthalonitrile and trithiochloroisophthalonitrile. These were present in about a 1:1 ratio. They may exist as the free sulfhydryl and as the methylated derivative.

CONCLUSIONS:

Male rats administered oral DS-2787 ring-labeled with $^{14}\text{-C}$ at 200 mg/kg produced urinary metabolites at 2.4 % of the administered dose. The metabolites were determined to be dithiodichlorophthalonitrile and trithiochlorophthalonitrile in an approximate ratio of 1:1. These may have existed as the free sulfhydryl and as the methylated form. The authors postulate that hepatic metabolism proceeds through conjugation with glutathione (GSH) followed by enzymatic degradation. The smaller conjugates are then transported via the bloodstream to the kidney where they are converted to thiol metabolites and excreted in the urine.

CORE RATING:

Minimum Data. Only four animals could be used instead of the original eight, and the urine samples were pooled.

DATA EVALUATION REPORT

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CHLOROTHALONIL

STUDY: Dermal Absorption Study in Male Rats

LABORATORY: Concord Woods Laboratories, Painesville, OH.

STUDY NUMBER & DATE: 649-4AM-84-0010-001 12/26/84

ACCESSION NUMBER: 258774

MRID: NA

MATERIAL TESTED: ^{14}C -Chlorothalonil, 99.7 % pure (117.4 mCi/mmol)

ANIMALS: Sprague-Dawley CD male rats, ca. 234 gm.

METHODS:

Husbandry: Standard GLP.

Diet and Feeding: Standard rat lab chow, fresh weekly.

Dosing:

Rats were assigned exposure groups and received 5 mg cold and "hot" CTN in 4 ml acetone, distributed over 25 cm² shaven skin, or an average dose of 46.7 $\mu\text{g}/\text{cm}^2$ skin; this was approximately equal to 112 $\mu\text{Ci}/\text{rat}$. The treated area was covered with a non-occlusive patch to prevent grooming of the application site.

Three rats per group were exposed for 2, 4, 8, 12, 24, 48, 72, 96 or 120 hours. Non-treated rats served as controls.

Sampling:

Blood was collected at termination and the amount of radioactivity was determined for blood and plasma by liquid scintillation chromatography (LSC).

Urine was collected at termination and analyzed by LSC on animals all exposed up to 24 hours, then at 24 hour periods thereafter from those remaining.

Fecal samples were collected along with the urine samples, but were frozen with dry ice, ground up and combusted for radioactive ^{14}C .

The protective patch was removed, extracted with acetone and the activity counted by LSC.

The treated and adjacent skin was removed and washed with acetone for counting for surface residues.

The skin was then chopped into small pieces, dry-frozen and homogenized and extracted twice with methanol and acetone for separate LSC determination of unbound residues.

The extracted skin was air-dried and combusted for determination of bound residues.

The intestinal tract less contents was assayed for radioactivity at termination as were the liver, kidneys and carcass.

RESULTS:

Blood:

Activity in the blood plateaued at ca. 72 hours, reaching a level of about 0.18% of the administered dose or approximately 140 ng-eq/ml. About 89 % of total blood activity was located in the plasma.

Liver and kidneys:

Concentration of activity in the liver plateaued similarly to that for the blood; the kidneys plateaued later (between 72 and 120 hours) and was somewhat higher in magnitude.

Carcass:

No apparent pattern was discernable for the carcass; only about 4 % of the administered dose was found there. This included all soft tissues and blood.

Urine:

Urinary excretion was determined to be a total of 6.04% of the total dose. The authors calculated that a constant rate of ca. 1.2 % of the total dose was excreted daily in the urine.

Feces:

Fecal radioactivity (plus gut contents) accounted for the greatest amount of material excreted. There was a close parallel between fecal excretion and blood concentration with time; whereas urinary excretion was independent of blood concentrations. This was attributed to dermal absorption and 17 excretion into the bile, thence into the feces.

Absorbed dose:

The authors observed that the rate of dermal absorption at 2 and four hours exposure was essentially the same (15.1 and 16.4 ug-equivalents, respectively), with the average daily absorption becoming constant after 24 hours and thereafter at a mean rate of $73.2 \pm 15.3 \text{ ug }^{14} \text{C-Chlorothalonil per day}$.

Skin Residues:

Skin residues, i.e., those washed off and those recovered from the dressing, dropped from 70.6 % at 24 hours to 44.5 % at 120 hours of the total applied dose. Residues penetrating the skin dropped from 60.3 to 19.6 % of the applied dose. Bound residues increased from 8.4 to 22.5% during this period, and the extractable activity remained at 2.5 % of the applied dose throughout the exposure period. Calculations indicated that 20% of the entire dose was lost at the time of application through evaporation.

CONCLUSIONS:

The rate of absorption from the skin is relatively constant (6.3 %) from 24 to 120 hours following a single dermal application in acetone of 5 mg/kg body weight. The principle route of excretion is via the feces (18 % of the total dose) with excretion in the urine (6 % of the total dose) being the secondary route of elimination. Fecal levels paralleled those for blood. There was substantial loss of activity during the application phase, indicating loss by evaporation.

The urinary excretion pattern, attaining constancy of 1.2 % of applied dose per day, suggested that the renal excretory mechanism for CTN and/or its metabolites is quickly saturated.

Surface residues constitute the bulk of activity, however.

DISCUSSION:

The above evidence suggesting that the renal excretory system for CTN is saturated at relatively low blood/plasma levels (e.g., 140 ng-equivalents) following dermal exposure may have relevance to the chronic renal toxicity of this material in light of the comparatively low oral doses used in earlier feeding studies (NOEL = 60 ppm in the diet of rats). Chronic effects on the kidneys included renal tubular necrosis and chronic glomerulonephritis (Tierney, 1981), and hyperplasia and tubular epithelial dilation, glomerulosclerosis and pigmentation (Paynter, 1976). This finding could also have implications for oncogenic effects on renal tubules reported in laboratory rodents (Campbell, 1978, and Tierney, ibid.).

The appearance of substantial activity in fecal matter strongly supports the conclusion that there is metabolism/secretion in the bile.

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CORE RATING:

Guideline.

Reviewer: D. Ritter # 47

TECH: 20 hours

DATA EVALUATION REPORT

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CHLOROTHALONIL

STUDY: Time Course of the Acute Effect of Technical Chlorothalonil on Hepatic and Renal Glutathione (GSH) Content in Male Rats.

LABORATORY: Safety Assessment Animal Facility, Painesville, OH.

STUDY NUMBER & DATE: 751-5TX-85-0032-001; E. M. Sadler, 6/27/85.

ACCESSION NUMBER: # 258776.

MATERIAL TESTED: SD-2787 Technical; 97.8% pure.

ANIMALS: Male Sprague-Dawley Rats, 41 days old at initiation of the study.

METHODS:

Husbandry: Standard GLP.

Feed: Withheld for sixteen hours prior to dosing, then offered ad libitum from four hours post-dosing.

Dosing: Animals were assigned 5/group in twelve groups. Six groups received vehicle (0.5% in methylcellulose) and six received 5000 mg/kg test material in 0.5% methylcellulose in a single oral dose.

One vehicle control group and one treatment group per time interval was killed at 1, 3, 9, 18, 24 or 48 hours post-dosing.

Animals were observed twice daily for signs of toxicity. Body weights were determined initially and at termination.

Determination of GSH:

At the appropriate time interval rats were killed and liver and kidneys were obtained, weighed, homogenized and analyzed for GSH content by an acceptable method. Data were statistically analyzed using Student's T-test.

RESULTS:

Observations:

Administration of vehicle did not induce toxic sequelae, nor did those animals whose exposure time was less than nine hours. Animals sacrificed nine to forty-eight hours post-dosing showed soft stools, and-genital staining and red nasal discharge.

Body weights:

Treated animals sacrificed at the 24 and 48 hour interval had reduced terminal body weights.

Liver Weights:

24 and 48 hour liver to body weight ratios were reduced in treated animals.

Kidney Weights:

Kidney to body weight ratios were increased in the 9, 18 and 48 hour groups.

GSH Content:

Hepatic GSH content was significantly decreased by 20% in the 9 hour group; 40% in the 18 hour group, and 25% in the 24 hour rats. No reduction was seen in the 48 hour rats.

Renal GSH was significantly increased by 21% at 9 hours; 45% at 18 hours; 38% at 24 hours and by 101% at 48 hours.

CONCLUSIONS:

Chlorothalonil given by gavage to male rats at a single oral dose of 5000 mg/kg induces significantly increased liver and kidney-body weight ratios, reduces hepatic GSH content up to twenty-four hours following challenge and increases renal GSH content significantly at up to 48 hours after treatment.

The investigators suggest that the hepatic GSH changes are related to its conjugation with chlorothalonil, but that the mechanism for renal reduction in GSH content is not known.

CORE RATING:

Guideline.

DATA EVALUATION REPORT

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CHLOROTHALONIL

STUDY: Acute Effect of Technical Chlorothalonil on Hepatic and Renal Glutathione (GSH) Content in Rats.

LABORATORY: Safety Assessment Animal Facility, Painesville, OH.

STUDY NUMBER & DATE: 732-5TX-85-0006-001; 6/19/85. J. A. Ignatoski.

ACCESSION NUMBER: #258776.

MATERIAL TESTED: Technical Chlorothalonil; 2,4,5,6-tetrachloroisophthalonitrile 97.8 % pure.

ANIMALS: Young Sprague Dawley male rats.

METHODS:

Husbandry: Standard GLP.

Dosing: 3 rats per group were selected. Group I received 1 mg/ml corn oil I.P. Group II received 5 mg/kg DS-2787 I.P. Group III received 1 mg/kg in 0.5 % aqueous methylcellulose P.O. Group IV received 5000 mg/kg P.O. in 0.5 % methylcellulose. Groups Va and VIa received the same treatments as Groups III and IV except each of these contained 5 rats/group.

Observations:

Animals were checked once daily prior to dosing and twice daily thereafter.

Body Weights:

Obtained initially only.

GSH Content Determination:

Groups I and II were killed at 2 hours post-dosing; the remaining groups were killed at 24 hours post-dosing.

At the pre-determined times, animals were killed and the livers and kidneys were prepared and analyzed for GSH content using standard laboratory wet-tissue procedures. GSH content was determined using a spectrophotometer.

Tissue GSH content values were analyzed using Student's "t" test.

RESULTS:

Observations for gross overt effects were negative in all groups.

No significant differences were reported in renal or hepatic GSH in rats dosed with 5 mg/kg i.p. of chlorothalonil in corn oil (Groups I and II).

Renal content of GSH was significantly increased in chlorothalonil - treated rats (Groups III vs. IV) at 24 hours following intubation with 5000 mg/kg in methyl cellulose. The increase was about 25 % more than the level of the corresponding control group. Hepatic GSH levels were reduced, but not significantly so.

Renal GSH content was significantly increased in the duplicate groups (Groups V and VI) but the hepatic GSH content was significantly reduced.

CONCLUSIONS:

1. 5 mg/kg BW of chlorothalonil given i.p. affects neither the renal nor hepatic GSH content when measured 2 hours after treatment.
2. 5000 mg/kg BW given by gavage reduces hepatic GSH content when measured at 24 hours following administration; the same dose increases renal GSH content.

It was suggested that this supports the proposed metabolic pathway which includes a GSH conjugate formed in the liver which is subsequently metabolized in the kidney to a sulfur containing, potentially nephrotoxic compound.

CORE RATING:

Acceptable.

DATA EVALUATION REPORT

004950

CHLOROTHALONIL

STUDY: Oral Distribution Metabolism in the Male Rat.

LABORATORY: Huntington Research Centre, Cambridgeshire, England.

STUDY NUMBER & DATE: 631-4AM-83-0011-002, Marciniszyn, J.P., 7/2/84.

ACCESSION NUMBER: # 258775

MATERIAL TESTED: Mixture of 14 -C-ring labeled and cold DS-2787, 99.7 % pure, specific activity = 85.9 mCi/mole in 0.75 % methylcellulose.

ANIMALS: 4 male Sprague-Dawley rats per dose level, 5, 50 or 200 mg/kg administered initially by intubation; termination at 2, 9, 24, 96 and 168 hours post-dosing.

METHODS:

Dosing:

Animals were intubated with test material at the stated dose levels. Urine and feces were collected from each animal at 24 hour intervals except those terminated at 2 and 9 hours. Blood was collected at termination. Urine, feces and blood samples were assayed for radioactivity. The following organs and tissues were removed and assayed for radioactivity:

Liver	Kidneys	Muscle
Fat	Muscle	Heart
Lungs	Stomach	Sm. Intestine
Lge. Intestines	Stomach contents	Intestinal Contents

RESULTS:

Animals receiving 200 mg/kg test material had loose stools which contaminated the urine samples to an undetermined degree.

Excretion of Radioactivity:

GI Tract

The major route of excretion was in the feces (ca. 83%). Three quarters of this occurred during the first 48 hours at the low and mid dose level; the high dose animals excreted about 60 % during this interval.

43 % of the low dose was found in the small gut at 2 hours, with 15 % in the stomach. By 9 hours the stomach had emptied and 57 % of the administered dose was found in the small gut.

At 2 hours the mid-dose group retained 30 % of the administered dose in the stomach; this had not changed significantly at 9 hours. At 24 hours the stomach had emptied and half the AD was found in the large gut.

56 % of the high dose remained in the stomach at 2 hours and 52 % remained at 9 hours. 13 % remained in the stomach at 24 hours.

Urine

Only 5 -7 % of AD appeared in the urine. Fecal contamination and reduced sample size resulted in equivocal results and cannot be further interpreted.

Blood

5 mg/kg groups showed their highest level at 2 hours (0.3 ug-eq/ml). This level persisted through the 9 hour period, then dropped to one fourth by 24 hours.

50 mg/kg groups showed their highest concentration at 9 hours (4.9 ug-eq/ml); these were essentially depleted to one fourth this level at 24 hours.

200 mg/kg groups showed peak blood levels at 9 hours (13.4 ug-eq/ml) with only half that at 24 hours.

Kidney and Liver

Low dose groups showed 0.55 % of AD in the kidneys and 0.72 % of AD in the livers. Renal levels expressed as ug-eq/gm were 3 to 30 times greater than those for the livers. Kidneys retained their activity longer than any other tissue. Renal and hepatic levels were not shown to be proportional to dose at any time.

Other Tissues

The investigators consider that tissue levels of radioactivity were not significant at any time; those for the stomach and large and small intestine were dependant on the activity of the their contents.

CONCLUSIONS:

The major route of excretion in this study is via the GI tract; of this, most is eliminated during the first 9 - 24 hours. Urinary excretion occurs at a low but continuous rate, indicating saturation of the renal excretory mechanism(s). Blood levels are low following dosing; these are dose-dependant with the highest levels attained at up to 9 hours, decreasing rapidly thereafter. Renal retention lasted longer than liver; tissues did not store activity.

C. R. Smith

00495

DATA EVALUATION REPORT

CHLOROTHALONIL

STUDY: Biliary Excretion of Radio-labeled ^{14}C -DS-2787 to Rats Following Oral Administration.

LABORATORY: Huntingdon Research Centre, Cambridgeshire, England

STUDY NUMBER & DATE: 633-4AM-83-0062-002; 1/3/85; J. A. Ignatoski.

ACCESSION NUMBER: #258775

MATERIAL TESTED: Mixture of ^{14}C -ring labeled and cold DS-2787, 99.7% pure; 27.9 $\mu\text{Ci}/\text{mg}$ in 0.75% methylcellulose suspending medium.

ANIMALS: 3 male and 4 female Sprague-Dawley rats (ave. 260 gm).

METHODS:

Husbandry:

Standard GLP.

Feed and Water:

Standard ad libitum.

Dosing:

Fasted except for water for 16 hours prior to bile duct cannulation procedure. 2-four male groups and 1-four female groups were used. Of these, two in each group had an additional cannula inserted into the duodenal bile tract; sodium tauracholate (a choleretic substance) was infused at a rate of 25 mg/hour into this fixture.

Animals were observed for a short time to insure adequate bile flow. Then the rats were intubated with ^{14}C -DS-2787 at 5 mg/kg in a single dose.

Sample Collection:

Animals were restrained and bile samples were collected at hourly intervals from 0 to 48 hours after dosing. Blood was sampled at 6 and 24 hours and at termination. Urine was collected in ^{20}C -chilled containers during the 0 - 6 hour period, the 6 - 24 hour period and the 24 - 48 hour period. Feces was collected during the 0 - 24 hour and the 24 - 48 hour periods.

Termination:

The animals were killed and the GI tract, stomach, large intestine and small intestine were excised, tied off and stored at -20° C. Cages were washed and the washings measured for activity.

Measurements:

Samples of bile, urine, cage washings, methanolic extracts of the carcasses were diluted with appropriate scintillator fluid and counted. Feces samples were homogenized and mixed with cellulose, combusted and counted. Whole blood samples also were combusted and counted.

The GI tract portions were separately minced, homogenized in acetone 50%, combusted in oxygen and counted. Carcasses were minced with rat chow and homogenized with methanol. The resultant supernatants were directly counted; the solids were air-dried, combusted and counted.

RESULTS:

Excretion of Radioactivity:

50.3 and 61.1% of the administered dose was excreted in the feces of the males and females respectively. 21.1 and 16.7% was excreted in the bile, males and females respectively. Urine, GI tract and carcasse contained 9.6% (males and females combined), 6.4 and 2.2% respectively of the administered dose. Overall recovery was said to be 91.2%.

No increase or decrease in the amount excreted in the bile was reported for those rats receiving taurocholate. The bulk of activity was excreted during the first 12 hours (e.g., 70 - 80%) in all groups. Of this, most was excreted during the 1 - 2 hour period for males and females irrespective of taurocholate administration.

The urine was next in order of magnitude of excretion, amounting to about 10% of the administered dose in both sexes. Taurocholate administration did not effect the renal excretion rate in either sex.

Blood concentrations were variable but were highest during the first 24 hours (ca. 200 ng-eq/ml at 6 hours and ca. 90 ng/eq/ml at 24 hours. Maximum blood concentration was 0.4% of administered dose. Taurocholate administration did not appear to effect these findings.

Fecal and residual GI tract content of activity accounted for approximately 60 % of the administered dose.

CONCLUSIONS:

The presence of activity in the blood, urine and bile clearly demonstrates that gut absorption occurs, and to a significant extent. Overall, the gut absorbed approximately 34 % of administered dose, with the remainder (67%) found in the feces and GI tract and represented non-absorbed material. Biliary excretion accounted for 17 -21 % of the administered dose, with maximum concentration eliminated within 2 hours of dosing.

Urinary excretion, at about 8 - 10 % of the labeled dose, shows this to be a significant route of elimination, but not a major one. No appreciable tissue binding is demonstrated as evidenced by low residual carcass levels, ca. 2 % of administered dose. Absorption via blood was also minimal, with maximum concentration less than 0.4% of the labeled dose.

CORE RATING:

Guideline.

DATA EVALUATION REPORTCHLOROTHALONIL

STUDY: Oral Distribution/Metabolism in the Female Rat.

LABORATORY: Huntington Research Centre, Cambridgeshire, England.

STUDY NUMBER & DATE: 631-4AM-84-0078-002, Marciniszyn, J.P., 7/10/85.

ACCESSION NUMBER: # 258775

MATERIAL TESTED: Mixture of 14 -C-ring labeled and cold DS-2787, 99.7 % pure, specific activity = 124.7 mCi/mmol in 0.75 % methylcellulose.

ANIMALS: 4 female Sprague-Dawley rats per dose level, 5, 50 or 200 mg/kg administered initially by intubation; termination at 2, 9, 24, 96 and 168 hours post-dosing.

METHODS:

Dosing:

Animals were intubated with test material at the stated dose levels. Urine and feces were collected from each animal at 24 hour intervals except those terminated at 2 and 9 hours. Blood was collected at termination. Urine, feces and blood samples were assayed for radioactivity. The following organs and tissues were removed and assayed for radioactivity:

Liver	Kidneys	Muscle
Fat	Muscle	Heart
Lungs	Stomach	Sm. Intestine
Lge. Intestines	Stomach contents	Intestine Contents

RESULTS:

Physical effects were limited to a finding of loose and watery stools in rats receiving 200 mg/kg during the first 24 hours, causing some contamination of urine.

Excretion of Radioactivity:

GI Tract

The major route of excretion was in the feces for all doses. At 5 mg/kg 79 % was eliminated during the first 48 hours and constituted 96 % of the total administered dose. 85 % of the 50 mg/kg dose was excreted during the first 72 hours, constituting 97 % of the administered dose. Animals receiving 200 mg/kg excreted 85 % in the feces, accounting for 93 % of the administered dose.

The stomach was essentially empty of radioactivity at 24 hours.

Urine

At 5 mg/kg about 11 % of the administered dose was excreted in the urine over the 7 day course of the study, with 92 % of this being lost during the first 24 hours. Those animals receiving 50 mg/kg excreted about 9 % of the administered dose with 80 percent of this gone by the end of the first 24 hours. Animals dosed with 200 mg/kg excreted 5.4 % of the AD; of this, 57 % was excreted in 24 hours, 85 % in 48 hours and 95 % in 72 hours. The increase in rate of excretion was not entirely dose-dependant at this level, suggesting that the urinary excretion mechanism was saturated.

Blood

5 mg/kg animals showed peak blood concentrations at 2 hours and 9 hours (630 and 616 ng-equivalents respectively); 50 mg/kg animals showed highest blood concentration at 9 hours (8190 ng-equivalents). Animals receiving 200 mg/kg showed peak blood concentrations at 9 and 24 hours (11,400 and 15,400 ng-equivalents). The authors consider that these data support a conclusion that the peak blood concentrations, seen at different times, could have been due to delayed stomach emptying.

Kidney

At the 5 mg/kg dose level the kidneys had the highest percentage of AD (0.71 %) with the bulk of this appearing at 2 hours (0.41 % AD/gm). At 50 mg/kg renal concentration was greatest at 9 hours (0.17 AD/gm). At 200 mg/kg the peak renal concentration occurred at 24 hours (0.07 % AD/gm). The authors consider that the delay in peak renal concentrations is due to delayed emptying time from the stomach as dose increased.

Liver

A similar pattern was seen in the liver. 5 mg/kg animals showed peak liver concentration at 2 hours (1.17 ug/gm), 50 mg/kg rats showed peak hepatic concentration at 9 hours (5.54 ug/gm) and at 200 mg/kg, the peak liver content occurred at 24 hours (9.25 ug/gm).

Other Tissues

Radioactivity remaining in these tissues was not considered to be remarkable.

CONCLUSIONS:

As in the male study, the major route of excretion in this study is via the GI tract; of this, most is eliminated during the first 9 - 24 hours. Urinary excretion occurs at a low but continuous rate, indicating saturation of the renal excretory mechanism(s). Blood levels are low following dosing; these are fairly dose-

dependant for the low and middle dose. The highest blood levels were attained at up to 9 to 24 hours for the high dose animals, decreasing rapidly thereafter.

Renal retention was low and lasted longer than liver; tissues did not store activity.

Taken together with the results of the Male study (631-4AM-83-0011-002, Marciniszyn, J.P., 7/2/85), these results support a tentative conclusion that the renal excretory mechanism is rate-limiting for elimination of Chlorthalonil absorbed into the blood-stream; that the bulk of activity remains in the gut or is re-excreted via the biliary apparatus into the feces, and that there is reason to believe that stomach evacuation is somewhat delayed at the 200 mg/kg dose level.

CORE Rating:

Guideline

DATA EVALUATION REPORTCHLOROTHALONIL

STUDY: Distribution of Radioactivity Following Repeated Oral Administration of ^{14}C -DS-2787 to Male Sprague Dawley Rats. Interim Report # I.

LABORATORY: Huntingdon Research Centre, Huntingdon, England.

STUDY NUMBER & DATE: 631-4AM-84-0079-001; M.C. Savides, 7/15/85.

ACCESSION NUMBER: # 258776.

MATERIAL TESTED: ^{14}C -labeled DS-2787 85.9 mCi/mmmole; ^{12}C 124.7 mCi/mmmole or 62.4 mCi/mmmole; 99.7 % purity.

ANIMALS: Young Male Sprague-Dawley male rats.

METHODS:

NOTE: This is a single-versus multiple dose study utilizing data reported in this Petition (See attached Bibliography) and certain data not yet officially submitted. Accordingly, We will only reiterate the Sponsor's summary here. Full and independent review awaits submission of all data pertaining to this analysis.

"Comparisons have been made of the data obtained from male and female rats after a single administration of ^{14}C -chlorothalonil at dose levels of 5, 50, or 200 mg/kg with data obtained from male rats administered ^{14}C -chlorothalonil at dose levels of 1.5, 5, 50 or 160 mg/kg/day for five consecutive days.

"Data from blood indicated that there were probable shifts in the times to peak blood concentrations with increasing single and multiple doses of chlorothalonil for both sexes. Significant depletion ($\geq 50\%$) of radiolabel from blood occurred by 24 hours post-dose for both sexes at dose levels less than or equal to 50 mg/kg. At 160 mg/kg/day, an apparent plateau in the concentration of radioactivity in the blood was reached after a single dose, which suggested that saturation of blood occurred between 50 and 160 mg/kg.

"The concentrations of radiolabel in kidneys after single dose administration showed no apparent sex-related differences, but the times to peak kidney concentrations did appear to increase with increased dose level for both sexes. With multiple doses, maximum kidney concentrations were found 2 hours after the fifth dose at all levels. The shift in time to peak concentrations, especially at 160 mg/kg/day, suggested that a plateau may have been reached by the final 160 mg/kg/day dose.

"After cessation of multiple dosing, the decreases in radiolabel in kidneys with time suggested that there was a trend toward slower depletion (or greater retention) in the kidneys with increased dose levels.

" Urinary data suggested a decreased rate of excretion for both sexes as single dose levels increased and a possible trend toward decreased urinary excretion (as a percent of the dose) as single or multiple dose levels increased. A possible change in urinary excretion may have occurred between doses of 5 and 50 mg/kg/day.

"It is suggested from the data that the apparent saturation of blood, the apparent plateau of radiolabel in kidneys, the trend toward slower depletion (or greater retention) of radiolabel from kidney and, possibly, the slight trend toward decreased urinary excretion are caused by increased and/or repeated doses of chlorothalonil. The effects on some of these parameters appear to occur at a dose between 50 and 160 mg/kg (blood saturation and kidney plateau) whereas the others appear to occur at a dose between 5 and 50 mg/kg. The data indicate that the effects are accompanied by an inability of the rat to respond to high doses of chlorothalonil in a manner similar as it would respond to low doses."

CORE RATING

Supplemental γ

REFERENCES

1. Study of the Distribution of Radioactivity Following Oral Administration of (^{14}C -SDS-2787) to Male Sprague-Dawley Rats. Document Number: 631-4AM-83-0011-002.
2. Study of the Distribution of Radioactivity Following Oral Administration of (^{14}C -SDS-2787) to Female Sprague-Dawley Rats. Document Number: 631-4AM-84-0078-002.
3. Study of the Distribution of Radioactivity Following Repeated Oral Administration of (^{14}C -SDS-2787) to Male Sprague-Dawley Rats. (Report in Preparation).
4. Levels of Radioactivity in Blood Following Oral Administration of ^{14}C -Chlorothalonil (^{14}C -SDS-2787) to Male Rats. Document Number: 621-4AM-83-0013-002.
5. Identification of Metabolites in Urine and Blood Following Oral Administration of ^{14}C -Chlorothalonil (^{14}C -SDS-2787) to Male Rats: The Thiol Metabolites in Urine. (Interim Report).
6. Pilot Study of the Biliary Excretion of Radioactivity Following Oral Administration of (^{14}C -SDS-2787) to Sprague-Dawley Rats. Document Number: 633-4AM-83-0062-002.
7. Study of the Dermal Absorption of ^{14}C -Chlorothalonil (^{14}C -SDS-2787) by Male Rats. Document Number: 649-4AM-84-0010-001.

BEST AVAILABLE COPY

Review of Mutagenicity Tests Performed with Chlorothalonil

1. Document No. 625-5TX-83-0029-002
In-Vivo Bone Marrow Chromosomal Aberration Assay in Mice
with Single Dose of Technical Chlorothalonil. C.E.R.T.I.
Laboratory Study No. 801, December 29, 1983.

The test compound, Technical Chlorothalonil, was administered orally to 3 groups of 10 male Swiss mice each (250, 1250, and 2500 mg/kg given as single treatment). The chromosome preparations were made 6, 24, and 48 hours after single treatment according to the method described by Kilian et al (Handbook of Mutagenicity Test Procedures, pp. 243-260, Elsevier Scientific Publishing Co., New York, 1977). Under the test conditions reported, the test compound (98.2% Purity) did not induce chromosomal aberrations in the mouse bone marrow cells at the dose levels tested. The positive control (MMS, 65 mg/kg) induced severe chromosomal damages in mice as expected. Therefore, Chlorothalonil was not considered to be clastogenic agent in this assay system.

2. Document No. 625-5TX-83-0028-002
In-Vivo Bone Marrow Chromosomal Aberration Assay in Rats
with A single Dose of Technical Chlorothalonil.
C.E.R.T.I. Laboratory Study No. 848, May 25, 1984.

The test compound, Technical Chlorothalonil, was administered by gavage in an aqueous suspension to 3 groups of 10 male Wistar rats each (500, 2500, and 5000 mg/kg given as single treatment). The chromosome preparations were made 6, 24, and 48 hours after single treatment according to the method described by Kilian et al (Handbook of Mutagenicity Test Procedures, pp. 243-260, Elsevier Scientific Publishing Co., New York, 1977). Under the test conditions reported, the only statistically significant effect observed was an increased number of total aberrations (e.g., gaps plus breaks) in the mid-dose group (2500 mg/kg) six hours after dosing. However, when only chromosomal breaks are evaluated, no statistically significant differences are present. The positive control (MMS, 130 mg/kg) induced severe chromosomal damages in mice as expected. Therefore, Chlorothalonil (98.2%) was not considered to be clastogenic agent in this assay system.

3. Document No. 625-5TX-83-0014-003
In-Vivo Bone Marrow Chromosomal Aberration Assay in
Chinese Hamsters with Technical Chlorothalonil
C.E.R.T.I. Laboratory Study No. 818, February 22, 1985.

The test compound, Technical Chlorothalonil, was administered by oesophagian loop to 6 groups of 10 male Chinese Hamsters each (500, 2500, and 5000 mg/kg given as single treatment and 50, 125 and 250 mg/kg given as five consecutive daily treatments). The chromosome slides were made from each animal 6, 24, 48 hours after single treatment and 6 hours after 5 consecutive daily treatments according to the method described by Kilian et al (Handbook of Mutagenicity Test Procedures, pp. 243-260, Elsevier Scientific Publishing Co., New York, 1977). Under the acute study, no clastogenic potentiality of Chlorothalonil could be detected by means of chromosomal aberration techniques in the male Chinese Hamsters after single dose of the test compound. However, under the subchronic study, significant increases in the percentage of mitosis with aberrations (gaps included) were observed in animal groups treated with 5 X 50 and 5 X 250 mg/kg of Chlorothalonil. Although a dose-response relationship was not established, gaps were present in a much higher than usual frequency (Control Group: 0.6%; Treated Groups: 5 X 50 mg/kg, 1.78%; 5 X 125 mg/kg, 1.44%; 5 X 250 mg/kg, 1.33%) in the treated animals. Therefore, Chlorothalonil was considered to be a weak clastogen in the bone marrow cells of treated Chinese Hamsters.

4. Document No. 694-5TX-84-0064-001
Salmonella/Mammalian-Microsome Plate Incorporation Assay
(Ames Test) with and without Renal Activation with
Technical Chlorothalonil
Microbiological Associates Study No. T2536.501., August
29, 1984.

The test compound, 2,4,5,6-Tetrachloroisophthalonitrile (chlorothalonil) was tested for mutagenic activity in Salmonella typhimurium strains TA98, TA100, TA1535, TA1537, and TA1538 according to the plate incorporation assay described by Ames et al (Mutation Res. 31:347-364, 1975). The following ten concentrations were selected for the mutation assay: 0.5, 2.5, 12.5, 25, and 50 ug/plate under activation and 0.16, 0.8, 4.0, 8.0, and 16.0 ug/plate under non-activation. Under the test conditions reported, the test compound failed to induce any significant increase in the number of revertant colonies over the negative control in the five strains of Salmonella typhimurium with and without renal metabolic activation at the dose levels tested. Therefore, Chlorothalonil was not mutagenic in the Ames test either with or without metabolic activation by the Aroclor induced rat kidney microsomes.

5. Document No. 694-5TX-84-0088-002
Salmonella/Mammalian-Microsome Plate Incorporation Assay
(Ames Test) with and without Renal Activation with 2,5,6-
Trichloro-3-Cyano-Benzamide Microbiological Associates
Study No. T2574.501., November 15, 1984.

inverted
8-74C

The test compound, 2,5,6-Trichloro-3-Cyano-Benzamide (SDS-47524) was tested for mutagenic activity in Salmonella typhimurium strains TA98, TA100, TA1535, TA1537, and TA1538 according to the plate incorporation assay described by Ames et al (Mutation Res. 31: 347-364, 1975). The following 5 concentrations were selected for the mutation assay with and without activation: 20, 100, 500, 1000, and 2000 ug/plate. Under the test conditions reported, the test compound (SDS-47424) failed to induce any significant increase in the number of revertant colonies over the negative control in the five strains of Salmonella typhimurium with and without renal metabolic activation at the dose levels tested. Therefore, the test compound was not mutagenic in the Ames test either with or without metabolic activation by the Aroclor induced rat kidney microsomes.

6. Document No. 694-5TX-84-0087-002
Salmonella/Mammalian-Microsome Plate Incorporation Assay
(Ames Test) with and without Renal Activation with
2,4,5,6-Tetrachloro-3-Cyano-Benzamide Microbiological
Associates Study No. T2573.501., October 17, 1984.

inverted
8-74C

The test compound, 2,4,5,6-Tetrachloro-3-Cyano-Benzamide (SDS-19221) was tested for mutagenic activity in Salmonella typhimurium strains TA98, TA100, TA1535, TA1537, and TA1538 according to the plate incorporation assay described by Ames et al (Mutation Res. 31:347-364, 1975). The following 10 concentrations were selected for the mutation assay: 10, 50, 250, 500, and 1000 ug/plate under activation and 6, 30, 150, 300, and 600 under nonactivation. Under the test conditions reported, the test compound (SDS-19221) failed to induce any significant increase in the number of revertant colonies over the negative control in the five strains of Salmonella typhimurium with and without renal metabolic activation at the dose levels tested. Therefore, the test compound was not mutagenic in the Ames test either with or without metabolic activation by the Aroclor induced rat kidney microsomes.

7. Document No. 694-5TX-84-0089-002

Salmonella/Mammalian-Microsome Plate Incorporation Assay (Ames Test) with and without Renal Activation with 2,5,6-Trichloro-4-Hydroxy-3-Cyano-Benzamide Microbiological Associates Study No. T2575.501., October 19, 1984.

inspected
676 -

The test compound, 2,5,6-Trichloro-4-Hydroxy-3-Cyano-Benzamide (SDS-47525) was tested for mutagenic activity in Salmonella typhimurium strains TA98, TA100, TA1535, TA1537, and TA1538 according to the plate incorporation assay described by Ames et al (Mutation Res. 31:347-364, 1975). The following 10 concentrations were selected for the mutation assay: 40, 400, 1000, 3000, 6000 ug/plate under activation and 20, 100, 400, 800, and 2000 ug/plate under nonactivation. Under the test conditions reported, the test compound (SDS-47525) failed to induce any significant increase in the number of revertant colonies over the negative control in the five strains of Salmonella typhimurium with and without renal metabolic activation at the dose levels tested. Therefore, the test compound was not mutagenic in the Ames test either with or without metabolic activation by the Aroclor induced rat kidney microsomes.

8. Document No. 694-5TX-84-0091-002

Salmonella/Mammalian-Microsome Plate Incorporation Assay (Ames Test) with and without Renal Activation with 2,3,5,6-Tetrachlorobenzonitrile Microbiological Associates Study No. T2577.501., December 28, 1984.

inspected
676 -

The test compound, 2,3,5,6-Tetrachlorobenzonitrile (SDS-3032) was tested for mutagenic activity in Salmonella typhimurium strains TA98, TA100, TA1535, TA1538 according to the plate incorporation assay described by Ames et al (Mutation Res. 31: 347-364, 1975). The following 5 concentrations were selected for the mutation assay with and without activation: 20, 100, 500, 1000, and 2000 ug/plate. Under the test conditions reported, the test compound (SDS-3032) failed to induce any significant increase in the number of revertant colonies over the negative control in the five strains of Salmonella typhimurium with and without renal metabolic activation at the dose levels tested. Therefore, the test compound was not mutagenic in the Ames test either with or without metabolic activation by the Aroclor induced rat kidney microsomes.

9. Document No. 694-5TX-84-0092-002
Salmonella/Mammalian-Microsome Plate Incorporation Assay
(Ames Test) with and without Renal Activation with 2,4,5,6-
Tetrachlorodibenzamide Microbiological Associates Study
No. T2578.501, December 28, 1984.

SDS-3133
The test compound, 2,4,5,6-Tetrachlorodibenzamide (SDS-3133) was tested for mutagenic activity in Salmonella typhimurium strains TA98, TA100, TA1535, TA1537, and TA1538 according to the plate incorporation assay described by Ames et al (Mutation Res. 31:347-364, 1975). The following 5 concentrations were selected for the mutation assay with and without activation: 100, 500, 2500, 5000, and 10000 ug/plate. Under the test conditions reported, the test compound (SDS-3133) failed to induce any significant increase in the number of revertant colonies over the negative control in the five strains of Salmonella typhimurium with and without renal metabolic activation at the levels tested. Therefore, the test compound was not mutagenic in the Ames test either with or without metabolic activation by the Aroclor induced rat kidney microsomes.

10. Document No. 694-5TX-84-0093-002
Salmonella/Mammalian-Microsome Plate Incorporation Assay
(Ames Test) with and without Renal Activation with 2,4,5-
Trichloro-3-Cyano-Benzamide Microbiological Associates
Study No. T2577.501., December 28, 1984.

SDS-47523
The test compound, 2,4,5-Trichloro-3-Cyano-Benzamide (SDS-47523) was tested for mutagenic activity in Salmonella typhimurium strains TA98, TA100, TA1535, TA1537, and TA1538 according to the plate incorporation assay described by Ames et al (Mutation Res. 31:347-364, 1975). The following 5 concentrations were selected for the mutation assay with and without activation: 20, 100, 500, 1000, and 2000 ug/plate. Under the test conditions reported, the test compound (SDS-47523) failed to induce any significant increase in the number of revertant colonies over the negative control in the five strains of Salmonella typhimurium with and without renal metabolic activation at the dose levels tested. Therefore, the test compound was not mutagenic in the Ames test either with or without metabolic activation by the Aroclor induced rat kidney microsomes.

11. Document No. 694-5TX-84-0124-002
Salmonella/Mammalian Microsome Plate Incorporation Assay
(Ames Test) with and with Renal Activation with 2,5,6-
Trichloro-4-Thio-Isophthalonitrile Microbiological
Associates Study No. T2683.501., March 29, 1985.

The test compound, 2,5,6-Trichloro-4-Thio-Isophthalonitrile (SDS-13353) was tested for mutagenic activity in Salmonella typhimurium strains TA98, TA100, TA1535, TA1537, and TA1538 according to the plate incorporation assay described by Ames et al (Mutation Res. 31:347-364, 1975). The following 7 concentrations were selected for the mutation assay with and without activation: 400, 630, 1000, 1600, 2500, 4000, and 5000 ug/plate. Under the test conditions reported, the test compound (SDS-13353) failed to induce any significant increase in the number of revertant colonies over the negative control in the five strains of Salmonella typhimurium with and without renal metabolic activation at the dose levels tested. Therefore, the test compound was not mutagenic in the Ames test either with or without metabolic activation by the Aroclor induced rat kidney microsomes.

12. Document No. 694-5TX-84-0139-002
Salmonella/Mammalian Microsome Plate Incorporation Assay
(Ames Test) with and without Renal Activation with 2,5,6-
Trichloro-3-Carboxy-Benzamide Microbiological Associates
Study No. T2810.501., March 28, 1985.

The test compound, 2,5,6-Trichloro-3-Carboxy-Benzamide (SDS-46851) was tested for mutagenic activity in Salmonella typhimurium strains TA98, TA100, TA1535, TA1537, and TA1538 according to the plate incorporation assay described by Ames et al (Mutation Res. 31:347-364, 1975). The following 5 concentrations were selected for the mutation assay with and without activation: 100, 500, 2500, 5000, and 10000 ug/plate. Under the test conditions reported, the test compound (SDS-46851) failed to induce any significant increase in the number of revertant colonies over the negative control in the five strains of Salmonella typhimurium with and without renal metabolic activation at the dose levels tested. Therefore, test compound was not mutagenic in the Ames test either with or without metabolic activation by the Aroclor induced rat kidney microsomes.

13. Document No. 694-5TX-84-0086-002

Salmonella/Mammalian-Microsome Plate Incorporation Assay
(Ames Test) with and without Renal Activation with 2,4,5-
Trichloroisophthalonitrile Microbiological Associates
Study No. T2572.501., October 19, 1984.

The test compound, 2,4,5-Trichloroisophthalonitrile (SDS-5473) was tested for mutagenic activity in Salmonella typhimurium strains TA98, TA100, TA1535, TA1537, and TA1538 according to the plate incorporation assay described by Ames et al (Mutation Res. 31:347-364, 1975). The following 5 concentrations were selected for the mutation assay with and without activation: 0.5, 2.5, 10.0, 35, and 70 ug/plate. Under the test conditions reported, the test compound (SDS-5473) failed to induce any significant increase in the number of revertant colonies over the negative control in the five strains of Salmonella typhimurium with and without renal metabolic activation at the dose levels tested. Therefore, the test compound was not mutagenic in the Ames test either with or without metabolic activation by the Aroclor induced rat kidney microsomes.

14. Document No. 694-5TX-84-0090-002

Salmonella/Mammalian-Microsome Plate Incorporation Assay
(Ames Test) with and without Renal Activation with
2,3,5,6-Tetrachloroterphthalonitrile Microbiological
Associates Study No. T2576.501., November 15, 1984.

The test compound, 2,3,5,6-Tetrachloroterphthalonitrile (SDS-2020) was tested for mutagenic activity in Salmonella typhimurium strains TA98, TA100, TA1535, TA1537, and TA1538 according to the plate incorporation assay described by Ames et al (Mutation Res. 31:347-364, 1975). The following 5 concentrations were selected for the mutation assay with and without activation: 4, 20, 100, 200, and 400 ug/plate. Under the test conditions reported, the test compound (SDS-2020) failed to induce any significant increase in the number of revertant colonies over the negative control in the five strains of Salmonella typhimurium with and without renal metabolic activation at the dose levels tested. Therefore, the test compound was not mutagenic in the Ames test either with or without metabolic activation by the Aroclor induced rat kidney microsomes.

15. Document No. 694-5TX-84-0094-002
Salmonella/Mammalian-Microsome Plate Incorporation Assay
(Ames Test) with and without Renal Activation with
Isophthalonitrile Microbiological Associates Study No.
T2580.501., December 28, 1984.

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The test compound, Isophthalonitrile (SDS-3176) was tested for mutagenic activity in Salmonella typhimurium strains TA98, TA100, TA1535, TA1537, and TA1538 according to the plate incorporation assay described by Ames et al (Mutation Res. 31:347-364, 1975). The following 5 concentrations were selected for the mutation assay with and without activation: 40, 200, 1000, 2000, and 4000 ug/plate. Under the test conditions reported, the test compound (SDS-3176) failed to induce any significant increase in the number of revertant colonies over the negative control in the five strains of Salmonella typhimurium with and without renal metabolic activation at the dose levels tested. Therefore, the test compound was not mutagenic in the Ames test either with or without metabolic activation by the Aroclor induced rat kidney microsome.

16. Document No. 694-5TX-84-0095-002
Salmonella Mammalian Microsome Plate Incorporation Assay
(Ames Test) with and without Renal Activation with
Pentachlorobenzonitrile Microbiological Associates Study
No. T2581.501., December 28, 1984.

The test compound, Pentachlorobenzonitrile (SDS-3297) was tested for mutagenic activity in Salmonella typhimurium strains TA98, TA100, TA1535, TA1537, and TA1538 according to the plate incorporation assay described by Ames et al (Mutation Res. 31:347-364, 1975). The following 5 concentrations were selected for the mutation assay with and without activation: 10, 50, 250, 500, and 1000 ug/plate. Under the test conditions reported, the test compound (SDS-3297) failed to induce any significant increase in the number of revertant colonies over the negative control in the five strains of Salmonella typhimurium with and without renal metabolic activation at the dose levels tested. Therefore, the test compound was not mutagenic in the Ames test either with or without metabolic activation by the Aroclor induced rat kidney microsomes.