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

JUN 19 2000

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OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

OPP OFFICIAL RECORD
HEALTH EFFECTS DIVISION
SCIENTIFIC DATA REVIEWS
EPA SERIES 361

MEMORANDUM:

SUBJECT: EPA ID#: 6F03344: Application for Exemption for Tolerance for the use of Dichlormid as Safener in/on Corn

DP CODE Nos.: D248305, D250746, D252242, D252248, D252251, D252298, D253129 & D253247
Submission No.: S546651
PC Nos.: 900497
Tox. Chem. No.: NA

From: Guruva B. Reddy
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6/6/2000

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I. CONCLUSIONS

The data base does not support the exemption from establishment of tolerance for the use of dichlormid as safener on corn.

A copy of the DERs are attached.

II. ACTION REQUESTED

Zeneca AG Products, has submitted an application for exemption from establishing tolerances for the use of dichlormid as safener on corn. The studies included in this package are listed below and the DERs are attached.

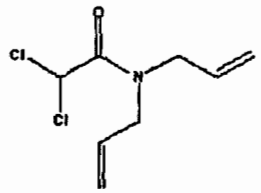
III. PRODUCT INFORMATION: Dichlormid

Updated: June 5, 2000

Dichlormid, N,N-diallyl-2,2-dichloroacetamide is used as safener. Formulations containing dichlormid are identified by names Fultime™, Surpass® 100, TopNotch™, Surpass® 7E, Surpass® 20-G, and Surpass® EC. The Fultime™, and Surpass® 100 contain atrazine and acetachlor as the active ingredients; all others contain acetachlor only as the active ingredient in the formulation. All products specify use on field corn, production seed corn, silage corn, and popcorn. The maximum rate of application is 0.54 lbs dichlormid/A [redacted] based on soil type and organic matter content.

Its chemical structure is as follows:

INERT INGREDIENT INFORMATION IS NOT INCLUDED



III. DATA REQUIREMENTS

Use Pattern: Terrestrial Food crop use as safener on corn
Action Type: Permanent Tolerance

Test		Technical	
		Required	Satisfied
870.1100	Acute Oral Toxicity	Y	Y
870.1200	Acute Dermal Toxicity	Y	Y
870.1300	Acute Inhalation Toxicity	Y	Y
870.2400	Primary Eye Irritation	Y	Y
870.2500	Primary Dermal Irritation	Y	Y
870.2600	Dermal Sensitization	Y	Y
870.6100	Acute Delayed Neurotox. (Hen)	N	-
870.6200a	Acute Neurotox. Screening Battery (Rat)	Y	N
870.3100	Oral Subchronic (Rodent)	Y	Y
870.3150	Oral Subchronic (Non-Rodent)	Y	Y
870.3200	21-Day Dermal	Y	N
870.3250	90-Day Dermal	N	-
870.3465	90-Day Inhalation	Y	Y
870.3700a	Developmental Toxicity (rodent)	Y	Y
870.3700b	Developmental Toxicity(non-rodent)	Y	Y
870.3800	Reproduction	Y	N
870.4100a	Chronic Toxicity (Rodent)	Y	Y
870.4100b	Chronic Toxicity (Non-rodent)	Y	N
870.4200a	Oncogenicity (Rat)	Y	Y
870.4200b	Oncogenicity (Mouse)	Y	Y
870.4300	Chronic/Oncogenicity	Y	Y
870.5100	Mutagenicity—Gene Mutation - bacterial	Y	Y
870.5300	Mutagenicity—Gene Mutation - mammalian	Y	Y
870.5395	Mutagenicity—Structural Chromosomal Aberrations	Y	Y
870.5550	Mutagenicity—Other Genotoxic Effects	N	Y
870.6100	90-Day Neurotoxicity (hen)	N	-
870.6200b	90 Day Neuro. Screening Battery (Rat)	Y	N
870.6300	Develop. Neuro	N	-
870.7485	General Metabolism	Y	N
870.7600	Dermal Penetration	N	-
870.7200	Companion Animal Safety	N	-
Special Studies for Ocular Effects		N	-
	Acute Oral (Rat)	N	-
	Subchronic Oral (Rat)	N	-
	Six-month Oral (Dog)	N	-

Y - Yes; N - no

IV. **TOXICOLOGY PROFILE** Updated: June 5, 2000

Guideline #	Study Identification and Classification	Results
81-1	Acute Oral Toxicity - Rats MRID 44606401 Study #:CTL/P/2197; Nov. 16, 1990 **Acceptable**	In an acute oral toxicity study, groups of fasted, young adult Wistar-derived albino rats/sex (5/sex) were given a single oral dose of dichlormid (97.2 % a.i.) in corn oil at doses of 500, 1,000, 2,000, 3,000, or 4,000 mg/kg and observed for 15 days. **Oral LD_{50} Males = 2,816 mg/kg (95% C.I. 2,143, 3,664 mg/kg)** **Females = 2,146 mg/kg (95% C.I. 1,478, 2,910 mg/kg)** Dichlormid is classified as **TOXICITY CATEGORY III** based on the LD_{50} in both sexes. No compound-related mortality occurred at 500 or 1,000 mg/kg. However, one female rat dosed at 500 mg/kg was killed *in extremis* on Day 5, but this was not thought to be compound related. Compound-related mortality occurred in 19/30 animals tested at ≥2,000 mg/kg within 5 days of administration. Clinical signs of toxicity seen in all dose groups included piloerection, upward curvature of the spine, decreased breathing rate, decreased activity, bizarre behavior and excessive grooming(500 and 1,000 mg/kg only), chromodacryorrhea, lachrymation, and salivation. Most animals dosed at 500 mg/kg and 1,000 mg/kg recovered by day 5. Animals from the 2,000, 3,000 or 4,000 mg/kg dose groups also exhibited tip toe gait, dehydration, reduced stability, and breathing abnormalities. All surviving animals in the ≥2,000 mg/kg dose groups recovered in 9 or 10 days after dosing. No treatment-related effects on body weight were observed in surviving animals. Gross necropsy of decedent animals revealed abnormal livers in 3 animals at 2,000 mg/kg, mottled liver and dark red intestines in one female at 3,000 mg/kg, and pale livers in 2 females at 4,000 mg/kg. Necropsy of animals sacrificed on day 15 revealed no abnormalities. This acute oral study is classified **ACCEPTABLE (§81-1)**. This study does satisfy the guideline requirement for an acute oral study (§81-1) in the rat.
81-2	Acute Dermal Toxicity - Rat MRID 44606402 Study #:CR2445; Nov. 16, 1990 **Acceptable**	In an acute dermal toxicity study , five Wistar-derived albino rats/sex were dermally exposed to dichlormid (97.2% a.i.) at 2,000 mg/kg (limit dose) for 24 hours. The test material was applied as received to approximately 10% of the total body surface area. Animals then were observed for clinical signs of toxicity and mortality for up to 14 days following administration. **Dermal LD_{50} Males >2,000 mg/kg (observed)** **Females >2,000 mg/kg (observed)** Dichlormid is classified as **TOXICITY CATEGORY III** based on the observed LD_{50} values for both sexes. All animals survived the 14-day observation period. No clinical signs of toxicity or skin irritation were seen in any of the animals. Initially (between Days 1 and 3), nine of ten animals showed a decrease in body weight. By Day 8, however, all animals showed an increased body weight compared to their initial weight (Day 1) and continued to gain weight until the end of the study. No macroscopic abnormalities were seen in any of the animals at gross necropsy. This acute dermal study is classified as **ACCEPTABLE (§81-2)**. It does satisfy the guideline requirement for an acute dermal study (§81-2) in the rat.

Guideline #	Study Identification and Classification	Results
81-3	Acute Inhalation Toxicity - Rat MRID 44606403 Report #:CTL/P/2305; Jan. 10, 1991 **Acceptable**	In an acute inhalation toxicity study, groups of young adult Wistar-derived (Alpk:APfSD) albino rats (5/sex) were exposed by inhalation route to dichlormid, 97.2% a.i.,for 4 hours to a nose only exposure concentration of 5.5 mg/L with a particle size of 4.35 (GSD = 2.10). Animals were then observed for 14 days. **LC_{50} for males and females is >5.5 mg/L (limit test).** **Dichlormid is classified as TOXICITY CATEGORY IV based on males and females.** None of the animals died during inhalation exposure or during the two week post-exposure period. Treatment-related clinical signs noted in test animals during exposure included salivation, lachrymation, and reduced response to sound. Immediately after exposure until Day 2, clinical signs indicative of neurological effects were seen and included head flicking, paw flicking, and salivation. During the 14-day observation period, abnormal respiratory noise (indicative of upper respiratory tract irritation) was noted in all males on Day 2 and continued in most males until Day 15, however, it was only present in 1-2 females from Days 2-5. Treated males and females had reductions in body weight and body weight gain initially after exposure on Day 1, however, by Day 8, weight gain was similar to or greater than that of controls. There were no treatment related necropsy findings. This acute inhalation study is classified as **ACCEPTABLE (§81-3)**, and satisfies the guideline requirement for an acute inhalation study (§81-3) in the rat.
81-4	Primary Eye Irritation - Rabbit MRID 44606404 Lab. Report #: T-13361; July 7, 1989 **Acceptable**	In a primary eye irritation study, 0.1 mL of R-25778 (dichlormid) (97.2% purity, Lot# WRC-4921-35-11) was instilled into the conjunctival sac of the left eye of each of nine Stauffland white rabbits. The treated eyes of three female rabbits were washed with water 20-30 seconds after exposure. The treated eyes of the remaining 6 rabbits (5 males, 1 female) were left unwashed. The right eye of each animal served as an untreated control. The animals were observed for ocular irritation at 1, 24, 48, 72, and 96 hours after treatment and eye irritation was scored by the Draize scheme. One hour following instillation, 5/6 rabbits whose eyes were left unwashed had mild to moderate conjunctival redness (scores of 1-2) and 3/6 rabbits had mild conjunctival chemosis (score of 1). In all three rabbits whose eyes were washed 20-30 seconds after treatment, mild conjunctival redness and chemosis (score of 1) were observed one hour following instillation. All irritation subsided by 24 hours. In this study, **R-25778 (dichlormid) is a mild ocular irritant**, and is classified as **TOXICITY CATEGORY IV** based on the mild to moderate ocular effects in 8/9 rabbits which subsided by 24 hours. This study is classified **ACCEPTABLE (§81-4)**, and satisfies the guideline requirement for a primary eye irritation study in the rabbit.

Guideline #	Study Identification and Classification	Results
81-5	Primary Dermal Irritation - Rabbit MRID 42807902 Study #:EB3495; Nov. 16, 1990 **Acceptable**	In a primary dermal irritation study, six male New Zealand white albino rabbits were dermally exposed to 0.5 mL of dichlormid for 4 hours. Animals were then observed for twelve days. Irritation was scored by the Draize scale. By 72 hours, 4/6 animals had well defined erythema. Very slight (2/6) or severe edema (3/6) was seen at 72 hours. Dermal irritation was reversible after day 5. In this study **dichlormid is a severe dermal irritant.** Dichlormid is classified as **TOXICITY CATEGORY II** for primary dermal irritation based on the degree of dermal effects observed at 72 hours. This study is classified as **ACCEPTABLE** and satisfies the guideline requirement for a primary dermal irritation study (§81-5) in the rabbit.
81-6	Dermal Sensitization - Guinea pig MRID 44606405 Study #:GG288; Nov. 16, 1990 **Acceptable**	In a dermal sensitization study with dichlormid (97.2% a.i., Lot # Y06015/002/007)) in corn oil, twenty female Dunkin Hartley albino guinea pigs were tested using the maximization test method of Magnusson and Kligman. Ten females were used for induction and challenge controls and 10 males were used as controls for rechallenge. Data from a positive control study using formaldehyde (40% aqueous solution) were provided. Following rechallenge with a 10% w/v preparation of dichlormid in corn oil, 4/20 (20%) test animals had scattered mild redness at 24 hours. No dermal irritation was observed in the control animals. The net percentage response was 20% indicating that rechallenge with a 10% w/v preparation of dichlormid in corn oil elicited a mild skin sensitization response (with no irritant response)in previously induced guinea pigs. Acceptable positive control data were provided to validate the test species and methods employed. **In this study, dichlormid (10% w/v in corn oil) is a mild dermal sensitizer.** This study is classified as **ACCEPTABLE** (§81-6), and does satisfy the guideline requirement for a dermal sensitization study (§81-6) in the guinea pig.

Guideline #	Study Identification and Classification	Results
82-1(a)	Subchronic Feeding - Rat MRID 44606406 Study #:PR0718; Aug. 14, 1989 **Acceptable**	In a subchronic toxicity study dichlormid (batch # WRC 4921-35-12:GG0101, 97.2% w/w) was administered in the diet for 90 days to 12 Alpk:APfSD rats/sex/dose at concentrations of 0, 20, 200, or 2000 ppm (intake of approximately 0, 1.4, 14, and 140 mg/kg/day for males and 0, 1.6, 16, and 150 mg/kg/day for females, respectively). No treatment-related mortality or clinical signs of toxicity occurred. Mid-dose females had lowered ($p \leq 0.05$ or 0.01) body weight gains (10-12%, weeks 6-12), food consumption (10-11%, weeks 8-10), and food efficiency (16%, weeks 5-8). High-dose males and females had lowered weight gain and food consumption throughout the study ($p \leq 0.05$ or 0.01), the most severe decreases occurring during the first treatment week, when weight gain was 62-72% lower than of controls and food consumption was 33-41% lower. For weeks 2-13, males had 21-30% lower weight gain and 11-21% lower food consumption than controls, whereas females had 33-40% lower weight gain and 15-24% lower food consumption. Food efficiency was only decreased in the high-dose females (15% overall, $p \leq 0.01$). Histopathological examination revealed that the liver was identified as a target organ in both male and female rats; males were affected to a greater extent than females. High-dose males had an increase in the combined incidence of liver centrilobular hypertrophy, eosinophilia, and chromatin margination (12/12 affected), bile duct pigmentation, and centrilobular vacuolation ($p \leq 0.05$ or 0.01). They also had a marginal but dose-related increase in the incidence of hepatic lipidosis ($p = 0.077$ at 2000 ppm; $p \leq 0.05$ for trend), and a slight decrease in plasma cholesterol and triglyceride levels (16-19%, $p \leq 0.05$ or 0.01). High-dose females also had an increased combined incidence of liver centrilobular hypertrophy, eosinophilia, and chromatin margination (6/12 affected), but did not develop lipidosis or have decreased plasma cholesterol and triglyceride levels. Liver weights were increased in 200 and 2000 ppm males, which both had 9% greater absolute weights than controls, and their relative (to body or brain) weights were increased by 16-32%, and 10-21%, respectively ($p \leq 0.01$; relative-to-brain weight not statistically analyzed). High-dose females had increased relative (to body or brain) liver weights (26%, $p \leq 0.01$; 12%, not analyzed). Aminopyrene N-demethylase (APDM) activity of liver tissue was significantly increased in mid and high-dose females (29% and 104%, respectively, $p \leq 0.01$), consistent with exposure to a xenobiotic; it is unclear why a similar effect was not seen in males. **Based on minor decreases in body weight gains and food efficiency in females and on increased liver weight and a slightly increased (NS) incidence of liver lipidosis in males, the LOAEL is 200 ppm under the conditions of this study (intake of approximately 14 mg/kg/day for males and 16 mg/kg/day for females). The NOAEL is 20 ppm (intake of approximately 1.4 mg/kg/day for males and 1.6 mg/kg/day for females).** This study is classified as acceptable (82-1a) and satisfies the guideline requirements for a subchronic oral toxicity study in rats.

Guideline #	Study Identification and Classification	Results
82-1(a)	Subchronic Feeding - Rat MRID 00058467 Study #:NA; May 17, 1972 **Unacceptable**	In a subchronic toxicity study R-25788 (dichlormid) (97.7% a.i.; Lot WRC 2012-9) was administered to 15 Sprague-Dawley rats/sex/dose in the diet at concentrations of 0, 60, 240 or 960 ppm (corresponding to target dose levels of 0, 10, 40, or 160 mg/kg/day) for 13 weeks. The clinical signs for the animals were not reported, although it was stated that there were no notable differences between the control and treated groups. There were no treatment-related deaths. At week 13, males in the 160 mg/kg/day group weighed 11.3% less than the controls ($p \leq 0.05$); females in the 40 and 160 mg/kg/day groups weighed 7.9% and 13.4% less than the controls ($p \leq 0.05$), respectively. Total weight gain was reduced by 14.4% in males at 160 mg/kg/day and by 10.0% and 19.5% in females at 40 and 160 mg/kg/day, respectively. With the exception of food intake values for the high dose females, there did not appear to be a compound-related effect on food intake. Weekly and total food efficiency did to appear to be affected by treatment. Clinical chemistry and hematology findings were not considered to be toxicologically significant and/or treatment-related. Statistically significant increases ($p \leq 0.05$) in absolute liver weights were seen in both male (40%) and female (22%) animals in the 160 mg/kg/day group. The relative weights were increased 40% over the control for both males and females. The high dose females showed a significantly decreased absolute kidney weight. Both male and females in the high dose group showed a significant (11%) reduction in adrenal weight. Neither the kidneys nor the adrenals showed any correlated histopathology. Significant histopathological correlates included a statistically significant increase in the incidence of increased liver cell size (centrolobular) in the 160 mg/kg/day males and females ($p \leq 0.004$) and degranulation of the liver cells in the 160 mg/kg/day males and females ($p \leq 0.004$) and the 40 mg/kg/day male group ($p \leq 0.02$). **The NOAEL is 10 mg/kg/day. Under the conditions of this study, the LOAEL is 40 mg/kg/day based on the liver histopathology in males.** This subchronic toxicity study is classified as **UNACCEPTABLE (82-1)** and not ungradable because numerous parameters required by 82-1 guidelines were missing (e.g. compound analysis in the diet, clinical chemistry, brain weight, histopathological evaluation of all animals in control and high dose groups and all target organs).

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Guideline #	Study Identification and Classification	Results
82-1(b)	Subchronic Feeding (capsule) - Dog MRID 41419401 Study #:PD0727; Dec. 14, 1988 **Acceptable**	In a subchronic toxicity study dichlormid (batch # WRC 4921-35-12 GGDO101, 97.2% w/w) was administered by capsule to 4 beagle dogs/sex/dose at doses of 0, 1, 5, 25, or 50 mg/kg/day for 90 days. One male and one female given 50 mg/kg/day dichlormid were sacrificed during week 7 due to poor physical condition and inappetence. These animals were thin, subdued, and the female salivated at dosing and had pale buccal membranes. The other animals survived to terminal sacrifice and had no consistent clinical signs. The 13-week body weight gain was substantially lower than of controls for dogs given 25 and 50 mg/kg/day: 37 to 54% lower in males and 57 to 68% lower in females, respectively ($p \leq 0.05$ for all but the 25 mg/kg/day males). Food consumption was generally unaffected (except in the two early-sacrifice animals); therefore the decrease in body weight gain for both sexes given 25 and 50 mg/kg/day was due to compound toxicity. Hematological changes consisted of decreased hemoglobin, hematocrit, and RBC count (10-17%, $p \leq 0.05$ or 0.01) at weeks 8 and/or 13 for both sexes of dogs given 50 mg/kg/day, and for females given 25 mg/kg/day. These were consistent with hemolytic anemia, although there were no correlated histopathological changes. The clinical chemistry alterations were consistent with voluntary muscle and liver toxicity. In both sexes of 25 and 50 mg/kg/day dogs, plasma creatine kinase levels were 7-107 times the control levels (weeks 6-8) and creatinine levels were 21-29% lower than controls ($p \leq 0.01$ for weeks 8 and/or 13). This was associated with microscopic voluntary muscle degeneration (with cellular infiltration) in all dogs given 25 and 50 mg/kg/day dichlormid (grossly, the muscle appeared pale in 50 mg/kg/day animals). Evidence of liver toxicity included increased ($p \leq 0.05$ or 0.01) aspartate aminotransferase (4-24 times controls for weeks 6-8) and alkaline phosphatase (38-70% increase at week 13) in both sexes of 25 and 50 mg/kg/day dogs, and decreased plasma albumin (8-14%) and urea (25-33%) in both sexes of 50 mg/kg/day dogs at week 13 and 4 or 8. The increase in alanine aminotransferase in males and females (levels were 3-14 times that of controls for weeks 6-8) could have been due to skeletal muscle damage and/or to liver toxicity. The levels of alanine and aspartate aminotransferase and of creatine kinase returned to baseline by week 13, possibly indicating that the dogs were beginning to recover from some of their treatment-induced injuries. Terminal absolute liver weight (and relative-to-brain weight) was markedly greater than of controls in both sexes given 25 and 50 mg/kg/day (16-25% and 50-56%, respectively; $p \leq 0.01$ for all except the 25 mg/kg/day females). Microscopic liver lesions were not common, suggesting that the liver weight increase might be an adaptive response. **Based on decreased body weight gains, hematological and clinical chemistry alterations, liver toxicity, and voluntary muscle pathological changes, the LOAEL is 25 mg/kg/day for both males or females under the conditions of this study.** The NOAEL is 5 mg/kg/day for both sexes. This study is classified as **acceptable (82-1b)** and satisfies the guideline requirements for a chronic oral toxicity study in dogs.

Guideline #	Study Identification and Classification	Results
82-1(b)	Subchronic Feeding - Dog MRID 00058468 Project #:45WR1862; May 17, 1972 **Unacceptable**	In a subchronic toxicity study dichlormid (R-25788) (lot # 1792-18: 99.5% pure; lot # WRC-2012-9 (unclear if this is a lot number): 97.7 ± 2% pure) was administered for 13 weeks to 4 beagle dogs/sex/dose in the diet at concentrations of 0, 80, 240, or 960 ppm (0, 2, 6, or 24 mg/kg/day, respectively, calculated using a dog food factor of 0.025). No animals died or exhibited treatment-related clinical signs or adverse effects on body weight gain, blood pressure, electrocardiograms, heart rates, or on hematology, ophthalmology, or urinalysis parameters. Necropsy revealed no treatment-related effects on organ weights or gross or microscopic pathology. The increase in serum alkaline phosphatase (AP) in high-dose animals for weeks 4-13, and in mid-dose males at week 4 were each largely due to increases in one of the four animals in each group. **Under the conditions of this study, a LOAEL cannot be assigned because there were no treatment-related toxicologically significant findings.** The NOAEL is ≥ 960 ppm (calculated as 24 mg/kg/day using the dog food factor of 0.025), the highest concentration tested. This study is classified as **unacceptable (82-1b) (Not ungradable)** and does not satisfy the guideline requirements for a chronic oral toxicity study in dogs because the animals were not adequately dosed (high dose was 960 ppm, or about 24 mg/kg/day), the test diets were not analyzed for stability, homogeneity, and concentration, and omission of much other required data. It is likely many of these deficiencies occurred because this study was conducted before GLP guidelines were developed.

Guideline #	Study Identification and Classification	Results
82-4	Subchronic Inhalation - Rat MRID 00155678 Lab. Report #:T-10773; Aug. 9, 1983 Acceptable	In a subchronic inhalation toxicity study, R-25788 (97.6% a.i.) was administered to 18 Sprague-Dawley rats/sex/dose by whole body exposure at concentrations of 0, 2, 19.9, and 192.5 mg/m³ (0, 2, 19.9, and 192.5 μg/L) for 6 hours per day, 5 days/week for a total of 65 days. The following parameters were measured: clinical signs, body weight, body weight change, food consumption, ophthalmology, organ weights, hematology, clinical chemistry, gross pathology, and histology. Clinical chemistry and hematological parameters were not effected by treatment with R-25788. One male in the 19.9 mg/m³ group died at day 17 of nontreatment related causes. The incidence of following clinical signs was significantly increased relative to the untreated control in both sexes in the 192.5 mg/m³ group: salivation, dull behavior, aggressive behavior, stained integument, rough coat, and wet hair. Although a common observation in inhalation studies, as shown in 83% incidence in controls, the day of onset for chromorhinorrhea was significantly earlier in the19.9 and 192.5 mg/m³ groups (day 15 and 1.5, respectively vs. day 54.5) relative to untreated control groups. Incidences of chromodacryrrhea (statistically significant) and closed eyes relative to the untreated control were observed in rats of the 19.9 and 192.5 mg/m³ groups. The daily total number of seminal plugs observed in the afternoon following exposure increased 328% and 289% in the19.9 and 192.5 mg/m³ groups, respectively compared to the control group. This increase in the daily total number of seminal plugs indicates that the rats were stressed from the treatment. Statistically significant body weights compared to the control were observed at pretest in the males and females making the interpretation of body weight data very difficult. Toxicologically and statistically significant decreases in body weight were observed in the 192.5 mg/m³ groups of both sexes (-17% in males; -10% in females) compared to control. Animals in the 192.5 mg/m³ group also consumed significantly less food relative to the control (-9.4%). Both sexes exhibited toxicologically significant increases relative to the control in liver organ to body weight ratios in the 19.9 and 192.5 mg/m³ groups (males +29% and +39%, respectively; females +7.4% and +23%, respectively). Females in the 192.5 mg/m³ exhibited a 10% increase in absolute liver weight relative to the control. Both sexes exhibited toxicologically significant increases relative to the control kidney organ to body weight ratios in the 19.9 and 192.5 mg/m³ groups (males +10% and +12%, respectively; females +35% and +47%). Differences in organ and organ to body weight ratios in heart, brain, lung, and adrenals relative to untreated control were the result of decreases in body weight and lack of feeding in the high dose group. In both sexes increases in the following non-neoplastic histological changes were observed in the nasal passages of the 19.9 and 192.5 mg/m³ groups : degeneration, necrosis, and/or sloughing of olfactory epithelium; attenuation of olfactory epithelium; intraepithelial cyst formation; and basal cell hyperplasia of olfactory epithelium. Rats exposed to 192.5 mg/m³ R-25788 exhibited increased incidence of lymphoid aggregates in the submucosa of the larynx, the prostate, lungs, and salivary glands relative to untreated control. Female rats in the 192.5 mg/m³ group exhibited increased lymphoid aggregates which were accompanied by cellular degeneration. **The LOAEL is 19.9 mg/m³ (19.9 μg/L), based on clinical signs, gross pathology, ophthalmology, liver and kidney weights, and non-neoplastic histology. The NOAEL is 2 mg/m³ (2 μg/L).** This study is considered acceptable-guideline and does satisfy the guideline requirement for a subchronic inhalation study (82-4; OPPTS 870.3465) in the rat.

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Guideline #	Study Identification and Classification	Results
83-3(a)	Developmental Toxicity - Rats MRID 44606408 Study #RR0406; July 18, 1989 **Acceptable**	In a developmental toxicity study, 24 presumed pregnant Wistar rats per group were administered Dichlormid (97.2% a.i.) orally by gavage at doses of 0, 10, 40, or 160 mg/kg/day on gestation days (GD) 7-16, inclusive. On GD 22, dams were sacrificed, subjected to gross necropsy, and all fetuses examined externally, viscerally, and skeletally for malformations/variations. Maternal toxicity in the 40 and 160 mg/kg/day groups is based on statistically significant decreases ($p<0.05$; 0.01) in absolute body weights, body weight gains, and food consumption. Final absolute body weights in the mid- and high-dose groups were 95% and 92% of controls, respectively. During the dosing interval (GD 7-16), mid- and high-dose dams gained 22% and 46% less weight, respectively, and consumed 13% and 31% less feed, respectively, than controls. The most pronounced reductions in body weight gain and food consumption occurred during the first three days of treatment (GD 7-10) in both groups: mid-dose females gained 39% less weight and consumed 15% less feed, while high-dose dams gained 144% less weight and consumed 38% less feed. In addition to decreases in body weight and food consumption, mid- and high-dose dams also had decreased food efficiency during the entire dosing interval (-9% and -22% of controls, respectively). No statistically significant decreases in absolute body weights, body weight gains, or food consumption were noted in the 10 mg/kg/day group dams. No treatment-related mortalities, clinical signs of toxicity, or gross pathological changes were observed in the study. **Therefore, the maternal toxicity LOAEL is 40 mg/kg/day based on decreased mean absolute body weights, body weight gains, and food consumption, and the maternal toxicity NOAEL is 10 mg/kg/day.** No dose- or treatment-related effects on pregnancy rates, number of corpora lutea, pre- or postimplantation losses, resorptions/dam, fetuses/litter, or fetal sex ratios were observed in the treated groups as compared with the controls. No treatment-related external or visceral malformations/variations were observed in any litter. Most treated and control litters contained fetuses with minor variations in skeletal ossification. The incidence of a misaligned 5th sternebra was statistically significantly ($p<0.05$) but marginally increased in fetuses (6/259 fetuses affected vs. 0/277 for control) and marginally increased in litters (4/22 litters affected vs. 0/22 for control: $p = 0.054$) from the high-dose group. Because no other potential treatment-related skeletal malformation/variations were identified, the marginal increase in this variation was used for the establishment of a threshold LOAEL. **Therefore, the developmental toxicity NOAEL is 40 mg/kg/day. The developmental toxicity LOAEL threshold is 160 mg/kg/day based on a marginal increase in skeletal anomalies.** This developmental toxicity study is classified as **Acceptable/guideline** and satisfies the subdivision F guideline requirements for a developmental toxicity study in rats (83-3a).

Guideline #	Study Identification and Classification	Results
83-3(a)	Developmental Toxicity - Rats MRID00058469 Study #NA; Jan. 28, 1972 **Unacceptable/not upgradable**	In a developmental toxicity study, 20 presumed pregnant Sprague-Dawley rats per group were administered R-25788 (97.7% a.i.) in the diet at doses of 0, 10, or 40 mg/kg/day on gestation days (GD) 6-15, inclusive. On GD 20, dams were sacrificed, subjected to gross necropsy, and all fetuses examined externally. One-third of the fetuses were examined viscerally, and the remaining fetuses were examined for skeletal malformations/variations. Maternal and developmental toxicity could not adequately be assessed because of several deficiencies in the conduct and reporting of the study, including: three test groups were not available (only 2 treatment groups), individual observations and summary tables addressing maternal clinical signs and gross necropsy were not provided, data analyses of the reported maternal data were not performed (means, statistical analyses), inadequate number of litters were available in the 10 and 40 mg/kg/day groups (18 instead of the guideline minimum of 20), no data on litter incidences were provided, no individual fetal examination data (external, visceral, or skeletal) were included, and statistical analyses of the reported fetal summary data were not conducted. Even if the missing data are supplied and support the study author's conclusions that the "three groups are entirely comparable," the study still will not have defined a maternal or developmental toxicity LOAEL. **Therefore, the maternal and developmental toxicity LOAEL and NOAEL could not be determined.** This study is classified as **Unacceptable/not upgradable** and does not satisfy the Subdivision F guideline requirements for a developmental toxicity study in rats (83-3a) because of many major deficiencies in the conduct of the study and reporting of data, including: an inadequate number of test doses (only 2 treatment groups), not known if technical form of the active ingredient was used, no information on the stability of the test compound, no analyses for test material stability, homogeneity, and concentration in dosing medium, no statistical analyses, no individual observations and summary tables addressing maternal antemortem/daily observations or gross necropsy, inadequate number of litters/dose (18 instead of 20), no data on litter incidences, and no individual fetal examination data (external, visceral, or skeletal). If the missing data are supplied and support the study author's conclusions that the "three groups are entirely comparable," the study will still be unacceptable because it will not have defined a maternal or developmental toxicity LOAEL.
83-3(b)	Developmental Toxicity - Rabbits MRID 44606407 Study #RB0414; Aug. 31, 1989 **Acceptable**	In a developmental toxicity study), 20 presumed pregnant New Zealand White rabbits per group were administered Dichlormid (97.2% a.i.) in corn oil orally by gavage at doses of 5, 30, or 180 mg/kg/day on gestation days (GD) 7-19, inclusive. A control group of 19 animals received corn oil alone. On GD 30, does were sacrificed, subjected to gross necropsy, and all fetuses examined externally, viscerally, and skeletally for malformations/variations.

Guideline #	Study Identification and Classification	Results
83-3(b)	MRID 44606407 Study #RB0414; Aug. 31, 1989 **Acceptable**	-Continued- Maternal toxicity in the 180 mg/kg/day group is based on an increased incidence of alopecia and decreases in body weight gains and food consumption. The incidence of alopecia was slightly increased in the high-dose group as compared with controls (no. animals/no. observations: 10/212 vs. 7/71 controls). In addition to the increased number of observations of alopecia in affected animals, affected high-dose animals generally had more severe hair loss than controls, and it tended to affect more than one area. No other treatment-related clinical signs were observed. Although no statistically significant differences in mean absolute maternal body weights were noted, high-dose females had significant decreases in body weight gains and food consumption during the entire dosing interval of GD 7-19 (-101% and -44% of controls, respectively; $p<0.01$). After dosing was stopped, high-dose females had compensatory increases in body weight gains (+206% and +33% for GD 19-22 and 19-30, respectively; $p<0.01$; 0.05) and food consumption (+17% for GD 22-26 and 26-30; $p<0.05$). Overall, high-dose does gained 26% less when compared with controls ($p<0.05$). No treatment-related differences in clinical signs, body weight gain, or food consumption were noted in the 5 or 30 mg/kg/day treatment groups as compared with the controls, and no clear treatment-related gross pathological changes were observed in any of the treated groups. One animal from the control group and two from the high-dose group were killed on GDs 29, 18, and 19, respectively, due to early delivery/abortions. One additional high-dose animal was killed *in extremis* on GD 8 due to morbidity. It is not certain if the morbidity was an effect of treatment. **Therefore, the maternal toxicity LOAEL is 180 mg/kg/day based on an increased incidence of alopecia and decreased mean maternal body weight gains and food consumption, and the maternal toxicity NOAEL is 30 mg/kg/day.** One control doe delivered early on GD 29, and two high-dose does aborted on GD 18 and 19, respectively. It is not clear if the abortions were treatment-related. In high-dose females, the percentage of post-implantation loss (23.1% vs. 7.2% for controls) was increased, statistically so ($p<0.01$) following transformation of the data for statistical analysis. The increase in post-implantation loss was accompanied by increases in the numbers of late resorptions ($p<0.05$ following transformation) and early resorptions (n.s.), decreased number of live fetuses/litter (6.67 vs. 8.18 for controls, n.s.), and decreased mean fetal body weight (37.70 g vs. 38.76 g for controls, n.s.). No clear, treatment-related effects were noted during Caesarian section in does from the 5 or 30 mg/kg/day groups. It is not known if any doe had complete litter resorption because nongravid uteri were not stained for this purpose. No treatment-related external or visceral malformations/variations were observed in any litter. Most treated and control litters contained fetuses with minor variations in skeletal ossification. The incidence of reduced ossification of frontal and parietal skull bones was statistically increased ($p<0.05$) in high-dose fetuses and was marginally increased in high-dose litters (5/12 litters affected vs. 2/11 for control; n.s.). **Therefore, the developmental toxicity LOAEL is 180 mg/kg/day based on increases in post-implantation loss accompanied by an increased number of resorptions/doe (both early and late resorptions), a decreased number of live fetuses/litter, and slightly decreased mean fetal body weights. The developmental toxicity NOAEL is 30 mg/kg/day.** This developmental toxicity study is classified as **Acceptable/guideline** and satisfies the subdivision F guideline requirements for a developmental toxicity study in rabbits (83-3b).

Guideline #	Study Identification and Classification	Results
83-5	Combined Chronic/Oncogenicity - Rat MRID 44529402 Lab. Project ID: MJLC-001; Jan. 29, 1999 **Acceptable**	In a combined chronic/oncogenicity study, dichlormid (97.9%, Lot #P12, inert ingredient) was administered via the diet to a total of 64 Alpk:AP$_f$SD rats/sex/group at 0, 20, 100, or 500 ppm (equivalent to 0, 1.3, 6.5, or 32.8 mg/kg/day in males and 0, 1.5, 7.5, or 37.1 mg/kg/day in females) for up to 104 weeks. Twelve rats/sex/dose were terminated after 52 weeks. Males in each dose group were terminated at week 100 when survival of males in the 20 ppm dose group reached 25%. Females in each dose group were terminated as scheduled. Survival, clinical signs, hematology and urinalysis parameters, ophthalmology, and gross findings for both sexes at all doses were unaffected by the treatment with dichlormid. No treatment-related findings were observed at the 20 or 100 ppm levels. Chronic toxicity was characterized at the 500 ppm dose level by minor reductions in mean body weights (↓3-9%; $p<0.01$) in both sexes throughout the study. Decreases ($p<0.01$ and 0.05) in food consumption were observed in the males (↓3-7%) through week 32 and the females (↓6-12%) through week 88. Mean food efficiency was decreased in the males during weeks 1-12 (↓3-7%, $p<0.01$ and 0.05) and in the females during weeks 1-4 (↓3%, $p<0.05$). Plasma triglycerides were decreased (↓20-36%) in the males at each sampling interval, with the decreases being significant ($p<0.05$ and 0.01) at each interval except week 79. In the females, triglyceride levels were decreased (↓16-45%, $p<0.05$ and 0.01) at weeks 27, 53, and 105, with non-significant decreases (↓5-9%) also occurring at weeks 14 and 79. Plasma γ-glutamyl transferase was increased (↑57-114%, $p<0.05$ and 0.01) in the males at weeks 27, 53, and 100, with non-significant increases (38-51%) also occurring during weeks 52 and 79. Absolute liver weights were increased ($p<0.05$ or 0.01) at the interim sacrifice (week 52) in the males (↑13%) and females (↑9%). At the terminal sacrifice (week 100/105), the males had increased absolute liver weights (↑8-9%, $p<0.05$). The males also showed increased incidences of hepatitis (38% treated vs 27% controls), hepatocyte vacuolation (89% treated vs 44% controls), and pigmentation 75% treated vs 38% controls). In addition, the males had an increased incidence of bilateral tubular degeneration in the testis (36% treated vs 19% controls). None of the non-neoplastic microscopic data were analyzed for statistical significance. In the uterus of high-dose females, there were slight increases over controls in the incidence of adenocarcinomas (7.8% treated vs 1.6% controls), adenomas (treated 3.1% vs 0% controls) and stromal cell sarcomas (3.1% treated vs 0% controls). The Sponsor stated that there was a significant trend ($p=0.026$) in the incidence of adenocarcinomas with increasing dose. However, the incidences of adenocarcinomas (7.8% treated vs historical controls 0-10.9%) and stromal cell sarcomas (treated 3.1% vs historical controls 0-3.1%) are within the range of historical controls. The increase for adenomas is slightly outside the range of historical controls (treated 3.1% vs historical controls 0-1.6%). Females exposed to 20 ppm dichlormid had an increased incidence of benign mammary gland fibroadenomas (15.6% treated vs controls 9.3%), but this increase was not seen at higher doses. Leiomyosarcoma was observed in the uterus of one high-dose female and in the cervix of one control female. Males dosed with 100 and 500 ppm dichlormid had slight increases in the number of follicular adenomas in the thyroid gland (100 ppm-4.7%, 500 ppm- 3.1% vs controls 0%). Data were presented to show that these values were within the range of historical control values (0-9.3%). The 20 ppm males had an increased incidence of benign pheochromocytoma of the adrenal gland (14% treated vs 7.8% controls), and the 20 ppm and 100 ppm males had increased incidences of benign leydig cell tumors in the testis (20 ppm - 12.5%, 100 ppm - 17.2% vs controls - 7.8%). Data were not presented to show that the incidence of these lesions were within the range of historical controls; however, increases in the occurrence of these lesions were not seen at the higher doses.

Guideline #	Study Identification and Classification	Results
83-5	MRID 44529402	-Continued- **The chronic LOAEL is 500 ppm (32.8 mg/kg/day and 37.1 mg/kg/day in males and females, respectively) based on non-neoplastic lesions in the liver associated with increased liver weights and changes in blood clinical chemistry parameters. The chronic NOAEL is 100 ppm (6.5 mg/kg/day and 7.5 mg/kg/day in males and females, respectively).** **Under the conditions of this study, there was no evidence of carcinogenic potential.** Dosing was considered adequate based on decreases in body weights, food consumption, food efficiency, triglycerides, and increases in gamma glutamyl transferase, and liver weights associated with increased incidence of hepatitis, hepatocyte vacuolation and pigmentation observed at 500 ppm. Subchronic rat feeding study (MRID 44606406) identified liver as the target organ at doses ≥ 16 mg/kg/day. The submitted study is classified as acceptable (§83-5) and does satisfy the guideline requirements for a chronic toxicity study (§83-1) and a carcinogenicity study (§83-2) in rats.

Guideline #	Study Identification and Classification	Results
83-2(b)	Oncogenicity - Mouse MRID 44529401 Lab. Project ID: CTL/P/5343/PM1000; Jan. 22, 1998 **Acceptable**	In a mouse oncogenicity study, dichlormid (97.9%, Lot #P12 (WRC13790-28-02), inert ingredient) was administered to C57BL/10J,CD-1 Alpk mice (55/sex/group) for up to 18 months at 0, 10, 50, or 500 ppm (equivalent to 0, 1.4, 7.0, or 70.7 mg/kg/day in males and 0, 1.8, 9.2, or 92.4 mg/kg/day in females). Survival, clinical signs, food consumption, differential leukocyte counts, and gross necropsy findings for both sexes at all doses were unaffected by treatment with dichlormid. Chronic toxicity was characterized at the high-dose by slight, statistically significant differences in body weights throughout the study in the males (↓2-6%; $p<0.01$) and in most weeks in the females (↓1-3%; $p<0.05$ or 0.01). At the end of the study, body weights in the high-dose males and females were lower than controls by 6% and 3%, respectively ($p<0.05$ or <0.01). In the males, food efficiency (g growth/100 g food) was lower than controls during weeks 1-4 (↓22%, $p<0.01$) and during weeks 1-12 (↓12%, $p<0.01$), the only periods in which food efficiency was determined. At 500 ppm dichlormid, male mice had an increase in the incidence of minimal to moderate tubular vacuolation within the kidney (31/55 males compared to 12-15/55 in the lower dose groups and the controls). This finding correlates with the increased absolute kidney weights in males (↑4%) in the 500 ppm group. The high-dose male mice also had an increased incidence of marked hyperplasia of Leydig cells within the testis (27/55 males compared to 1-11/55 in controls and 13/55 in each of the lower treatment groups. In the females, there was an increase in the incidence of ovarian atrophy, with 23/55 animals affected in this group compared to 10 or 11 animals in the control groups and 12 or 14 animals in the 10 ppm and 50 ppm treatment groups, respectively. These non-neoplastic changes, although marked at the highest dose, were not analyzed for statistical significance. There were small changes in tumor incidence in the male mice, including an increased ($p<0.05$) incidence in harderian gland adenomas (9.1%) at 500 ppm and histiocytic sarcomas at 50 ppm (9.1%). However, data were presented to show that the numbers of tumors found in treated animals were consistent with tumor incidence in historical control animals (0-8% harderian gland adenomas and 0-16% histiocytic sarcomas). **The LOAEL for chronic toxicity is 500 ppm (equivalent to 70.7 mg/kg/day for male mice and 92.4 mg/kg/day for females), based on changes in reproductive organs and kidney changes in males. The chronic NOAEL is 50 ppm (equivalent to 7.0 and 9.2 mg/kg/day for males and female mice, respectively).** **Under the conditions of this study, there was no evidence of carcinogenic potential.** Dosing was considered adequate based on slight decreased body weights in both high-dose males and females, decreased food efficiency in males, and changes in reproductive organs in males and females. This oncogenicity study is determined to be **acceptable** [§83-2(b)] and does satisfy the guideline requirement for an oncogenicity study in mice.

Guideline #	Study Identification and Classification	Results
84-2	Gene Mutation - Ames MRID 41561404 **Study #T-13178; Dec. 31, 1987** **Acceptable**	In independently performed microbial reverse gene mutation assays, Salmonella typhimurium strains TA1535, TA1537, TA98 and TA100 were exposed to Dichlormid as R-25788 (97.2%) at doses ranging from 188-3000 µg/plate without or without S9 fractions from two species (both trials). The S9 fractions were derived from Aroclor 1254-induced rat livers and mouse livers. The test material was delivered to the test system in dimethylsulfoxide. Cytotoxicity and compound insolubility were evident at 5000 µg/plate -/+ rat or mouse S9 in the preliminary study; R-25788 was, therefore, tested to a high soluble and sub-cytotoxic dose in the main assay (both trials). All strains responded in the expected manner to the appropriate positive controls. **There was, however, no evidence that R-25788 induced a mutagenic effect under any test condition in either assay.** This study is classified as **Acceptable** and satisfies the guideline requirements for a bacterial gene mutation assay (84-2).
84-2	Gene Mutation - Mammalian cell culture MRID 41561405 Study #T-13179; Dec. 31, 1987 **Acceptable**	In independently *in vitro* mammalian cell gene mutation assays, L5178Y mouse lymphoma cells were exposed to Dichlormid as R-25788 (97.2%) at nonactivated doses ranging from 80-600 µg/mL (initial trial) or 200-600 µg/mL (repeat trial). Trials performed with S9 activation included S9 fractions from two species and investigated doses of 1.0-7.5 µg/mL + rat liver S9 or 2.5-40.0 µg/mL + mouse liver S9. The S9 fractions were derived from Aroclor 1254-induced rat livers or mouse livers, and the test material was delivered to the test system in dimethylsulfoxide. Under nonactivated conditions, levels ≥600 µg/mL were severely cytotoxic. **The evidence of a mutagenic effect at 600 µg/mL in the first trial was confirmed in the repeat assay using a narrow range of doses (200, 300, 400, 550, and 600 µg/mL). Increased mutant colonies and mutation frequencies (MF) were seen at all assayed levels. However, the three highest concentrations induced clear dose-related increases in the MF; MFs were 2.1-, 2.6-, and 2.9-fold higher than the solvent control at 400, 550, and 600 µg/mL, respectively. Relative total growth (RTG) at these levels ranged from 25% at 400 to 9% at 600 µg/mL.** In the presence of both S9 activation systems, R-25788 was more cytotoxic as indicated by the appreciably lower doses that were selected for these trials compared to the nonactivated phase of testing: 1.0, 2.5, 5.0, and 7.5 µg/mL + rat S9 and 2.5, 5.0, 7.5, 10.0, 25.0, and 40.0 µg/mL + mouse S9. The results indicated that increased mutant colony counts and MFs accompanied exposure to 5.0 and 7.5 µg/mL + rat liver S9 and 25 and 40 µg/mL + mouse liver S9. At 5.0 and 7.5 µg/mL/+ rat S9, MFs were 1.8- and 2.8-fold higher than the solvent control; RTGs at these concentrations were ≥ 12%. In the presence of mouse liver S9 activation, the 25- and 40-µg/mL treatment levels induced 3.2- and 4.7-fold increases in the MF, respectively; RTG was 9% at 25.0 µg/mL and 5% at 40.0 µg/mL. Although the study authors stated that the findings were inconclusive because of cytotoxicity, we assess that there was sufficient justification to include the cytotoxic levels in the interpretation of the results (see Section D-- Reviewers Discussion and Interpretation of Results). **We assess, therefore, that R-25788 is mutagenic in L5178Y mouse lymphoma cells both with and without S9 activation at doses that extend into the cytotoxic range.** The study is classified as **Acceptable** and satisfies the requirements for an in vitro mammalian forward gene mutation study (84-2).

Guideline #	Study Identification and Classification	Results
84-2	Structural chromosome aberration - *In vivo* mouse lymphocytes MRID 41561403 Study # T-13182; Dec. 31, 1987 **Acceptable**	In an in vivo mouse micronucleus assay, groups of five male CD-1 mice received single oral gavage administration of 1000, 1500 or 2000 mg/kg Dichlormid as R-25788 (97.2%) and five females per group received 500, 1000 or 1200 mg/kg. Bone marrow cells were collected 24, 48, or 72 hours after compound administration and were examined for micronucleated polychromatic erythrocytes (MPEs). The test material was delivered to the test animal in corn oil. Deaths were observed in high-dose males and females, and a slight cytotoxic effect on the target organ (bone marrow cells) was seen in the high-dose groups at the 72-hour sacrifice. The positive control induced the expected high yield of MPEs. **There was, however, no evidence that R-25788 was clastogenic or aneugenic at any dose or harvest time.** The study is classified as **Acceptable** and satisfies the guideline requirement for a mouse micronucleus assay (84-2).
84-2	Structural chromosome aberration - *In vivo* human lymphocytes MRID 41561407 Study # SV0311; June 7, 1989 **Acceptable**	In an *in vitro* chromosome aberration assay (MRID No. 41561407), human lymphocytes obtained from one male and one female donor were exposed to Dichlormid (R-25788) (97.2%) at doses ranging from 2.5-1200 µg/mL in both the absence and presence of S9 activation. Cells were harvested and metaphases were analyzed from cells dosed with 75, 500 or 750 µg/mL +/-S9. The S9 was derived from Aroclor 1254-induced Alpk:APfSD male rat livers, and the test material was delivered to the test system in dimethylsulfoxide. The high dose with or without S9 activation induced a ≥50% decrease in the mitotic index. The positive controls induced the expected high yield of cells with structural chromosome aberrations. **There was, however, no evidence that Dichlormid induced a clastogenic response at any nonactivated or S9-activated dose.** The study is classified as **Acceptable** and satisfies the requirements for an in vitro mammalian cell cytogenetic assay (84-2).

Guideline #	Study Identification and Classification	Results
84-2	Structural chromosome aberration - *In vitro* mouse lymphocytes MRID 41561406 Study # SV0311; June 7, 1989 **Unacceptable**	In an *in vitro* chromosome aberration assay , mouse lymphoma L5178Y cells were exposed to Dichlormid as R-25788 (97.2%) at doses ranging from 200-500 µg/mL in the absence of S9 activation and doses of 8-60 µg/mL in the presence of S9 activation. Cells were harvested 12 hours post-treatment and metaphases were analyzed for structural chromosome aberrations. The S9 was derived from Aroclor 1254-induced Sprague Dawley male rat livers, and the test material was delivered to the test system in dimethylsulfoxide. Under nonactivated conditions, R-25788 did not increase the frequency of structural or numerical chromosome aberrations in cells harvested 12 hours posttreatment. Cytotoxicity as indicated by a marked reduction in the mitotic index was apparent at the highest assayed level (500 µg/mL); higher concentrations were severely cytotoxic. **The findings indicate that nonactivated R-25788 was tested over an adequate range of test material concentrations but failed to induce a clastogenic effect.** **No conclusions can be reached; however, for the S9-activated phase of testing.** No significant or dose-related increase in the percentage of cells with structural or numerical aberrations was seen. However, the presence of rare complex aberrations (i.e., triradials, quadriradials, and translocations) at the majority of the doses, although not sufficient to conclude that R-25788 is a clastogen, should have prompted the performance of a repeat test to resolve this issue. Similarly, the lack of a marked cytotoxic effect on high-dose cultures suggest that higher concentrations could have been evaluated. The study author claimed that the number of cells completing two cell cycles (M_2) was reduced at all S9-activated assayed levels in parallel cultures incubated with BrdU for 16 hours; no data were provided to support this statement. If cell cycle delay was suspected, the rationale for proceeding with the analysis of 12-hour posttreatment cultures, presumably with a high proportion of M_1 cells, is unclear. Based on the above considerations, we assess that conditions may not have been optimal to detect the potential, if any, of S9-activated R-25788 to induce clastogenesis. STUDY CLASSIFICATION: The study is **Unacceptable** and does not satisfy the requirements for an in vitro mammalian cell cytogenetic assay (84-2)because definitive conclusions can not be reached. It is recommended that the S9-activated assay be repeated using either higher test material levels and/or a prolonged cell harvest time.
84-2	DNA Damage - Rat hepatocytes MRID No. 41561408 Study # SV0350; Aug. 18, 1989 **Acceptable/non-guideline**	In independently conducted *in vivo/in vitro* unscheduled DNA synthesis (UDS) assay, groups of male Fischer-344 rats were administered single oral gavage doses of 350, 700 or 1400 mg/kg Dichlormid (97.2%) prepared in corn oil. Animals were sacrificed at 2 hours (2 males/dose in the first trial; 3 males/dose in the second trial) or 16 hours (3 males/dose in the first trial; 2 males/ dose in the second trial) posttreatment, and hepatocytes were analyzed for UDS. Clinical signs of toxicity (e.g., weakness, loss of balance, and low body temperature), adverse effects on the target organ (i.e., mottled livers), and cytotoxic effects on the hepatocyte cultures were apparent in the high-and/or mid-dose groups. The results obtained with the positive controls confirmed the sensitivity of the test system to detect UDS. **There was no evidence that Dichlormid at the selected doses increased the frequency of UDS in treated hepatocytes at either sacrifice interval.** The study is classified as **Acceptable** (Nonguideline) and does not satisfy the requirements for FIFRA Test Guideline 84-2 for UDS mutagenicity data. It can, however, be considered supplemental information.

Guideline #	Study Identification and Classification	Results
84-2	DNA Damage - Rat hepatocytes MRID 44606409 Final Report # T-13181; April 12, 1988 **Acceptable**	In an unscheduled DNA synthesis assay, primary rat hepatocyte cultures were exposed to R-25788 in DMSO at concentrations of 20-60 ug/mL for 19-20 hours. In addition to untreated (medium) and solvent (DMSO) controls, additional cultures were exposed to a known mutagen, dimethylnitrosamine (DMN) to serve as a positive control. R-25788 was tested up to a cytotoxic concentration (60 ug/mL). **The positive controls induced the appropriate response. There was no consistent, reproducible evidence that unscheduled DNA synthesis, as determined by radioactive tracer procedures [nuclear silver grain counts] was induced.** This study is classified as **Acceptable**, and satisfies the requirement for FIFRA Test Guideline 84-2 for other genotoxic mutagenicity data.

V. DATA GAPS

The toxicity data requirements for establishment of permanent tolerance for use of dichlormid as safener on corn is incomplete. Following are the DATA GAPS:

Acute Neurotoxicity
Subchronic Neurotoxicity
21-Day Dermal Toxicity
Chronic Toxicity in Dog
2-Generation Reproduction
Metabolism

The HIARC considered the requirement for developmental neurotoxicity as a data gap, however, it was placed in reserve pending receipt and review of the findings of the acute and subchronic neurotoxicity studies. This is because that the 2-generation reproduction toxicity study is a data gap and there is a qualitative evidence of increased susceptibility of fetal effects more severe than those observed in maternal animals in the developmental toxicity study in rabbits.

VI. ACTION BEING TAKEN TO OBTAIN ADDITIONAL INFORMATION OR CLARIFICATION:

The sponsor should be notified of the issues discussed under Section V and will be required to rectify for full registration of this chemical.

VII. REFERENCE DOSE (RfD):

The Hazard Identification Assessment Review Committee (HIARC) met on August 5, 1999 (HED Doc. #013614) and selected doses and endpoints for dietary and non-dietary exposure risk assessments. **The decisions made at this meeting are only for the TIME LIMITED**

TOLERANCE.

A. Acute Reference Dose (RfD)

For females 13+ and as well as the general population, the HIARC selected a rat developmental NOAEL of 10 mg/kg/day based on decreased body weight gain, food consumption (most significant on days 7 - 10 of dosing), and maternal toxicity NOAEL of 40 mg/kg/day. An acute RfD of 0.10 mg/kg/day was calculated using above NOAEL and an uncertainty factor (UF) of 100 to account for inter-species extrapolation (10X) and intra-species variability (10X). This 100 fold uncertainty factor is considered adequate for protecting children and infants under FQPA.

B. Chronic Reference Dose (RfD)

A chronic RfD of 0.022 mg/kg/day was calculated using a NOAEL of 6.5 mg/kg/day based on liver weights, clinical pathology and liver histopathology at the LOAEL of 32.8 mg/kg/day from the chronic toxicity in rat. An uncertainty factor (UF) of 100 was applied to account for inter-species extrapolation (10X) and intra-species variability (10X). In addition, an extra 3X UF factor was applied because chronic study in dog is a DATA GAP. This 300 fold uncertainty factor is considered adequate for protecting children and infants under FQPA.

C. FQPA Safety Factor

Based solely on the hazard assessment (with no consideration of the exposure assessments), the HIARC recommended that the FQPA safety factor be retained at 10X since: 1) there is qualitative evidence of increased susceptibility in the developmental study in rabbits; 2) the toxicity data base is incomplete: there is a data gap for the 2-generation reproduction study in rats; 3) the recommendation for a developmental neurotoxicity toxicity study in rats is placed in reserve pending receipt and review of the findings of the acute and subchronic neurotoxicity studies.

VIII. PENDING REGULATORY ACTIONS:

The Registration Action Branch I is unaware of any pending regulatory action against this chemical.

IX. TOXICOLOGY ISSUES PERTINENT TO THIS REQUEST:

A. Available data indicate no toxicity concerns at this time. Several toxicity studies (subchronic dog and rat and chronic rat) identified liver as the target organ. There is no difference in toxicity between males and females. It is not a development toxicant, although there is slight increased susceptibility in the developmental rabbit study. It is not

a carcinogen in in vivo bioassays in 2 species.

B. **Mutagenicity** - The current Mutagenicity guidelines require 3 studies: Ames and mammalian mutation assays as well as an acceptable chromosomal aberration assay. The data base is considered adequate based on 1991 mutagenicity guidelines. These genetic toxicology studies do not present a Mutagenicity concern at this time. It has been found found to be negative in various Mutagenicity assays except a positive response in the mouse lymphoma assay including in vivo carcinogenicity in rat and mouse.

C. **Risk Concerns:**

Dietary

See Section VII for acute and chronic dietary exposures.

Worker Exposure

There is no concern regarding the residential exposure since dichlormid is being used as safener on corn. Since 21-day dermal toxicity is a DATA GAP a 100% default is used for dermal absorption. The HIARC used rat developmental for Short-Term Dermal and chronic rat toxicity for Intermediate-Term Dermal. There is minimal concern for Long-Term dermal exposure/risk.

Inhalation exposure of all duration utilized a NOAEL of 2 mg/m^3 based on clinical signs, increased liver and kidney weights, gross pathology and liver histopathology at a LOAEL of 19.9 mg/m^3 from the subchronic rat inhalation study.

A MOE of 100 is adequate for occupational exposure.

QA/QC evaluation

014199

DATE: June 5, 2000

MEMORANDUM

Subject: QA/QC Evaluation of DERs for DICHLORMID (PC Code 900497)

From: Guruva B. Reddy (QA/QC)
RAB1

6/5/2000

To: Melba Morrow
RAB1

Study ID type and MRID number	DER acceptable	Study acceptable	Comments
2-Yr Feeding/Carcinogenicity Rat MRID 44529402	Yes	Yes	
18-MN Carcinogenicity Mouse MRID 44529401	Yes	Yes	
1-Yr Feeding Dog			DATA GAP
2-Gen Repro Rat			DATA GAP
Developmental Tox Rat MRID 44606408	Yes	Yes	
Developmental Tox Rat MRID 00058469	Yes	No	
Developmental Tox Rabbit MRID 44606407	Yes	Yes	
13-Week Feeding Rat MRID 44606406	Yes	Yes	
13-Week Feeding Rat MRID 000558467	Yes	No	
13-Week Feeding (Capsule) Dog MRID 41419401	Yes	Yes	
13-Week Feeding (Capsule) Dog MRID 00058468	Yes	No	

QA/QC evaluation

Study ID type and MRID number	DER acceptable	Study acceptable	Comments
90-Day Inhalation Rat MRID 00155678	Yes	Yes	
Acute Neurotoxicity Rat			DATA GAP
90-Day Neurotoxicity Rat			DATA GAP
21-Day Dermal Toxicity			DATA GAP
Mutagenicity/Gene Mutation MRID 41561404	Yes	Yes	
Mutagenicity/Gene Mutation MRID 41561405	Yes	Yes	
Mutagenicity/Structural Chromosomal Aberration MRID 41561403	Yes	Yes	
Mutagenicity/Structural Chromosomal Aberration MRID 41561406	Yes	No	
Mutagenicity/Structural Chromosomal Aberration MRID 41561407	Yes	Yes	
Mutagenicity/Other MRID 44606409	Yes	Yes	
Mutagenicity/Other MRID 41561408	Yes	Yes	
Metabolism			DATA GAP
Acute Oral MRID 44606401	Yes	Yes	
Acute Dermal MRID 44606402	Yes	Yes	
Acute Inhalation MRID 44606403	Yes	Yes	
Primary Eye Irritation MRID 44606404	Yes	Yes	
Primary Dermal Irritation MRID 42807902	Yes	Yes	

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Study ID type and MRID number	DER acceptable	Study acceptable	Comments
Dermal Sensitization MRID 44606405	Yes	Yes	

014199

DATA EVALUATION RECORD

DICHLORMID

Study Type: §83-5; Combined Chronic/Oncogenicity Study - Rats

Work Assignment No. 1-01-14B (MRIDs 44529402 and 44751801)

Prepared for

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

Prepared by

Pesticides Health Effects Group
Sciences Division
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Primary Reviewer:
Laurie E. Roszell, Ph.D.

Signature: Laurie E. Roszell
Date: 18 June 99

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Signature: Mary L. Menetrez
Date: 6/21/99

Program Manager:
Mary L. Menetrez, Ph.D.

Signature: Mary L. Menetrez
Date: 6/20/99

Quality Assurance:
Steve Brecher, Ph.D., D.A.B.T.

Signature: Steven Brecher
Date: 6/24/99

Disclaimer

This Data Evaluation Record may have been altered by the Health Effects Division subsequent to signing by Dynamac Corporation personnel.

DICHLORMID **Combined Chronic/Carcinogenicity (§83-5)**

EPA Reviewer: William Greear, MPH, D.A.B.T. William Greear 7/8/99
Registration Action Branch 3 (7509C)
Work Assignment Manager: Marion Copley, D.V.M., D.A.B.T. MCopley 7/13/99
Registration Action Branch 1 (7509C)

014199

DATA EVALUATION RECORD

STUDY TYPE: Combined Chronic/Carcinogenicity-Rats
OPPTS Number: 870.4300 OPP Guideline Number: §83-5

DP BARCODE: D250746 SUBMISSION CODE: S546651
P.C. CODE: 999999 TOX. CHEM. NO.: None

TEST MATERIAL (PURITY): Dichlormid (97.9%, inert ingredient.)

SYNONYMS: N, N-diallyl-2-2-dichloroacetamide (IUPAC)

CITATIONS: Horner, S.A., (1998) Dichlormid: 2 Year Dietary Toxicity and Oncogenicity Study in Rats. Central Toxicology Laboratory, Cheshire, UK, Laboratory Project ID: CTL/P/5554; PR09991, February 10, 1998. MRID 44529402. Unpublished

Clapp, M.J.L., (1999) Historical control supplement to MRID 44529402: Dichlormid: 2 Year Dietary Toxicity and Oncogenicity Study in Rats. Central Toxicology Laboratory, Cheshire, UK, Laboratory Project ID: MJLC-001, January 29, 1999. MRID 44751801. Unpublished

SPONSOR: Zeneca Ag Products, Wilmington, DE

44751801

EXECUTIVE SUMMARY: In a combined chronic/oncogenicity study (MRID 44529402), dichlormid (97.9%, Lot #P12, inert ingredient) was administered via the diet to a total of 64 Alpk:AP$_f$SD rats/sex/group at 0, 20, 100, or 500 ppm (equivalent to 0, 1.3, 6.5, or 32.8 mg/kg/day in males and 0, 1.5, 7.5, or 37.1 mg/kg/day in females) for up to 104 weeks. Twelve rats/sex/dose were terminated after 52 weeks. Males in each dose group were terminated at week 100 when survival of males in the 20 ppm dose group reached 25%. Females in each dose group were terminated as scheduled.

Survival, clinical signs, hematology and urinalysis parameters, ophthalmology, and gross findings for both sexes at all doses were unaffected by the treatment with dichlormid. No treatment-related findings were observed at the 20 or 100 ppm levels.

Chronic toxicity was characterized at the 500 ppm dose level by minor reductions in mean body weights (↓3-9%; $p<0.01$) in both sexes throughout the study. Decreases ($p<0.01$ and 0.05) in

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food consumption were observed in the males (↓3-7%) through week 32 and the females (↓6-12%) through week 88. Mean food efficiency was decreased in the males during weeks 1-12 (↓3-7%, $p<0.01$ and 0.05) and in the females during weeks 1-4 (↓3%, $p<0.05$). Plasma triglycerides were decreased (↓20-36%) in the males at each sampling interval, with the decreases being significant ($p<0.05$ and 0.01) at each interval except week 79. In the females, triglyceride levels were decreased (↓16-45%, $p<0.05$ and 0.01) at weeks 27, 53, and 105, with non-significant decreases (↓5-9%) also occurring at weeks 14 and 79. Plasma γ-glutamyl transferase was increased (↑57-114%, $p<0.05$ and 0.01) in the males at weeks 27, 53, and 100, with non-significant increases (38-51%) also occurring during weeks 52 and 79. Absolute liver weights were increased ($p<0.05$ or 0.01) at the interim sacrifice (week 52) in the males (↑13%) and females (↑9%). At the terminal sacrifice (week 100/105), the males had increased absolute liver weights (↑8-9%, $p<0.05$). The males also showed increased incidences of hepatitis (38% treated vs 27% controls), hepatocyte vacuolation (89% treated vs 44% controls), and pigmentation 75% treated vs 38% controls). In addition, the males had an increased incidence of bilateral tubular degeneration in the testis (36% treated vs 19% controls). None of the non-neoplastic microscopic data were analyzed for statistical significance.

In the uterus of high-dose females, there were slight increases over controls in the incidence of adenocarcinomas (7.8% treated vs 1.6% controls), adenomas (treated 3.1% vs 0% controls) and stromal cell sarcomas (3.1% treated vs 0% controls). The Sponsor stated that there was a significant trend ($p=0.026$) in the incidence of adenocarcinomas with increasing dose. However, the incidences of adenocarcinomas (7.8% treated vs historical controls 0-10.9%) and stromal cell sarcomas (treated 3.1% vs historical controls 0-3.1%) are within the range of historical controls. The increase for adenomas is slightly outside the range of historical controls (treated 3.1% vs historical controls 0-1.6%). Females exposed to 20 ppm dichlormid had an increased incidence of benign mammary gland fibroadenomas (15.6% treated vs controls 9.3%), but this increase was not seen at higher doses. Leiomyosarcoma was observed in the uterus of one high-dose female and in the cervix of one control female.

Males dosed with 100 and 500 ppm dichlormid had slight increases in the number of follicular adenomas in the thyroid gland (100 ppm-4.7%, 500 ppm- 3.1% vs controls 0%). Data were presented to show that these values were within the range of historical control values (0-9.3%). The 20 ppm males had an increased incidence of benign pheochromocytoma of the adrenal gland (14% treated vs 7.8% controls), and the 20 ppm and 100 ppm males had increased incidences of benign leydig cell tumors in the testis (20 ppm - 12.5%, 100 ppm - 17.2% vs controls - 7.8%). Data were not presented to show that the incidence of these lesions were within the range of historical controls; however, increases in the occurrence of these lesions were not seen at the higher doses.

The chronic LOAEL is 500 ppm (32.8 mg/kg/day and 37.1 mg/kg/day in males and females, respectively) based on non-neoplastic lesions in the liver associated with increased liver weights and changes in blood clinical chemistry parameters. The chronic NOAEL is 100 ppm (6.5 mg/kg/day and 7.5 mg/kg/day in males and females, respectively).

Under the conditions of this study, there was no evidence of carcinogenic potential.

Dosing was considered adequate based on decreases in body weights, food consumption, food efficiency, triglycerides, and increases in gamma glutamyl transferase, and liver weights associated with increased incidence of hepatitis, hepatocyte vacuolation and pigmentation observed at 500 ppm. Subchronic rat feeding study (MRID 44606406) identified liver as the target organ at doses ≥ 16 mg/kg/day.

The submitted study is classified as **acceptable (§83-5)** and does satisfy the guideline requirements for a chronic toxicity study (§83-1) and a carcinogenicity study (§83-2) in rats.

COMPLIANCE: Signed and dated GLP, Quality Assurance, Data Confidentiality, and Flagging statements were provided.

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I. MATERIALS AND METHODS

A. Materials:

1. Test material: Dichlormid
   Description: Brown liquid
   Lot/Batch #: P12
   Purity: 97.9%, inert ingredient
   Stability of compound: The test substance is stable in the diet at room temperature for up to 20 days (MRID 44529401).
   CAS #: 37764-25-3
   Structure: Not provided

2. Vehicle: Diet

3. Test animals: Species: Rat
   Strain: Alpk:AP$_f$SD (Wistar derived)
   Age and weight at study initiation: 4 weeks old; 112-113 g (males) and 102-103 g (females)
   Source: The Rodent Breeding Unit, Zeneca Pharmaceuticals, Alderley Park, UK
   Housing: Multiple rat racks; 4 rats/cage
   Diet: Powdered CT1 diet, ad libitum, Manufacturer: Special Diet Services Limited, Stepfield, Witham, Essex, UK)
   Water: Tap water, ad libitum
   Environmental conditions:
   Temperature: 21±2° C
   Humidity: 55±15%
   Air changes: ≥15 changes/hour
   Photoperiod: 12 h dark/12 h light
   Acclimation period: 7 days

B. Study design:

1. In life dates - start: February 1995 end: March 1997.

2. Animal assignment: Animals were assigned randomly to the treatment groups indicated in Table I.

Table 1. Study design

Test Group	Dose in diet (ppm)	Compound concentration M/F (mg/kg/day)	Number of Animals[a]			
			Main Study 24 months		Interim Study 12 months	
			Males	Females	Males	Females
1	0 (control)	0/0	52	52	12	12
2	20	1.3/1.5	52	52	12	12
3	100	6.5/7.5	52	52	12	12
4	500	32.8/37.1	52	52	12	12

a Each treatment group consisted of 64 animals, with 12 animals of each sex designated for interim kill after 1 year.

3. Dose selection: Dose levels were based on the results of subchronic feeding studies carried out in the same strain of rat in the same laboratory. No further information was provided.

4. Dose preparation, administration, and analysis: Experimental diets were prepared in 60 kg batches. The appropriate amount of dichlormid was ground with 500 g of milled diet and then mixed with 59.5 kg of diet. The prepared diets were utilized within 2 weeks of preparation. Samples from all dose levels were taken at 1-3 month intervals throughout the study and analyzed quantitatively for dichlormid. Homogeneity was also determined by analyzing top, middle, and bottom samples from the first 2 batches prepared for the low and high dose groups. The dose was administered by food continuously throughout the study.

   Results - Homogeneity analysis: Mean concentrations of dichlormid in the diets sampled on 17 February 1995 were within 3.5% of the nominal concentrations of 20 or 500 ppm. Diets sampled on 2 March 1995 were within 7% of the nominal concentrations.

   Concentration analysis: Concentrations of dichlormid in each preparation were within 10% of the nominal concentration, with the exception of the 100 ppm sample prepared on 2 March 1995. This sample was within 14% of the nominal concentration. The overall mean concentration for each dose group was within 2% of the nominal concentration.

   Stability analysis: Chemical stability was not analyzed in the current study. Data from the concurrently submitted oncogenicity study (MRID 44529401) using the same batch of dichlormid indicated that dichlormid was stable in the diet for at least 20 days at room temperature.

The analytical data indicated that the mixing procedure was adequate and that the variance between nominal and actual dosage to the study animals was acceptable.

5. Statistics - Data were evaluated using SAS (1989) statistical software. All tests were 2-sided, and were run separately for males and females. Analyses were carried out using the GLM procedure. Least-squares means for each group were calculated using the LSMEAN option in SAS PROC GLM. Estimates of differences from control were provided by the difference between the least-squares mean of each treatment group and the control group. Significant differences between the control and each treated group were determined by comparing the least-squares mean of each treatment group with the control group, using a Student's t-test, based on the error mean square in the analysis.

   Body weights were considered by analysis of covariance (ANCOVA) on week 1 body weights. Food consumption, food utilization, hematology, and blood and urine clinical chemistries were considered by analysis of variance (ANOVA). Organ weights were considered both by ANOVA and ANCOVA on final body weights. Kaplan-Meier survival estimateswere calculated for each treatment group. Intergroup comparisons of mortality were done using a logrank test (Peto and Pike, 1973). Fisher's Exact Test was used to analyze the incidence of each tumor and the overall incidence of each tumor type. The Cochran-Armitage Test was used to test for trend with group number.

C. Methods:

1. Observations: Prior to the start of the study, all animals were examined to ensure that they were normal. Cage-side observations were made daily. These observations included changes in clinical condition or behavior. Detailed clinical observations were made once per week. Animals found dead or requiring euthanasia were subjected to a post mortem examination.

2. Body weight: Each animal was weighed immediately before the commencement of the study. Subsequent to the start of the study, animals were weighed once per week for the first 14 weeks, and biweekly thereafter until the termination of the study.

3. Food consumption: Food consumption for each cage of rats was determined weekly for the first 14 weeks, on week 16, and every 4 weeks thereafter. Food utilization was calculated as the body weight gained by the rats in the cage per 100 g of food eaten.

4. Ophthalmoscopic examination: Ophthalmoscopic examinations were performed on all animals prior to dose initiation. All surviving animals from the control and high dose groups were examined during week 52 and prior to termination (week 99 or 100 for males and week 102 or 103 for females).

5. Blood analyses: Blood was collected from the tail vein of thirteen male and thirteen female rats per group at weeks 14, 27, 53 and 79. Different animals were used for hematology and clinical chemistry samples. The CHECKED (X) parameters below were examined in each blood sample.

a. Hematology:

X	Hematocrit (HCT)	X	Leukocyte differential count
X	Hemoglobin (HGB)	X	Mean corpuscular HGB (MCH)
X	Leukocyte count (WBC)	X	Mean corpusc. HGB conc.(MCHC)
	Corrected leukocyte count (Cor WBC)	X	Mean corpusc. volume (MCV)
X	Erythrocyte count (RBC)	X	Reticulocyte count*
X	Platelet count	X	Erythrocyte morphology (distribution width)
X	Blood clotting measurements (Thromboplastin time) (Prothrombin time)		

* Reticulocyte films examined when significant increases in red cell distribution width was noted.

b. Clinical chemistry:

	ELECTROLYTES		OTHER
X	Calcium	X	Albumin
X	Chloride	X	Blood creatinine
	Magnesium	X	Blood urea nitrogen
X	Phosphorus (as phosphate)	X	Total Cholesterol
X	Potassium		Globulins
X	Sodium	X	Glucose
	Carbon dioxide	X	Total bilirubin
		X	Total serum protein
		X	Triglycerides
			Albumin/globulin ratio
	ENZYMES		
X	Alkaline phosphatase (AP)		
	Plasma cholinesterase (PL-ChE)		
	Erythrocyte cholinesterase (RBC-CHE)		
	Brain cholinesterase (BR-CHE)		
X	Creatinine kinase		
	Lactate dehydrogenase (LDH)		
X	Serum alanine aminotransferase (ALT)		
X	Serum aspartate aminotransferase (AST)		
X	Gamma glutamyl transferase (GGT)		
	Glutamate dehydrogenase (GLDH)		

6. Urinalysis: Urinalyses were performed on the same animals as were used for blood clinical chemistry analyses. Samples were collected at weeks 13, 26, 52 and 78, and prior to termination (weeks 99 and 104 for males and females, respectively). The following CHECKED (X) parameters were examined.

	Transparency	X	Glucose*
X	Volume	X	Ketones*
X	Specific gravity		Bilirubin
X	pH	X	Blood*
X	Sediment (microscopic)	X	Color
X	Protein*	X	Urobilinogen*
	Osmolality		Chloride
X	Appearance		Creatinine
			Potassium
			Sodium
			Urea

* Semi-quantitative assessment

7. Sacrifice and pathology: All animals that died or were killed *in extremis* and those sacrificed on schedule (interim and final termination) were subjected to gross pathological examination and the CHECKED (X) tissues were collected. Additionally, the (XX) organs were weighed for those animals sacrificed on schedule.

	DIGESTIVE SYSTEM		CARDIOVASC./HEMAT.		NEUROLOGIC
X	Oral cavity	X	Aorta	XX	Brain (3 levels)
	Tongue	X	Heart	X	Sciatic nerve
X	Salivary glands	X	Bone marrow	X	Spinal cord (3 levels)
X	Esophagus	X	Lymph nodes	X	Pituitary
X	Stomach	X	Spleen	X	Eyes
X	Duodenum	X	Thymus		GLANDULAR
X	Jejunum		UROGENITAL	XX	Adrenal glands
X	Ileum	XX	Kidneys	X	Hardarian gland
X	Cecum	X	Urinary bladder	X	Mammary gland
X	Colon	XX	Testes	X	Parathyroids
X	Rectum	X	Epididymides	X	Thyroid
XX	Liver	X	Prostate		OTHER
	Gall bladder	X	Seminal vesicles	X	Bone
X	Pancreas	X	Ovaries	X	Skeletal muscle
	RESPIRATORY		Oviducts		Mandibular lymph nodes
X	Trachea	X	Uterus	X	Skin
X	Lungs		Vagina		Lacrimal gland
X	Nasal cavity	X	Cervix	X	All gross lesions and masses
X	Pharynx		Ureter		
	Larynx		Urethra		

All tissues were submitted for histology except oral and nasopharyngeal cavities which were stored. All submitted tissues were examined by light microscopy.

II. RESULTS

A. Observations:

1. Toxicity - No treatment-related clinical signs were noted during the study.

2. Mortality - Dichlormid had no apparent effects on survival of the rats. All male groups were terminated in week 100 when the survival in the males receiving 20 ppm dichlormid dropped to 25%. At this time survival in the remaining male groups was as follows: control- 42%; 100 ppm-28%; 500 ppm-39%. Female groups were terminated as scheduled in week 105. At this time, survival was as follows: control-54%; 20 ppm-60%; 100 ppm-57%; 500 ppm-58%.

B. Body weight:

Dichlormid resulted in reduced body weights in both sexes at dietary levels of 100 and 500 ppm. Mean body weight, adjusted for initial weight, was reduced ($p<0.01$) in males (↓4-8%) and females (↓3-9%) in the 500 ppm dose group throughout the study. At the 100 ppm level, mean body weight, adjusted for initial weight, was similar to that of controls during the first half of the study, but was reduced ($p<0.05$ and 0.01) consistently between weeks 57-91 for males (↓2-6%) and weeks 71-97 for females (↓3-6%). Body weights of animals dosed with dichlormid at 20 ppm were similar to control values. Selected mean body weights are presented in Table 2.

C. Food consumption and efficiency:

Throughout much of the study, mean food consumption of high-dose males and females was reduced ($p<0.01$ and 0.05) when compared to controls. Significant decreases in food consumption were observed in high-dose males (↓3-7%) through week 32 and in high-dose females (↓6-12%) through week 88. In addition, mean food efficiency was also decreased in high-dose males during weeks 1-8 (↓3-7%, $p<0.01$ and 0.05) and weeks 1-12 (↓4%, $p<0.01$), and in high-dose females during weeks 1-4 (↓3%, $p<0.05$). Food consumption and efficiency in the 20 and 100 ppm dose groups were similar to that of controls. Selected data on food consumption and efficiency are presented in Table 2.

Table 2. Select body weights, food consumption and food efficiency in rats fed dichlormid for up to 2 years[a].

Week[b]	Male				Female			
	Dietary Level (ppm)							
	0	20	100	500	0	20	100	500
	Body Weight (g)[c]							
1	112.7	112.2	113.4	112.3	102.8	102.4	102.6	102.2
2	172.9	171.9	172.2	166.8** (↓4%)	143.0	141.6* (↓1%)	141.9	138.5** (↓3%)
25	583.1	580.4	574.8	545.9** (↓6%)	300.3	297.5	296.5	282.1** (↓6%)
51	654.8	649.7	643.6	619.0** (↓5%)	349.6	344.6	337.7** (↓3%)	322.8** (↓8%)
75	666.3	663.3	635.6** (↓5%)	621.2** (↓7%)	398.1	396.7	384.3* (↓3%)	364.1** (↓9%)
88/91	622.7	617.0	583.3** (↓6%)	570.7** (↓8%)	408.3	403.7	385.8** (↓6%)	370.1** (↓9%)
100/105	583.0	554.5	566.1	551.7** (↓5%)	373.2	386.4	371.8	365.8
	Food Consumption (g/rat/day)[c]							
1	23.1	23.0	23.3	21.5** (↓7%)	19.5	19.2	19.1	17.9** (↓8%)
24	30.3	29.9	30.5	29.4* (↓3%)	19.7	19.7	20.0	18.1** (↓8%)
52	28.7	28.9	29.1	28.2	19.7	19.8	20.1	19.0
72	29.1	28.3	28.6	27.4* (↓6%)	23.2	22.5	21.6** (↓7%)	20.6** (↓11%)
88	27.4	26.3	26.4	26.8	22.8	20.9* (↓8%)	22.5	20.1** (↓12%)
96/104	24.5	25.0	25.8	24.4	19.5	19.6	18.9	19.0
	Food Efficiency (g growth/100g food)[d]							
1-4	26.43	26.30	26.11	25.57** (↓3%)	17.91	17.60	17.70	17.39* (↓3%)
5-8	11.25	11.12	11.14	10.50* (↓7%)	6.32	6.48	6.32	6.41
9-12	6.95	6.97	6.92	6.72	3.13	3.44	3.18	3.39
1-12	14.46	14.35	14.29	13.84** (↓4%)	9.05	9.09	8.99	8.99

a These data were extracted from Tables 8, 9, and 10, pages 88-110 of MRID 44529402.

b Where two weeks are listed, the week #/# are for males and females, respectively.

c Body weight data are adjusted for initial body weights and are the average of 61-64 rats/sex for weeks 1-52; 43-49 rats/sex for week 75; 28-36 males and 40-43 females for week 88/91; and 13-19 males and 27-30 females for week 100/105.

d Food consumption and efficiency were based on the average of 16 cages (4 rats/cage) for ≤52 weeks and 12 or 13 cages (~4 rats/cage) for >52 weeks.

* $p<0.05$; ** $p<0.01$

D. Ophthalmoscopic examination: There were no treatment related ophthalmological findings detected in rats dosed with 500 ppm dichlormid.

E. Blood analyses:

1. Hematology - Although there were small differences in several hematological parameters compared to controls, they were not considered to be treatment-related in the absence of a dose-response relationship and corroborating evidence.

Red blood cell (RBC) counts were decreased at week 14 in females dosed at 20 ppm (↓2%, $p<0.05$) or 100 ppm (↓2%, $p< 0.01$). Similar changes were seen during weeks 27, 53, and 79, but were not statistically significant. A significant decrease (↓4%, $p<0.05$) in RBC counts was also observed at week 27 in males dosed at 100 ppm. However, these changes were not dose dependent.

Mean cell volume was increased ($p<0.05$ and 0.01) during weeks 14 and 27 in females dosed at 20 ppm (↑2-3%) and 100 ppm (↑2%), and during week 27 in females dosed at 500 ppm (↑2%). Mean cell hemoglobin was also slightly increased (↑2%, $p<0.05$ and 0.01) at weeks 14 and/or 27 in females dose with dichlormid. In addition, decreases were noted in platelet counts in females dosed at 100 and 500 ppm at week 14 (↓7-9%, $p<0.05$), and in females dosed at 20 ppm at week 52 (↓10%, $p<0.01$). However, these changes were not dose-dependent and were not observed at later intervals or in males.

Males in the 500 ppm dose group had substantial increases ($p<0.05$) in white blood cells (↑20%), lymphocytes (↑22%), and large unstained cells (↑30%) at week 52, and 100 ppm females also had a 32% increase ($p<0.05$) in large unstained cells at week 52, but these changes were not seen at any other sampling intervals.

Other minor or isolated differences in hematology that were statistically significant included: a decrease (↓1%, $p<0.05$) in hemoglobin in 500 ppm females at week 14; a decrease (↓3%, $p<0.05$) in hematocrit in 100 ppm males at week 27; a 4% decrease ($p<0.05$) in mean cell hemoglobin in 500 ppm males at week 79; 1% decreases ($p<0.05$ and 0.01) in cell hemoglobin concentrations in 20 and 100 ppm males at weeks 52 and 53 and in 100 ppm females at week 27; a 4% decrease ($p<0.05$) in RBC distribution width in 500 ppm females at week 52; and a 6% decrease in prothrombin time in 20 ppm males at week 100.

2. Blood clinical chemistry -Select blood clinical chemistry parameters are presented in Table 3.

Plasma cholesterol was increased in females dosed at 100 and 500 ppm at each sampling interval except week 105. Initial increases (↑2-6%) at weeks 14 and 27 were not significant, but significant increases (↑16-25%, $p<0.05$ and 0.01) were noted in both dose

groups at week 52 and in the 500 ppm females at week 53. Subsequent increases in the 100 ppm females (↑2-9%) at weeks 53 and 79 and in the 500 ppm females (↑9%) at week 79 were not statistically significant.

Plasma triglycerides were decreased (↓20-36%) in males dosed at 500 ppm at each week, with the decreases being significant ($p<0.05$ and 0.01) at each sampling interval except week 79. Non-significant decreases in triglyceride levels were also noted in males dosed at 100 ppm (↓6-14%) at each interval except week 53 and in males dosed at 20 ppm (↓4-16%) at weeks 52, 53, and 79. In the 500 ppm females, triglyceride levels were decreased (↓16-45%, $p<0.05$ and 0.01) at weeks 27, 53, and 105, with non-significant decreases (↓5-9%) also occurring at weeks 14 and 79. Non-significant decreases (10-21%) in triglyceride levels were observed in females dosed at 100 ppm at weeks 14, 53, 79, and 105, along with an increase (↑30, $p<0.05$) at week 52. Decreases (↓20-25%) were also observed in females dosed at 20 ppm at weeks 53-105, with the decrease at week 53 being significant ($p<0.05$).

Plasma γ-glutamyl transferase was increased (↑57-114%, $p<0.05$ and 0.01) in males dosed at 500 ppm at weeks 27, 53, and 100, with non-significant increases (38-51%) also occurring during weeks 52 and 79. At weeks 27, 52, 79 and 100, increases were also observed in males dosed at 100 ppm (↑7-76%, $p<0.05$ at week 79) and in males dosed at 20 ppm (↑16-55%).

Plasma alanine aminotransferase was initially decreased (↓17-19%, $p<0.01$) at week 14 for males dosed at 100 and 500 ppm, but was subsequently increased (↑6-49%) in high-500 ppm males, with significant increases (↑48-49%, $p<0.05$) occurring at weeks 52 and 100. In females in the 500 ppm dose group, this enzyme was decreased during all sampling periods (↓4-37%) with significant decreases (↓23-37%, $p<0.01$) occurring at week 14, 27, and 52. Decreases (↓15-20%, $p<0.05$) were also observed in 100 ppm females at week 14 and 20 ppm females at week 27.

At week 14, aspartate aminotransferase (AST) was decreased in males (↓15-17%, $p<0.01$) dosed at 100 and 500 ppm. In high-dose males, this enzyme subsequently increased (↑33-40%, $p<0.05$ at week 52) at weeks 52, 53, and 100, but changes in AST were erratic for the 100 ppm males. In females, AST decreased for the 100 ppm (↓9-13%, $p<0.05$) and 500 ppm (↓17-37%, $p<0.01$) groups during weeks 14 and 27, and then increased (↑10-21%) in these groups from week 52 to 105. A similar pattern was also observed in females dosed at 20 ppm.

Other minor or isolated differences in blood chemistry that were statistically significant included: increases ($p<0.05$) in plasma urea at week 52 in 100 ppm males (↑16%) and 500 ppm females (↑13%); 3-4% increases ($p<0.05$) in plasma albumin in 500 ppm females at week 53 and 79; a 10% decrease ($p<0.05$) in plasma alkaline phosphatase in 500 ppm males at week 14; 1% increases ($p<0.05$ and 0.01) in plasma sodium at week 79 in males dosed at 20, 100, and 500 ppm; increases ($p<0.05$ and 0.01) in plasma calcium in

males (↑2%) at week 27 and females (↑1-3%) at weeks 14 and 27 from the 500 ppm dose group; and increases ($p<0.05$) in plasma phosphorus in 500 ppm males (↑9%) at week 52 and 500 ppm females (↑15%) at week 14. These changes were considered minor and of equivocal biological significance.

Table 3. Select blood clinical chemistry data from rats fed dichlormid for up to 2 years[a].

Week	Male				Female			
	Dietary Level (ppm)							
	0	20	100	500	0	20	100	500
	Cholesterol (mmol/l)							
14	2.75	2.76	2.87	2.66	2.39	2.33	2.53	2.50
27	3.37	3.49	3.40	3.34	2.82	2.78	2.87	2.92
52[b]	5.51	5.63	6.61	4.93	2.88	2.88	3.61** (↑25%)	3.39** (↑18%)
53	5.24	4.77	5.26	5.28	3.16	3.23	3.46	3.67* (↑16%)
79	7.19	7.28	7.85	8.21	3.82	3.62	3.88	4.15
	Triglyceride (mmol/l)							
14	1.47	1.52	1.35	1.14** (↓22%)	1.09	1.01	0.98	0.99
27	1.37	1.40	1.18	0.87** (↓36)	1.29	1.28	1.28	1.08* (↓16%)
52[b]	2.16	1.82	1.91	1.61* (↓25)	1.01	1.19	1.31* (↑30%)	1.03
53	1.87	1.80	1.88	1.22** (↓35)	1.77	1.33* (↓25%)	1.53	1.34* (↓24%)
79	2.23	1.89	1.93	1.79 (↓20)	2.20	1.76	1.91	2.09
100/105[c]	1.93	1.98	1.81	1.34* (↓31)	2.63	2.00	2.08	1.45** (↓45%)
	γ-Glutamyl Transferase (IU/l)							
27	1.4	1.8	1.5	2.2* (↑57%)	1.6	1.2	1.6	1.3
52[b]	2.9	4.3	4.7	4.4 (↑51%)	1.8	0.6* (↓67%)	1.3	1.0
53	2.6	2.5	2.4	4.9** (↑88%)	2.2	1.9	2.1	1.8
79	5.0	5.8	8.8* (↑76%)	6.9 (↑38%)	2.7	2.9	2.7	3.0
100/105[c]	2.9	4.5	3.9	6.2* (↑114%)	1.7	1.9	1.8	2.2

(*continued*)

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Table 3. Continued.

Week	Male				Female			
	Dietary Level (ppm)							
	0	20	100	500	0	20	100	500
	Alanine Aminotransferase (IU/l)							
14	102.2	95.8	82.9** (↓19%)	84.8** (↓17%)	77.5	76.2	66.2* (↓15%)	59.7** (↓23%)
27	133.2	122.3	133.2	141.5	85.5	68.2* (↓20%)	72.2	58.0** (↓32%)
52[b]	86.0	92.1	101.0	128.0* (↑49%)	90.9	70.3	92.6	57.0** (↓37%)
53	127.9	118.0	121.5	146.4	74.0	80.3	76.7	66.9
100/105[c]	51.6	51.5	67.3	76.5* (↑48%)	64.5	63.6	65.9	62.0
	Aspartate Aminotransferase (IU/l)							
14	109.1	100.8	90.8** (↓17%)	92.2** (↓15%)	89.5	92.6	81.3* (↓9%)	74.3** (↓17%)
27	154.9	151.5	140.5	155.9	129.4	99.5* (↓23%)	112.5	81.3** (↓37%)
52[b]	121.1	129.7	132.6	169.4* (↑40%)	147.6	123.8	172.0	126.9
53	143.1	136.7	143.6	189.8	129.4	133.1	154.8	138.8
100/105[c]	83.4	82.1	100.2	113.6	147.8	159.7	164.0	147.0

a Data are the average of 12 or 13 rats/sex and were obtained from Table 12, pages 153-156 and 161-166 of MRID 44529402.
b Interim sacrifice was at 52 weeks
c Males were killed during week 100 of the study; females were killed during week 105.
* $p<0.05$; ** $p<0.01$

3. Urine clinical chemistry and sediment -There were reductions seen in urine pH for males receiving 100 or 500 ppm dichlormid. Urinary pH was decreased ($p<0.05$) in the 500 ppm males at week 13 (↓7%) and in the 100 and 500 ppm males at week 26 (↓6%). In the absence of similar changes during later weeks, these reductions were not considered to be of toxicological significance. There was no evidence of treatment-related changes in the urinary sediment.

G. Sacrifice and pathology:

1. Organ weights - Changes in organ weights were seen following both the interim and terminal sacrifices in male and female rats (Table 4). Following the interim sacrifice (week 52),absolute liver weights were increased ($p<0.01$) for males receiving 500 ppm

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dichlormid (↑13%), and females receiving 100 ppm (↑10%) or 500 ppm (↑9%, p<0.05) dichlormid. Absolute kidney weights were also increased (↑9%, p<0.01) in females receiving 100 or 500 ppm dichlormid. The differences in female liver and kidney weights were not observed at the terminal sacrifice.

Following the terminal sacrifice (week 100/105), males dosed at 500 ppm had increased absolute liver weights (↑8-9%, p<0.05) and males dosed at 20 ppm had minor increases in absolute brain weights (↑3%, p<0.05 and 0.01). In females, absolute adrenal gland weights were decreased (↓13-16%, p<0.05 and 0.01) over all doses of dichlormid. However, these decreases in adrenal weights were not associated with histopathological findings and the values were within historical control ranges (treated 0.083-0.086g vs historical controls 0.796-0.100g).

Table 4. Absolute organ weights (g) adjusted for body weights of rats exposed to dichlormid[a]

Organ	Male				Female				Historical controls
	Dietary Level (ppm)								
	0	20	100	500	0	20	100	500	
	Interim sacrifice								
Kidney	4.04	4.27	4.22	4.31	2.43	2.38	2.66** (↑9%)	2.66** (↑9%)	-[b]
Liver	23.5	24.4	24.7	26.5** (↑13%)	11.7	11.6	12.9** (↑10%)	12.8* (↑9%)	-
	Terminal sacrifice								
Brain	2.25	2.32** (↑3%)	2.27	2.29	2.09	2.08	2.10	2.09	-
Liver	22.8	23.4	22.9	24.8* (↑9%)	15.5	15.2	15.4	15.7	-
Adrenal gland[c]	0.079	0.074	0.072	0.075	0.099	0.086* (↓13%)	0.084** (↓15%)	0.083** (↓16%)	0.796±0.036-0.100±0.063

a Data are the average of 11 or 12 rats/sex/dose for the interim sacrifice and 13-19 males/dose and 24-29 females/dose for the terminal sacrifice. Data were obtained from Table 16, pages 193, 194, 197, 199, 202, and 204 of MRID 44529402.

b Historical data not given.

c Absolute weights (not adjusted for body weights) of adrenal glands are given, for comparison with historical control data.

* p<0.05; ** p<0.01

2. Gross pathology - There were no increases in abnormal gross pathology findings reported related to the treatment of dichlormid.

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3. Microscopic pathology at necropsy:

   a) Non-neoplastic: When all animals were combined, including all animals that died and those sacrificed on schedule, an increased incidence of liver pigmentation, hepatitis, and hepatocyte vacuolation was observed (Table 5). At the high-dose, males showed increased incidences of hepatitis (500 ppm - 38% vs. control - 27%), hepatocyte vacuolation (500 ppm - 89% vs. control - 44%), and pigmentation (500 ppm - 75% vs. control - 38%), and high-dose females showed a slight increase in the incidences of liver pigmentation (500 ppm - 36% vs. control - 17%). These data were not analyzed for statistical significance, although the study report did state that the increase in hepatocyte vacuolation in male rats receiving 500 ppm dichlormid was significant.

   In addition, high-dose males had an increased incidence of bilateral tubular degeneration in the testis (500 ppm - 36% vs. control 19%). Males and females also had increased incidences of epithelial hyperplasia of the thymus at all levels of dichlormid (males, treated 11-17% vs controls 5%; females, treated 38-50% vs controls 28%), but the differences were not dose-related.

Table 5. Selected incidences of microscopic changes observed in rats fed dichlormid for up to 104 weeks[a].

Severity of Effect	Male				Female			
	Dietary Level Dichlormid (ppm)							
	0	20	100	500	0	20	100	500
Liver - Hepatitis								
minimal	10	16	17	9	6	4	4	3
slight	4	4	3	8	0	0	3	1
moderate	3	0	1	7	0	1	1	0
total	17	20	21	24	6	5	8	4
Liver - Hepatocyte Vacuolation								
minimal	15	19	22	26	12	10	16	19
slight	7	14	11	17	7	2	4	4
moderate	6	7	8	9	2	0	1	0
marked	0	0	0	5	0	0	0	0
total	28	40	41	57	21	12	21	23
Liver - Pigmentation								
minimal	21	18	25	28	9	12	15	23
slight	3	7	5	20	2	4	3	0
total	24	25	30	48	11	16	18	23
Testis Bilateral Tubular Degeneration								
minimal	4	2	4	9	-	-	-	-
slight	3	3	2	4	-	-	-	-
moderate	1	2	2	3	-	-	-	-
marked	4	6	8	7	-	-	-	-
total	12	13	16	23	-	-	-	-
Epithelial Hyperplasia of the Thymus								
minimal	2	4	6	7	16	16	26	27
slight	0	2	5	2	2	8	2	5
moderate	1	1	0	0	0	0	0	0
total	3	7	11	9	18	24	28	32

a Data taken from Table 19, pages 323 - 348 of MRID 44529402; n=64 rats/sex/dose.

b) Neoplastic: Selected neoplastic lesions are presented in Table 6. In the uterus of high-dose females, there were slight increases in the incidence of adenocarcinomas (500 ppm - 7.8% vs. control - 1.6%), adenomas (500 ppm - 3.1% vs. control - 0%)

and stomal cell sarcomas (500 ppm - 3.1% vs. control - 0%). The Sponsor stated that there was a significant trend in the incidence of adenocarcinomas with increasing dose (p=0.026). However, the incidences of adenocarcinomas (500 ppm 7.8% vs historical controls 0-10.9%) and stromal cell sarcomas (500 ppm - 3.1% vs historical controls 0-3.1%) are within the range of historical controls. The increase for adenomas is slightly outside the range of historical controls (500 ppm - 3.1% vs historical controls 0-1.6%). Females exposed to 20 ppm dichlormid had increased incidence of benign mammary gland fibroadenomas (15.6% vs controls 9.3%), but this increase was not seen at higher doses. Data were not presented to show that this incidence of fibroadenomas was within the range of historical controls. Leiomyosarcoma was observed in the uterus of one high-dose female and in the cervix of one control female.

Males dosed with 100 and 500 ppm dichlormid had slight increases in the number of follicular adenomas in the thyroid gland (100 ppm-4.7%, 500 ppm- 3.1% vs controls 0%). Data were presented to show that these values were within the range of historical control values (0-9.3%), which included follicular adenomas, follicular cell adenomas and/or follicular cystadenomas. Low-dose males had an increased incidence of benign pheochromocytoma of the adrenal gland (20 ppm - 14% vs controls - 7.8%), and low- and mid-dose males had increased incidents of benign leydig cell tumors in the testis (20 ppm - 12.5%, 100 ppm - 17.2% vs controls - 7.8%). Data were not presented to show that the incidence of these lesions were within the range of historical controls; however, increases in the occurrence of these lesions were not seen at the higher doses.

Table 6. Total incidence of select neoplasms in rats (64/sex) dosed with dichlormid at 20, 100 or 500 ppm for approximately 24 months.[a]

Dose (ppm)	0	20	100	500	Historical Controls
Males					
Follicular adenomas (Thyroid gland)	0	0	3	2	0/85-6/64 [b]
% incidence	0	0	4.7	3.1	0-9.3
trend	p=0.072				
Pheochromocytoma (Adrenal gland)	5	9	6	3	-[c]
% incidence	7.8	14	9.4	4.7	
Leydig cell tumor (Testis)	5	8	11	5	-
% incidence	7.8	12.5	17.2	7.8	
Females					
Adenocarcinoma (Uterus)	1	0	1	5	0/64-7/64 [d]
% incidence	1.6	0	1.6	7.8	0-10.9
trend	p=0.026				
Adenoma (Uterus)	0	1	0	2	0/84-1/64 [d]
% incidence	0	1.6	0	3.1	0-1.6
trend	p=0.195				
Stromal cell sarcoma (Uterus)	0	0	0	2	0/64-2/64
% incidence	0	0	0	3.1	0-3.1
trend	p=0.057				
Fibroadenoma (Mammary gland)	6	10	6	7	-
% incidence	9.3	15.6	9.3	10.9	

a These data represent total number of tumors from intercurrent deaths and interim and terminal sacrifice. Data were obtained from Tables 21 and 22, pages 366-378 of MRID 44529402.

b Historical control data include follicular adenomas, follicular cell adenomas and/or follicular cystadenomas.

c Historical control data not presented.

d Historical control data for adenoma and adenocarcinoma were obtained from Table 1, page 5 of MRID 44751801.

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III. DISCUSSION

A. Investigators conclusions - Treatment with dichlormid for up to two years at dose levels of 0-500 ppm showed no evidence of oncogenicity. At the high-dose, there were treatment-related effects on growth, food consumption and efficiency, and reductions in plasma triglyceride levels. In male rats, there were also increases in liver weights and the incidence and severity of hepatocyte vacuolation and pigmentation.

The chronic LOAEL is 500 ppm and the NOAEL is 100 ppm.

B. Reviewer's discussion/conclusions - In this combined chronic/oncogenicity study, male and female rats were fed diets containing dichlormid at 0, 20, 100 or 500 ppm (equivalent to 0, 1.3, 6.5 or 32.8 mg/kg/day in males and 0, 1.5, 7.5 or 37.1 mg/kg/day in females) for up to 104 weeks. Dietary analyses at select study intervals confirmed that nominal diet concentrations of dichlormid were achieved.

No clinical signs of toxicological significance were observed and the ophthalmology findings were normal. All male groups were terminated in week 100 of the 104 week study when survival in the 20 ppm dichlormid group dropped to 25%. Female rats were terminated on schedule. There were no significant increases in mortality observed in any of the treated groups when compared to control groups. Hematology and urinalysis parameters, ophthalmology, and gross findings for both sexes at all doses were unaffected by the treatment with dichlormid.

Dichlormid resulted in reduced body weights in both sexes at dietary levels of 100 and 500 ppm. Mean body weight was reduced (↓3-9%; $p<0.01$) in the 500 ppm dose group throughout the study. At the 100 ppm level, mean body weight was similar to that of controls during the first half of the study, but was reduced ($p<0.05$ and 0.01) consistently between weeks 57-91 for males (↓2-6%) and weeks 71-97 for females (↓3-6%). Body weights of animals dosed with dichlormid at 20 ppm were similar to control values.

Significant decreases ($p<0.01$ and 0.05) in food consumption were observed in high-dose males (↓3-7%) through week 32 and in high-dose females (↓6-12%) through week 88. Mean food efficiency was decreased in high-dose males during weeks 1-8 (↓3-7%, $p<0.01$ and 0.05) and weeks 1-12 (↓4%, $p<0.01$), and in high-dose females during weeks 1-4 (↓3%, $p<0.05$). Food consumption and efficiency in the 20 and 100 ppm dose groups were similar to that of controls.

Plasma triglycerides were decreased (↓20-36%) in males dosed at 500 ppm at each sampling interval, with the decreases being significant ($p<0.05$ and 0.01) at each sampling interval except week 79. In the 500 ppm females, triglyceride levels were decreased (↓16-45%, $p<0.05$ and 0.01) at weeks 27, 53, and 105, with non-significant decreases (↓5-9%) also occurring at weeks 14 and 79. The decreases in triglyceride levels in the other treated groups

compared to the concurrent controls were generally not statistically significant and judged to be not of toxicological concern.

Plasma γ-glutamyl transferase was increased (↑57-114%, $p<0.05$ and 0.01) in males dosed at 500 ppm at weeks 27, 53, and 100, with non-significant increases (38-51%) also occurring during weeks 52 and 79. The differences in enzyme levels in the other treated groups compared to the concurrent controls were generally not statistically significant and judged to be not of toxicological concern. Differences from controls in levels of plasma alanine aminotransferase and aspartate aminotransferase were erratic and were considered of no toxicological significance. Plasma cholesterol levels were increased in the 100 and 500 ppm females (↑16-25%, $p<0.05$ and 0.01) on weeks 52/53. However, these differences were not observed at subsequent time points.

Absolute liver weights were increased ($p<0.05$ or 0.01) at the interim sacrifice (week 52) in the 500 ppm males (↑13%) and females (↑9%). At the terminal sacrifice (week 100/105), the 500 ppm males had increased absolute liver weights (↑8-9%, $p<0.05$).

When all animals were combined, including all animals that died and those sacrificed on schedule, the 500 ppm males showed increased incidences of hepatitis (38% treated vs 27% controls), hepatocyte vacuolation (89% treated vs 44% controls), and pigmentation 75% treated vs 38% controls). The high-dose females showed only a slight increase in the incidences of liver pigmentation (36% treated vs 17% controls). In addition, high-dose males had an increased incidence of bilateral tubular degeneration in the testis (36% treated vs 19% controls). None of these data were analyzed for statistical significance.

In the uterus of high-dose females, there were slight increases in the incidence of adenocarcinomas (7.8% treated vs 1.6% controls), adenomas (treated 3.1% vs 0% controls) and stomal cell sarcomas (3.1% treated vs 0% controls). The Sponsor stated that there was a significant trend in the incidence of adenocarcinomas with increasing dose ($p=0.026$). However, the incidences of adenocarcinomas (7.8% treated vs historical controls 0-10.9%) and stromal cell sarcomas (treated 3.1% vs historical controls 0-3.1%) are within the range of historical controls. The increase for adenomas is slightly outside the range of historical controls (treated 3.1% vs historical controls 0-1.6%). Females exposed to 20 ppm dichlormid had increased incidence of benign mammary gland fibroadenomas (15.6% vs controls 9.3%), but this increase was not seen at higher doses. Leiomyosarcoma was observed in the uterus of one high-dose female and in the cervix of one control female.

Males dosed with 100 and 500 ppm dichlormid had slight increases in the number of follicular adenomas in the thyroid gland (100 ppm-4.7%, 500 ppm- 3.1% vs controls 0%). Data were presented to show that these values were within the range of historical control values (0-9.3%), which included follicular adenomas, follicular cell adenomas and/or follicular cystadenomas. The 20 ppm males had an increased incidence of benign pheochromocytoma of the adrenal gland (14% treated vs 7.8% controls), and the 20 ppm and

100 ppm males had increased incidences of benign leydig cell tumors in the testis (20 ppm - 12.5%, 100 ppm - 17.2% vs controls - 7.8%). Data were not presented to show that the incidence of these lesions were within the range of historical controls; however, increases in the occurrence of these lesions were not seen at the higher doses.

Under the conditions of this study, no treatment-related increases in the incidences of any neoplasm were observed in the dosed animals.

The chronic LOAEL is 500 ppm (32.8 mg/kg/day and 37.1 mg/kg/day in males and females, respectively) based on non-neoplastic lesions in the liver associated with increased weights and changes in blood clinical chemistry parameters. The chronic NOAEL is 100 ppm (6.5 mg/kg/day and 7.5 mg/kg/day in males and females, respectively).

The submitted study is classified as **acceptable (§83-5)** and does satisfy the guideline requirements for a chronic toxicity study (§83-1) and a carcinogenicity study (§83-2) in rats.

C. Study deficiencies - A dose rationale was not submitted; however, this deficiency does not affect the acceptability of the study since a LOAEL was established.

ATTACHMENTS

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014199

DATA EVALUATION RECORD

DICHLORMID

Study Type: §83-2 (b); Oncogenicity Study in Mice

Work Assignment No. 1-01-14A (MRID 44529401)

Prepared for

Health Effects Division
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Disclaimer

This Data Evaluation Record may have been altered by the Health Effects Division subsequent to signing by Dynamac Corporation personnel.

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Registration Action Branch 3 (7509C)

Work Assignment Manager: Marion Copley, D.V.M., D.A.B.T. MCopley 7/13/99
Registration Action Branch I (7509C)

DATA EVALUATION RECORD

STUDY TYPE: Oncogenicity Study in Mice
OPPTS Number: 870.4200 OPP Guideline Number: §83-2 (b)

DP BARCODE: D250746 SUBMISSION CODE: S546651
P.C. CODE: 999999 TOX. CHEM. NO.: None

TEST MATERIAL (PURITY): Dichlormid (97.9%, inert ingredient)

SYNONYMS: Not provided

CITATION: Tinston, D.J. Brammer, A., (1998) Dichlormid: 80 Week Oncogenicity Study in Mice. Central Toxicology Laboratory, Cheshire, UK. Lab Project ID. CTL/P/5343/PM1000, January 22, 1998. MRID 44529401. Unpublished.

SPONSOR: Zeneca Ag Products, Wilmington, DE

EXECUTIVE SUMMARY: In a mouse oncogenicity study (MRID 44529401), dichlormid (97.9%, Lot #P12 (WRC13790-28-02), inert ingredient) was administered to C57BL/10J$_f$CD-1 Alpk mice (55/sex/group) for up to 18 months at 0, 10, 50, or 500 ppm (equivalent to 0, 1.4, 7.0, or 70.7 mg/kg/day in males and 0, 1.8, 9.2, or 92.4 mg/kg/day in females).

Survival, clinical signs, food consumption, differential leukocyte counts, and gross necropsy findings for both sexes at all doses were unaffected by treatment with dichlormid.

Chronic toxicity was characterized at the high-dose by slight, statistically significant differences in body weights throughout the study in the males (↓2-6%; $p<0.01$) and in most weeks in the females (↓1-3%; $p<0.05$ or 0.01). At the end of the study, body weights in the high-dose males and females were lower than controls by 6% and 3%, respectively ($p<0.05$ or <0.01). In the males, food efficiency (g growth/100 g food) was lower than controls during weeks 1-4 (↓22%, $p<0.01$) and during weeks 1-12 (↓12%, $p<0.01$), the only periods in which food efficiency was determined.

At 500 ppm dichlormid, male mice had an increase in the incidence of minimal to moderate tubular vacuolation within the kidney (31/55 males compared to 12-15/55 in the lower dose

groups and the controls). This finding correlates with the increased absolute kidney weights in males (↑4%) in the 500 ppm group. The high-dose male mice also had an increased incidence of marked hyperplasia of Leydig cells within the testis (27/55 males compared to 1-11/55 in controls and 13/55 in each of the lower treatment groups. In the females, there was an increase in the incidence of ovarian atrophy, with 23/55 animals affected in this group compared to 10 or 11 animals in the control groups and 12 or 14 animals in the 10 ppm and 50 ppm treatment groups, respectively. These non-neoplastic changes, although marked at the highest dose, were not analyzed for statistical significance.

There were small changes in tumor incidence in the male mice, including an increased ($p<0.05$) incidence in harderian gland adenomas (9.1%) at 500 ppm and histiocytic sarcomas at 50 ppm (9.1%). However, data were presented to show that the numbers of tumors found in treated animals were consistent with tumor incidence in historical control animals (0-8% harderian gland adenomas and 0-16% histiocytic sarcomas).

The LOAEL for chronic toxicity is 500 ppm (equivalent to 70.7 mg/kg/day for male mice and 92.4 mg/kg/day for females), based on changes in reproductive organs and kidney changes in males. The chronic NOAEL is 50 ppm (equivalent to 7.0 and 9.2 mg/kg/day for males and female mice, respectively).

Under the conditions of this study, there was no evidence of carcinogenic potential.

Dosing was considered adequate based on slight decreased body weights in both high-dose males and females, decreased food efficiency in males, and changes in reproductive organs in males and females.

This oncogenicity study is determined to be **acceptable [§83-2(b)]** and does satisfy the guideline requirement for an oncogenicity study in mice.

COMPLIANCE: Signed and dated GLP, Quality Assurance, Data Confidentiality, and Flagging statements were provided.

I. MATERIALS AND METHODS

A. MATERIALS:

1. Test material: Dichlormid
   Description: Brown liquid
   Lot/Batch #: P12 (WRC 13790-28-02)
   Purity: 97.9%, inert ingredient
   Stability of compound: The compound is stable in the diet at room temperature for up to 20 days.
   CAS #: 37764-25-3
   Structure: Not Provided

2. Vehicle: Diet

3. Test animals: Species: Mouse
   Strain: C57BL/10J$_f$CD-1 Alpk
   Age and weight at study initiation: 5-6 weeks old; 22.4 - 22.8 g (males) and 18.0 - 18.2 g (females)
   Source: Barriered Animal Breeding Unit, Zeneca Pharmaceuticals, Alderley Park
   Housing: Multiple mouse cages suitable for animal of this strain, 5 mice/cage, segregated by sex. Mice were transferred to clean cages and racks as necessary.
   Diet: Powdered CT1 diet, ad libitum, (Special Diet Services Limited, Stepfield, Witham, Essex, UK)
   Water: Tap water, ad libitum
   Environmental conditions:
   Temperature: 21±3°C
   Humidity: 55±15%
   Air changes: ≥15/hr
   Photoperiod: 12 h dark/12 h light
   Acclimation period: At least 10 days

B. STUDY DESIGN:

1. In life dates - start: 3/27/95 end: 10/18/96.

2. Animal assignment: Animals were randomly assigned to treatment groups as indicated in Table 1.

Table 1. Study design

Test Group	Dose in diet (ppm)	Compound Consumption M/F (mg/kg/day)	Number of Animals	
			Males	Females
1	0 (control)	0	55	55
2	0 (control)	0	55	55
3	10	1.4/1.8	55	55
4	50	7.0/9.2	55	55
5	500	70.7/92.4	55	55

3. Dose rationale: It was stated that based upon the results of preliminary studies in the same strain of mice, the dosages presented in Table 1 were selected for this 18 month oncogenicity study. Data for the preliminary studies were not submitted.

4. Diet preparation and analysis: The test diets were prepared every three weeks by mixing appropriate amounts of dichlormid with the 500 g of milled diet. This premix was then added to 29.5 kg of the diet, and stored at room temperature. Samples of the treated food from each dose level (including controls) were collected from the top, middle and bottom at intervals of up to three months throughout the study and analyzed quantitatively for dichlormid. The homogeneity of dichlormid in the diet was determined by analyzing samples from the 10 and 500 ppm levels. The chemical stability of dichlormid in diet stored at room temperature was determined for these diets over a period of up to 20 days.

   Results:

   Homogeneity and concentration analyses: Overall mean concentrations of the samples collected during the study interval were found to be within 5% of the nominal concentration. No dichlormid was detected in the control diet (detection limit 0.1 ppm). of the mean concentrations. The homogeneity of dichlormid in diet at concentrations of 10 and 500 ppm was within 4% and 2%, respectively.

   Stability analysis: Stored samples contained 92.0-101.2% of the nominal test article concentration; therefore, the test substance is stable in the diet at room temperature for up to 20 days.

   The analytical data indicated that the mixing procedure was adequate and that the variance between nominal and actual dosage to the study animals was acceptable.

5. Statistics: With the exception of hematology, all analyses were run separately for males and females. To assess significance of intergroup differences, body weights were

analyzed by analysis of covariance on initial body weight. Food consumption, food utilization, organ weights and hematology were analyzed by ANOVA. Hematology percentage were first subjected to the double arcsine transformation of Freeman and Tukey. Organ weights were also considered by analysis of covariance on final body weight. All statistical tests were two-sided. Differences from pooled controls were tested statistically by comparing each treatment group least-squares mean with the pooled control group least-squares mean using a Student's t-test, based on the error mean square in the analysis. Kaplan-Meier survival estimates were calculated separately for each treatment group. Intergroup comparisons of mortality comparing each treatment group with the pooled control groups and an overall test for trend were performed using a log rank test according to the method of Peto and Pike. Fisher's Exact Test was used to analyze the incidence of each tumor and the overall incidence of each tumor type by comparing each treated group with the control group. A test for trend with group number was done using the Cochran-Armitage Test. Control groups 1 and 2 were pooled. Statistical analyses were not performed for microscopic non-neoplastic findings.

C. METHODS:

1. Observations: Prior to the start of the study, all animals were observed to ensure they were physically normal and exhibited normal activity. Animals were checked daily for changes in clinical condition and behavior. Clinical examinations, including the finding of no abnormalities detected, were made weekly.

2. Body weight: Animals were weighed weekly for the first 13 weeks and approximately biweekly thereafter. All animals surviving until study termination were weighed prior to necropsy.

3. Food consumption: Food consumption was recorded for a 7-day period and was determined weekly for the first 12 weeks and every fourth week thereafter. Weekly mean food consumption was calculated as g food/mouse/day for each cage. Food efficiency (body weight gain/100 g of food) was calculated for the first twelve weeks of the study only.

4. Ophthalmoscopic examination: Ophthalmoscopic examinations were not performed in this study. These data are not required for carcinogenicity studies based on Subdivision F Guidelines.

5. Blood analyses: At week 53, blood smears were prepared from the controls and the high-dose animals (11/sex/dose) and red cell morphology and differential white blood cell counts were performed. At the end of the study, the checked (X) parameters below were examined on blood samples from all surviving animals.

X	Hematocrit (HCT)	X	Leukocyte differential count
X	Hemoglobin (HGB)	X	Mean corpuscular HGB (MCH)
X	Leukocyte count (WBC)	X	Mean corpusc. HGB conc.(MCHC)
	Corrected leukocyte count (Cor WBC)	X	Mean corpusc. volume (MCV)
X	Erythrocyte count (RBC)	X	Reticulocyte count*
X	Platelet count	X	Erythrocyte morphology
	Blood clotting measurements	X	Erythrocyte distribution width
	(Thromboplastin time)		
	(Prothrombin time)		

* reticulocyte count was taken where there was a significant increase in red cell distribution width and the blood film appeared abnormal.

6. Urinalysis: Data on urinalyses were not submitted. These data are not required for carcinogenicity studies based on Subdivision F Guidelines.

7. Sacrifice and pathology: All animals that died or were killed *in extremis* and those surviving until study termination (18 months) were subjected to gross pathological examination and the CHECKED (X) tissues were collected. Additionally, the (XX) organs were weighed for those animals sacrificed on schedule.

	DIGESTIVE SYSTEM		CARDIOVASC./HEMAT		NEUROLOGIC
X	Oral Cavity	X	Aorta	XX	Brain (3 levels)
	Tongue	X	Heart		Peripheral nerve
X	Salivary glands	X	Bone marrow	X	Sciatic nerve
X	Esophagus	X	Lymph nodes	X	Spinal cord (3 levels)
X	Stomach	X	Spleen	X	Pituitary
X	Duodenum	X	Thymus	X	Eyes and optic nerve
X	Jejunum				GLANDULAR
X	Ileum		UROGENITAL	XX	Adrenal glands
X	Cecum	XX	Kidneys	X	Harderian gland
X	Colon	X	Urinary bladder	X	Mammary gland
X	Rectum	XX	Testes	X	Parathyroids
XX	Liver	X	Epididymides	X	Preputial gland
X	Gall bladder	X	Prostate	X	Thyroid
X	Pancreas	X	Seminal vesicles		OTHER
		X	Ovaries	X	Bone and joint
	RESPIRATORY		Oviducts	X	Skeletal muscle
X	Trachea	X	Uterus	X	Skin
X	Lungs		Vagina		Lacrimal gland
	Nasopharynx	X	Cervix	X	All gross lesions and masses
	Pharynx		Ureter		Auricles
	Larynx		Urethra		
X	Nasal Passages				

The nasal passages and oral cavities were stored. Tissues from the control and all treated groups were examined by light microcopy.

II. RESULTS

A. Observations

1. Toxicity - A variety of clinical findings typically seen in mice of this age and strain was seen in all groups of mice during the study. No treatment related clinical signs of toxicity were observed.

2. Mortality - There were no statistically significant differences in mortality between treated and control groups. At the end of the study, percent survival (calculated by the reviewers from the number of animals surviving to terminal sacrifice) ranged from 85.4 to 96.4% for male mice and 78.1 to 92.7% for females. These values are within the guideline requirements for an 18 month mouse study.

B. Body weight - From week 2 of the study onward, body weights of the high-dose males were lower (↓2-6%; $p<0.01$) than the controls. Body weights in the high-dose females were also statistically significantly lower than controls for most of the study (↓1-3%; $p<0.05$ or 0.01). There were no treatment-related changes to body weight in either sex at 10 or 50 ppm dichlormid. Select data for body weights and food efficiency are presented in Table 2.

Table 2. Select body weights and food efficiency in mice fed dichlormid for up to 18 months[a].

Interval (Week)	Male					Female				
	Dietary Level (ppm)									
	0	0	10	50	500	0	0	10	50	500
	Body Weight (g)									
1	22.7	22.8	22.4	22.7	22.5	18.2	18.1	18.2	18.0	18.1
2	23.6	23.8	23.7	23.6	23.1**	18.5	18.6	18.6	18.8**	18.6
5	26.5	26.5	26.5	26.0*	25.5**	21.7	21.9	21.6	21.8	21.5*
10	28.9	28.8	28.9	28.5	27.8**	23.7	23.7	23.3	23.9	23.2**
41	34.7	34.5	34.9	34.5	32.9**	26.9	26.5	26.7	26.7	26.2
81	35.7	35.4	35.9	35.7	33.7**	29.0	28.3	28.8	28.8	28.0*
	Food Efficiency (g growth/100g food)									
1-4	3.11	3.14	3.08	2.68	2.43**	2.83	3.00	2.81	2.97	2.76
1-12	1.99	2.01	1.96	1.92	1.76**	1.60	1.64	1.55	1.66	1.55

a These data were extracted from the study report, Tables 7 and 9, pages 66-77 and 84; n=55 for body weight data and 10-11 for food efficiency.

* $p<0.05$; ** $p<0.01$

C. Food consumption and compound intake

1. Food consumption - At the highest level of dichlormid, there were sporadic incidences of lower food consumption (g/animal/day) in male mice compared to concurrent controls. These decreases occurred during weeks 1, 4, and 16 (↓4-5%, $p<0.05$). Additionally, at 10 ppm, week 12, and 50 ppm, week 64, there were increases in food consumption (↑5 and 8%, $p<0.05$ or 0.01, respectively) in the male animals. Female mice had decreased food consumption at 500 ppm dichlormid from weeks 3-6 and week 9 (↓6-9%, $p<0.05$ or 0.01). On week 2 there was an increase in food consumption in the 50 ppm females (↑7%, $p<0.05$). These changes in food consumption were not consistent and are not of toxicological concern.

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2. Compound consumption - Time-weighted average compound consumption in mg/kg/day is summarized in Table 1.

3. Food efficiency - In males fed 500 ppm dichlormid, food efficiency (g growth/100 g food) was lower than controls during weeks 1-4 (↓22%, $p<0.01$) and during weeks 1-12 (↓12%, $p<0.01$), the only periods in which food efficiency was determined. Food efficiency in females was not significantly changed during these same intervals. Selected data on food efficiency are presented in Table 2.

D. Blood analyses - Blood smears taken at week 53 showed no differences in the differential white blood cell count between the controls and the 500 ppm dichlormid group. At the termination of the study, at 50 and 500 ppm dichlormid, male mice had decreases in the lymphocyte count, reflected in a decreased white blood cell count (↓23-28%, $p<0.05$ or 0.01). In the 10 ppm males, there was a slight, but significant decrease (↓1%, $p<0.05$) in the mean cell volume. The high-dose females had a slight, but significant decrease (↓5%, $p<0.01$) in red blood cell count and increases in mean cell volume and mean cell hemoglobin (↑2%, $p<0.01$). Increases in red cell distribution width were detected at 50 ppm dichlormid (↑5%, $p<0.05$). These differences from control animals were not corroborated by other data and are not of toxicological concern.

E. Sacrifice and pathology

1. Organ weights - At sacrifice, the 500 ppm males had decreased absolute brain weights (↓2%, $p<0.01$) and increased ($p<0.01$) absolute kidney and liver weights (↑4% and ↑6%, respectively). Absolute liver weights in females at 500 ppm were slightly increased, but the difference was not significant even when adjusted for body weights. However, when two high liver weights in control females were excluded from statistical analysis, the absolute liver weight when adjusted for bodyweight was significantly higher in the high-dose females compared to the controls (↑12%, $p<0.01$).

2. Gross pathology - There were no macroscopic findings of toxicological concern in any of the treatment groups. In general, necropsy findings were observed both in the control and treated groups with comparable frequency and are commonly seen in this strain/age of mice.

3. Microscopic pathology

a) Non-neoplastic - Non-neoplastic changes were not evaluated for statistical significance. At 500 ppm dichlormid, male mice had an increase in the incidence of minimal to moderate tubular vacuolation within the kidney (31/55 males compared to 12-15/55 in the lower dose groups and the controls). This finding correlates with the increased kidney weights in males. Males also had increased marked hyperplasia of Leydig cells within the testis at 500 ppm (27/55 males compared to 1-11/55 in controls and 13/55 in each of the lower treatment groups. In female mice fed 500

ppm dichlormid, there was an increase in the incidence of ovarian atrophy, with 23/55 animals affected in this group compared to 10 or 11 animals in the control groups and 12 or 14 animals in the 10 ppm and 50 ppm treatment groups, respectively. Of the females in the 500 ppm group, 21/43 animals exhibited ovarian atrophy at the terminal sacrifice; the remaining 2 animals were intercurrent removals. No information was provided describing the severity of the atrophy. There was also a slight increase in the total incidence of ovarian mononuclear cell infiltration in these females, with 12/55 affected compared to 2 and 7 in the control groups and 6 and 3 in the 10 ppm and 50 ppm treatment groups, respectively. Infiltration was described as being minimal or slight, with approximately equal incidences in severity. Data for select non-neoplastic lesions are presented in Table 3.

Table 3. Select non-neoplastic lesions observed at final necropsy in mice fed dichlormid for up to 18 months[a].

Finding	Male					Females				
	Dietary Level (ppm)									
	0	0	10	50	500	0	0	10	50	500
Kidneys										
Tubular vacuolation-total	12	15	15	14	31	0	0	0	0	0
minimal	11	10	13	10	15	0	0	0	0	0
slight	1	5	2	3	15	0	0	0	0	0
moderate	0	0	0	1	1	0	0	0	0	0
Ovaries										
Atrophy	-[b]	-	-	-	-	10	11	12	14	23
Mononuclear cell infiltration-total	-	-	-	-	-	2	7	6	3	12
minimal						0	2	5	2	5
slight						2	5	1	1	7
Testis										
Leydig cell hyperplasia- total	46	48	49	53	51	-	-	-	-	-
minimal	5	5	1	10	2	-	-	-	-	-
slight	19	13	10	10	7	-	-	-	-	-
moderate	21	19	25	20	15	-	-	-	-	-
marked	1	11	13	13	27	-	-	-	-	-

a These data were extracted from the study report, Table 15, pages 157, 163, and 172; n-53-55.
b - = not applicable.

b) Neoplastic - Select neoplastic lesions are presented in Table 4a. There was an increase (5/55 or 9.1%, $p<0.05$) in the incidence of harderian gland adenomas in male mice fed 500 ppm dichlormid. This compared to an incidence of 0-1.8% in control animals and 0 and 5.5% in male mice dosed with 10 or 50 ppm, respectively. The Sponsor stated that there was also a significant trend in the incidence of these tumors with increasing dose ($p=0.003$). Hardarian gland adenomas were a contributory factor in the deaths of 2 high-dose males and in one each of the 50 ppm and control males. Male mice dosed with 50 ppm dichlormid had a significant increase (5/55 or 9.1%, $p<0.05$) in the number of histiocytic sarcomas; there was also an increased incidence compared to controls at the 10 and 500 ppm levels (7.3% each). The Sponsor stated that there was also a significant trend in the incidence of these tumors with increasing dose ($p=0.031$). The provided historical control data showed that the incidence of harderian gland adenomas ranged from 0 to 8%, while the incidence of histiocytic sarcomas ranged from 0 to 16% in males and 0 to 23% in females. Overall, there was a slight increase in the number of tumor bearing animals in the dosed groups (Table 4b). When broken into categories, both male and female mice had a slight increase in the number of malignant tumors. Male mice also had an increased number of benign, multiple, single, and metastatic tumors, while female mice had an increased incidence of single and metastatic tumors. These increased incidences were not statistically significant.

Table 4a. Select neoplastic lesions observed at final necropsy in male mice fed dichlormid for up to 18 months[a].

Finding	Dietary Level (ppm)					
	0	0	10	50	500	Historical controls
Harderian Gland Adenoma	0/53 (0%)	1/55 (1.8%)	0/55 (0%)	3/55 (5.5%)	5/55* (9.1%)	0/50-4/50 (0-8%)
Trend	p=0.003					
Histiocytic Sarcoma	1/55 (1.8%)	0/55 (0%)	4/55 (7.3%)	5/55* (9.1%)	4/55 (7.3%)	0/60-9/55 (0-16%)
Trend	p=0.031					

a Data were extracted from the study report, Table 19 (pp. 185 and 186), and Appendix F (pp. 200 and 201).
* $p<0.05$

Table 4b. Tumor incidences (number of animals) in mice fed dichlormid for up to 18 months[a].

Finding	Males					Females				
	Dietary Level (ppm)									
	0	0	10	50	500	0	0	10	50	500
Total tumor bearing animals	16	14	15	20	22	19	23	26	23	30
malignant tumors	14	12	14	17	16	15	17	20	20	25
benign tumors	2	3	3	4	9	6	8	7	5	7
multiple tumors	0	1	4	2	5	4	4	1	5	2
single tumors	16	13	11	18	17	15	19	25	18	28
multiple malignant tumors	0	0	2	1	1	2	1	0	3	0
multiple benign	0	0	0	0	1	1	1	0	0	0
metastatic tumors	13	10	14	16	15	15	15	20	20	22

a These data were extracted from the study report, Table 18, page 184; n-55.

III. DISCUSSION

A. Investigators Conclusions - The study report concluded that oral administration of dichlormid to animals at 500 ppm was associated with reduced body weights in both male and female mice, and reduced food efficiency in male mice. Body weight differences were explained by changes in food intake and food efficiency. The NOAEL was determined to be 50 ppm and was based on reduced body weights.

Although there were small changes in tumor incidence at 500 ppm dichlormid, the numbers of tumors seen were considered to be within the range of spontaneous tumor incidence seen in animals of this strain. Therefore, there is no clear evidence for oncogenicity in this study.

B. Reviewer's Discussion/Conclusions - Male and female mice were fed diets containing dichlormid at 0, 10, 50, or 500 ppm (equivalent to 0, 1.4, 7.0, and 70.7 mg/kg/day in males and 0, 1.8, 9.2, or 92.4 mg/kg/day in females) for 18 months. Dietary analyses at select study intervals confirmed that nominal diet concentrations of dichlormid were achieved. No clinical signs of toxicological significance were observed. No significant increases in mortality rates were observed in any of the treated groups throughout the study when compared to control groups.

There were no changes of toxicological concern in food consumption or gross pathology.

At 500 ppm dichlormid, male mice had an increase in the incidence of minimal to moderate tubular vacuolation within the kidney (31/55 males compared to 12-15/55 in the lower dose groups and the controls). This finding correlates with the increased absolute kidney weights in males (↑4%) in the 500 ppm group. Also at 500 ppm dichlormid, male mice with marked hyperplasia of Leydig cells within the testis was increased (27/55 males compared to 1-11/55 in controls and 13/55 in each of the lower treatment groups. In female mice fed 500 ppm dichlormid, there was an increase in the incidence of ovarian atrophy, with 23/55 animals affected in this group compared to 10 or 11 animals in the control groups and 12 or 14 animals in the 10 ppm and 50 ppm treatment groups, respectively. Other non-neoplastic changes were minor, and are not considered to be of toxicological significance. Non-neoplastic lesions were not evaluated for statistical significance.

At the highest level of dichlormid (500 ppm) there was a decrease in the body weights of males and females (↓6% and 3%, respectively, $p<0.05$ or 0.01). These changes were associated with decreases in food efficiency in males during the first 12 weeks of the study (↓12%, $p<0.01$) and decreased food consumption in females, significant during the first 9 weeks of the study (↓6-9%, $p<0.05$ or 0.01). These changes were not seen in males or females at the 10 or 50 ppm dose levels.

Other changes seen were increases in relative liver weights (↑6%, $p<0.01$) in the 500 ppm males and a significant decrease in lymphocyte counts in males fed 50 and 500 ppm dichlormid. In the absence of any histopathological changes, these changes were not considered to be of toxicological significance.

There were small changes in tumor incidence in male mice. There was an increased ($p<0.05$) incidence in harderian gland adenomas at 500 ppm with 5/55 (9.1%) animals affected vs 0/53 or 1/55 (0-1.8%) controls affected. However, data were presented to show that the numbers of tumors found in treated animals were consistent with tumor incidence in historical control animals (0/50-4/50, or 0-8%). There was also an increase in the incidence of histiocytic sarcomas at 50 ppm ($p<0.05$) with 5/55 (9.1%) animals affected vs 0/55 or 1/55 controls affected (0-1.8%). As with the harderian gland adenomas, data were presented to show that the numbers of tumors found in treated animals were consistent with tumor incidence in historical control animals (0/60-9/55, or 0-16%). Therefore, these increases are not of toxicological significance.

In conclusion, the dose levels employed in this study were adequate to characterize the oncogenic potential of dichlormid in both sexes of C57BL/10J$_f$CD-1 Alpk mice. Chronic toxicity was characterized in mice by slight decreases in body weights in both high-dose males and females, decreased food efficiency in males, changes in reproductive organs in males and females and changes in the kidney in males.

Under the conditions of this study, no increases in the incidences of any neoplasm were observed in the dosed animals.

The LOAEL for chronic toxicity is 500 ppm (equivalent to 70.7 mg/kg/day for male mice and 92.4 mg/kg/day for females), based on changes in reproductive organs, and kidney changes in males. The chronic NOAEL is 50 ppm.

This oncogenicity study is determined to be **acceptable [§83-2(b)]** and does satisfy the guideline requirement for an oncogenicity study in mice.

C. Study deficiencies - No rationale was given for the dose levels selected, beyond the statement that the levels were based on the results of preliminary feeding studies. However, as it was possible to determine a LOAEL from the submitted data, this deficiency does not affect the acceptability of the study.

ATTACHMENTS

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DATA EVALUATION REPORT **014199** 68

DICHLORMID

STUDY TYPE: DEVELOPMENTAL TOXICITY– RAT (83-3a)

Prepared for

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Office of Pesticide Programs
U.S. Environmental Protection Agency
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Chemical Hazard Evaluation Group
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014199

DATA EVALUATION RECORD

STUDY TYPE: Developmental Toxicity - Rat
OPPTS 870.3700 [83-3a]

DP BARCODE: D248305
P.C. CODE: N/A

SUBMISSION CODE: S546651
TOX. CHEM. NO.: none stated

TEST MATERIAL (PURITY): Dichlormid (97.2% a.i.)

SYNONYMS: R-25788

CITATION: Wickramaratne, G. (1989) Dichlormid: teratogenicity study in the rat. ICI Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, U.K. Report No. CTL/P/2383, Study No. RR0406, July 18, 1989. MRID 44606408. Unpublished.

SPONSOR: ICI Americas Inc., Agricultural Products, Wilmington, DE 19897.

EXECUTIVE SUMMARY: In a developmental toxicity study (MRID 44606408), 24 presumed pregnant Wistar rats per group were administered dichlorimid (97.2% a.i.) orally by gavage at doses of 0, 10, 40, or 160 mg/kg/day on gestation days (GD) 7-16, inclusive. On GD 22. dams were sacrificed, subjected to gross necropsy, and all fetuses examined externally, viscerally, and skeletally for malformations/variations.

Maternal toxicity in the 40 and 160 mg/kg/day groups is based on statistically significant decreases ($p<0.05$; 0.01) in absolute body weights, body weight gains, and food consumption. Final absolute body weights in the mid- and high-dose groups were 95% and 92% of controls, respectively. During the dosing interval (GD 7-16), mid- and high-dose dams gained 22% and 46% less weight, respectively, and consumed 13% and 31% less feed, respectively, than controls. The most pronounced reductions in body weight gain and food consumption occurred during the first three days of treatment (GD 7-10) in both groups: mid-dose females gained 39% less weight and consumed 15% less feed, while high-dose dams gained 144% less weight and consumed 38% less feed. In addition to decreases in body weight and food consumption, mid- and high-dose dams also had decreased food efficiency during the entire dosing interval (-9% and -22% of controls, respectively). No statistically significant decreases in absolute body weights, body weight gains, or food consumption were noted in the 10 mg/kg/day group dams.

No treatment-related mortalities, clinical signs of toxicity, or gross pathological changes were observed in the study.

Therefore, the maternal toxicity LOAEL is 40 mg/kg/day based on decreased mean absolute body weights, body weight gains, and food consumption, and the maternal toxicity NOAEL is 10 mg/kg/day.

No dose- or treatment-related effects on pregnancy rates, number of corpora lutea, pre- or postimplantation losses, resorptions/dam, fetuses/litter, or fetal sex ratios were observed in the treated groups as compared with the controls.

No treatment-related external or visceral malformations/variations were observed in any litter. Most treated and control litters contained fetuses with minor variations in skeletal ossification. The incidence of a misaligned 5th sternebra was statistically significantly ($p<0.05$) but marginally increased in fetuses (6/259 fetuses affected vs. 0/277 for control) and marginally increased in litters (4/22 litters affected vs. 0/22 for control: $p = 0.054$) from the high-dose group. Because no other potential treatment-related skeletal malformation/variations were identified, the marginal increase in this variation was used for the establishment of a threshold LOAEL. **Therefore, the developmental toxicity NOAEL is 40 mg/kg/day. The developmental toxicity LOAEL threshold is 160 mg/kg/day based on a marginal increase in skeletal anomalies.**

This developmental toxicity study is classified as **Acceptable/guideline** and satisfies the subdivision F guideline requirements for a developmental toxicity study in rats (83-3a).

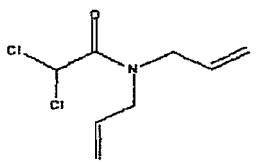
COMPLIANCE: Signed and dated Quality Assurance, Good Laboratory Practice, Data Confidentiality, and Flagging statements were included.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: dichlormid
   Description: clear, amber-colored, viscous liquid
   Lot/Batch No.: WRC4921-35-12: GGD0101
   Purity: 97.2% a.i.
   Stability of compound: data not provided
   CAS No.: 37764-25-3

   Structure:



Vehicle and/or positive control

Corn oil (Kraft-Wesson 100% corn oil supplied by Kraft Foods Limited, UK) was used as the control article and the vehicle for the preparation of the dosing formulations. No positive control was used in this study.

3. Test animals

Species: rat
Strain: Wistar (Alpk:APfSD)
Age and weight at study initiation: approximately 12 weeks; 218-297 g
Source: Barriered Animal Breeding Unit, ICI Pharmaceuticals, Alderley Park, Macclesfield, Cheshire, UK.
Housing: Animals were housed individually in suspended stainless steel cages
Diet: Special Diets Services Limited (Stepfield, Witham, Essex, UK) and tap water were available *ad libitum*
Environmental conditions:
Temperature: 17-25°C
Humidity: 51-60%
Air changes: at least 12 air changes/hour
Photoperiod: 12 hr light/dark
Acclimation period: not stated

B. PROCEDURES AND STUDY DESIGN

The purpose of this study was to investigate the developmental toxicity potential of dichlormid when administered by gavage to pregnant rats on gestation days 7 through 16, inclusive.

1. In life dates

Start: May 16, 1988; end: June 17, 1988

2. Mating

Females were paired overnight with unrelated males of the same strain and source for breeding. On the following morning, vaginal smears from these females were examined for the presence of sperm. The day on which spermatozoa were detected was designated Day 1 of gestation (GD 1).

3. Animal assignment and dose selection are presented in Table 1. Bred females were assigned by random block design to one of four groups containing 24 rats each.

TABLE 1. Animal assignment		
Test group	**Dose (mg/kg/day)**	**Number females assigned**
Control	0	24
Low dose	10	24
Mid dose	40	24
High dose	160	24

Data taken from Text Figure on p. 16, MRID 44606408.

4. Dose selection rationale

The dose levels selected for this study were based on the results of an embryotoxicity study in the rat (referenced as Kinsey, 1989, ICI Central Toxicology Labortory Report No. CTL/P/2292, but data was not provided).

5. Dose solution preparation and analysis

Dichlormid was formulated in corn oil. An appropriate amount of corn oil was added to a weighed amount of dichlormid to provide one preparation per dose level. Each preparation was handshaken until a solution was formed before being subdivided into aliquots. The control article was also dispensed into aliquots. The aliquots were stored in the dark at 4°C and fresh bottles were used for each day of the study.

A sample of each solution was analyzed prior to the start of dosing to verify the achieved concentrations of dichlormid in corn oil. The chemical stability of dichlormid in corn oil was determined by re-analysis of the lowest and highest dosing formulations (nominally 1 and 16 mg/mL) after intervals of 0, 6 (16 mg/mL only), and 27 days. It is assumed that the dosing solutions reanalyzed were from those stored in the dark at 4°C.

Results:

Concentration: Absence of test material was confirmed in the vehicle. The mean concentrations of the samples as a percentage of nominal concentrations for the 10, 40, and 160 mg/kg/day test solutions were 96.0%, 98.5%, and 101.9%, respectively.

Homogeneity: Homogeneity of the dosing solutions was not measured.

Stability Analysis: After 0 and 27 days, the percentage of the initial concentration of the 1 mg/mL (10 mg/kg/day) dosing solution was 100% and 101.1%, respectively. After 0, 6, and 27 days, the percentage of the initial

concentration of the 16 mg/mL (160 mg/kg/day) dosing solution was 100%, 96.9%, and 95.1%, respectively.

The analytical data of the 10, 40, and 160 mg/kg/day test solutions demonstrated that the test substance was stable in corn oil for at least 27 days, and that the variance between nominal and actual doses to the animals was acceptable. It is not known if the mixing procedure was adequate because homogeneity analysis was not conducted.

6. Dosing

All doses were in a volume of 1 mL/100g/day prepared daily during the dosing period (GD 7-16, inclusive). Dosing by gavage was based on the most recently recorded body weights.

C. OBSERVATIONS

1. Maternal observations and evaluations

All animals were checked on arrival to ensure that they were physically normal externally and were subsequently observed daily. Any changes in behavior or clinical condition were recorded daily during the dosing period and on those days when the animals were weighed. Maternal body weights were recorded on GD 1, and 4, 7-16 (inclusive), and 19 and 22. Food consumption over three day periods was measured on GD 4, 7, 10, 13, 16, 19, and 22. Females were euthanized on GD 22 by halothane BP and were subjected to gross necropsy. The uterus was weighed and the uterus and ovaries of each dam were examined to determine the number of corpora lutea, numbers of viable and dead fetuses, and number of implantation and resorption sites.

2. Fetal evaluations

At necropsy, the fetuses were weighed and then killed with injection of pentobarbitone. Each fetus was examined for external abnormalities and for cleft palate. All fetuses were then examined internally for visceral abnormalities under magnification, sexed, eviscerated, and fixed in methanol. The head of each fetus was cut along the fronto-parietal suture line and the brain was examined for macroscopic abnormalities. All carcasses were then returned to methanol for subsequent processing and stained with Alizarin Red S for skeletal examination. Ossification of the individual bones of the manus and pes was assessed and the results converted to a four point scale.

D. DATA ANALYSIS

1. Statistical analysis

Maternal body weight gain, maternal food consumption, numbers of implantations and live fetuses/dam, percentage of pre- and post-implantation loss, percentage of male fetuses, gravid uterine weights, litter weights, mean fetal weights, mean manus and pes score/fetus, and the percentage of fetuses with minor external/visceral defects only, minor skeletal defects only, and external/visceral variants were analyzed by analysis of variance. Each treatment group mean was compared with the control group using Student's t-test, based on the error mean square in the analysis. The proportion of females with pre- or post-implantation loss and early or late intrauterine deaths, the proportion of male fetuses, and the proportion of fetuses with major or minor (only) external/visceral defects, external/visceral variants, minor (only) skeletal defects, skeletal variants, and each individual finding were analyzed by Fisher's Exact Test. The proportion of fetuses with each individual finding was analyzed on a litter basis. All statistical tests were one-sided with the following exceptions which were two-sided: maternal body weight gain, maternal food consumption and the proportion of male fetuses.

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

II. RESULTS

A. MATERNAL TOXICITY

1. Mortality and clinical signs

All mated females survived until sacrifice. No definitive treatment-related signs of toxicity were noted in any of the treated dams. Although the incidence of alopecia appeared to exhibit a dose-related increase in the mid- and high-dose groups when compared with the control group (1/1, 1/2, 1/2, 8/14 animals affected/number of observations for the 0, 10, 40, and 160 mg/kg/day groups, respectively), the occurrence was generally transient, and several animals exhibited the slight hair loss pre- or post-dosing. Piloerection was generally only observed in the affected animals once or twice and was only slight. Two high-dose dams exhibited multiple clinical signs (one had tip toe gait, hunched posture, piloerection, sides pinched in; the other had tail erection, splayed gait, tip toe gait, piloerection, urinary incontinence, and alopecia); however, these observations again were only transient.

2. Body weight

Statistically significant decreases in mean maternal absolute body weights and body weight gains were observed in mid- and high-dose groups (see Table 2). In the mid-dose group, mean maternal body weights were statistically significantly decreased on GD 15, 16, 19, and 22 (-5% to -8% as compared with controls: $p<0.05$), and mean body weight gain was statistically significantly reduced ($p<0.05$; 0.01) during the dosing intervals of GD 7-10 and 13-16 (-39% and -26%. respectively, as compared with controls), the entire dosing interval (GD 7-16; -22%), and for the entire study (GD 1-22; -11%). In the high-dose group, statistically significant decreases ($p<0.05$) in mean maternal body weights occurred on GD 9-22 (-6% to -9% as compared with controls), and statistically significant reductions ($p<0.05$; 0.01) in mean maternal body weight gain as compared with controls occurred during all measured dosing intervals (GD 7-10: -144%; GD10-13: -35%; GD 13-16: -17%), the entire dosing interval (GD 7-16: -46%), and the entire study period (GD 1-22: -18%).

TABLE 2: Selected mean maternal body weights and body weight gains during gestation (g)				
GD	0 mg/kg/day	10 mg/kg/day	40 mg/kg/day	160 mg/kg/day
Mean maternal body weights (g) [a]				
1	265.6 ± 19.9	258.8 ± 20.9	255.8 ± 19.0	258.3 ± 15.3 (-3) [b]
7	295.9 ± 23.7	288.6 ± 21.0	286.8 ± 22.4	286.9 ± 16.7 (-3)
10	304.2 ± 25.0	298.9 ± 21.3	291.9 ± 23.2	283.0* ± 20.7 (-7)
13	320.8 ± 26.6	316.2 ± 23.9	307.0 ± 23.6	293.9* ± 25.4 (-8)
16	342.6 ± 27.3	337.3 ± 26.1	323.0* ± 24.1 (-6)	312.1* ± 24.2 (-9)
19	380.7 ± 29.9	374.6 ± 29.5	351.2* ± 25.2 (-8)	346.4* ± 24.9 (-9)
22	409.1 ± 32.2	408.6 ± 31.0	383.6* ± 29.2 (-6)	376.9* ± 30.6 (-8)
Adjusted body weight [c]	322.1	320.1	305.8 (-5)	295.6 (-8)
Mean maternal body weight gains (g)				
1-7 (predosing)	30.5	29.9	30.9	28.3
7-10	8.4	10.3	5.1* (-39)	-3.7** (-144)
10-13	16.4	17.3	15.2 (-7)	10.7** (-35)
13-16	21.7	21.2	16.0** (-26)	18.0* (-17)
7-16 (during dosing)	46.4	48.7	36.3** (-22)	25.0** (-46)
16-22 (postdosing)	66.4	71.3	60.6	64.6
1-22 (overall)	143.3	149.9	127.8** (-11)	117.9** (-18)
1-22 adjusted [d]	56.5	61.3	50 (-12)	37.1 (-34)

Data taken from Table 5 and Appendix 3, p. 43 and 123-126, MRID 44606408.
[a] Statistical analysis of mean maternal body weights conducted by reviewer using ANOVA followed by Dunnett's, statistical analysis of mean maternal body weight gain calculated by study author: Significantly different from controls: *p<0.05; **p<0.01.
[b] Numbers in parentheses represent the percent change compared with controls; calculated by reviewer.
[c] Calculated by reviewer: GD 22 mean maternal body weight - mean gravid uterine weights (p. 48; MRID 44606408)
[d] Calculated by reviewer: adjusted body weight - GD 1 body weight

3. Food consumption

Maternal food consumption was statistically significantly decreased ($p<0.05$; 0.01) for all measured intervals during the dosing period in both the mid-dose group (-10% to -15% as compared with controls) and high-dose group (-24% to -38% as compared with controls) (see Table 3). The decreases in food consumption during the dosing period were decreased in a dose-, but not time-, related manner. Food consumption was also statistically significantly decreased in these groups during the first part of the post-dosing interval of GD 16-19 (-9%

and -10%, respectively; $p<0.05$), but was not decreased during the latter part (GD 19-22), or for the entire pre- or post-dosing periods (GD 1-7 or GD 16-22).

Food efficiency was estimated by the reviewer for the predosing, dosing, and postdosing intervals (see Table 3). Food efficiency during the dosing period was decreased by 9% and 22% in the mid- and high-dose dams, respectively, as compared with controls. Food efficiency during the pre- and post-dosing intervals was not affected in any of the treatment groups as compared with the controls.

TABLE 3: Mean maternal food consumption and food efficiency during gestation (g)

GD	0 mg/kg/day	10 mg/kg/day	40 mg/kg/day	160 mg/kg/day
Food consumption (g/day)				
1-7 (predosing)	24.6	24.2	24.2	24.1
7-10	20.1	20.1	17.0** (-15) [a]	12.4** (-38)
10-13	22.2	22.4	19.9* (-10)	14.7** (-34)
13-16	23.8	23.8	20.7** (-13)	18.2** (-24)
7-16 (during dosing)	22.0	22.1	19.2** (-13)	15.1** (-31)
16-19	29.5	30.4	26.8* (-9)	26.5* (-10)
16-22 (post dosing)	28.5	29.6	26.4	26.7
Food efficiency [b]				
1-7	21	21	21	20
7-16	23	24	21 (-9)	18 (-22)
16-22	39	40	38	40

Data from Table 6, p. 44, MRID 44606408.
[a] Numbers in parentheses represent the percent change compared with controls; calculated by reviewer.
[b] Crude estimate calculated by reviewer: [g body weight change per unit time/g food consumed per unit time] x 100
Significantly different from controls: *p<0.05; **p<0.01.

4. Gross pathology

Gross necropsy was unremarkable. Gross pathological findings occurred in low incidence or in a nondose-related manner. One high-dose dam each had distention of the colon or cecum with gas, two had distention of the rectum with gas, one had small cysts on the spleen, and one had clear fluid in the stomach and yellow, viscous fluid in the ileum.

5. Cesarean section data

Data collected at cesarean section are summarized in Table 4. There were no apparent treatment-related differences in the pregnancy rate, mean numbers of viable fetuses, early and late resorptions, implantation sites, copora lutea, fetal sex

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ratios, or fetal body weights. The 40 mg/kg/day group had a statistically significantly lower mean gravid uterine weight as compared with controls ($p<0.05$). It is not known if any dams had complete litter resorption because nongravid uteri were not stained for this purpose.

TABLE 4. Cesarean section observations

Observation	0 mg/kg/day	10 mg/kg/day	40 mg/kg/day	160 mg/kg/day
No. Animals Assigned	24	24	24	24
No. Animals Pregnant	22	24	24	22
Pregnancy Rate (%)	92	100	100	92
Maternal Mortality	0	0	0	0
Aborted	0	0	0	0
Total Corpora Lutea	300	333	311	293
Mean Corpora Lutea	13.6 ± 1.9	13.9 ± 2.1	13.0 ± 2.1	13.3 ± 2.4
Total Implantations	283	306	283	268
Preimplantation Loss (%)	5.7	8.1	9.0	8.5
Postimplantation Loss (%)	2.1	2.0	4.2	3.4
Gravid Uterine Weight (g)	87.0	88.5	77.8*	81.3
Placental Weight (g)	-	-	-	-
Total Live Fetuses	277	300	271	259
Live Fetuses/Litter [a]	12.6	12.5	11.3	11.8
Mean Fetal Weight (g)	4.96	5.01	4.90	4.90
Sex Ratio (%Male)	48	52	48	48
Total Dead Fetuses	0	0	0	0
Dams with all Resorptions	-	-	-	-
Total Resorptions	6	6	12	9
Resorptions/Dam	0.3 ± 0.6	0.3 ± 0.5	0.5 ± 1.1	0.4 ± 0.7
Early Resorptions	0.2 ± 0.5	0.3 ± 0.5	0.4 ± 1.1	0.3 ± 0.6
Late Resorption	0.0 ± 0.2	0.0 ± 0.0	0.1 ± 0.3	0.1 ± 0.4

Data from Table 8 and Appendix 5, pp. 46-48 and 133-136, MRID 44606408.
[a] Calculated by reviewer.
Statistically different from controls: *$p<0.05$.

B. DEVELOPMENTAL TOXICITY

The overall incidence rates for litters containing fetuses with major malformations in the 0, 10, 40, or 160 mg/kg/day groups were 0/22, 2/24, 1/24, and 2/22, respectively.

1. External examination

No treatment-related malformations/variations were noted during external examination of fetuses. Incidental external abnormalities included subcutaneous hemorrhage in one fetus from the 10 and 160 mg/kg/day group, and macroglossia in another 160 mg/kg/day fetus from a different litter.

2. Soft tissue examination

Examination of fetal soft tissue did not reveal any treatment-related soft tissue malformations/variations. Soft tissue malformations/variations that occurred equally in all groups or at low incidences included dilated and/or kinked ureter, distended bladder, hemorrhagic or pale kidney, a white area on the stomach wall. or a blood clot attached to the intestines. Additionally, one 40 mg/kg/day fetus had situs inversus of the abdomen and one160 mg/kg/day fetus had white rimmed eyes.

3. Skeletal examination

Most treated and control litters contained fetuses with minor variations in skeletal ossification. The fetal incidence of unossified vertebral cervical centrum was statistically significantly increased ($p<0.05$) in the 10 and 40 mg/kg/day group for the 3rd, 4th, and 5th centrum, and in the 160 mg/kg/day group for the 2nd and 3rd centrum (see Table 5). Additionally, the 160 mg/kg/day group had a statistically significantly increased ($p<0.05$) litter incidence of unossified vertebral cervical 5th centra, and statistically significant increases in the fetal incidence of a misaligned 5th sternebra ($p<0.05$) and a short extra rib ($p<0.01$). The litter incidence of a misaligned 5th sternebra approached statistical significance ($p = 0.054$ as calculated by the reviewer using Fischer's Exact Test). The only other statistically significant difference between treated groups and the control group was an increase in the fetal incidence of reduced ossification of the 4th lumbar ($p<0.05$) in the 10 mg/kg/day group. Other skeletal malformations/variations occurred equally in all groups or at low incidences.

No treatment-related effects were noted during the assessment of ossification of the individual bones of the manus and pes.

TABLE 5. Selected skeletal malformations/variations				
Observations	0 mg/kg/day	10 mg/kg/day	40 mg/kg/day	160 mg/kg/day
No. Fetuses (litter) examined	277 (22)	300 (24)	271 (24)	259 (22)
Unossified vertebral cervical centrum: 2nd	98 (22)	116 (21)	106 (23)	111* (19)
3rd	15 (11)	30* (9)	30* (13)	28* (14)
4th	7 (5)	19* (7)	22** (11)	13 (9)
5th	1 (1)	7* (5)	7* (3)	6 (6*)
Misaligned 5th sternebra	0 (0)	1 (1)	2 (2)	6* (4)
Extra ribs - short length	56 (16)	73 (22)	40 (15)	80** (19)

Data taken from Table 11, pp. 53-63, MRID 44606408.
Significantly different from control: *p < 0.05; **p<0.01

III. DISCUSSION

A. INVESTIGATOR'S CONCLUSIONS

Administration of 160 or 40 mg/kg/day resulted in maternal toxicity, manifested as a reduction in weight gain and food consumption during the dosing period. No maternal toxicity was seen in the 10 mg/kg/day group. There was no evidence that dichlormid is teratogenic to the rat at any of the dose levels tested and 160 mg/kg/day is, therefore, considered to be the no-effect level for teratogenicity. A marginal developmental effect was noted at 160 mg/kg/day, with findings consisting of slight misalignment of the 5th sternebra, unossified 2nd cervical centra, and extra 14th ribs. However, these findings alone are considered to be of doubtful toxicological significance.

B. REVIEWER'S DISCUSSION

1. Maternal toxicity

All females survived until sacrifice. Although it initially appeared that the clinical signs alopecia and piloerection were related to treatment, closer examination of the data did not support this conclusion because the signs were transient, also occurred before or after dosing, and were generally only slight. The multiple clinical signs (including altered gait, piloerection, hunched posture) reported in two of the high-dose dams were also only transient.

Treatment with 40 and 160 mg dichlormid/kg/day resulted in dose-related, statistically significant decreases in absolute body weights, body weight gains. and food consumption. Although the statistically significant decreases in maternal absolute body weights were of marginal biological significance in the 40 mg/kg/day group (only -5 to -8% of controls), these decreases in absolute weights

were accompanied by more severe reductions in body weight gain (up to 39% reduction compared with controls), and corresponding decreases in food consumption. Absolute body weights, body weight gains, and food consumption were decreased to a greater extent in high-dose dams. The decreased food efficiency during the dosing interval in the mid- and high-dose groups indicated that the decreased food consumption by itself did not explain the decreased body weights.

Therefore, we agree with the investigator that the maternal toxicity LOAEL is 40 mg/kg/day based on decreased mean absolute body weights, body weight gains, and food consumption, and the maternal toxicity NOAEL is 10 mg/kg/day.

2. Developmental toxicity

a. Deaths/resorptions

Treatment with dichlormid did not result in increased embryonic or fetal death in the treatment groups receiving up to 160 mg/kg/day. The number of resorptions was also not significantly affected by treatment.

b. Altered growth

No treatment-related alterations in fetal growth were observed. Although statistically significant increases were noted in the fetal incidence of unossified cervical centrum (3rd, 4th and 5th) in most treated groups, this reduction in ossification was not considered an effect of treatment because it was not related to dose and was not accompanied by increases in litter incidence. The statistically significant increase in the litter incidence of unossified 5th cervical centra and fetal incidence of unossified 2nd cervical centra in the high-dose group was also not considered treatment related because a dose-response was lacking.

c. Developmental variations

The statistically significant increase in the fetal incidence of short extra ribs in the high-dose group was not considered an effect of treatment because it was not related to dose and was not accompanied by increases in litter incidence. Additionally, this variation is a common occurrence in rats. The statistically significant increase in the fetal incidence of misaligned 5th sternebra in the high-dose group may represent a marginal effect of treatment with dichlormid. The number of fetuses and litters affected did show a slight dose-response, and the litter incidence approached statistical significance ($p = 0.054$). Although the number affected was only marginally increased when compared with the total number of fetuses or litters examined, it is possible that this variation may be a compound-related effect observed at this dose (LOAEL), and hence 40 mg/kg/day may represent the clear NOAEL for

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fetotoxicity (developmental toxicity). Other variations noted occurred equally in all groups or at low incidences.

d. Malformations

No major fetal malformations could be attributed to maternal treatment with dichlormid.

C. STUDY DEFICIENCIES

Homogeneity of the dosing solutions was not determined; therefore, it is not known if the mixing procedure was adequate. Because concentration and stability analysis were acceptable, however, the lack of homogeneity analysis is not believed to have compromised the study. A minor deficiency was that non-gravid uteri were not stained to check for complete litter resorption (but only 2 control and 2 high-dose dams were nongravid). Additionally, historical control data in the form of graphs were only provided for fetal incidences of a few variations. Historical control data for litter incidences would have be beneficial, particularly for misaligned 5th sternebra.

D. CORE CLASSIFICATION

This developmental toxicity study is classified as **Acceptable/guideline** and satisfies the subdivision F guideline requirements for a developmental toxicity study in rats (83-3a).

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DATA EVALUATION REPORT

DICHLORMID 014199

STUDY TYPE: DEVELOPMENTAL TOXICITY– RAT (83-3a)

Code No. 225002431 (MRID No. 00058469)

Prepared for

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

Prepared by

Chemical Hazard Evaluation Group
Toxicology and Risk Analysis Section
Life Sciences Division
Oak Ridge National Laboratory
Oak Ridge, TN 37831
Task Order No. 98-36C

Primary Reviewer: Claudia M. Troxel, Ph.D.	Signature:	Claudia M Troxel
	Date:	4-16-99
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Robert H. Ross, M.S., Group Leader	Signature:	
	Date:	APR 15 1999
Quality Assurance: Eric B. Lewis, M.S.	Signature:	Eric B. Lewis
	Date:	APR 15 1999

Disclaimer

This review may have been altered subsequent to the contractor's signatures above.

Managed by Lockheed Martin Energy Research Corp. for the U.S. Department of Energy under Contract No. DE-AC05-96OR22464.

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DICHLORMID **Developmental Toxicity Study (83-3a)**

EPA Reviewer: Irving Mauer, Ph.D. Date: 05/27/99
Reregistration Action Branch 3, HED (7509C)
EPA Secondary Reviewer: Stephen C. Dapson, Ph.D. Date: 06/01/99
Reregistration Action Branch 3, HED (7509C)
EPA Work Assignment Manager: Sanjivani Diwan, Ph.D. Date: 6/2/99
Toxicology Branch 1, HED (7509C)

014199

DATA EVALUATION RECORD

STUDY TYPE: Developmental Toxicity - Rat
OPPTS 870.3700 [83-3a]

DP BARCODE: not provided
P.C. CODE: not provided

SUBMISSION CODE: not provided
TOX. CHEM. NO.: none stated

TEST MATERIAL (PURITY): Dichlormid (97.7% a.i.)

SYNONYMS: R-25788

CITATION: (1972) A safety evaluation by a teratology study in rats (R-25788). Woodard Research Corporation. January 28, 1972. Code No. 225002431 (MRID No. 00058469). Unpublished.

SPONSOR: Stauffer Chemical Company

EXECUTIVE SUMMARY: In a developmental toxicity study (Code No. 225002431; MRID No. 00058469), 20 presumed pregnant Sprague-Dawley rats per group were administered R-25788 (97.7% a.i.) in the diet at doses of 0, 10, or 40 mg/kg/day on gestation days (GD) 6-15, inclusive. On GD 20, dams were sacrificed, subjected to gross necropsy, and all fetuses examined externally. One-third of the fetuses were examined viscerally, and the remaining fetuses were examined for skeletal malformations/variations.

Maternal and developmental toxicity could not adequately be assessed because of several deficiencies in the conduct and reporting of the study, including: three test groups were not available (only 2 treatment groups), individual observations and summary tables addressing maternal clinical signs and gross necropsy were not provided, data analyses of the reported maternal data were not performed (means, statistical analyses), inadequate number of litters were available in the 10 and 40 mg/kg/day groups (18 instead of the guideline minimum of 20). no data on litter incidences were provided, no individual fetal examination data (external, visceral, or skeletal) were included, and statistical analyses of the reported fetal summary data were not conducted. Even if the missing data are supplied and support the study author's conclusions that the "three groups are entirely comparable," the study still will not have defined a maternal or developmental toxicity LOAEL.

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This study is classified as **Unacceptable/not upgradable** and does not satisfy the Subdivision F guideline requirements for a developmental toxicity study in rats (83-3a) because of many major deficiencies in the conduct of the study and reporting of data, including: an inadequate number of test doses (only 2 treatment groups), not known if technical form of the active ingredient was used, no information on the stability of the test compound, no analyses for test material stability, homogeneity, and concentration in dosing medium, no statistical analyses, no individual observations and summary tables addressing maternal antemortem/daily observations or gross necropsy, inadequate number of litters/dose (18 instead of 20), no data on litter incidences, and no individual fetal examination data (external, visceral, or skeletal). If the missing data are supplied and support the study author's conclusions that the "three groups are entirely comparable," the study will still be unacceptable because it will not have defined a maternal or developmental toxicity LOAEL.

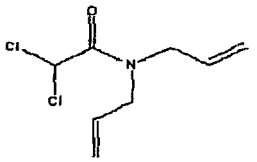
COMPLIANCE: The study was conducted prior to the establishment of GLPs. Signed and dated Quality Assurance, Good Laboratory Practice Statements, Data Confidentiality, and Flagging statements were not included.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: R-25788

Description: clear amber liquid
Lot/Batch No.: not provided
Purity: 97.7% a.i.
Stability of compound: data not provided
CAS No.: 37764-25-3
Structure:



2. Vehicle and/or positive control

The vehicle was Purina Laboratory Chow (meal) supplemented with 0.5% USP cod liver oil. Negative controls received untreated diet. No positive control was used in this study.

3. Test animals

Species: rat

Strain: Sprague-Dawley
Age and weight at study initiation: age not provided; 170-235 g
Source: Charles River Breeding Laboratories, Inc.
Housing: Animals were housed individually in cages
Diet: Purina Laboratory Chow (meal) supplemented with 0.5% USP cod liver oil and water were available *ad libitum*
Environmental conditions:
Temperature: not stated
Humidity: not stated
Air changes: not stated
Photoperiod: 12 hr light/dark
Acclimation period: 6 weeks

B. PROCEDURES AND STUDY DESIGN

This developmental toxicity study was designed to evaluate the safety of R-25788 when administered by feed to pregnant rats on gestation days 6 through 15, inclusive.

1. In life dates

Start: not stated; end: not stated

2. Mating

Females were paired with a resident male from the same strain and source for breeding. Positive evidence of mating was confirmed by the presence of a vaginal plug. The day on which evidence of mating was identified was designated as gestation day 0 (GD 0).

3. Animal assignment and dose selection are presented in Table 1. Bred females were assigned in rotation to one of three groups containing 20 rats each.

TABLE 1. Animal assignment

Test group	Dose (mg/kg/day)	Number females assigned
Control	0	20
Low dose	10	20
High dose	40	20

Data taken from Text Figure on p. 283, Code No. 225002431 (MRID No. not assigned).

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4. Dose selection rationale

No rationale was given for the doses selected in the study.

5. Dose solution preparation and analysis

Appropriate aliquots of R-25788 were dissolved in 50 ml of acetone and thoroughly mixed with a small quantity of feed. The feed containing the test compound was then mechanically mixed with the bulk feed after the acetone had evaporated. No further information was provided. The study author did not state if diets were adjusted to maintain the stated daily dietary concentrations as the animals gained weight during the study.

Data regarding the verification of the stability, homogeneity, and concentration of the test material in the feed were not provided.

C. OBSERVATIONS

1. Maternal observations and evaluations

No information about clinical signs was reported. Maternal body weights and feed consumption were recorded on GD 0, 6, 15, and 20. Females were euthanized on GD 20 by chloroform and the uterus and ovaries of each dam were examined to determine the number of corpora lutea, numbers of viable and dead fetuses, and number of implantation and resorption sites. Uteri with no evidence of macroscopic implantation sites were considered to have been nonpregnant.

2. Fetal evaluations

At necropsy, the total body weights of the fetuses and any external signs of fetal abnormality were recorded. Approximately one-third of the fetuses from each dam were examined after preservation in Bouin's fixative. The head of each fetus was sectioned and examined for changes. Body cavities were opened and any changes in the viscera were noted. The remaining fetuses of each litter were cleared and stained by the KOH-Alizarin Red S technique, and the specimens examined for skeletal malformations under low-power magnification.

D. DATA ANALYSIS

1. Statistical analysis

Statistical analyses of the data were not conducted.

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

II. RESULTS

A. MATERNAL TOXICITY

1. Mortality and clinical signs

All mated females survived until sacrifice. No information about clinical signs was provided.

2. Body weight

Mean body weights and body weight gains were not calculated by the study author.

3. Food consumption

Complete maternal food consumption data were not provided. The study author stated in the text that mean food consumption amounts during the days of compound administration were 211, 197, and 180 g for the control, low-dose, and high-dose dams, respectively. No further details were provided.

4. Gross pathology

No data were provided regarding the results of gross necropsy of the dams.

5. Cesarean section data

Data collected at cesarean section are summarized in Table 2. No treatment-related differences were apparent between the treated groups and the control group in the pregnancy rate, mean numbers of viable fetuses, mean fetal body weights, total resorption and implantation sites, total number copora lutea, or fetal sex ratios. Gravid uterine weights, and the numbers of resorptions/dam, early and late resorptions, and dams with all resorptions were not provided.

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TABLE 2. Cesarean section observations

Observation	0 mg/kg/day	10 mg/kg/day	40 mg/kg/day
No. Animals Assigned	20	20	20
No. Animals Pregnant	20	18	18
Pregnancy Rate (%)	100	90	90
Maternal Mortality	0	0	0
Aborted	-[a]	-	-
Total Corpora Lutea	243	245	235
Total Implantations	241	234	216
Preimplantation Loss (%)	-	-	-
Postimplantation Loss (%)	-	-	-
Gravid Uterine Weight (g)	-	-	-
Placental Weight (g)	-	-	-
Total Live Fetuses	227	219	200
Live Fetuses/Litter	11.4	12.2	11.1
Mean Fetal Weight (g)	3.73	3.84	3.54
Sex Ratio (%Male) [a]	50	51	48
Total Dead Fetuses	0	0	0
Dams with all Resorptions	-	-	-
Total Resorptions	14	15	16
Resorptions/Dam	-	-	-
Early Resorptions	-	-	-
Late Resorptions	-	-	-

Data from p. 286. Code No. 225002431 (MRID No. not assigned).
[a] - Data not provided.

B. DEVELOPMENTAL TOXICITY

Developmental toxicity could not adequately by assessed. Three test groups were not available, an inadequate number of litters were available in the 10 and 40 mg/kg/day groups (18 instead of the guideline minimum of 20), no data on litter incidences were provided, no individual fetal examination data (external, visceral, or skeletal) were included, and statistical analyses of the reported summary data were not conducted. The data presented below are from summary tables which reported fetal incidences only.

1. External examination

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The study author stated that no gross abnormalities were seen in the fetuses examined at delivery.

2. Soft tissue examination

Visceral malformations/variations were reported only as fetal incidences, and included dilated lateral ventricles, dilated ureters, undulated ureters, and lobulated kidneys.

3. Skeletal examination

Skeletal malformations/variations were reported only as fetal incidences, and included unequal bilateral ossification, irregular contours of the interparietal and parietals, incomplete ossification of the centra, non-fused centra, and unequal ossification of the 13th ribs. The fetal incidences (number affected/number examined) of irregular contours of the supraoccipital were 2/151, 7/145, and 9/137 for the control, 10, and 40 mg/kg/day groups, respectively.

III. DISCUSSION

A. INVESTIGATOR'S CONCLUSIONS

The study authors concluded that the three groups (0, 10, and 40 mg/kg/day groups) were entirely comparable to one another and that R-25788 at doses of 40 mg/kg/day is not teratogenic for the rat.

B. REVIEWER'S DISCUSSION

1. Maternal toxicity

Maternal toxicity could not adequately be assessed because of several deficiencies in the conduct and reporting of the study, including: three treatment groups were not available (only 2 treatment groups), individual observations and summary tables addressing maternal clinical signs and gross necropsy were not provided, and data analyses (means and statistical analyses) of the reported data were not performed (i.e., body weights, food consumption).

Therefore, the maternal toxicity LOAEL and NOAEL could not be determined.

2. Developmental toxicity

Developmental toxicity could not adequately be assessed because of several deficiencies including three treatment groups were not available, an inadequate number of litters were available in the 10 and 40 mg/kg/day groups (18 instead of the guideline minimum of 20), no data on litter incidences were provided, no

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individual fetal examination data (external, visceral, or skeletal) were included, and statistical analyses of the reported summary data were not conducted.

Because of these deficiencies, no conclusions could be made regarding the effect of treatment with dichlormid on fetal deaths and resorptions, growth, developmental variations, or malformations.

Therefore, the developmental toxicity LOAEL and NOAEL could not be determined.

C. STUDY DEFICIENCIES

There were many major deficiencies in the conduct of this study: inadequate number of doses (only 2 tested instead of 3), not known if technical form of the active ingredient was used, no information on the stability of the test compound, no analyses for test material stability, homogeneity, and concentration in dosing medium, no statistical analyses, no individual or summary tables of maternal antemortem/daily observations or necropsy data, inadequate number of litters/dose (18 instead of 20), no data on litter incidences, and no individual fetal examination data (external, visceral, or skeletal).

D. CORE CLASSIFICATION

This study is classified as **Unacceptable/not upgradable** and does not satisfy the Subdivision F guideline requirements for a developmental toxicity study in rats (83-3a) because of many major deficiencies in the conduct of the study and reporting of data, including: an inadequate number of test doses, not known if technical form of the active ingredient was used, no information on the stability of the test compound, no analyses for test material stability, homogeneity, and concentration in dosing medium, no statistical analyses, no individual observations and summary tables addressing maternal antemortem/daily observations or gross necropsy. inadequate number of litters/dose (18 instead of 20), no data on litter incidences, and no individual fetal examination data (external, visceral, or skeletal). If the missing data are supplied and support the study author's conclusions that the "three groups are entirely comparable," the study will still be unacceptable because it will not have defined a maternal or developmental toxicity LOAEL.

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DATA EVALUATION REPORT

DICHLORMID

STUDY TYPE: DEVELOPMENTAL TOXICITY– RABBIT (83-3b)

Prepared for

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

Prepared by

Chemical Hazard Evaluation Group
Toxicology and Risk Analysis Section
Life Sciences Division
Oak Ridge National Laboratory
Oak Ridge, TN 37831
Task Order No. 99-22B

Primary Reviewer: Claudia M. Troxel, Ph.D.	Signature:	Claudia M Troxel
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	Date:	3/29/99
Robert H. Ross, M.S., Group Leader	Signature:	Robert H Ross
	Date:	3/29/99
Quality Assurance: Eric B. Lewis, M.S.	Signature:	Eric B Lewis
	Date:	3/29/99

Disclaimer

This review may have been altered subsequent to the contractor's signatures above.

Managed by Lockheed Martin Energy Research Corp. for the U.S. Department of Energy under Contract No. DE-AC05-96OR22464.

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DICHLORMID **Developmental Toxicity Study (83-3b)**

EPA Reviewer: Irving Mauer, Ph.D. Date 06/01/99
Reregistration Action Branch 3, HED (7509C)
EPA Secondary Reviewer: Stephen C. Dapson, Ph.D. Date: 06/01/99
Reregistration Action Branch 3, HED (7590C)
EPA Work Assignment Manger: Sanjivani Diwan, Ph.D. Date 6/2/99
Toxicology Branch 1 HED (7509C)

DATA EVALUATION RECORD

STUDY TYPE: Developmental Toxicity - Rabbit
OPPTS 870.3700 [83-3b]

DP BARCODE: D248305
P.C. CODE: N/A

SUBMISSION CODE: S546651
TOX. CHEM. NO.: none stated

TEST MATERIAL (PURITY): Dichlormid (97.2% a.i.)

SYNONYMS: R-25788

CITATION: Wickramaratne, G. (1989) Dichlormid: teratogenicity study in the rabbit. ICI Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, U.K. Report No. CTL/P/2442, Study No. RB0414, August 31, 1989. MRID 44606407. Unpublished.

SPONSOR: ICI Americas Inc., Agricultural Products, Wilmingon, DE 19897.

EXECUTIVE SUMMARY: In a developmental toxicity study (MRID 44606407), 20 presumed pregnant New Zealand White rabbits per group were administered dichlorimid (97.2% a.i.) in corn oil orally by gavage at doses of 5, 30, or 180 mg/kg/day on gestation days (GD) 7-19, inclusive. A control group of 19 animals received corn oil alone. On GD 30, does were sacrificed, subjected to gross necropsy, and all fetuses examined externally, viscerally, and skeletally for malformations/variations.

Maternal toxicity in the 180 mg/kg/day group is based on an increased incidence of alopecia and decreases in body weight gains and food consumption. The incidence of alopecia was slightly increased in the high-dose group as compared with controls (no. animals/no. observations: 10/212 vs. 7/71 controls). In addition to the increased number of observations of alopecia in affected animals, affected high-dose animals generally had more severe hair loss than controls, and it tended to affect more than one area. No other treatment-related clinical signs were observed. Although no statistically significant differences in mean absolute maternal body weights were noted, high-dose females had significant decreases in body weight gains and food consumption during the entire dosing interval of GD 7-19 (-101% and -44% of controls, respectively; $p<0.01$). After dosing was stopped, high-dose females had compensatory increases in body weight gains (+206% and +33% for GD 19-22 and 19-30, respectively; $p<0.01$; 0.05) and food consumption (+17% for GD 22-26 and 26-30; $p<0.05$). Overall, high-dose does gained

26% less when compared with controls ($p<0.05$). No treatment-related differences in clinical signs, body weight gain, or food consumption were noted in the 5 or 30 mg/kg/day treatment groups as compared with the controls, and no clear treatment-related gross pathological changes were observed in any of the treated groups. One animal from the control group and two from the high-dose group were killed on GDs 29, 18, and 19, respectively, due to early delivery/abortions. One additional high-dose animal was killed *in extremis* on GD 8 due to morbidity. It is not certain if the morbidity was an effect of treatment.

Therefore, the maternal toxicity LOAEL is 180 mg/kg/day based on an increased incidence of alopecia and decreased mean maternal body weight gains and food consumption, and the maternal toxicity NOAEL is 30 mg/kg/day.

One control doe delivered early on GD 29, and two high-dose does aborted on GD 18 and 19, respectively. It is not clear if the abortions were treatment-related. In high-dose females, the percentage of post-implantation loss (23.1% vs. 7.2% for controls) was increased, statistically so ($p<0.01$) following transformation of the data for statistical analysis. The increase in post-implantation loss was accompanied by increases in the numbers of late resorptions ($p<0.05$ following transformation) and early resorptions (n.s.), decreased number of live fetuses/litter (6.67 vs. 8.18 for controls, n.s.), and decreased mean fetal body weight (37.70 g vs. 38.76 g for controls, n.s.). No clear, treatment-related effects were noted during Caesarian section in does from the 5 or 30 mg/kg/day groups. It is not known if any doe had complete litter resorption because nongravid uteri were not stained for this purpose.

No treatment-related external or visceral malformations/variations were observed in any litter. Most treated and control litters contained fetuses with minor variations in skeletal ossification. The incidence of reduced ossification of frontal and parietal skull bones was statistically increased ($p<0.05$) in high-dose fetuses and was marginally increased in high-dose litters (5/12 litters affected vs. 2/11 for control; n.s.).

Therefore, the developmental toxicity LOAEL is 180 mg/kg/day based on increases in post-implantation loss accompanied by an increased number of resorptions/doe (both early and late resorptions), a decreased number of live fetuses/litter, and slightly decreased mean fetal body weights. The developmental toxicity NOAEL is 30 mg/kg/day.

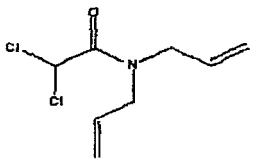
This developmental toxicity study is classified as **Acceptable/guideline** and satisfies the subdivision F guideline requirements for a developmental toxicity study in rabbits (83-3b).

COMPLIANCE: Signed and dated Quality Assurance, Good Laboratory Practice, Data Confidentiality, and Flagging statements were included.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: dichlormid
   Description: clear, amber-colored, liquid
   Lot/Batch No.: WRC4921-35-12: GGD0101
   Purity: 97.2% a.i.
   Stability of compound: data not provided
   CAS No.: 37764-25-3
   Structure:



2. Vehicle and/or positive control

   Corn oil (Kraft-Wesson 100% corn oil supplied by Kraft Foods Limited, UK) was used as the control article and the vehicle for the preparation of the dosing formulations. No positive control was used in this study.

3. Test animals

   Species: rabbit
   Strain: New Zealand White
   Age and weight at study initiation: age of rabbits not provided; 2779-3827 g
   Source: Interfauna UK, Huntington, Cambridgeshire, UK
   Housing: Animals were housed individually in suspended mobile rabbit units
   Diet: CRB pellets supplied by Labsure Animal Diets (Poole, Dorset, UK) and tap water were available *ad libitum*
   Environmental conditions:
   - Temperature: 20-27°C
   - Humidity: 45-81%
   - Air changes: not stated
   - Photoperiod: 12 hr light/dark

   Acclimation period: at least 6-7 days

B. PROCEDURES AND STUDY DESIGN

The purpose of this study was to investigate the developmental toxicity potential of dichlormid when administered by gavage to pregnant rabbits on gestation days 7 through 19, inclusive.

1. In life dates

Start: June 1988; end: July, 1988

2. Mating

The supplier gave virgin female rabbits an intravenous injection of 25 IU chorionic gonadotrophin approximately 2 weeks before delivery to the performing laboratory in order to promote ovulation prior to insemination. At the performing laboratory, a total of 8 male rabbits of the same source and strain were used for the collection of semen using an artificial vagina. The sample of semen was diluted with sufficient saline to inseminate one replicate (the study was divided into 20 replicates with each replicate containing one inseminated female from each dose group). Each replicate of females was inseminated with semen from one male. After insemination, each female was given an intravenous injection of 25 IU PROFASI to promote ovulation. The day of insemination was designated as gestation day 1 (GD 1).

3. Animal assignment and dose selection are presented in Table 1. Animals were individually identified by an ear tattoo. The study consisted of three treated groups containing 20 rabbits each and a control group containing 19 rabbits. The study was divided into 20 replicates with each replicate containing one inseminated female from each dose group. Cages within the replicates were assigned one of the four groups using computer-generated random number permutations. Following insemination, each female was allocated to a cage randomly within the replicate. Replicates were filled sequentially.

TABLE 1. Animal assignment

Test group	Dose (mg/kg/day)	Number females assigned
Control	0	19
Low dose	5	20
Mid dose	30	20
High dose	180	20

Data taken from Text Figure on p. 17, MRID 44606407.

4. Dose selection rationale

The dose levels selected for this study were based on the results of a developmental toxicity study in the rabbit (referenced as Kinsey, 1989, ICI Central Toxicology Laboratory Report No.: CTL/P/2293, but data not provided).

5. Dose solution preparation and analysis

Dichlormid was formulated in corn oil. An appropriate amount of corn oil was added to a weighed amount of dichlormid to provide approximately 2 liters of solution of the required concentration for each dose level. Before being subdivided into aliquots, each preparation was handshaken until a solution was formed. The control article was also dispensed into aliquots. The aliquots were stored in the dark at 4°C and fresh bottles were used for each day of the study.

A sample of each bulk solution was analyzed prior to the start of dosing to verify the achieved concentrations of dichlormid in corn oil. The chemical stability of dichlormid in corn oil was determined by re-analysis of the nominal 5 and 180 mg/mL dosing solutions after an interval of 27 days and 28 days. It is assumed that the dosing solutions reanalyzed were from those stored in the dark at 4°C.

Results:

Concentration: Absence of test material was confirmed in the vehicle. The mean concentrations of the samples as a percentage of nominal concentrations for the 5, 30, and 180 mg/kg/day test solutions were 97.6%, 99.7%, and 98.9%, respectively.

Homogeneity: Homogeneity of the dosing solutions was not measured.

Stability Analysis: After 0 and 27 days, the percentage of the initial measured concentration of the 5 mg/ml dosing solution was 100% and 106.8%, respectively. After 0 and 28 days, the percentage of the initial measured concentration of the 180 mg/mL dosing solution was 100% and 104.5%, respectively.

The analytical data of the 5, 30, and 180 mg/kg/day test solutions demonstrated that the test substance was stable in corn oil for at least 27 days, and that the variance between nominal and actual doses to the animals was acceptable. It is not known if the mixing procedure was adequate because homogeneity analysis was not conducted.

6. Dosing

All doses were in a volume of 1 mL/kg/day prepared daily during the dosing period (GD 7-19, inclusive). Dosing by gavage was based on the most recently recorded body weights.

C. OBSERVATIONS

1. Maternal observations and evaluations

All animals were checked on arrival to ensure that they were physically normal externally and were subsequently checked daily. Details of changes in behavior or clinical condition including no abnormalities detected were recorded daily during

the study. Maternal body weights were recorded on GD 1 and 4, 7-19 (inclusive), and 22, 26 and 30. Food consumption over three day periods was measured on GD 4, 7, 10, 13, 16, 19, and 22, and over four day periods on GD 26 and 30. Females were killed on GD 30 by an intravenous injection of EUTHATAL and were subjected to gross necropsy. The uterus was weighed and the uterus and ovaries of each dam were examined to determine the number of corpora lutea, numbers of viable fetuses, and number of implantation and resorption sites.

2. Fetal evaluations

At necropsy, the fetuses were weighed and then killed with an intracardiac injection of EUTHATAL. Each fetus was examined for external abnormalities and for cleft palate. All fetuses were then examined internally for visceral abnormalities, sexed, eviscerated, and fixed in methanol. After approximately 24 hours, the head of each fetus was cut along the fronto-parietal suture line and the brain was examined for macroscopic abnormalities. The carcasses were then returned to methanol for subsequent processing and staining with Alizarin Red S for skeletal examination. Ossification of the individual bones of the manus and pes was assessed and the result converted to a five-point scale.

D. DATA ANALYSIS

1. Statistical analysis

Maternal body weight gain, maternal food consumption, numbers of implantations and live fetuses/female, percentage of pre- and post-implantation loss, the percentage of implantations which were early or late intrauterine deaths, the percentage of male fetuses, gravid uterus weights, litter weights, mean fetal weights, mean manus and pes score/fetus, and the percentage of fetuses with minor external/visceral defects only and minor skeletal defects only were analyzed by analysis of variance. Fetal data were converted to the litter level before calculating group means or carrying out ANOVA. Percentages were transformed before analysis using the double arcsine transformation of Freeman and Tukey. Following ANOVA, each treatment group mean was compared with the control group mean using Student's t-test, based on the error mean square in the analysis.

The proportion of females with pre- or post-implantation loss and early or late intrauterine deaths, the proportion of male fetuses, and the proportion of fetuses with major or minor (only) external/visceral defects, major or minor (only) skeletal defects, skeletal variants, and each individual finding were analyzed by Fisher's Exact Test. The proportion of fetuses with each individual finding was also analyzed on a litter basis.

All statistical tests were one-sided with the following exceptions which were two-sided: maternal body weight gain, maternal food consumption and the proportion of male fetuses.

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

II. RESULTS

A. MATERNAL TOXICITY

1. Mortality and clinical signs

One animal from the control group and two from the high-dose group were killed on GDs 29, 18, and 19, respectively, due to early delivery/abortions. One additional high-dose animal was pale, subdued, thin, cold to the touch, and had labored breathing on GD 8, and was consequently killed *in extremis*.

A slight increase in the incidences of alopecia and body thinness was noted in high-dose does (see Table 2). Upon closer examination of alopecia, affected high-dose females generally had more severe hair loss than controls, and it tended to affect more than one area. Body thinness was generally only slight in both the high-dose and control group. Other clinical signs occurred in low incidence and were not dose-related.

TABLE 2. Summary of selected clinical signs [Number animals/number observations]

Observation	0 mg/kg/day	5 mg/kg/day	30 mg/kg/day	180 mg/kg/day
n =	19	20	20	20
Mortality	1	0	0	3
Alopecia	7/71	2/22	4/124	10/212
Thin	5/39	1/12	3/7	9/69

Data taken from Table 4, pp. 37-40, MRID 44606407.

2. Body weight

Mean absolute body weights were not calculated by the study author; therefore, the reviewer calculated mean absolute body weights for GDs 1, 7, 10, 13, 16, 19, and 30 (see Table 3) and conducted statistical analysis of these means using ANOVA. No statistically significant differences in mean body weights were noted between the treated groups as compared with the control group. Mean maternal body weight gain in the high-dose group, however, was affected by treatment. High-dose females had a loss in body weight during the first six days of dosing (GD 7-13), and a decreased body weight gain during GD 13-19 when compared with the controls. These reductions in mean body weight gain were statistically significant ($p<0.05$; 0.01) for GDs 10-13, 13-16, and 7-19 (-124%, -52%, and -101%, respectively). Following dosing, body weight gain was statistically increased in the high-dose group (+206% and +33% for GD 19-22 and

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19-30, respectively; p<0.01; 0.05) when compared with controls. High-dose does gained 26% less overall when compared with controls (p<0.05). In the 30 mg/kg/day group, the only statistically significant difference in maternal body weight gain occurred during GD 22-26 (+60%; p<0.05). No statistically significant differences in body weight gains were noted in the 5 mg/kg/day group.

Two high-dose does had unusually low growth for Days 7-10 and 16-19, respectively. Their inclusion or exclusion in the statistical analysis of body weight gain for these respective intervals did not affect the results of the statistical analysis.

TABLE 3: Selected mean maternal body weights and body weight gains during gestation (g)

GD	0 mg/kg/day	5 mg/kg/day	30 mg/kg/day	180 mg/kg/day
Mean maternal body weights (g) [a]				
1	3234 ± 174.3	3282 ± 204.9	3257 ± 252.8	3353 ± 200.5
7	3417 ± 204.5	3443 ± 240.0	3473 ± 234.3	3537 ± 198.5
10	3425 ± 223.8	3453 ± 232.5	3510 ± 247.2	3485 ± 196.5
13	3514 ± 222.2	3519 ± 242.3	3565 ± 239.0	3464 ± 186.1
16	3637 ± 212.1	3643 ± 245.0	3719 ± 260.4	3524 ± 228.3
19	3689 ± 223.0	3676 ± 251.6	3740 ± 255.0	3533 ± 186.8
30	3953 ± 297.3	3968 ± 276	4008 ± 320.1	3884 ± 168.7
Mean maternal body weight gains (g)				
1-7 (predosing)	183.1	160.6	216.1	163.6
7-10	8.5	9.4	36.1	-39.5
10-13	88.5	66.1	55.3	-20.8** (-124) [b]
13-16	123.2	123.6	154.2	59.4* (-52)
7-19 (during dosing)	272.1	232.8	267.1	-3.8** (-101)
19-30 (postdosing)	264.5	291.6	267.9	351.7* (+33)
1-30 (overall)	719.7	685.0	751.0	531.0* (-26)

Data taken from Table 5A and Appendix 3, pp. 42 and 140-148, MRID 44606407.
[a] Calculations of mean absolute body weights and statistical analysis of mean maternal body weights using ANOVA performed by reviewer
[b] Numbers in parentheses represent the percent change compared with controls; calculated by reviewer.
Significantly different from controls: *p<0.05; **p<0.01.

3. Food consumption

Maternal food consumption was statistically significantly decreased ($p<0.01$) during all measured intervals during the dosing period in the high-dose group (-36% to -48% as compared with controls; see Table 4), and was also statistically significantly increased ($p<0.05$) in the high-dose groups during most of the post-dosing intervals (+17% for GD 22-26 and 26-30).

Food efficiency was estimated by the reviewer for the predosing, dosing, and postdosing intervals. Food efficiency in the high-dose females was decreased by 101% during the dosing period and slightly increased (+27%) in the post-dosing interval as compared with controls. Food efficiency during the pre-dosing interval was not affected in any of the treatment groups as compared with the controls.

TABLE 4: Mean maternal food consumption and food efficiency during gestation (g)

GD	0 mg/kg/day	5 mg/kg/day	30 mg/kg/day	180 mg/kg/day
Food consumption (g/day)				
1-7 (predosing)	159.2	162.4	163.9	162.1
7-10	120.0	119.9	136.0	70.4** (-41) [a]
13-16	127.6	121.8	128.7	69.1** (-46)
16-19	134.7	135.4	122.6	86.7** (-36)
7-19 (during dosing)	133.4	127.2	126.3	74.5** (-44)
22-26	157.0	168.5	164.0	184.4* (+17)
19-30 (post dosing)	155.4	162.3	160.4	170.2
Food efficiency [b]				
1-7	19	17	22	17
7-19	17	15	18	-0.43 (-103)
19-30	15	16	15	19 (+27)

Data from Table 6, p. 44, MRID 44606407.
[a] Numbers in parentheses represent the percent change compared with controls; calculated by reviewer.
[b] Crude estimate calculated by reviewer: [g body weight change per unit time/g food consumed per unit time] x 100
Significantly different from controls: *$p<0.05$; **$p<0.01$.

4. Gross pathology

Gross necropsy of the two high-dose does that aborted on GD 18 and 19, respectively, revealed possible liver effects. The lobes of one doe had a prominent reticular pattern, were thickened and misshapen, and had white speckles covering the dorsal and ventral surfaces. This doe also had excessive pericardial fluid. The liver from the other doe had brown discoloration throughout, with two areas of white mottling on papillary processes, and a small,

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brown, fluid filled cyst was attached to one lobe. This doe also had a bladder filled with red fluid, and semisolid, brown/red contents in the stomach and large intestines. Gross necropsy was not performed on the high-dose doe sacrificed on GD 8. Gross necropsy of the other does, including the control doe that delivered early, was unremarkable.

5. Cesarean section data

Data collected at cesarean section are summarized in Table 5. One control doe delivered early on GD 29, and two high-dose does aborted on GD 18 and 19, respectively. High-dose females had a number of changes in cesarean section parameters as compared with the controls. The percentage of post-implantation loss (23.1% vs. 7.2% for controls) was increased in high-dose females, statistically so following double arcsine transformation ($p<0.01$). The increase in post-implantation loss was accompanied by increases in late resorptions ($p<0.05$ following double arcsine transformation), increases in early resorptions (n.s.), decreased number of live fetuses/litter (6.67 vs. 8.18 for controls, n.s.), decreased mean fetal body weight (37.70 g vs. 38.76 g for controls, n.s.), and decreased gravid uterine weights (-14% of controls, n.s.). The percentage of pre-implantation loss was elevated, but after double arcsine transformation did not follow a dose-response pattern or achieve statistical significance.

Treatment with 30 mg/kg/day resulted in marginal effects. The increased number of late resorptions was statistically significant ($p<0.05$) following double arcsine transformation. The number of live fetuses/litter was slightly decreased (n.s.), but was accompanied by an increase in mean fetal body weight (42.29 g vs. 38.76 g for controls).

No statistically significant changes were observed in the 5 mg/kg/day treatment group. It is not known if any does had complete litter resorption because nongravid uteri were not stained for this purpose.

TABLE 5. Cesarean section observations

Observation	0 mg/kg/day	5 mg/kg/day	30 mg/kg/day	180 mg/kg/day
No. animals assigned	19	20	20	20
No. animals pregnant (%)	12 (63)	15 (75)	17 (85)	15 (75)
Aborted/early delivery	1	0	0	2
Mortality	0	0	0	1 [a]
Total number of litters	11	15	17	12
Total corpora lutea	115	159	182	132
Mean corpora lutea	10.5 ± 2.6	10.6 ± 2.9	10.7 ± 2.6	11.0 ± 1.9
Total implantations	97	132	144	104
Mean implantations	8.8 ± 2.6	8.8 ± 3.0	8.5 ± 3.6	8.7 ± 2.7
Preimplantation loss (%)	15.7	17.0	20.9	21.2
Mean transformed value [b]	0.373	0.408	0.473	0.398
Postimplantation loss (%)	7.2	14.4	16.7	23.1
Mean transformed value [b]	0.306	0.391	0.439	0.531**
Gravid uterine weight (g)	438.2	443.1	424.6	375.1
Total live fetuses	90	113	120	80
Live fetuses/litter	8.18	7.53	7.06	6.67
Mean fetal weight (g)	38.76	41.48	42.29	37.70
Sex ratio (% male)	44.1	53.2	61.3	52.7
Total dead fetuses	-	-	-	-
Total resorptions	7	19	24	24
Resorptions/doe	0.6 ± 1.0	1.3 ± 1.5	1.4 ± 1.5	2.0 ± 1.3
Early resorptions	0.5 ± 0.9	0.9 ± 1.4	0.7 ± 1.3	1.3 ± 1.3
Late resorption	0.1 ± 0.3	0.4 ± 0.6	0.7 ± 0.9	0.8 ± 0.9

Data from Table 8 and Appendix 5, pp. 47-49 and 155-158, MRID 44606407.
[a] Sacrificed moribund on GD 8.
[b] Percentages were transformed before analysis using the double arcsine transformation of Freeman and Tukey.
Statistically different from controls: **p<0.01.

B. DEVELOPMENTAL TOXICITY

The overall incidence rates for litters containing fetuses with major malformations in the 0, 5, 30, or 180 mg/kg/day groups were 1/11, 2/15, 0/17, and 2/12, respectively.

The number of fetuses(litters) examined were 90(11), 113(15), 120(17), and 80(12), respectively.

1. External examination

No treatment-related malformations/variations were noted during external examination of fetuses. Incidental external malformations included agnathia of the jaw in one high-dose fetus, and bilateral anophthalmia in another high-dose fetus from a different litter.

2. Soft tissue examination

Soft tissue malformations/variations occurred equally in all groups or at low incidences. The fetus with agnathia also had an extremely reduced pulmonary artery, while the fetus with bilateral anophthalmia had moderately dilated lateral ventricles in the brain. Additionally, one 5 mg/kg/day fetus was missing a kidney.

3. Skeletal examination

Most treated and control litters contained fetuses with minor variations in skeletal ossification. The high-dose group exhibited a dose-related increase in the fetal and litter incidence of partially ossified frontal and interparietal skull bones as compared with controls, although only the fetal incidence achieved statistical significance. The number of fetuses(number of litters) affected with partially ossified skeletal bones in the 0, 5, 30, and 180 mg/kg/day groups were 3(2), 5(3), 10(4), and 19*(5), respectively (*$p<0.05$).

Statistically significant increases ($p<0.05$; 0.01) in fetal incidences also occurred for the following variations/malformations: a misshapen 6th sternebra in the high-dose group, asymmetrical development of the 1st sacral vertebra in all treated groups and of the 2nd sacral vertebra in the mid-dose group, partially unossified 6th sternebra in the low-dose group, and extra 13th rib and 27th presacral vertebrae in low- and high-dose groups. Although the increases in the fetal incidences of these various skeletal variations/malformations were statistically significant, they were not supported by significant increases in the litter incidence and/or were generally not dose-related. The study author cited an increased incidence of reduced ossification of the pubes in the high-dose group, but neither the fetal nor litter incidences were statistically different. Major skeletal malformations included major vertebral column defects in one control fetus, extremely fused ribs in one low-dose fetus, and scoliosis and fused maxillae in the high-dose fetus with anophthalmia and dilated lateral ventricles.

No treatment-related effects were noted during the assessment of ossification of the individual bones of the manus and pes.

III. DISCUSSION

A. INVESTIGATOR'S CONCLUSIONS

Administration of 180 mg dichlormid/kg/day resulted in maternal toxicity manifested as a reduction in weight gain and food consumption. There was evidence of developmental toxicity at a dose of 180 mg/kg/day shown as increases in early intra-uterine deaths and overall post-implantation loss, a reduction in ossification of the skull and pubes, and increased incidence of misshapen 6th sternebrae and fusion of the 3rd/4th and 4th/5th sternebrae. The small increase in early intra-uterine deaths and overall post-implantation loss observed in the 30 mg/kg/day group were considered by the investigator to be unrelated to treatment because they were stated "to be within historical control incidences."

B. REVIEWER'S DISCUSSION

1. Maternal toxicity

One animal from the control group was killed on GD 29 due to early delivery while two does from the high-dose group were killed on GDs 18, and 19, respectively, due to abortions. One additional high-dose animal was killed *in extremis* due to morbidity (pale, subdued, thin, cold to the touch, labored breathing). It is not known if the morbidity was an effect of treatment because postmortem examination was not conducted on this animal, and because details from the range-finding study were not included to provide information on the clinical signs characteristic of the test substance. However, because the moribund condition occurred so soon after the commencement of dosing (GD 8), it is unlikely that the moribundity was an adverse effect of treatment; rather, the cause may have been gavage trauma.

The incidence of alopecia in the high-dose group is considered to be an effect of treatment. Although the number of affected animals was not substantially increased in the high-dose group as compared with the control group (10/20 animals compared with 7/19 controls), the number of observations was greatly increased (212 compared with 71 for controls), affected high-dose animals generally had more severe hair loss than controls, and it tended to affect more than one area. Body thinness may have been related to treatment, but it was generally only slight and transient in both the high-dose and control group.

Although no statistically significant decreases in maternal body weights were noted. high-dose females showed consistent reductions in body weight gain and food consumption during dosing, and exhibited slight compensatory increases in body weight gain and food consumption during post-dosing. Estimates of food efficiency revealed a similar pattern with a decrease over the dosing period and slight increase post-dosing. High-dose does gained 26% less overall when compared with controls.

No treatment-related differences in clinical signs, body weight gain, or food consumption were noted in the 5 or 30 mg/kg/day treatment groups as compared with the controls, and no treatment-related difference in gross pathological changes were noted in any of the treated groups.

Therefore, we agree with the investigator that the maternal toxicity LOAEL is 180 mg/kg/day based on an increased incidence of alopecia and decreased mean maternal body weight gains and food consumption, and the maternal toxicity NOAEL is 30 mg/kg/day.

2. Developmental toxicity

a. Deaths/resorptions

The control doe that aborted on GD 29 was considered to have delivered early. It is not clear if the abortions by the two high-dose does (on GD 18 and 19) were an effect of treatment. Possible liver toxicity was noted in these dams. Results from the range-finding study would have been helpful, in that it would be known if abortions were a characteristic of treatment with diclormid at similar or higher doses.

High-dose females had a treatment-related increase in the percentage of post-implantation loss. The increase in post-implantation loss was supported by an increased number of resorptions/doe caused by both early and late resorptions, and a decreased number of live fetuses/litter in combination with slightly decreased mean fetal body weights. Decreased gravid uterine weights accompanied the decreased number of live fetuses/litter and mean fetal body weights. Unfortunately, apparently nongravid uteri were not stained for detection of early resorptions. Therefore, it is not known if any of the five apparently nongravid high-dose does also exhibited early resorptions. Had this been done, an even higher incidence of post-implantation loss may have been confirmed.

In contrast to the high-dose group, the slightly elevated increase in post-implantation loss observed in the mid-dose group was not clearly supported by other biologically significant changes. The total number of resorptions/doe was comparable to the low-dose group, and the decreased number of live fetuses/litter was accompanied by a compensatory increase in fetal body weight. No significant changes were observed in the 5 mg/kg/day treatment group.

b. Altered growth

Partially ossified frontal and interparietal skeletal bones in the high-dose group may have been a marginal effect of treatment, possibly a response to the maternal toxicity observed at this dose. The litter incidence did not

achieve statistical significance, but a slight dose-response was apparent (18, 20, 24, and 42% litters affected in the 0, 5, 30, and 180 mg/kg/day groups, respectively). Unfortunately, historical control data of this variation were not available to determine if the litter incidence was within the normal range for this strain of rabbit. The reduced ossification of the pubes cited by the study author did not achieve statistical significance for either the fetal or litter incidence, and the increase in the litter incidence was not dose-related.

c. Developmental variations

No treatment-related developmental variations were noted. Although several statistically significant increases in the fetal incidence of various skeletal variations were noted, they were not supported by significant increases in the litter incidence and were generally not dose-related. The increase in the incidences of a misshapen 6th sternebra and fused sternebrae in the high-dose group were determined by the study author to be treatment-related. However, the litter incidences were not statistically significantly increased as compared with the controls, and only a small number of fetuses and litters were affected. Additionally, the incidence of fused sternebrae was not dose-related. Again, no historical control data were available to further determine the relevance of these variations.

d. Malformations

No major fetal malformations could be attributed to maternal treatment with dichlormid.

Therefore, we agree with the investigator that the developmental toxicity LOAEL is 180 mg/kg/day based on increases in post-implantation loss accompanied by an increased number of resorptions/doe (both early and late resorptions), a decreased number of live fetuses/litter, and slightly decreased mean fetal body weights. The developmental toxicity NOAEL is therefore 30 mg/kg/day.

C. STUDY DEFICIENCIES

A major deficiency of this study was that nongravid uteri were not stained to check for complete litter resorption. This oversight may have resulted in an under reporting of the number of resorptions noted in the high-dose group. Homogeneity of the dosing solutions was also not determined; therefore, it is not known if the mixing procedure was adequate. Because concentration and stability analysis were acceptable, however, the lack of homogeneity analysis is not believed to have compromised the study. A minor deficiency is that sufficient historical control data were not provided. Historical control data would have aided in further assessment of the questionable developmental variations observed in the high-dose group. A minor deficiency is that the age of the rabbits at study initiation was not provided.

D. CORE CLASSIFICATION

This developmental toxicity study is classified as **Acceptable/guideline** and satisfies the subdivision F guideline requirements for a developmental toxicity study in rabbits (83-3b).

06

DATA EVALUATION REPORT

014199

DICHLORMID

STUDY TYPE: SUBCHRONIC ORAL TOXICITY FEEDING - RAT (82-1a)

Prepared for

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Office of Pesticide Programs
U.S. Environmental Protection Agency
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Prepared by

Chemical Hazard Evaluation Group
Toxicology and Risk Analysis Section
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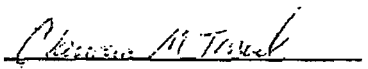
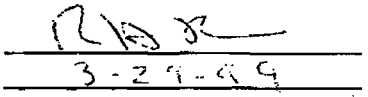
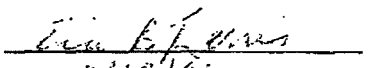
Oak Ridge, TN 37831
Task Order No. 99-22A

Primary Reviewer: Sylvia Milanez, Ph.D., D.A.B.T.	Signature: Date:	 3-24-99
Secondary Reviewers: Claudia M. Troxel, Ph.D.	Signature: Date:	 3-24-99
Robert H. Ross, M.S., Group Leader	Signature: Date:	 3-29-99
Quality Assurance: Eric B. Lewis, M.S.	Signature: Date:	 3/29/99

Disclaimer

This review may have been altered subsequent to the contractor's signatures above.

Oak Ridge National Laboratory, managed by Lockheed Martin Energy Research Corp. for the U.S. Department of Energy under contract number DE-AC05-96OR22464.

DICHLORMID **Subchronic Oral Study (82-1a)**

EPA Reviewer: Irving Mauer, Ph.D. Date 05/28/99
Reregistration Action Branch 3 HED (7509C)
EPA Secondary Reviewer: Stephen C. Dapson, Ph.D. Date: 06/01/99
Reregistration Action Branch 3 HED (7509C)
EPA Work Assignment Manager: Sanjivani Diwan, Ph.D. Date 6/2/99
Toxicology Branch 1 HED (7509C)

014199

DATA EVALUATION RECORD

STUDY TYPE: Subchronic Oral Toxicity Feeding-Rat
OPPTS 870.3100 [§82-1a]

DP BARCODE: D248305
P.C. CODE: N/A

SUBMISSION CODE: S546651
TOX. CHEM. NO.:

TEST MATERIAL (PURITY): Dichlormid (97.2%, w/w)

SYNONYMS: R-25788

CITATION: Milburn, G. 1989. Dichlormid: 90 day feeding study in rats. ICI Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, U.K. Report No. CTL/P/2363. Study No. PR0718, August 14, 1989. MRID 44606406. Unpublished.

SPONSOR: ICI Americas Inc., Agricultural Products, Wilmington, DE 19897.

EXECUTIVE SUMMARY: In a subchronic toxicity study (MRID 44606406) dichlormid (batch # WRC 4921-35-12:GG0101,97.2% w/w) was administered in the diet for 90 days to 12 Alpk:APfSD rats/sex/dose at concentrations of 0, 20, 200, or 2000 ppm (intake of approximately 0, 1.4, 14, and 140 mg/kg/day for males and 0, 1.6, 16, and 150 mg/kg/day for females, respectively).

No treatment-related mortality or clinical signs of toxicity occurred. Mid-dose females had lowered ($p \leq 0.05$ or 0.01) body weight gains (10-12%, weeks 6-12), food consumption (10-11%, weeks 8-10), and food efficiency (16%, weeks 5-8). High-dose males and females had lowered weight gain and food consumption throughout the study ($p \leq 0.05$ or 0.01), the most severe decreases occurring during the first treatment week, when weight gain was 62-72% lower than of controls and food consumption was 33-41% lower. For weeks 2-13, males had 21-30% lower weight gain and 11-21% lower food consumption than controls, whereas females had 33-40% lower weight gain and 15-24% lower food consumption. Food efficiency was only decreased in the high-dose females (15% overall, $p \leq 0.01$).

Histopathological examination revealed that the liver was identified as a target organ in both male and female rats; males were affected to a greater extent than females. High-dose males had an

increase in the combined incidence of liver centrilobular hypertrophy, eosinophilia, and chromatin margination (12/12 affected), bile duct pigmentation, and centrilobular vacuolation ($p \leq 0.05$ or 0.01). They also had a marginal but dose-related increase in the incidence of hepatic lipidosis ($p = 0.077$ at 2000 ppm; $p \leq 0.05$ for trend), and a slight decrease in plasma cholesterol and triglyceride levels (16-19%, $p \leq 0.05$ or 0.01). High-dose females also had an increased combined incidence of liver centrilobular hypertrophy, eosinophilia, and chromatin margination (6/12 affected), but did not develop lipidosis or have decreased plasma cholesterol and triglyceride levels. Liver weights were increased in 200 and 2000 ppm males, which both had 9% greater absolute weights than controls, and their relative (to body or brain) weights were increased by 16-32%, and 10-21%, respectively ($p \leq 0.01$; relative-to-brain weight not statistically analyzed). High-dose females had increased relative (to body or brain) liver weights (26%, $p \leq 0.01$; 12%, not analyzed). Aminopyrene N-demethylase (APDM) activity of liver tissue was significantly increased in mid and high-dose females (29% and 104%, respectively, $p \leq 0.01$), consistent with exposure to a xenobiotic; it is unclear why a similar effect was not seen in males.

Based on minor decreases in body weight gains and food efficiency in females and on increased liver weight and a slightly increased (NS) incidence of liver lipidosis in males, the LOAEL is 200 ppm under the conditions of this study (intake of approximately 14 mg/kg/day for males and 16 mg/kg/day for females). The NOAEL is 20 ppm (intake of approximately 1.4 mg/kg/day for males and 1.6 mg/kg/day for females).

This study is classified as **acceptable (82-1a)** and satisfies the guideline requirements for a subchronic oral toxicity study in rats.

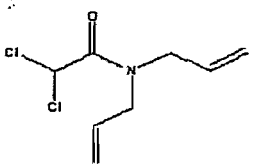
COMPLIANCE: Signed and dated Quality Assurance, Data Confidentiality, GLP and Flagging statements were provided. The only deviation from GLP was failure to give the stability of the test substance.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: dichlormid

Description: amber liquid
Batch #: WRC 4921-35-12: GG0101; CTL reference no. Y06015/002/001-002
Purity: 97.2% w/w
Stability of compound: not given; compound was stored at ~ 20°C in a ventilated cupboard
CAS #: 37764-25-3
Structure:



2. Vehicle and/or positive control

The test material was given in the feed.

3. Test animals

Species: rat
Strain: Alpk:APfSD (Wistar-derived)
Age and weight at study initiation: age: 5-6 weeks; weight of males: 110-175 g: females: 100-138 g
Source: Barriered Animal Breeding Unit, ICI Pharmaceuticals, Alderley Park, Macclesfield, Cheshire, UK
Housing: Initially housed in litters, sexes separately, in stainless steel rat cages (All Type Tools (Woolwich) Ltd., London, UK). Housed in groups of 4 after being assigned to study groups. Food was given in glass jars of 300 g capacity within the cage.
Diet: CT1 diet (Special Diets Services Ltd., Stepfield, Witham, Essex, UK), *ad libitum*.

Water: mains water was supplied *ad libitum*
Environmental conditions:
Temperature: 21.2 ± 2°C (nominal)
Humidity: 40-75% (nominal)
Air changes: ≥ 15 per hour
Photoperiod: 12 hours light/dark alternated

Acclimation period: 10 days

B. STUDY DESIGN

1. In life dates

Start: May 23-25, 1988; end: August 24, 1988.

2. Animal assignment

Animals were assigned to the test groups in Table 1 by randomization so that each litter would be equally represented in all dose groups, including the control group. The study was divided into six, single-sex replicates, each replicate consisting of four cages.

TABLE 1: Study design

Test Group	Exposure concentration (ppm)	Mean Dose to Animal (mg/kg/day)[1]		Number of Animals	
		Male	Female	Male	Female
1	0	0	0	12	12
2	20	1.4	1.6	12	12
3	200	14	16	12	12
4	2000	140	150	12	12

Data taken from page 15, MRID 44606406.
[1]Estimated by reviewer as the intake during week 7 from Figure 1 on p. 28, MRID 44606406.

3. Dose selection rationale

Dose selection was stated to have been based on the results of a previous dichlormid feeding study in the same laboratory, using the same strain of rats, but neither reference nor details of this study were provided.

4. Diet preparation and analysis

The test diets were prepared every 3 weeks in 30-kg batches by mixing 0.5 kg premix with 29.5 kg ground CT1 diet. Mixing was with a TKF PMA 150S mixer for 2 minutes, or a TKF PMA 100S for 4 minutes. The premixes were prepared by combining appropriate amounts of test substance (0.617, 6.17, or 61.6 g for 20, 200, and 2000 ppm, respectively) with 0.5 kg ground CT1 diet. The control diets were prepared identically except no dichlormid was included. Samples of the diets were assayed for concentration (ethyl acetate extraction followed by gas chromatography) prior to being fed to the animals and twice during the study (samples were taken from the animals' feeding jars and are presumably at room temperature). Stability of the test

substance was determined using the 20 and 2000 ppm diets. Homogeneity was tested for the first prepared batch of 20 and 2000 ppm diets; details were not provided of how the diets were sampled.

Results –

Homogeneity Analysis: Sampling of trays 1, 3, and 6 (at 20 and 2000 ppm) showed that the dichlormid concentration for all samples deviated from the overall mean concentration by < 2%. It was not clearly explained in the study report what was meant by "trays."

Stability Analysis: Twenty-three days after preparation of the 20 and 2000 ppm diets, the mean concentration was 91.8 and 93.7%, respectively, of the initial concentrations. After 36 days, concentrations were 77.1 and 84.4% of the initial concentration (20 and 2000 ppm diets, respectively).

Concentration Analysis: On the two sampling dates, the mean dietary concentration as a fraction of the target concentration was 92.0 and 112.0% at 20 ppm, 97.5 and 109.0% at 200 ppm, and 96.7 and 107.2% at 2000 ppm.

The analytical data indicated that the mixing procedure was adequate and that the variance between nominal and actual dosage to the animals was acceptable.

5. Statistics

Analysis of variance (ANOVA) was used to analyze weekly and final body weight gain, weekly food consumption, total food consumption, and food efficiency, separately for males and females. Clinical biochemistry, urinalysis, and hematology were analyzed using the combined male and female data (except for urine protein) by ANOVA, and the results examined to see if there were any consistent differences between the sexes in the control and treatment animals. Hepatic aminopyrene N-demethylase activity was assessed by ANOVA after a natural logarithmic transformation, separately for males and females. Organ weights were analyzed using ANOVA and analysis of covariance (ANCOVA) on the final body weight, separately for males and females. A two-sided Student's t-test was used to compare treatment means with control means and statistical significance was noted at the 5% and 1% levels. The ANOVA and ANCOVA allowed for the replicate structure of the study design and for the litter of origin (except for food consumption and food efficiency). The data were also analyzed after excluding "unusual" values, but no differences were found in the outcome of these analyses.

C. METHODS

1. Observations

Animals were inspected at least once/day for evidence of clinical and behavioral abnormalities. A more detailed clinical examination was given weekly during the study, usually at the time that body weight was measured.

2. Body weight

All animals were weighed immediately before the first feeding of the experimental diets, on the same day of each subsequent week throughout the study, and at termination.

3. Food consumption and compound intake

Food consumption for each cage of four animals was determined weekly throughout the experiment. The dose received for each level was calculated from the group mean body weight and the food consumed. Food utilization/cage (i.e. food efficiency) was calculated for weeks 1-4, 5-8, 9-13, and 1-13 based on the amount of weight gained per cage for each 100 g food consumed.

4. Ophthalmoscopic examination

A direct ophthalmoscopic examination (after instillation of 0.5% tropicamide to dilate the pupils) was conducted on control and high-dose rats prior to treatment and at week 12.

5. Blood was collected by cardiac puncture from all animals at termination for hematology and clinical chemistry analysis. Blood for clinical chemistry analysis was collected in lithium-heparinized tubes, and blood for the hematology analysis was collected in tubes containing EDTA or 0.11 M trisodium citrate. The CHECKED (X) parameters were examined.

a. Hematology

X		X	
x	Hematocrit (HCT)* (packed cell volume)	x	Leukocyte differential count*
x	Hemoglobin (HGB)*	x	Mean corpuscular HGB (MCH)
x	Leukocyte count (WBC)*	x	Mean corpuscular HGB concentration (MCHC)
x	Erythrocyte count (RBC)*	x	Mean corpuscular volume (MCV)
x	Platelet count*		Reticulocyte count
x	Blood clotting measurements*		
	(Thromboplastin time)		
x	(Kaolin-cephalin time)		
x	(Prothrombin time)		

* Required for subchronic studies based on Subdivision F Guidelines.

b. Clinical chemistry

X	ELECTROLYTES	X	OTHER
x	Calcium*	x	Albumin*
	Chloride*	x	Plasma creatinine*
	Magnesium	x	Blood urea nitrogen*
x	Phosphorus*	x	Total Cholesterol
x	Potassium*		Globulins
x	Sodium*	x	Glucose*
		x	Total bilirubin
	ENZYMES	x	Total serum protein (TP)*
x	Alkaline phosphatase (ALK)	x	Triglycerides
	Cholinesterase (ChE)		Serum protein electrophores
x	Creatine phosphokinase		
	Lactic acid dehydrogenase (LDH)		
x	Plasma alanine amino-transferase (also Plasma alanine transaminase)*		
x	Plasma aspartate amino-transferase (also plasma aspartate transaminase)*		
	Gamma glutamyl transferase (GGT)		
	Glutamate dehydrogenase		

* Required for subchronic studies based on FIFRA Subdivision F Guidelines

6. Liver enzyme activity

The activity of aminopyrene N-demethylase (APDM) was measured in liver samples taken at termination from 6 males and 6 females per group for all dose groups.

7. Urinalysis

Urine was collected from all rats in the week prior to study termination, following a 16-18 hour food and water deprivation. The CHECKED (X) parameters were examined.

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

8. Sacrifice and pathology

At the end of the treatment period, all rats were sacrificed by exsanguination under halothane BP vapor anesthesia and subjected to a full pathological examination. The

CHECKED (X) tissues were fixed, processed, embedded in paraffin wax, sectioned, and stained with hematoxylin and eosin. The tissues were examined by light microscopy in all control and 2000 ppm rats. Additionally, the liver, kidney, lung, voluntary muscle, and any abnormalities were examined in the rats fed 20 or 200 ppm dichlormid. Frozen liver sections were prepared from all animals for fat content evaluation after staining with Oil Red 0. The (XX) organs, in addition, were weighed.

X	DIGESTIVE SYSTEM	X	CARDIOVASC./HEMAT.	X	NEUROLOGIC
	Tongue	x	Aorta*	xx	Brain*
x	Salivary glands*	x	Heart*	x	Peripheral nerve*
x	Esophagus*	x	Bone marrow*	x	Spinal cord (3 levels ??)
x	Stomach*	x	Lymph nodes*	x	Pituitary*
x	Duodenum*	x	Spleen*	x	Eyes (optic nerve)
x	Jejunum*	x	Thymus*		
x	Ileum*				GLANDULAR
x	Cecum*		UROGENITAL	xx	Adrenal gland*
x	Colon*	xx	Kidneys*+		Lacrimal gland
x	Rectum*	x	Urinary bladder*	x	Mammary gland (females only)
xx	Liver*+	xx	Testes*+	x	Parathyroids*
	Gall bladder*	x	Ovaries	x	Thyroids*
x	Pancreas*	x	Epididymides		
		x	Prostate		OTHER
	RESPIRATORY	x	Seminal vesicle	x	Bone
x	Trachea*	x	Uterus*	x	Skeletal muscle
x	Lung*	x	Cervix	x	Skin
x	Nose			x	All gross lesions
	Pharynx				and masses*
	Larynx				

* Required for subchronic studies based on FIFRA Subdivision F Guidelines
\+ Organ weight required in subchronic and chronic studies.

II. RESULTS

A. OBSERVATIONS

1. Toxicity

There were no treatment-related clinical signs of toxicity in any dose group.

2. Mortality

None of the animals died prematurely due to dichlormid treatment. One 20 ppm male with dental malocclusion was sacrificed during week 11, and one 200 ppm male with a twisted snout was sacrificed during week 10.

B. BODY WEIGHT AND WEIGHT GAIN

Weekly body weight gains of males and females given 20 ppm and males given 200 ppm dichlormid were comparable to controls (weekly body weights were not provided). Body weight gains of females given 200 ppm were 10-12% lower than of controls for weeks 6-12 ($p \leq 0.05$ or 0.01). Body weight gains of the high-dose rats of both sexes were substantially lower than of controls, gains being only 28-38% of controls for week 0-1 and 57-63% for week 0-2. During subsequent weeks, the high-dose males gained 70-79% as much weight as the controls, whereas the high-dose females gained only 60-67% as much weight as controls. Mean body weights and body weight gains for selected time intervals are shown in Table 2.

TABLE 2. Body weights (BW) (g) and weight gains (g) of rats fed dichlormid for 13 weeks [1]

Time interval	Exposure concentration (ppm)			
	0	20	200	2000
Males				
Initial BW	143.0	147.8	143.8	139.4
Final BW	484.8	498.5	490.5	409.4** (84)
week 0-4 gain	185.8	192.8	182.8	137.1** (74)
week 0-7 gain	255.9	267.1	256.2	193.3** (76)
week 0-10 gain	309.0	313.5	309.2	240.4** (78)
week 0-13 gain	341.8	352.1	346.1	270.0** (79)
Females				
Initial BW	119.3	122.0	121.3	122.8
Final BW	268.1	265.3	258.2	221.1** (82)
week 0-4 gain	85.8	84.4	81.4	55.7** (65)
week 0-7 gain	115.1	113.0	103.5* (90)	74.8** (65)
week 0-10 gain	139.9	132.9	124.0** (89)	92.6** (66)
week 0-13 gain	148.8	143.3	136.8* (92)	98.3** (66)

Data taken from Table 5, pp. 40-41, MRID 44606406.
Significantly different from controls: *$p \leq 0.05$; **$p \leq 0.01$.
[1]Numbers in parentheses are the percent of controls, calculated by reviewer.

C. FOOD CONSUMPTION AND COMPOUND INTAKE

1. Food consumption

Rats given 20 ppm dichlormid ate a similar amount of food as the controls. Males given 200 ppm dichlormid ate slightly less food than controls during weeks 2 and 3 (4-5%, $p \leq 0.05$), and females ate less during weeks 8-10 (10-11%, $p \leq 0.05$ or 0.01). Both sexes of high-dose rats ate less food than controls throughout the study: 33-41% less during week 1, and 11-21% (males) and 15-24% (females) lower during weeks 2-

13. Overall (week 1-13) food consumption of animals given 200 ppm dichlormid was 2% (males) and 6% lower (females) than controls (NS), but consumption by rats given 2000 ppm was 18 and 23% lower, respectively, than of controls ($p \leq 0.01$).

2. Compound consumption

Animals were given the test compound in the feed to achieve target dietary concentrations of 20, 200, or 2000 ppm. The dose to the animals (mg/kg/day), as shown in Table 1, was estimated by the reviewer as the intake during week 7 in Figure 1 (p. 28 MRID 44606406); Figure 1 was a plot of the compound intake by the animals throughout the 13-week study. [Week 7 was chosen because it appeared to be a median for the intake.] It was not stated whether the amount of dichlormid given to the animals was adjusted for compound purity (97.2%); there was no adjustment for body weight.

3. Food efficiency

Food efficiency of the dichlormid-treated males was comparable to that of controls. Food efficiency of the females administered 20 ppm was unaffected, and of females given 200 ppm was lowered for weeks 5-8 (16% lower than controls, $p \leq 0.01$). The food efficiency of females given 2000 ppm dichlormid was lower than of controls throughout the study: 17% for weeks 1-4 ($p \leq 0.01$), 11% for weeks 5-8 ($p \leq 0.05$), 14% for weeks 9-13 (NS), and 15% overall ($p \leq 0.01$).

D. OPHTHALMOSCOPIC EXAMINATION

There were no treatment-related ophthalmological findings.

E. BLOOD WORK

1. Hematology

No treatment-related effects were observed on hematology parameters. The decreases in the hemoglobin, hematocrit, and RBC count in one or both sexes were small (3-6%. $p \leq 0.05$ or 0.01), within the range of the laboratory's historic controls, and not toxicologically significant. The elevated (18%, $p \leq 0.01$) platelet count in females given 2000 ppm was within historic control values. The difference from the control group of the neutrophil count of 2000 ppm males (144% of controls, $p \leq 0.05$) was due to one animal with a high value and to the low levels in the controls (the neutrophil count of 20 ppm males was 130% of controls, NS). The decreased monocyte counts of the 20 and 200 ppm group males were not dose-related.

2. Clinical chemistry

A number of minor clinical chemistry alterations occurred in one or both sexes of rats. High-dose males had increased plasma urea (11% greater than controls, $p \leq 0.05$). Plasma cholesterol and triglyceride levels were decreased 16-19% in the high-dose males ($p \leq 0.05$ or 0.01), but were elevated 19-25% in high-dose females ($p \leq 0.05$). Calcium levels were slightly increased in high-dose males and females (3%, $p \leq 0.05$ and 4%, $p \leq 0.01$, respectively). The slight increase in plasma albumin in high-dose females (4.5% increase, $p \leq 0.05$) and the 18% ($p \leq 0.01$) decrease in plasma aspartate transaminase in high-dose females were not considered biologically significant.

TABLE 3. Clinical chemistry changes and liver tissue aminopyrene N-demethylase (APDM) activity in rats fed dichlormid for 13 weeks[1]

Parameter (mg/100 mL)	Exposure concentration (ppm)			
	0	20	200	2000
Males				
Plasma urea	48.0	49.5	50.1	53.2* (111)
Plasma cholesterol	73.4	67.0	72.7	59.6** (81)
Plasma triglycerides	144	163	156	121* (84)
Plasma calcium	10.4	10.5	10.4	10.7* (103)
Tissue APDM activity[2]	21.1	22.2	24.8	17.3** (82)
Females				
Plasma cholesterol	70.0	65.8	69.0	83.3** (119)
Plasma triglycerides	79	90	88	99* (125)
Plasma calcium	10.6	10.6	10.6	11.0** (104)
Tissue APDM activity[2]	8.5	11.7* (119)	14.8** (129)	17.3** (204)

Data taken from Tables 11 and 14, pp. 54-58, and p. 65, respectively, MRID 44606406.
Significantly different from controls: *$p \leq 0.05$; **$p \leq 0.01$.
[1]Numbers in parentheses are the percent of controls, calculated by reviewer.
[2]Units given as µmol formaldehyde/h/g liver tissue.

F. LIVER ENZYME ACTIVITY

The activity of aminopyrene N-demethylase (APDM) of liver samples taken at termination was 18% lower in the high-dose males than in controls ($p \leq 0.01$). APDM activity was elevated in all three groups of dichlormid-treated females, as shown in Table 3. The value obtained for the 20 ppm group females (11.7, $p \leq 0.05$) was within the range of historic controls, and the value for the 200 ppm females (14.8, $p \leq 0.01$) was only slightly outside the range of historic controls (historic range was given as 5.4-14.7 by study author on p. 23, MRID 44606406). APDM activity of the high-dose females was roughly twice that of the control females ($p \leq 0.01$), and outside the range of historic controls. The results are shown in Table 3.

G. URINALYSIS

High-dose females had a 37% lower urine volume than the control group ($p \leq 0.05$). High-dose males had slightly lower urine pH (6.30 vs. 6.49 in controls; $p \leq 0.01$) and elevated urine protein levels (21%, $p \leq 0.05$). RBCs were seen in the urine sediment of 3 high-dose females (not in other groups), although RBCs were not detected by dip-stick tests for blood in the remainder of the sample, according to the study author (p. 26, MRID 44606406).

H. SACRIFICE AND PATHOLOGY

1. Organ weight

The most marked change was the dose-related increase in liver weight in both sexes of rats. Males given 200 and 2000 ppm dichlormid, respectively, had a 9 and 16% increase ($p \leq 0.01$) in the absolute liver weight, a 9 and 32% increase in the relative (to body weight) liver weight ($p \leq 0.01$), and a 10% and 21% increase in the liver-to-brain weight ratio (latter was calculated by reviewer and not statistically analyzed). The increase in liver weight of mid-dose females was not statistically significant, but high-dose females had increased relative liver weight (26%, $p \leq 0.05$) and relative-to-brain weight (12%, not statistically analyzed). Kidney weight (adjusted for body weight) was increased 13% in 2000 ppm males ($p \leq 0.05$) but the absolute kidney weight and the kidney-to-brain weight ratio were similar to controls. High-dose females had decreased absolute kidney weight (10%, $p \leq 0.01$) but increased adjusted kidney weight (3%, NS). Absolute adrenal weight was lowered 22% in high-dose males ($p \leq 0.01$), although the adjusted (for body weight) adrenal weight was not statistically different from the controls. The organ weight changes are summarized in Table 4.

TABLE 4: Organ weights: absolute, adjusted for body weight, and as a percent (%) of brain weight of rats given dichlormid for 13 weeks[1]

Organ and absolute or relative weight	Exposure concentration (ppm)			
	0	20	200	2000
Males				
Brain: absolute (g)	2.099	2.083	2.093	2.005* (96)
Liver : absolute (g)	18.4	18.9	20.1** (109)	21.3** (116)
Adjusted for BW (g)	17.8	17.8	19.4** (109)	23.5** (132)
% of absolute brain weight[2]	877	907	960 (110)	1062 (121)
Kidney: absolute (g)	3.02	3.19	3.10	2.99 (99)
Adjusted for BW (g)	2.93	3.03	2.98	3.32** (113)
% of absolute brain weight[2]	144	153	148	149 (104)
Adrenals: absolute (g)	0.068	0.067	0.062	0.053** (78)
Adjusted for BW (g)	0.067	0.065	0.061	0.057 (85)
% of absolute brain weight[2]	3.23	3.22	2.96	2.64 (82)
Females				
Brain: absolute (g)	1.930	1.907	1.904	1.848** (96)
Liver : absolute (g)	9.7	9.8	10.0	10.4 (107)
Adjusted for BW (g)	9.2	9.4	9.8	11.6** (126)
% of absolute brain weight[2]	503	513	525	563 (112)

Kidney: absolute (g)	1.89	1.83	1.83	1.71**	(90)
Adjusted for BW (g)	1.82	1.77	1.81	1.87	(103)
% of absolute brain weight[2]	97.9	96.0	96.1	92.5	(94)

Data taken from Table 16, pages 67-69, MRID 44606406.
Significantly different from controls: *p ≤ 0.05; **p ≤ 0.01.
[1]Numbers in parenthesis are the percent of controls, calculated by the reviewer.
[2]Calculated by reviewer; not statistically analyzed.

2. Gross pathology

Only a few findings were reported; none of these were clearly treatment-related. The 20 ppm male sacrificed during week 11 had dental malocclusion, and the 200 ppm male sacrificed during week 10 had a twisted snout (and marked periodontal hemorrhage).

3. Microscopic pathology

Treatment-related microscopic lesions were seen in the liver of both male and female rats. Both sexes of high-dose animals had a dose-related increase in the incidence of centrilobular hypertrophy, eosinophilia, and/or chromatin margination ($p \leq 0.01$; only the combined incidence of these three lesions were given). High-dose males also had an increased incidence of bile duct pigmentation ($p \leq 0.05$), centrilobular vacuolation ($p \leq 0.01$), and a marginal but dose-related increase in the incidence of hepatic lipidosis detected using Oil Red 0 ($p = 0.077$ at 2000 ppm; $p \leq 0.05$ for trend). There were small increases in the incidence of kidney tubular basophilia and interstitial mononuclear cell infiltration in mid-dose males ($p \leq 0.05$) and hyaline droplet formation in high-dose males (NS). Voluntary muscle myodegeneration was evident in mid-dose males (NS). The vast majority of the lesions were of minimal or slight severity (the exception was two high-dose males that had moderate or marked centrilobular hypertrophy/eosinophilia/chromatin margination). The incidences of the microscopic lesions are summarized in Table 5.

TABLE 5: Microscopic findings in rats given dichlormid for 13 weeks				
Organ: lesion	Exposure concentration (ppm)			
	0	20	200	2000
Males				
Liver : Centrilobular hypertrophy/ eosinophilia/chromatin margination	0/12[1]	0/12	0/12	12/12**
Bile duct pigmentation	0/12[1]	0/12	0/12	5/12*
Centrilobular vacuolation	2/12[1]	3/12	2/12	9/12**
Hepatic lipidosis (Oil Red 0)	1/12[1]	3/12[2]	4/12[2]	5/12
Kidney: Tubular basophilia	1/12	2/12[2]	6/11*	4/12
Interst. mononuclear cell infiltration	0/12	2/12[2]	4/12*	3/12
Hyaline droplet formation	0/12	0/12	1/12	3/12
Voluntary muscle: Myodegeneration	1/12	1/12	4/12	1/12
Females				
Kidney: Tubular basophilia	0/12	0/12	0/12	2/12
Liver : Centrilobular hypertrophy/ eosinophilia/chromatin margination	0/12[1]	0/12	0/12	6/12**

Data taken from Table 18, pages 75-84, MRID 44606406.
Significantly different from controls: *p ≤ 0.05, **p ≤ 0.01, calculated by reviewer using the Fisher exact test.
[1]A statistically significant trend with dose (p≤ 0.05) was obtained using the Cochran-Armitage trend test (conducted by reviewer).
[2]Of the given incidence, 1/12 is due to a premature decedent (sacrificed at week 10 or 11).

III. INVESTIGATOR'S CONCLUSIONS

Based on minor decreases in body weight gains and food efficiency in females and on increased liver weight and a slight (not statistically significant) increase in liver lipidosis in males the investigator concluded that the LOAEL is 200 ppm under the conditions of this study (intake of approximately 14 mg/kg/day for males and 16 mg/kg/day for females), and the NOAEL is 20 ppm for both sexes (intake of approximately 1.4 mg/kg/day for males and 1.6 mg/kg/day for females).

IV REVIEWER' DISCUSSION

A. None of the animals died prematurely or had clinical signs of toxicity due to dichlormid treatment. Two males were sacrificed during weeks 10 and 11 due to tooth malocclusion or a physical injury. Mid-dose females had lowered body weight gains (10-12%, weeks 6-12), food consumption (10-11%, weeks 8-10), and food efficiency (16%, weeks 5-8) (p≤ 0.05 or 0.01). Both sexes of high-dose rats had lowered weight gain and food consumption throughout the study (p≤ 0.05 or 0.01), with females being affected more than males. In both sexes, the most severe decreases occurred during the first treatment week, when weight gain was 62-72% lower than of controls and food consumption was 33-41% lower. For weeks 2-13, males had 21-30% lower weight gain and 11-21% lower food consumption than controls, whereas females had 33-40%

lower weight gain and 15-24% lower food consumption. Food efficiency was only decreased in the high-dose females (15% overall, $p \leq 0.01$).

There were no treatment-related ophthalmological findings. The statistically significantly altered hematology parameters were within historic control values and/or not clearly dose-related. Several clinical chemistry changes ($p \leq 0.05$ or 0.01) that were seen in high-dose animals were not considered toxicologically significant because they were marginally different from controls and had no histopathological correlates. These include increased plasma urea in males and decreased aspartate transaminase in females (11%, 18%, respectively), the 3-4% increase in plasma calcium in both sexes, the 4.5% increase in plasma albumin in high-dose females, and the 19-25% increase in plasma cholesterol and triglycerides in females.

The liver was identified as a target organ in both male and female rats, males being affected to a greater degree than females. High-dose males had an increased combined incidence of liver centrilobular hypertrophy, eosinophilia, and chromatin margination (12/12 affected), bile duct pigmentation, and centrilobular vacuolation ($p \leq 0.05$ or 0.01). High-dose males also had a marginal but dose-related increase in the incidence of hepatic lipidosis detected using Oil Red 0 ($p = 0.077$ at 2000 ppm; $p \leq 0.05$ for trend). The lipidosis may have been related to the slight decrease in plasma cholesterol and triglyceride levels (16-19%, $p \leq 0.05$ or 0.01) of this dose group. The vast majority of the lesions in all dose groups were of minimal or slight severity. Consistent with liver toxicity, the liver absolute weight, relative to body weight, and relative to brain weights were increased in 200 and 2000 ppm males (9%, 16-32%, and 10-21%, respectively).

High-dose females also had an increase in the combined incidence of liver centrilobular hypertrophy, eosinophilia, and chromatin margination (6/12 affected) and increased liver weight (26% and 12% relative to body or brain weight, respectively) ($p \leq 0.01$). However, females did not have an increase in the incidence of hepatic lipidosis or a decrease in plasma cholesterol and triglyceride levels.

Aminopyrene N-demethylase (APDM) activity of liver tissue was significantly increased in mid and high-dose females (29% and 104%, respectively, $p \leq 0.01$), consistent with exposure to a xenobiotic. High-dose males, however, had slightly decreased APDM activity (18% ppm, $p \leq 0.01$). It is unclear why APDM activity of males was not also elevated; it may be a due to liver toxicity, which was more extensive (i.e. occurred in more animals; more lesions seen) in males than in females.

A number of histopathological findings ($p \leq 0.05$ or 0.01) did not appear to be treatment-related or toxicologically significant because they were marginally different from controls, not dose-related, and/or lacked histopathological correlates. These included an increased incidence of kidney interstitial mononuclear cell infiltration and tubular basophilia in 200 ppm males (the latter is reportedly a common finding in this strain of rats), the 21% increase in urine protein and slight decrease in urine pH of 2000

ppm males, and the 37% decrease in urine volume of 2000 ppm females (possibly due to dehydration, consistent with their slightly increased (4.5%) plasma albumin). Alterations in the kidney weights of 2000 ppm males (13% increase in relative-to-body weight) and females (10% decrease in absolute weight) and in the adrenals of high-dose males (22% lower absolute weight) were not supported by similar changes in the organ-to-brain weight ratios. RBCs seen in the urine sediment of 3 high-dose females were not corroborated by dip-stick tests for blood in the remainder of the sample.
A slight, statistically non-significant increase occurred in the incidence of voluntary muscle myodegeneration in mid-dose males (4/12 vs. 1/12 for controls). Although this lesion does not appear to be treatment-related in rats, it is notable that in another study (MRID 41419401), muscle degeneration was one of the primary findings in dogs given 25 or 50 mg/kg/day dichlormid by capsule for 90 days.

Therefore we agree with the investigator's conclusion that the LOAEL for dichlormid in this 90-day subchronic rat study is 200 ppm (intakes of 14 mg/kg/day for males and 16 mg/kg/day for females), with a NOAEL of 20 for both sexes (1.4 mg/kg/day for males and 1.6 mg/kg/day for females).

B. STUDY DEFICIENCIES

There were no major study deficiencies. Minor shortcomings include failure to provide (1) the stability of the test substance (2) details of the method used to determine test material homogeneity (3) details of the "dip-stick" method used to test the urine for RBC content, (4) the plasma chloride content, and (5) statistical analysis of the histopathology results. Establishing the significance of the urinalysis and histopathology findings would have been facilitated by inclusion of historical control data.

DATA EVALUATION REPORT 014199

DICHLORMID

STUDY TYPE: SUBCHRONIC ORAL TOXICITY FEEDING - RAT (82-1)

Prepared for

Health Effects Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
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Prepared by

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Task Order No. 98-36

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Disclaimer

This review may have been altered subsequent to the contractor's signatures above.

Oak Ridge National Laboratory, managed by Lockheed Martin Energy Research Corp. for the U.S. Department of Energy under contract number DE-AC05-96OR22464.

[DICHLORMID] Subchronic Oral Study (82-1)

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014199

DATA EVALUATION RECORD

STUDY TYPE: Subchronic Oral Toxicity Feeding-Rat
OPPTS 870.3100 [§82-1]

DP BARCODE: D252251 SUBMISSION CODE: S546651
P.C. CODE: N/A TOX. CHEM. NO.: N/A

TEST MATERIAL (PURITY): Dichlormid (97.7%)

SYNONYMS: R-25788; N, N-diallyl dichloroacetamide; 2,2-dichloro-N,N-di-2-propenyl-acetamide; N,N-diallyl-2,2-dichloroacetamide

CITATION: B. Vos (1971) R-25788 Safety Evaluation by Dietary Feeding to Rats for 13 Weeks. Woodard Research Corporation, 12310 Pinecrest Road, Herndon, Virginia 22070. Report No. not given, May 17, 1972. MRID 00058467 Unpublished

SPONSOR: Stauffer Chemical Company (no address given)

EXECUTIVE SUMMARY: In a subchronic toxicity study (MRID 00058467) R-25788 (dichlormid) (97.7% a.i.; Lot WRC 2012-9) was administered to 15 Sprague-Dawley rats/sex/dose in the diet at concentrations of 0, 60, 240 or 960 ppm (corresponding to target dose levels of 0, 10, 40, or 160 mg/kg/day) for 13 weeks.

The clinical signs for the animals were not reported, although it was stated that there were no notable differences between the control and treated groups. There were no treatment-related deaths. At week 13, males in the 160 mg/kg/day group weighed 11.3% less than the controls ($p \leq 0.05$); females in the 40 and 160 mg/kg/day groups weighed 7.9% and 13.4% less than the controls ($p \leq 0.05$), respectively. Total weight gain was reduced by 14.4% in males at 160 mg/kg/day and by 10.0% and 19.5% in females at 40 and 160 mg/kg/day, respectively. With the exception of food intake values for the high dose females, there did not appear to be a compound-related effect on food intake. Weekly and total food efficiency did to appear to be affected by treatment. Clinical chemistry and hematology findings were not considered to be toxicologically significant and/or treatment-related. Statistically significant increases ($p \leq 0.05$) in absolute liver weights were seen in both male (40%) and female (22%) animals in the 160 mg/kg/day group. The relative weights were increased 40% over the control for both males and females. The high dose females showed a significantly decreased absolute kidney weight. Both male and females in the high dose group showed a significant (11%) reduction in adrenal weight. Neither the kidneys nor the adrenals showed any correlated histopathology. Significant histopathological correlates included a statistically significant increase in the incidence of increased liver cell size (centro-lobular) in the 160 mg/kg/day males and females ($p \leq 0.004$) and

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degranulation of the liver cells in the 160 mg/kg/day males and females ($p \leq 0.004$) and the 40 mg/kg/day male group ($p \leq 0.02$).

The NOAEL is 10 mg/kg/day. Under the conditions of this study, the LOAEL is 40 mg/kg/day based on the liver histopathology in males.

This subchronic toxicity study is classified as **UNACCEPTABLE (82-1)** and not upgradeable because numerous parameters required by 82-1 guidelines were missing (e.g. compound analysis in the diet, clinical chemistry, brain weight, histopathological evaluation of all animals in control and high dose groups and all target organs).

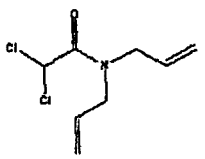
COMPLIANCE: GLP, Data Confidentiality, Quality Assurance and Flagging statements were not present.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: R-25788 (Dichlormid)

   Description: amber liquid
   Lot: WRC 2012-9
   Purity: 97.7 ± 2%
   Stability of compound: not given
   CAS #: 37764-25-3
   Structure:



2. Vehicle and/or positive control

   The test material was dissolved in acetone and combined with feed. The acetone was allowed to evaporate.

3. Test animals

   Species: rat
   Strain: albino
   Age and weight at study initiation: unspecified weanling; individual animal weights were given and the mean weights of the four groups of males were 47 g and of females were 50 g at week -1. At treatment initiation (week 0) the male and females weights, respectively, ranged from 64-104 g, 73-95 g (control), 62-100 g,

68-91 g (10 mg/kg/day), 67-98 g, 68-106 g, (40 mg/kg/day), and 66-121 g, 68-93 g (160 mg/kg/day).
Source: Charles River Breeding Laboratories
Housing: Individual cages
Diet: Purina lab chow meal, *ad libitum*
Water: Drinking water was available, *ad libitum*
Environmental conditions:
Temperature: Controlled, values not given
Humidity: Not specified
Air changes: Not specified
Photoperiod: Not specified
Acclimation period: One week (week -1 to week 0)

B. STUDY DESIGN

1. In life dates

Start: Approximately November 8, 1971 (estimated by reviewer)
End: Approximately February 8-15, 1972 (13 to 14 weeks) (estimated by reviewer)

2. Animal assignment

The method used to assign the animals to the dose groups was not given but the reviewer observed that the animals were assigned to groups to standardize mean weights. The group assignments are given in Table 1.

TABLE 1: Study design

Test group	Target Dosage (mg/kg/day)[a]	Concentration in diet (ppm)[b]	Number of animals	
			Male	Female
I	0 (Control)	0	15	15
II	160	960	15	15
III	40	240	15	15
IV	10	60	15	15

Data taken from pp. 46-47, MRID 225002429.
[a]Actual intake not given (no dietary analysis provided)
[b]Beginning concentration, dietary concentration adjusted at weeks 3,7, and 11 for body weight changes.

3. Diet preparation and analysis

The test material was mixed with an appropriate quantity of acetone to give 50 ml. This was poured over 6000 g of control diet, allowed to evaporate for 15 minutes, and then mixed with a mechanical mixer. The stability and homogeneity of R-25788 in the diet were not reported. The concentrations in the diet were adjusted over the term of the experiment to maintain the approximate dose. See Table 1 for the adjustments.

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The exact preparation formulas were not given. It is not known if acetone was poured on the control diet.

Results -
Homogeneity Analysis: not reported
Stability Analysis: not reported
Concentration Analysis: not reported

4. Statistics

The only statistical analyses reported were the calculated means for all the measured parameters. The *t*-test was inappropriately applied as a repeated measure to body weights, organ weights, and relative organ weights. The reviewer analyzed the body weight, liver, kidney, and adrenal weights, and selected hematological results by ANOVA and Dunnet's comparison test. The reviewer used the *t*-test for comparison of glucose, prothrombin time, and SGPT. The reviewer analyzed the histopathology data using Fisher's exact test.

C. METHODS

1. Observations

Animals were inspected daily for signs of toxicity and mortality.

2. Body weight

Animals were weighed initially (week -1 and at the initiation of treatment week 0) and weekly through treatment week 13.

3. Food consumption and compound intake

Food consumption for each animal was determined weekly for treatment weeks 1 through 13. Compound intake was maintained by periodically adjusting the compound concentration in the feed to maintain the dosage by body weight. The reviewer calculated the food efficiency as weekly body weight gain (g)/weekly food intake (g).

4. Ophthalmoscopic examination

An ophthalmoscopic examination was not performed; the Subdivision F Guidelines suggest that it should have been performed. The eyes were preserved and examined.

5. Blood was collected from 5 animals/sex/ in the control and 160 mg/kg/day groups at treatment weeks 4,8 and 13 (not specified how blood was collected) and from 5 animals/sex in the 10 and 40 mg/kg/day groups at week 13 for hematology and clinical chemistry analysis. The CHECKED (X) parameters were examined.

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a.. Hematology

X		X	
x	Hematocrit (HCT)* (packed cell vol.)	x	Leukocyte differential count*
x	Hemoglobin (HGB)*		Mean corpuscular HGB (MCH)
x	Leukocyte count (WBC)*		Mean corpusc. HGB conc.(MCHC)
	Erythrocyte count (RBC)*		Mean corpusc. volume (MCV)
x	Platelet count*		Reticulocyte count
	Blood clotting measurements*		
	(Thromboplastin time)		
x	(Clotting time)		
x	(Prothrombin time)		

* Required for subchronic studies based on Subdivision F Guidelines

b. Clinical chemistry

X	ELECTROLYTES	X	OTHER
	Calcium*		Albumin*
	Chloride*		Blood creatinine*
	Magnesium		Blood urea nitrogen*
	Phosphorus*		Total Cholesterol
	Potassium*		Globulins
	Sodium*	x	Glucose*
			Total bilirubin
	ENZYMES		Total serum protein (TP)*
	Alkaline phosphatase (ALK)		Triglycerides
	Cholinesterase (ChE)		Serum protein electrophores
	Creatine phosphokinase		
	Lactic acid dehydrogenase (LDH)		
x	Serum alanine amino-transferase (also SGPT)*		
	Serum aspartate amino-transferase (also SGOT)*		
	Gamma glutamyl transferase (GGT)		
	Glutamate dehydrogenase		

* Required for subchronic studies based on Subdivision F Guidelines

6. Urinalysis

No urinalysis was performed.

7. Sacrifice and pathology

All animals that survived the treatment period were sacrificed after week 13 by guillotine. It was not specified if the animals were fasted prior to sacrifice. They were all subjected to gross pathological examination. The CHECKED (X) tissues were collected for histological examination. Tissues from all control and high-dose rats were examined histologically, as well as all lesions from animals with grossly observed alterations. The (XX) organs, in addition, were weighed. Only results from 5 rats per sex/treatment group were reported.

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X	DIGESTIVE SYSTEM	X	CARDIOVASC./HEMAT	X	NEUROLOGIC
	Tongue		Aorta*	x	Brain*+
	Salivary glands*	xx	Heart*		Periph. nerve*
	Esophagus*	x	Bone marrow*		Spinal cord (3 levels)T
x	Stomach*	x	Lymph nodes*	xx	Pituitary*
x	Duodenum*	xx	Spleen*	x	Eyes (optic n.)T
	Jejunum*		Thymus*		
	Ileum*				GLANDULAR
	Cecum*		UROGENITAL	xx	Adrenal gland*+
	Colon*	xx	Kidneys*+		Lacrimal gland T
	Rectum*	x	Urinary bladder*	x	Mammary gland T
xx	Liver*+	xx	Testes*-		Parathyroids*
	Gall bladder*	xx	Ovaries	xx	Thyroids*
x	Pancreas*		Epididymides		
		xx	Prostate		OTHER
	RESPIRATORY		Seminal vesicle		Bone
	Trachea*	xx	Uterus*	x	Skeletal muscle
xx	Lung*				Skin
	Nose			x	All gross lesions
	Pharynx				and masses*
	Larynx				

* Required for subchronic studies based on Subdivision F Guidelines
+ Organ weight required in subchronic and chronic studies.
T = required only when toxicity or target organ.

II. RESULTS

A. OBSERVATIONS

1. Toxicity

The clinical signs for the animals were not given. The study author, however, stated that there were no differences in behavior or appearance between the control and treated groups.

2. Mortality

One female was removed from the 40 mg/kg/day group during week two of treatment and died the following week from a brain abscess. All other animals survived until the termination of the study.

B. BODY WEIGHT AND WEIGHT GAIN

The body weights of males at 160 mg/kg/day were significantly lower than the controls at week 13 (11.3% reduction, $p \leq 0.05$). The week 13 body weights of the females at 160 mg/kg/day and 40 mg/kg/day were significantly less than the controls (13.4% and 7.9% reductions, respectively, $p \leq 0.05$). The effect on body weight appears to be treatment related. Overall weight gain at 160 mg/kg/day was reduced by 14.4% in males and by 19.5% in females. The results are summarized in Table 2.

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TABLE 2. Selected week group mean body weights (g), body weight decreases, total body weight gains (g), and terminal weights (g) of rats fed R-25788 for 13 weeks[1]

Study week	Dose (mg/kg/day)			
	0	10	40	160
Males				
0	82	78	82	84
4	274	275 (+)	268 (-2.2)	258 (-5.9)
8	375	366 (-2.4)	351 (-6.4)	345 (-8.0)
13	450	443 (-1.6)	420 (-6.7)	399 (-11.3)*
0-13 (total weight gain)	368	365 (-0.8)	338 (-8.2)	315 (-14.4)
terminal wt.	440	442 (+)	424 (-3.6)	412 (-6.3)
Females				
0	85	82	82	84
4	191	191 (0)	180 (-5.8)	174 (-8.9)
8	228	220 (-3.5)	209 (-8.3)	198 (-13.2)
13	254	249 (-2.0)	234 (-7.9)*	220 (-13.4)*
0-13 (total weight gain)	169	167 (-1.1)	152 (-10.0)	136 (-19.5)
terminal wt.	255	251 (-1.6)	234 (-8.2)	220 (-13.7)

Data taken from pp. 54-62 and 92, MRID 225002429.
*Indicates a significant difference from control: $p \leq 0.05$ by Dunnet's Comparison (reviewer calculated)
[1]Numbers in parenthesis are the percent decrease relative to untreated controls, calculated by the reviewer .
+ Indicates a weight gain compared to control.

C. FOOD CONSUMPTION AND COMPOUND INTAKE

1. Food consumption

Selected weekly food consumption is reported in Table 3. Overall food consumption in the high dose group was reduced 6.8% in males and 14.6% in females compared to the control group. With the exception of food intake values (weekly and overall) in females at 160 mg/kg/day, there did not appear to be a compound-related effect on food intake.

2. Compound consumption

Animals were given the test compound in the diet as shown in Table 1.

3. Food efficiency

Food efficiency is reported in Table 4. Weekly and total food efficiency did not appear to be affected by treatment. In males, there is a decrease in food efficiency reported, but it does not appear to be of toxicological significance. In the 10 mg/kg/day males, total food efficiency (Weeks 0-13) was 5.9% lower than controls. In the 40 and 160 mg/kg/day males, total food efficiency was 11.8% lower than controls for both dose groups. However, the actual values from control to high dose

are within 0.02, ranging from 0.15 (40 and 160 mg/kg/day) to 0.17 (controls). For females, there is no real change in food efficiency that would be of toxicological significance.

TABLE 3. Selected weekly group food intake (g) of rats fed R-25788 for 13 weeks[1]

Study week	Dose (mg/kg/day)			
	0	10	40	160
Males				
1	123	126 (+2)	118 (-4)	114 (-7)
4	177	179 (+1)	174 (-2)	160 (-10)
8	173	184 (+6)	168 (-3)	170 (-2)
12	172	164 (-5)	170 (-1)	152 (-12)
1-13 (total food intake)	2185	2216 (+1)	2192 (+0.3)	2037 (-6.8)
Females				
1	122	123 (+1)	113 (-7)	104 (-15)
4	134	135 (+1)	126 (-6)	106 (-21)
8	126	123 (-2)	112 (-11)	101 (-20)
12	118	123 (+4)	106 (-10)	99 (-16)
1-13 (total food intake)	1653	1645(-0.5)	1510 (-8.7)	1412 (-14.6)

[1]Numbers in parenthesis are the percent increase or decrease relative to untreated controls, calculated by the reviewer

TABLE 4. Selected weekly group food efficiency of rats fed R-25788 for 13 weeks[1]

Study week	Dose (mg/kg/day)			
	0	10	40	160
Males				
1	0.41	0.42	0.40	0.40
4	0.27	0.27	0.28	0.28
8	0.05	0.11	0.09	0.09
13	0.04	0.05	0.05	0.04
0-13 (total food efficiency)	0.17	0.16 (-5.9)	0.15 (-11.8)	0.15 (-11.8)
Females				
1	0.36	0.37	0.35	0.38
4	0.14	0.16	0.15	0.12
8	0.09	0.06	0.05	0.04
13	0.03	0	0.02	0
0-13 (total food efficiency)	0.10	0.10	0.10	0.096 (-4)

Calculated by reviewer from weekly group means. Overall food efficiency = total gain/total feed intake
[1]Numbers in parenthesis are the percent decrease relative to untreated controls, calculated by the reviewer

D. OPHTHALMOSCOPIC EXAMINATION

An ophthalmoscopic examination was not performed but eyes were maintained for histopathologic evaluation.

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E. BLOOD WORK

1. Hematology

No toxicologically significant effects were seen in either sex at any dose for hemoglobin or hematocrit. At week 13 the white blood cell count was significantly higher than the control in the 40 mg/kg/day and 160 mg/kg/day males (27% and 28.6%, respectively, $p \leq 0.05$). The effects were also evident at four weeks (34.5% increase in high dose males, no statistical analysis). The males did not show any other treatment effects. The female 40 mg/kg/day treatment group at 13 weeks had significantly higher numbers of segmented granulocytes (175% increase) and fewer lymphocytes than any other group (7.4% reduction) ($p \leq 0.05$ for both). The coagulation time in the 10 mg/kg/day and 40 mg/kg/day female treatment groups was significantly less than the control group (10.4% reduction for both groups, $p \leq 0.05$), however, there were no differences in prothrombin time in the high dose group. (Selected findings are shown in Table 5.

TABLE 5. Hematology - Group Mean Values

Parameters	Dose (mg/kg/day)			
	0	10	40	160
Males				
WBC (1 K/CMM) (week 4)	14.2	--	--	19.1 (+34.5)
WBC (1 K/CMM) (week 13)	14	15.7 (+12)	17.8* (+27)	18*(+28.6)
Females				
Segmented granulocytes[a] (week 13)	4	5 (+25)	11* (+175)	4
Lymphocytes[a] (week 13)	95	96 (+1)	88 * (-7.4)	94 (-1)
Coagulation Time[a] (week 13)	4.8	4.3* (-10.4)	4.3* (-10.4)	4.4 (-8.3)
Prothrombin Time (seconds) (week 13)	14.5	--	--	14.3 (-1)

Data taken from pp. 72-79, 84-87 MRID 225002429.
*Indicates a significant difference from control: $p \leq 0.05$ (reviewer calculated)
[1]Numbers in parenthesis are the percent increase or decrease relative to untreated controls, calculated by the reviewers.
[a]Units were not provided for various parameters.

2. Clinical chemistry

Serum alanine amino transferase (SGPT) levels were generally lower than the controls in the 160 mg/kg/day males at weeks 4 (-16%) and 13 (-14%) of the study. The reduction approaches statistical significance ($p \leq 0.05$) by the two tail *t*-test and is

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significant by a one-tailed test at week 13. This effect was not seen in the female high treatment group. The blood glucose levels were significantly elevated in the males at 160 mg/kg/day compared to the controls (10.7% increase, $p \leq 0.05$, *t*-test) at week 13, with the effect seen throughout the 13 weeks of the study. In females at 160 mg/kg/day, blood glucose was elevated, but it was not statistically significant. Selected findings are shown in Table 6.

Numerous clinical chemistry parameters required for subchronic studies by the Subdivision F Guidelines were not assayed in this study, including blood electrolytes (calcium, chloride, magnesium, phosphorus, potassium, sodium), alkaline phosphatase, serum aspartate amino transferase (SGOT), serum albumin and total protein, and blood creatinine, urea nitrogen, bilirubin and cholesterol.

TABLE 6. Clinical Chemistry - Group Mean Values

Parameters	Dose (mg/kg/day)			
	0	10	40	160
Males				
SGPT (sigma-frankel units per mL) (week 4)	49	--	--	41*(-16)
SGPT (sigma-frankel units per mL) (week 13)	44	--	--	38*(-14)
Glucose (mg/100 mL) (week 13)	84	--	--	93* (+10.7)
Females				
SGPT (sigma-frankel units per mL) (week 4)	40	--	--	42 (+5)
SGPT (sigma-frankel units per mL) (week 13)	40	--	--	39 (-2.5)
Glucose (mg/100 mL) (week 13)	86	--	--	92 (+7)

Data taken from pp. 80-83, 88-91 MRID 225002429.
*Indicates a significant difference from control: $p \leq 0.05$ (reviewer calculated)
[1]Numbers in parenthesis are the percent increase or decrease relative to untreated controls, calculated by the reviewers.

F. SACRIFICE AND PATHOLOGY

1. Organ weight

Statistically significant increases or decreases ($p \leq 0.05$) were seen in the absolute weight of a number of organs in one or both sexes of rats. Selected organ weights are shown in Table 7. Alterations seen in both sexes include [1] increased absolute and/or relative liver weight at one or more doses (160 mg/kg/day male and female livers were significantly larger than control (males 40%, females 22%), with relative weights increased over 40% of control for both sexes), [2] decreased absolute kidney

weight in high-dose females compared to control (8% reduction), and [3] significantly decreased absolute adrenal weight in high-dose males and females (11% reduction in both sexes). There were no other obvious trends in the other organs. There was no wet brain weight or separate brain region weights other than the pituitary gland.

TABLE 7. Selected mean organ weights of rats fed R-25788 for 13 weeks[1]

Organ	Dose (mg/kg/day)			
	0	10	40	160
Males				
Absolute Liver (g)	15.8	17.0 (+7%)	17.5 (+11%)	22.1* (+40%)
Relative Liver (g)	3.61	3.86 (+7)	4.10 (+14)	5.35 (+48)
Absolute Kidney (g)	3.22	3.44 (+7%)	3.35 (+4%)	3.54 (+10%)
Absolute Adrenals (mg)	53.7	52.6 (-2%)	49.6 (-8%)	47.6* (-11%)
Females				
Absolute Liver (g)	8.9	9.4 (+6%)	9.3 (+4%)	10.9* (+22%)
Relative Liver (g)	3.49	3.74 (+7)	3.97 (+14)	4.95 (+42)
Absolute Kidney (g)	2.02	2.05 (+1%)	1.92 (-5%)	1.85 (-8%)
Absolute Adrenals (mg)	65.5	64.8 (-1%)	60.3 (-8%)	58.1* (-11%)

From MRID 225002429, pages 92-93.
[1]Numbers in parenthesis are the percent increase or decrease relative to untreated controls, calculated by the reviewer
*Statistically different at $p \leq 0.05$.

2. Gross pathology

There were no treatment-related findings. Lesions (pneumatosis intestinalis) were found in the colon of two 40 mg/kg/day males. The etiology was not apparent and was not described as treatment related.

3. Microscopic pathology

The most notable histopathological findings are those involving the liver (centrolobular zone). Selected findings are shown in Table 8 and include a statistically significant increase in the incidence of increased liver cell size in the 160 mg/kg/day treatment groups in both the males and the females ($p \leq 0.004$) and a statistically significant increased incidence of degranulation in the liver cells in the high dose groups of males and females ($p \leq 0.004$) and also in the 40 mg/kg/day male group ($p \leq 0.02$). There were no other indicators of liver cell necrosis, inflammation or cholestasis. In the kidney there was a significant increase in the number of hyaline droplets present in the convoluted tubules of the male rats in the high dose group when compared to the controls ($p \leq 0.02$), but hyaline droplets were seen in all groups of the male rats. All other histopathologic observations were not treatment related. There were no evaluations of aorta, caecum, small intestine other than duodenum, peripheral nerves, esophagus, rectum, salivary gland and thymus.

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TABLE 8. Selected microscopic findings of rats fed R-25788 for 13 weeks[1]				
Tissue/Response	Dose (mg/kg/day)			
	0	10	40	160
Males				
Liver/ increased cell size	0/5	0/5	1/5	5/5*
Liver/degranulation of hepatic cell	0/5	0/5	4/5**	5/5*
Kidney/hyaline droplets (granulation)	1/5	1/5	3/5	5/5**
Females				
Liver/increased cell size	0/5	0/5	0/5	5/5*
Liver/degranulation of hepatic cell	0/5	0/5	0/5	5/5*
Kidney/hyaline droplets (granulation)	0/5	0/5	0/5	0/5

Data taken from MRID 225002429, page 113.
[1]Numbers represent incidence in animals examined (n=5 per group/sex)
* $p \leq 0.004$, compared to control
** $p \leq 0.02$ compared to control

III. DISCUSSION

A. DISCUSSION (Note: The EPA reviewer did not redo contractor statistics.)

The clinical signs for the animals were not reported, although it was stated that there were no notable differences between the control and treated groups. There were no treatment-related deaths; one female (40 mg/kg/day) died in week 3 from unrelated causes. Treatment- related effects on body weight were observed. The week 13 body weights of both males and females in the high dose groups were significantly reduced compared to the controls. The males in the 160 mg/kg/day group weighed 11.3% less than the controls ($p \leq 0.05$). The females in the 40 mg/kg/day and 160 mg/kg/day groups weighed significantly less ($p \leq 0.05$) than the controls (7.9% and 13.4%, respectively). Overall weight gain was reduced by 14.4% in the high dose group males and by 19.5% in the high dose group females. The 10% reduction in overall weight gain in the 40 mg/kg/day female group is not considered to be toxicologically significant. The effects on weight gain were directly related to the decreased food consumption in females at 160 mg/kg/day. Males showed decreased food consumption, but it did not appear to be compound related. Weekly and total food efficiency did not appear to be affected by treatment.

A variety of hematological changes were noted, but these are of questionable clinical significance, lack dose-response, and are probably not treatment related. For example, the white blood cell (WBC) count in males at 40 mg/kg/day and 160 mg/kg/day was significantly elevated at 13 weeks (27% and 28.6% increase, respectively). If the WBC count were treatment-related at 40 mg/kg/day, then similar or more exaggerated findings for WBC components would be expected at 160 mg/kg/day, which was not the case. In fact, the WBC count at the high dose had actually decreased from Week 4 to Week 13. At week 13, females in the 40 mg/kg/day treatment group showed significantly higher

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numbers of segmented granulocytes and fewer lymphocytes than controls (175% increase and 7.3% decrease, respectively, $p \leq 0.05$). The week 13 coagulation time in the 10 mg/kg/day and 40 mg/kg/day treatment groups was also significantly reduced compared to controls (1.4% for both treatments, $p \leq 0.05$), but given that there were no differences in prothrombin time in the high dose group, this is incidental to treatment.

Most of the clinical chemistry parameters required for subchronic studies by today's EPA Subdivision F Guidelines were not assayed in MRID 225002429, which was conducted in 1971. Missing parameters included blood electrolytes (calcium, chloride, magnesium, phosphorus, potassium, sodium), alkaline phosphatase, SGOT, serum albumin, total protein, blood creatinine, urea nitrogen, bilirubin, and cholesterol. Urinalysis was not performed. SGPT levels in males were generally reduced (14-16%) in the 160 mg/kg/day group throughout the study and approached statistical significance ($p \leq 0.05$). The blood glucose levels in the high dose group males and females were slightly elevated compared to controls (10.7% increase, $p \leq 0.05$ in males). The values ranged from 78-105 mg/100 mL blood. This change is probably not biologically significant because the blood glucose values were within the biologically normal range.

Statistically significant ($p \leq 0.05$) increases in absolute liver weights were seen in both male (40%) and female (22%) animals in the 160 mg/kg/day treatment group. The relative weights in both sexes were increased over 40% of the control. Absolute kidney weight was decreased in high dose females (8%) and adrenal weight was significantly decreased in male and female high dose groups (both 11%, $p \leq 0.05$). The kidney and adrenal weight changes were of small magnitude, lacked histopathological correlates, and, therefore, were not considered to be treatment related. No wet brain weight was taken and, therefore, no organ weights were related to brain weight.

There were no treatment related gross pathologies. Significant histopathological findings included a statistically significant ($p \leq 0.004$) increase in the incidences of increased liver cell size (centro-lobular) in the 160 mg/kg/day males and females. The incidence of degranulation of the liver cell was also increased for the 40 mg/kg/day ($p \leq 0.02$) and 160 mg/kg/day males ($p \leq 0.004$) and 160 mg/kg/day females ($p \leq 0.004$). Hyaline droplets were found in the kidney convoluted tubules in all groups of male rats, but the incidence was significant ($p \leq 0.02$) in only the high dose group. All other observations were not specific to the treatment.

The NOAEL is 10 mg/kg/day for males and 40 mg/kg/day for female. Under the conditions of this study, the LOAEL is 40 mg/kg/day based on the liver histopathology in males and 160 mg/kg/day based on liver histopathology of the females. The effects on weight gain in the 40 mg/kg/day females are not considered to be toxicologically significant. Based on the findings in both sexes, the NOAEL and LOAEL have been set at 10 mg/kg/day and 40 mg/kg/day, respectively.

B. STUDY DEFICIENCIES

The most serious deficiencies of this study are the lack of analysis of the stability, homogeneity, and concentration in the diet, data for numerous clinical chemistry parameters (blood electrolytes, alkaline phosphatase, SGOT, serum albumin, total protein, blood creatinine, urea nitrogen, bilirubin, and cholesterol). Wet weights of brains were not recorded. There was no histopathological examination of the aorta, caecum, small intestine (other than duodenum), esophagus, rectum, salivary gland, thymus, or peripheral nerve and not all animals of control and high dose groups had histopathological examinations.

Minor deficiencies that did not likely affect the interpretation or outcome of the study included failure to give the study in life dates, to provide signed and dated compliance statements, the initial age of animals, and to provide a description of the method used to collect blood for hematology and clinical analysis.

This subchronic toxicity study is classified as **UNACCEPTABLE (82-1) and not upgradeable** because numerous parameters required by 82-1 guidelines (e.g. compound analysis in the diet, clinical chemistry, brain weight, histopathological evaluation of all animals in control and high dose groups and all target organs) were not measured.

DATA EVALUATION REPORT

014199

DICHLORMID

STUDY TYPE: SUBCHRONIC ORAL TOXICITY [CAPSULE] - DOG (82-1b)

Prepared for

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

Prepared by

Chemical Hazard Evaluation Group
Toxicology and Risk Analysis Section
Life Sciences Division
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Task Order No. 98-36A

Primary Reviewer: Sylvia Milanez, Ph.D., D.A.B.T.	Signature: Date:	Sylvia Milanez 11-25-98
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Robert H. Ross, M.S., Group Leader	Signature: Date:	Robert H. Ross 11-25-98
Quality Assurance: Eric B. Lewis, M.S.	Signature: Date:	Eric B. Lewis 11-25-98

Disclaimer

This review may have been altered subsequent to the contractor's signatures above.

Oak Ridge National Laboratory, managed by Lockheed Martin Energy Research Corp. for the U.S. Department of Energy under contract number DE-AC05-96OR22464.

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014199

DATA EVALUATION RECORD

STUDY TYPE: Subchronic Oral Toxicity [capsule]-Dog
OPPTS 870.3150 [§82-1b]

DP BARCODE: D252251
P.C. CODE: 900497

SUBMISSION CODE: S546651
TOX. CHEM. NO.:

TEST MATERIAL (PURITY): Dichlormid (97.2%, w/w)

SYNONYMS: R-25788

CITATION: Foster, P. 1988. Dichlormid: 90 day oral dosing study in dogs. ICI Central Toxicology Laboratory, Alderley Park, Macclesfield., Cheshire, U.K. Report No. CTL/P/2482, Study No. PD0727, December 14, 1988. MRID 41419401. Unpublished.

SPONSOR: ICI Americas Inc., Agricultural Products, Wilmington, DE 19897.

EXECUTIVE SUMMARY: In a subchronic toxicity study (MRID 41419401) dichlormid (batch # WRC 4921-35-12 GGDO101, 97.2% w/w) was administered by capsule to 4 beagle dogs/sex/dose at doses of 0, 1, 5, 25, or 50 mg/kg/day for 90 days.

One male and one female given 50 mg/kg/day dichlormid were sacrificed during week 7 due to poor physical condition and inappetence. These animals were thin, subdued, and the female salivated at dosing and had pale buccal membranes. The other animals survived to terminal sacrifice and had no consistent clinical signs. The 13-week body weight gain was substantially lower than of controls for dogs given 25 and 50 mg/kg/day: 37 to 54% lower in males and 57 to 68% lower in females, respectively ($p \leq 0.05$ for all but the 25 mg/kg/day males). Food consumption was generally unaffected (except in the two early-sacrifice animals); therefore the decrease in body weight gain for both sexes given 25 and 50 mg/kg/day was due to compound toxicity. Hematological changes consisted of decreased hemoglobin, hematocrit, and RBC count (10-17%, $p \leq 0.05$ or 0.01) at weeks 8 and/or 13 for both sexes of dogs given 50 mg/kg/day, and for females given 25 mg/kg/day. These were consistent with hemolytic anemia, although there were no correlated histopathological changes.

The clinical chemistry alterations were consistent with voluntary muscle and liver toxicity. In both sexes of 25 and 50 mg/kg/day dogs, plasma creatine kinase levels were 7-107 times the control levels (weeks 6-8) and creatinine levels were 21-29% lower than controls ($p \leq 0.01$ for

weeks 8 and/or 13). This was associated with microscopic voluntary muscle degeneration (with cellular infiltration) in all dogs given 25 and 50 mg/kg/day dichlormid (grossly, the muscle appeared pale in 50 mg/kg/day animals). Evidence of liver toxicity included increased ($p \leq 0.05$ or 0.01) aspartate aminotransferase (4-24 times controls for weeks 6-8) and alkaline phosphatase (38-70% increase at week 13) in both sexes of 25 and 50 mg/kg/day dogs, and decreased plasma albumin (8-14%) and urea (25-33%) in both sexes of 50 mg/kg/day dogs at week 13 and 4 or 8. The increase in alanine aminotransferase in males and females (levels were 3-14 times that of controls for weeks 6-8) could have been due to skeletal muscle damage and/or to liver toxicity. The levels of alanine and aspartate aminotransferase and of creatine kinase returned to baseline by week 13, possibly indicating that the dogs were beginning to recover from some of their treatment-induced injuries. Terminal absolute liver weight (and relative-to-brain weight) was markedly greater than of controls in both sexes given 25 and 50 mg/kg/day (16-25% and 50-56%, respectively; $p \leq 0.01$ for all except the 25 mg/kg/day females). Microscopic liver lesions were not common, suggesting that the liver weight increase might be an adaptive response.

Based on decreased body weight gains, hematological and clinical chemistry alterations, liver toxicity, and voluntary muscle pathological changes, the LOAEL is 25 mg/kg/day for both males or females under the conditions of this study. The NOAEL is 5 mg/kg/day for both sexes.

This study is classified as **acceptable (82-1b)** and satisfies the guideline requirements for a chronic oral toxicity study in dogs.

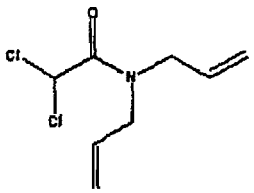
COMPLIANCE: Signed and dated Quality Assurance, Data Confidentiality, GLP and Flagging statements were provided. The only deviation from GLP was failure to give the stability of the test substance.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: Dichlormid (97.2% w/w)

Description: amber liquid
Batch #: WRC 4921-35-12 GGDO101
Purity: 97.2% w/w
Stability of compound: not given; compound was stored at room temperature in a glass container in the dark
CAS #: 37764-25-3
Structure:



2. Vehicle and/or positive control

The test material was given in gelatin capsules (controls received empty capsules).

3. Test animals

Species: dog
Strain: beagle
Age and weight at study initiation: age: 16-21 weeks; weight of males: 8.5-13.0 kg; females: 7.0-16.9 kg
Source: ICI Pharmaceuticals, Alderley Park, Macclesfield, Cheshire, UK
Housing: individually in indoor pens (345 x 115 cm floor) with a heated sleeping area and a separate exercise area
Diet: an expanded dry diet (Laboratory Diet A; Special Diets Services Ltd., Stepfield, Witham, Essex, UK); males received 400 g and females 350 g per day.
Water: potable water was supplied *ad libitum*
Environmental conditions:
Temperature: 19-23°C (16-27°C on occasion)
Humidity: not specified
Air changes: 10 per hour

Photoperiod: 11 hours continuous light in each 24 hours
Acclimation period: 5-6 weeks

B. STUDY DESIGN

1. In life dates

Start: September 6, 1988; end: December 14, 1988 (final necropsy).

2. Animal assignment

Animals were assigned to the test groups in Table 1 by randomization so there would be an even distribution according to litter and body weight.

TABLE 1: Study design

Test group	Mean dose to animal (mg/kg/day)[1]		Number of animals	
	Male	Female	Male	Female
1	0	0	4	4
2	1	1	4	4
3	5	5	4	4
4	25	25	4	4
5	50	50	4	4

Data taken from page 15, MRID 41419401.
[1] Test material was given by capsule.

3. Dose selection rationale

Dose selection was based on the results of a previous 6-week oral dose range-finding study conducted at Central Toxicology Laboratory. No details of this study were provided.

4. Diet preparation and analysis

Animals were fed an expanded dry diet not containing any test material. Dichlormid was administered in 6 mL gelatin capsules immediately prior to feeding (approximately 10 am) daily. The amount of dichlormid was adjusted according to the most recent body weight and for a purity of 97.2% w/w. A Gilson Autodispenser Model 231/401 system was used to fill the capsules. Controls were given empty gelatin capsules.

Results –
Homogeneity analysis: not relevant; dichlormid was given by capsule.

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Stability analysis: stability of the dichlormid given by capsule was not given.

Concentration analysis: not relevant; dichlormid was given by capsule (adjusted for animal body weight and dichlormid purity)

5. Statistics

Analysis of variance (ANOVA) was used to analyze body weight gain, separately for males and females. Organ weights were assessed using ANOVA and analysis of covariance (ANCOVA) on the final body weight, separately for males and females. Clinical biochemistry and hematology were analyzed at each sampling time for the combined male and female data by ANCOVA on the pre-experimental values, with a covariate adjustment based on the separate sex pre-experimental group means. The plasma alanine transaminase, aspartate transaminase, and creatine kinase were additionally analyzed by excluding animals in the 25 and 50 mg/kg/day groups because of the large increases in variation between animals in these two groups. A two-sided Student's t-test was used to compare treatment means with control means and statistical significance was noted at the 5% and 1% levels. The data was also analyzed after excluding "unusual" values, but because no differences in the conclusions were found, the results of this analysis were not given.

C. METHODS

1. Observations

Animals were inspected daily at dosing and usually twice more during the day for evidence of clinical and behavioral abnormalities. Fecal consistency was recorded daily. A full clinical examination including cardiac and pulmonary auscultation was given at week -2 and prior to termination.

2. Body weight

All animals were weighed once a week during the pre-experimental period, on study day 1, and weekly thereafter.

3. Food consumption and compound intake

Food consumption for each animal was determined daily by measuring the amount of food residue prior to the next feeding (residual food was discarded). The daily food residues were measured starting at or before week -2 and continued throughout the experiment. The daily compound intake, given in gelatin capsules, was adjusted for animal body weight and dichlormid purity. Food efficiencies were not calculated.

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4. Ophthalmoscopic examination

An (indirect) ophthalmoscopic examination was given at week -2 and prior to termination.

5. Blood was collected before feeding from the jugular vein from all animals at weeks -1, 4, 8, and 13 for hematology and clinical chemistry analysis. Plasma alanine transaminase, aspartate transaminase, and creatine kinase were additionally measured at weeks 2, 6, and 10. Blood for clinical chemistry analysis was collected in lithium heparin tubes, and blood for the hematology analysis was collected in tubes containing EDTA or 0.11 M trisodium citrate. The CHECKED (X) parameters were examined.

a. Hematology

X		X	
x	Hematocrit (HCT)* (packed cell vol.)	x	Leukocyte differential count*
x	Hemoglobin (HGB)*	x	Mean corpuscular HGB (MCH)
x	Leukocyte count (WBC)*	x	Mean corpusc. HGB conc.(MCHC)
x	Erythrocyte count (RBC)*	x	Mean corpusc. volume (MCV)
x	Platelet count*		Reticulocyte count
x	Blood clotting measurements*		
	(Thromboplastin time)		
x	(Kaolin-cephalin time)		
x	(Prothrombin time)		

* Required for subchronic studies based on Subdivision F Guidelines.

b. Clinical chemistry

X	ELECTROLYTES	X	OTHER
x	Calcium*	x	Albumin*
	Chloride*	x	Plasma creatinine*
	Magnesium	x	Blood urea nitrogen*
x	Phosphorus*	x	Total Cholesterol
x	Potassium*		Globulins
x	Sodium*	x	Glucose*
		x	Total bilirubin
	ENZYMES	x	Total serum protein (TP)*
x	Alkaline phosphatase (ALK)	x	Triglycerides
	Cholinesterase (ChE)		Serum protein electrophores
x	Creatine phosphokinase		
	Lactic acid dehydrogenase (LDH)		
x	Plasma alanine amino-transferase (also Plasma alanine transaminase)*		
x	Plasma aspartate amino-transferase (also plasma aspartate transaminase)*		
	Gamma glutamyl transferase (GGT)		
	Glutamate dehydrogenase		

* Required for subchronic studies based on Subdivision F Guidelines

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6. Urinalysis

Urine was collected from all dogs prior to the beginning of treatment and at study termination. The CHECKED (X) parameters were examined.

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

7. Sacrifice and pathology

At the end of the 90-day treatment period, all dogs were sacrificed by exsanguination under sodium pentobarbitone anesthesia and subjected to gross pathological examination. The CHECKED (X) tissues were examined histologically in all animals after being fixed, processed, embedded in paraffin wax, sectioned, and stained with hematoxylin and eosin. Additional liver samples were taken as fresh frozen sections for fat content evaluation and treated with Rossman's fixative for glycogen analysis. The (XX) organs, in addition, were weighed.

X	DIGESTIVE SYSTEM	X	CARDIOVASC./HEMAT	X	NEUROLOGIC
	Tongue	x	Aorta*	xx	Brain*
x	Salivary glands*	x	Heart*	x	Periph. nerve*
x	Esophagus*	x	Bone marrow*	x	Spinal cord (3 levels ??)
x	Stomach*	x	Lymph nodes*	x	Pituitary*
x	Duodenum*	x	Spleen*	x	Eyes (optic n.)
x	Jejunum*	x	Thymus*		
x	Ileum*				GLANDULAR
x	Cecum*		UROGENITAL	xx	Adrenal gland*
x	Colon*	xx	Kidneys*+		Lacrimal gland
x	Rectum*	x	Urinary bladder*	x	Mammary gland
xx	Liver*+	xx	Testes*+	x	Parathyroids*++
x	Gall bladder*	x	Ovaries	xx	Thyroids*++
x	Pancreas*	xx	Epididymides		
		x	Prostate		OTHER
	RESPIRATORY		Seminal vesicle	x	Bone
x	Trachea*	x	Uterus*	x	Skeletal muscle
x	Lung*	x	Cervix	x	Skin
	Nose			x	All gross lesions and masses*
	Pharynx				
	Larynx				

* Required for subchronic studies based on Subdivision F Guidelines
+ Organ weight required in subchronic and chronic studies.
++ Organ weight required for non-rodent studies.

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II. RESULTS

A. OBSERVATIONS

1. Toxicity

There were no treatment-related signs in dogs given 0, 1, 5, or 25 mg/kg/day dichlormid. The two animals sacrificed at week 7 (one male and one female given 50 mg/kg/day) were thin, had subdued behavior, and the female salivated at dosing and had pale buccal membranes. Another female given 50 mg/kg/day had tremors on one occasion during week 8.

2. Mortality

One male (# 733) and one female (#739) given 50 mg/kg/day were sacrificed during week 7 due to poor physical condition and inappetence.

B. BODY WEIGHT AND WEIGHT GAIN

For weeks 1-13, the weight gain of both sexes of dogs given 1 or 5 mg/kg/day dichlormid was comparable to the controls. Males given 25 or 50 mg/kg/day gained somewhat more weight than the controls during the first 5 weeks of the study, but gained substantially less weight than controls during weeks 5-13 and overall (63% and 46% of controls for 25 and 50 mg/kg/day dogs, respectively). Females given 25 or 50 mg/kg/day dichlormid gained less weight than controls throughout the study; their overall gain was 43% and 32% of controls, respectively. Statistical analysis by the study author showed that the decreases were statistically significant for several time intervals and overall for the 50 mg/kg/day males and for many time intervals and overall for the 25 and 50 mg/kg/day females. The weekly body weights of the animals were not given for weeks 1-12 (although they could be calculated from the weight gain data) and they were not statistically analyzed. The final body weights (week 13) were significantly lower than controls for the 50 mg/kg/day males (90% of controls, $p \leq 0.05$) and for the 25 and 50 mg/kg/day females (90 and 89% of controls, respectively, $p \leq 0.05$). The mean body weights and body weight gains at selected time intervals are shown in Table 2.

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TABLE 2. Body weights (BW) (kg) and cumulative body weight gains (gain) (kg) of dogs fed dichlormid for 13 weeks [1]

Time interval	Dose (mg/kg/day)						
	0	1	5	25		50	
Males							
Initial BW	14.13	13.92	14.07	14.13		13.63	
Final BW	15.60	16.09	15.90	15.11	(97)	14.09*	(90)
week 0-2 gain	0.27	0.32	0.47	0.35	(130)	0.61*	(226)
week 0-5 gain	0.86	1.09	0.99	0.90	(105)	1.08	(126)
week 0-9 gain	1.48	1.77	1.64	1.09	(74)	0.67*	(45)
week 0-13 gain	1.71	1.84	1.88	1.07	(63)	0.79*	(46)
week 5-13 gain[2]	0.61	1.08	0.84	0.08	(13)	-0.62	(–)
Females							
Initial BW	12.22	11.82	11.45	11.47		11.60	
Final BW	13.75	13.20	13.26	12.39*	(90)	12.26*	(89)
week 0-2 gain	0.28	0.29	0.39	0.25	(89)	0.05	(18)
week 0-5 gain	0.82	0.75	0.91	0.58	(71)	0.51	(62)
week 0-9 gain	1.34	1.41	1.32	0.47*	(35)	0.39*	(29)
week 0-13 gain	1.64	1.56	1.78	0.71*	(43)	0.52*	(32)
week 5-13 gain[2]	0.71	0.63	0.90	0.34	(48)	0.15	(21)

Data taken from Table 5, pp. 46-47, MRID 41419401.
[1]Numbers in parentheses are the percent of controls, calculated by reviewer.
[2]Calculated by the reviewer; no statistical analysis conducted.

C. FOOD CONSUMPTION AND COMPOUND INTAKE

1. Food consumption

The vast majority of the dogs ate all of their daily food rations every day. The two animals given 50 mg/kg/day and sacrificed during week 7 due to poor physical condition and inappetence (male # 733 and female # 739) left all their food for 1-3 days during weeks 6-7. One male given 1 mg/kg/day left 9-29% of his food at weeks 8-11 and one male given 25 mg/kg/day left 2-4% of his food at weeks 8-9.

2. Compound consumption

Animals were given the test compound by capsule to achieve target doses of 1, 5, 25, or 50 mg/kg/day, as shown in Table 1. The amount given was adjusted for animal body weight and compound purity.

3. Food efficiency

Food efficiency was not calculated since the diet was restricted.. However, since all dose groups ate virtually all of their allocated food (except for the two sacrificed dogs) males and females given 25 or 50 mg/kg/day dichlormid gained less weight, the food efficiencies of these groups is likely lower than of the controls.

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D. OPHTHALMOSCOPIC EXAMINATION

There were no treatment-related ophthalmological findings.

E. BLOOD WORK

1. Hematology

The most significant hematological changes were the decreases in hemoglobin, hematocrit, and RBC count in the 50 mg/kg/day males and females ($p \leq 0.05$ or 0.01). The hemoglobin and hematocrit were 11-17% lower than controls at weeks 8 and 13 and the RBC count was 10-14% lower at weeks 8 and/or 13. These three parameters were also decreased (12-14%, $p \leq 0.05$ or 0.01) in females given 25 mg/kg/day dichlormid. Results are summarized in Table 3. Other statistically significant changes ($p \leq 0.05$ or 0.01) were of small magnitude and/or not clearly dose-related and not biologically significant. These included decreased MCV and MCH (4-6% at 50 mg/kg/day for males and/or females), increased platelet count (29-54% in 50 mg/kg/day males and 19-25% in 25 mg/kg/day females), decreased lymphocyte count (34% in 50 mg/kg/day females), decreased monocyte count (43-60% in 1 and 5 mg/kg/day females), increased eosinophil count (118% in 50 mg/kg/day males; 299% in 1 mg/kg/day females), and decreased prothrombin and kaolin-cephalin time (6-14% in 5 and 50 mg/kg/day males).

TABLE 3. Hematology parameters altered in dogs given dichlormid by capsule for 13 weeks [1]

Parameter	week	Dose (mg/kg/day) 0	1	5	25	50[2]
Males						
Hemoglobin (g/dL)	0	15.3	15.2	14.7	14.8	15.0
	4	14.9	15.2	15.0	15.2	14.2
	8	14.6	16.0* (110)	15.3	14.2	12.2** (84)
	13	15.1	15.8	15.6	14.9	13.3* (88)
Hematocrit	0	0.426	0.420	0.418	0.413	0.416
	4	0.407	0.430	0.415	0.414	0.399
	8	0.380	0.415*	0.395	0.371	0.315** (83)
	13	0.414	(109) 0.436	0.429	0.413	0.370* (89)
RBC count ($\times 10^{12}$/L)	0	6.44	6.29	6.27	6.13	6.27
	4	6.22	6.67	6.37	6.25	6.20
	8	5.97	6.54* (110)	6.26	5.79	5.16* (86)
	13	6.28	6.44	6.47	6.22	5.87
Females						
Hemoglobin (g/dL)	0	16.4	15.0	14.9	15.5	16.0
	4	15.5	15.1	15.3	15.2	15.0
	8	15.4	14.8	14.8	13.6** (88)	13.4** (87)
	13	15.8	16.0	15.4	14.9	13.8** (87)
Hematocrit	0	0.460	0.408	0.413	0.428	0.454
	4	0.426	0.420	0.422	0.430	0.409
	8	0.403	0.385	0.384	0.353* (88)	0.348** (86)
	13	0.435	0.442	0.425	0.416	0.381** (88)
RBC count ($\times 10^{12}$/L)	0	6.93	6.18	6.13	6.45	6.72
	4	6.65	6.38	6.39	6.59	6.40
	8	6.47	6.04	6.02	5.59** (86)	5.63** (87)
	13	6.60	6.59	6.36	6.26	5.96* (90)

Data taken from Table 6, pp. 49-55, MRID 41419401.
Significantly different from controls: *$p \leq 0.05$; **$p \leq 0.01$.
[1]Numbers in parentheses are the percent of controls, calculated by reviewer.
[2]There were 4 dogs/sex/dose except at the 8 and 13 week time points there were only 3 dogs/sex at 50 mg/kg/day (due to sacrifice).

2. Clinical chemistry

Results for the clinical chemistry evaluations are presented in Tables 4 (males) and 5 (females). Plasma alanine transaminase, aspartate transaminase, and creatine kinase were markedly elevated in 25 and 50 mg/kg/day dogs of both sexes (statistical analysis was not conducted) for weeks 4-10. The most extreme changes occurred at weeks 6 and 8: alanine transaminase was 3 to 14 times the control levels, aspartate transaminase was 4-24 times the control levels, and creatine kinase was 7-107 times the control levels. Levels of all three enzymes returned to baseline by week 13. Alkaline phosphatase (AP) activity was moderately increased (38-70%, $p \leq 0.05$ or 0.01) at week 13 in both sexes of 25 and 50 mg/kg/day dogs; the latter animals also had increased AP at week 4 (males) or 8 (females).

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Several other clinical chemistry values differed significantly ($p \leq 0.05$ or 0.01) from that of the control groups. Plasma albumin was decreased 8-14% ($p \leq 0.05$) in males and females given 50 mg/kg/day dichlormid at week 13 and at week 4 in males and week 8 in females. Plasma urea was lowered 16-33% in 1, 5, and 50 mg/kg/day males and 17-25% in 25 and 50 mg/kg/day females at week 13 ($p \leq 0.05$ or 0.01). Creatinine levels were 11-29% lower than controls at weeks 8 and 13 for $\geq$ 5 mg/kg/day for males and/or females. Cholesterol levels were increased 33-77% in all groups of treated males at week 8 and/or 13, and were increased 41% in 50 mg/kg/day females at week 8. Statistically significant alterations in other parameters were sporadic, of small magnitude, and considered incidental to treatment.

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TABLE 4. Clinical chemistry parameters in male dogs given dichlormid by capsule for 13 weeks [1]

Parameter	week	Dose (mg/kg/day)				
		0	1	5	25	50[2]
Albumin (g/100 mL)	0	3.70	3.72	3.70	3.65	3.72
	4	3.83	3.92	3.75	3.69	3.46* (90)
	8	3.74	3.86	3.77	3.78	3.50
	13	3.85	3.88	3.76	3.74	3.31* (86)
Urea (mg/100 mL)	0	28.5	32.8	34.5	25.5	28.3
	4	31.4	30.6	25.0	31.0	29.5
	8	31.0	35.1	29.5	33.0	26.7
	13	31.5	26.5* (84)	25.8* (82)	29.5	21.0** (67)
Creatinine (mg/100 mL)	0	1.02	1.05	1.07	0.92	0.88
	4	0.92	0.95	0.88	0.92	0.99
	8	0.91	1.02	0.90	0.81	0.65** (71)
	13	0.99	1.07	0.92	0.81** (82)	0.78** (79)
Cholesterol (mg/100 mL)	0	154	140	151	150	156
	4	140	184	172	182	183
	8	132	187* (142)	175* (133)	182* (138)	233** (177)
	13	127	164	167	175* (138)	203** (160)
Alkaline phosphatase (mU/mL)	0	203	187	186	188	191
	4	142	172	175	170	183* (129)
	8	139	168	169	174	190
	13	112	143	146	155* (138)	182** (163)
Plasma alanine transaminase[3] (mU/mL)	0	20.8	23.5	25.5	25.5	20.0
	2	24.3	26.3	28.8	18.8	14.8
	4	23.3	25.5	27.8	16.5	20.8
	6	24.3	23.8	28.0	69.0 (284)	320.3 (1318)
	8	20.5	20.0	24.8	155.0 (756)	164.0 (800)
	10	25.5	25.3	28.0	62.3 (244)	38.7 (152)
	13	24.3	21.8	26.0	19.5	15.0
Plasma aspartate transaminase[3] (mU/mL)	0	16.8	16.0	17.0	18.0	16.3
	2	18.5	15.3	17.8	17.5	19.3
	4	17.0	17.5	17.5	18.8	22.5
	6	17.5	17.5	17.0	107.3 (613)	427.8 (2445)
	8	16.0	13.8	13.8	131.0 (819)	138.3 (864)
	10	19.5	16.0	17.5	40.0 (205)	27.7 (142)
	13	17.3	15.0	15.5	18.5	16.3
Creatine kinase[3] (mU/mL)	0	51.3	46.5	46.8	44.5	50.3
	2	70.5	44.5	48.5	55.0	67.8
	4	73.3	49.3	50.5	58.5	93.8
	6	41.3	39.0	48.8	1030.0 (2494)	4453.8 (10784)
	8	46.3	55.8	36.0	936.8 (2023)	901.3 (1947)
	10	46.0	49.5	37.5	181.8 (395)	91.0 (198)
	13	47.5	36.3	45.3	47.3	37.3

Data taken from Tables 8-11, pp. 56-68, MRID 41419401.
Significantly different from controls: *p ≤ 0.05; **p ≤ 0.01.
[1]Numbers in parentheses are the percent of controls, calculated by reviewer.
[2]There were 4 dogs/sex/dose except at the 8 and 13 week time points there were only 3 dogs/sex (due to sacrifice).
[3]Alanine and aspartate transaminase and creatine kinase were not statistically analyzed.

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TABLE 5. Clinical chemistry parameters in female dogs given dichlormid by capsule for up to 13 weeks[1]

Parameter	week	Dose (mg/kg/day)				
		0	1	5	25	50[2]
Albumin (g/100 mL)	0	3.65	3.57	3.60	3.52	3.52
	4	3.85	3.82	3.79	3.82	3.88
	8	3.64	3.68	3.66	3.56	3.34* (92)
	13	3.78	3.71	3.66	3.49	3.30* (87)
Urea (mg/100 mL)	0	28.3	31.3	30.0	28.0	28.5
	4	28.2	29.5	32.6	29.8	31.1
	8	31.7	32.8	33.4	30.0	32.8
	13	31.1	31.3	28.5	25.8* (83)	23.3** (75)
Creatinine (mg/100 mL)	0	0.82	1.02	1.10	1.00	0.75
	4	0.97	0.98	0.92	0.96	1.02
	8	1.02	1.03	0.91	0.78** (76)	0.76** (75)
	13	1.06	1.10	0.94* (89)	0.79** (75)	0.82** (77)
Cholesterol (mg/100 mL)	0	149	139	140	135	138
	4	150	149	162	158	172
	8	128	140	150	165	180* (141)
	13	128	142	147	155	168
Alkaline phosphatase (mU/mL)	0	203	213	197	184	180
	4	194	172	183	194	216
	8	153	168	170	190	260** (170)
	13	122	146	142	188** (154)	207** (170)
Plasma alanine transaminase[3] (mU/mL)	0	18.5	18.8	18.8	22.0	18.3
	2	19.8	24.5	21.8	21.3	18.3
	4	17.8	21.3	18.3	20.8	17.5
	6	20.0	23.5	21.0	137.0 (685)	276.3 (1382)
	8	19.0	21.8	17.8	154.8 (815)	115.3 (607)
	10	19.8	23.8	19.5	34.8 (176)	25.7 (130)
	13	17.8	24.5	19.5	21.5	19.3
Plasma aspartate transaminase[3] (mU/mL)	0	18.0	16.3	16.0	15.0	15.5
	2	18.0	20.8	24.0	21.8	16.0
	4	15.3	20.5	21.3	19.5	21.3
	6	17.5	18.8	19.0	182.8 (1044)	363.0 (2074)
	8	14.5	16.5	17.8	71.3 (492)	59.0 (407)
	10	16.8	19.5	18.5	19.8	17.0
	13	14.5	17.8	17.3	16.8	14.3
Creatine kinase[3] (mU/mL)	0	43.3	61.8	56.8	53.5	47.5
	2	51.3	75.5	62.3	85.3	67.0
	4	44.0	61.5	49.5	60.0	134.0 (305)
	6	44.8	74.5	52.0	2287.0 (5105)	3915.3 (8739)
	8	41.8	58.8	105.5	546.5 (1307)	302.0 (722)
	10	36.5	51.0	(252)	64.3	43.7
	13	33.8	61.8	41.3	41.5	36.7
				50.8		

Data taken from Tables 8-11, pp. 56-68, MRID 41419401.
Significantly different from controls: *p ≤ 0.05; **p ≤ 0.01.
[1]Numbers in parentheses are the percent of controls, calculated by reviewer.
[2]There were 4 dogs/sex/dose except at the 8 and 13 week time points there were only 3 dogs/sex (due to sacrifice).
[3]Alanine and aspartate transaminase and creatine kinase were not statistically analyzed.

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F. URINALYSIS

Urinalysis revealed no notable differences between treated and control dogs. The only statistically significant change ($p \leq 0.01$) was a 2% decrease in urine specific gravity at week 13 in males given 50 mg/kg/day dichlormid.

G. SACRIFICE AND PATHOLOGY

1. Organ weight

The most marked change was the dose-related increase in liver weight (absolute and adjusted for body weight) in both sexes. The increase was statistically significant for the 25 and 50 mg/kg/day animals; the absolute weight increase was 16-25% and 50-56%, respectively. Kidney and thyroid weights were statistically significantly greater than of controls ($p \leq 0.05$ or 0.01) in the 25 and/or 50 mg/kg/day females; the thyroid weight increases were not dose-related. Males given 50 mg/kg/day dichlormid had approximately 20% lower weight of epididymides than the control group. Results are summarized in Table 6. The relative-to-brain weights for the above-mentioned organs, (calculated by the reviewer; results not shown) yielded very similar results.

TABLE 6: Organ weights, absolute and adjusted to final body weights, of dogs given dichlormid for 13 weeks[1]

Organ and absolute or relative weight	Dose (mg/kg/day)				
	0	1	5	25	50[2]
Males					
Liver : absolute (g)	471	478	518	590** (125)	707** (150)
Adjusted for BW (g)	463	473	509	597** (129)	727** (157)
Kidney: absolute (g)	66.5	64.0	64.3	70.7	76.9
Adjusted for BW (g)	66.0	63.6	63.7	71.2	78.2
Thyroid: absolute (g)	1.001	1.064	1.133	1.200	1.268 (127)
Adjusted for BW (g)	0.988	1.055	1.119	1.211	1.301 (132)
Epididymides: absolute (g)	4.24	4.49	4.24	4.17	3.28* (77)
Adjusted for BW (g)	4.17	4.44	4.17	4.24	3.46 (83)
Females					
Liver : absolute (g)	405	395	425	468 (116)	632** (156)
Adjusted for BW (g)	370	381	413	504** (136)	666** (180)
Kidney: absolute (g)	55.2	54.0	54.5	57.3	67.6** (122)
Adjusted for BW (g)	54.5	53.7	54.2	58.0	68.3* (125)
Thyroid: absolute (g)	0.910	0.911	0.905	1.167* (128)	1.018 (112)
Adjusted for BW (g)	0.879	0.899	0.894	1.199* (136)	1.048 (119)

Data taken from Table 14, pages 73-75, MRID 41419401.
Significantly different from controls: *$p \leq 0.05$; **$p \leq 0.01$.
[1]Numbers in parenthesis are the percent of controls, calculated by the reviewer.
[2]There were only 3 dogs/sex in this group due to the early sacrifice of one animal.

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2. Gross pathology

The most common finding in males was pale voluntary muscle: this was seen at 50 mg/kg/day in the premature decedent and in 2/3 males at terminal sacrifice. Other lesions in the early sacrifice male included a pale and/or discolored heart, liver, and tonsils (also enlarged). Terminal sacrifice males had a reduced prostate gland (2/3 at 50 mg/kg/day), discolored lungs (1/4 at 25 mg/kg/day; 1/3 at 50 mg/kg/day), a pituitary gland cyst, a reduced/flaccid testis, and red spots on the urinary bladder mucosa (1/3 at 50 mg/kg/day for each); the control incidence was 0/4 for these lesions.

The 50 mg/kg/day female sacrificed early had a pale and/or discolored heart, liver, and duodenum and jejunum contents. Lesions found in the females (1/3 or 1/4 each) included pale and atrophied voluntary muscle, discolored lungs (25 and 50 mg/kg/day), pale liver, stomach thickening (50 mg/kg/day), and urinary bladder red mucosal spots (5 and 50 mg/kg/day).

3. Microscopic pathology

Degeneration of the voluntary muscle (with varying amounts of cellular infiltration) was the most notable finding in both sexes of dogs given 25 or 50 mg/kg/day dichlormid; this was the only lesion with a statistically significantly greater incidence than in controls ($p \leq 0.05$). It was found in all the treated 25 and 50 mg/kg/day animals (both the early sacrifice and terminal sacrifice dogs) and its severity was greatest in the early-sacrifice 50 mg/kg/day male. Microscopic lesions were also seen in the liver, and included glycogen depletion and portal inflammation (early-sacrifice animals only), increased periportal fat (early and terminal sacrifice animals; more severe early), and Kupffer cell pigmentation (terminal-sacrifice animals only). The incidence of the liver lesions was only slightly greater in the treated than the control groups and was not clearly dose-related. Several animals had chronic (lung) pneumonitis and/or peribronchial inflammation; in males the incidence was not dose-related. Lesions were seen sporadically in other organs of both the treated and control animals; these were not considered treatment-related. The microscopic findings are summarized in Table 7.

TABLE 7: Microscopic findings in dogs (premature decedents and terminal sacrifice) given dichlormid for 13 weeks

Organ: lesion	Dose (mg/kg/day)				
	0	1	5	25	50
Males					
Liver : Glycogen depletion	0/4	0/4	0/4	0/4	1/4†
Periportal fat increase	1/4	0/4	0/4	0/4	1/4†
Kupffer cell pigmentation	1/4	1/4	2/4	1/4	2/4
Lung: Chronic pneumonitis	1/4	0/4	0/4	0/4	1/4
Peribronchial inflammation	1/4	1/4	0/4	3/4	1/4
Voluntary muscle: Myodegeneration; cellular infiltrate	0/4	0/4	0/4	4/4*	4/4† *
Females					
Liver : Glycogen depletion	0/4	0/4	0/4	0/4	1/4†
Periportal fat increase	0/4	1/4	0/4	1/4	1/4†
Portal inflammation	0/4	0/4	0/4	0/4	1/4†
Kupffer cell pigmentation	1/4	1/4	1/4	2/4	2/4
Lung: Peribronchial inflammation	0/4	0/4	1/4	2/4	3/4
Voluntary muscle: Myodegeneration; cellular infiltrate	0/4	0/4	0/4	4/4*	4/4† *

Data taken from Table 16, pages 82-89, MRID 41419401.
Significantly different from controls: *p ≤ 0.05 (calculated by reviewer using Fisher exact test)
†Of the given incidence, 1/4 is due to the premature decedent (sacrificed at week 7).

III. DISCUSSION

A. DISCUSSION

In a subchronic toxicity study (MRID 41419401) dichlormid was administered by capsule to 4 beagle dogs/sex/dose at doses of 0, 1, 5, 25, or 50 mg/kg/day for 90 days.

One male and one female given 50 mg/kg/day dichlormid were sacrificed during week 7 due to poor physical condition and inappetence. These animals were thin, subdued, and the female salivated at dosing and had pale buccal membranes. All other animals survived to the terminal sacrifice. One female given 50 mg/kg/day dichlormid had tremors once during week 8.

The weight gain of dogs given 1 or 5 mg/kg/day dichlormid was comparable to the controls, but was substantially lower in dogs given 25 and 50 mg/kg/day. The body weight gain of the 25 and 50 mg/kg/day males was lowered during weeks 6-13 and was 37-54% lower overall; the decrease was statistically significant ($p \leq 0.05$) for the 50 mg/kg/day males. The 25 and 50 mg/kg/day females gained 57-68% less weight overall than controls; lowered weight gain was seen throughout the study ($p \leq 0.05$ for many time intervals and overall). Week 13 body weights were

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significantly lower than controls (10-11%, $p \leq 0.05$) for both sexes at 50 mg/kg/day and for the 25 mg/kg/day females.

There were no treatment-related ophthalmological findings. Urinalysis revealed no notable differences between treated and control dogs; the 2% decrease in urine specific gravity of 50 mg/kg/day males at week 13 ($p \leq 0.01$) appeared to be incidental to treatment.

Treatment-related hematological changes consisted of decreased hemoglobin, hematocrit, and RBC count (10-17%, $p \leq 0.05$ or 0.01). The decrease was seen at weeks 8 and/or 13 in both sexes of dogs given 50 mg/kg/day, and in females given 25 mg/kg/day. These changes are indicative of hemolytic anemia, although there were no correlated histopathological alterations.

There were numerous clinical chemistry changes, the most marked being the increases in plasma alanine transaminase, aspartate transaminase, and creatine kinase in 25 and 50 mg/kg/day dogs of both sexes. The enzyme levels were increased for weeks 4-10 (no statistical analysis), but the most extreme changes occurred at weeks 6 and 8: alanine transaminase, aspartate transaminase, and creatine kinase were, respectively, 3-14, 4-24, and 7-107 times the control levels. Unexpectedly, levels of all three enzymes were back to baseline by week 13, suggesting that some injury to the dogs occurred at week 6-8 and the animals had recovered by week 13. Alkaline phosphatase (AP) activity was moderately increased (38-70%, $p \leq 0.05$ or 0.01) in both sexes of 25 and 50 mg/kg/day dogs at week 13 (and week 4 or 8). Plasma albumin, urea, and creatinine levels were decreased (8-14%, 25-33%, and 21-29%, respectively; $p \leq 0.05$ or 0.01) in both sexes primarily in the 50 mg/kg/day groups. Cholesterol levels were elevated at week 8 and/or 13 in both sexes given 50 mg/kg/day (41-77%; $p \leq 0.05$ or 0.01); increases seen in other groups of males were not dose-related.

The clinical chemistry changes were consistent with the observed liver and skeletal muscle pathology. The extreme increase in creatine kinase and the decrease in creatinine are consistent with skeletal muscle damage, which was evident microscopically in all dogs given 25 or 50 mg/kg/day dichlormid as myodegeneration with varying amounts of cellular infiltrate infiltration. At the gross level, the voluntary muscle appeared pale in the 50 mg/kg/day animals (3/4 males and 1/4 females). A few males and females given 50 mg/kg/day dichlormid (and occasionally 5 or 25 mg/kg/day) had a pale and/or discolored heart, liver, lungs, tonsils, duodenum and jejunum contents and/or and red spots on the urinary bladder mucosa (1/4 each vs. 0/4 for controls). It is unknown whether dichlormid caused these "discolorations" as well, although their incidences are too low to draw definitive conclusions.

Indicators of liver toxicity included decreased plasma albumin and urea and increased cholesterol, aspartate aminotransferase and alkaline phosphatase. The increase in alanine aminotransferase (3-14 times controls) could have been be due to either skeletal muscle damage and/or to liver toxicity. There was a marked increase in the absolute liver weight (and relative-to-brain weight) in both sexes given 25 and 50 mg/kg/day (16-25% and 50-56%, respectively; $p \leq 0.01$ for all except the 25 mg/kg/day females). Microscopic liver lesions were seen in only a few animals and were not clearly dose-related (glycogen depletion, portal inflammation, increased periportal fat, and Kupffer cell pigmentation); the increased periportal fat and glycogen depletion were more severe in the two early-sacrifice dogs than in the terminal sacrifice dogs. Because the incidence and severity of the microscopic lesions were not consistent with the large increase in liver weights, it appears that the liver weight increase was an adaptive response to dichlormid treatment (e.g., caused by induction of detoxification enzymes).

Peribronchial inflammation was seen microscopically in several dogs of both sexes; males also had chronic pneumonitis. The incidence of the lesions in males was not dose-related; in females the incidence of peribronchial inflammation increased with dose. It is unclear whether the lung lesions in females were caused by dichlormid treatment.

The statistically significant increased kidney and thyroid weights in the 25 and/or 50 mg/kg/day females lacked histopathological correlates and did not appear to be treatment-related. The "reduced" prostate gland, reduced/flaccid testis, and 20% decrease in weight of epididymides in 50 mg/kg/day males also lacked histopathological correlates and may not be toxicologically significant.

Based on decreased body weight gains, hematological and clinical chemistry alterations, liver toxicity, and voluntary muscle pathological changes, the LOAEL is 25 mg/kg/day for both males or females under the conditions of this study. The NOAEL is 5 mg/kg/day for both sexes.

B. STUDY DEFICIENCIES

There were no serious study deficiencies. Minor shortcomings include the lack of test compound stability data and of calculated food efficiencies; these did not affect the validity of the study. Presumably the compound stability is available from the study sponsor.

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DATA EVALUATION REPORT 014199

DICHLORMID

STUDY TYPE: SUBCHRONIC ORAL TOXICITY FEEDING - DOG (82-1b)

Prepared for

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

Prepared by

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Toxicology and Risk Analysis Section
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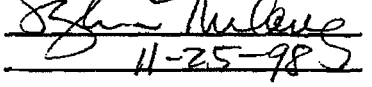
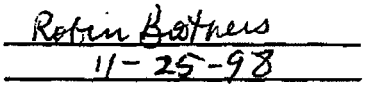
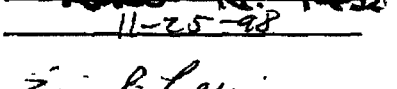
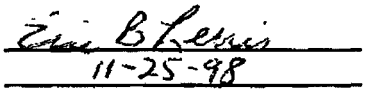
Task Order No. 98-36D

Primary Reviewer: Sylvia Milanez, Ph.D., D.A.B.T.	Signature: Date:	 11-25-98
Secondary Reviewers: Robin A. Brothers, Ph.D., D.A.B.T.	Signature: Date:	 11-25-98
Robert H. Ross, M.S., Group Leader	Signature: Date:	 11-25-98
Quality Assurance: Eric B. Lewis, M.S.	Signature: Date:	 11-25-98

Disclaimer

This review may have been altered subsequent to the contractor's signatures above.

Oak Ridge National Laboratory, managed by Lockheed Martin Energy Research Corp. for the U.S. Department of Energy under contract number DE-AC05-96OR22464.

DICHLORMID Subchronic Oral Study (82-1b)

EPA Reviewer: Laurence D. Chitlik, DABT Laurence Chitlik, Date 5/24/99
Toxicology Branch 1 (7509C)
EPA Work Assignment Manager: S. Diwan, Ph.D. Sanjiv Diwan, Date 6/17/99
Toxicology Branch 1 (7509C)

014199

DATA EVALUATION RECORD

STUDY TYPE: Subchronic Oral Toxicity Feeding-Dog
OPPTS 870.3150 [§82-1b]

DP BARCODE: D248305
P.C. CODE: 900497

SUBMISSION CODE: S546651
TOX. CHEM. NO.:

TEST MATERIAL (PURITY): Dichlormid (one lot: 99.5% a.i.; second lot 97.7 ± 2% a.i.)

SYNONYMS: R-25788

CITATION: Woodard, M. et al. 1972. R-25788. Safety evaluation by dietary feeding to dogs for 13 weeks. Woodard Research Corporation, 12310 Pinecrest Road, Herndon, VA 22070. Project 45WR1862. May 17, 1972. MRID 00058468 Unpublished.

SPONSOR: Stauffer Chemical Company, Research Laboratories, Mountain View, CA.

EXECUTIVE SUMMARY: In a subchronic toxicity study (MRID 00058468) dichlormid (R-25788) (lot # 1792-18: 99.5% pure; lot # WRC-2012-9 (unclear if this is a lot number): 97.7 ± 2% pure) was administered for 13 weeks to 4 beagle dogs/sex/dose in the diet at concentrations of 0, 80, 240, or 960 ppm (0, 2, 6, or 24 mg/kg/day, respectively, calculated using a dog food factor of 0.025).

No animals died or exhibited treatment-related clinical signs or adverse effects on body weight gain, blood pressure, electrocardiograms, heart rates, or on hematology, ophthalmology, or urinalysis parameters. Necropsy revealed no treatment-related effects on organ weights or gross or microscopic pathology. The increase in serum alkaline phosphatase (AP) in high-dose animals for weeks 4-13, and in mid-dose males at week 4 were each largely due to increases in one of the four animals in each group.

Under the conditions of this study, a LOAEL cannot be assigned because there were no treatment-related toxicologically significant findings. The NOAEL is ≥ 960 ppm (calculated as 24 mg/kg/day using the dog food factor of 0.025), the highest concentration tested.

This study is classified as **unacceptable (82-1b) (Not upgradeable)** and does not satisfy the guideline requirements for a chronic oral toxicity study in dogs because the animals were not adequately dosed (high dose was 960 ppm, or about 24 mg/kg/day), the test diets were not analyzed for stability, homogeneity, and concentration, and omission of much other required data. It is likely

many of these deficiencies occurred because this study was conducted before GLP guidelines were developed.

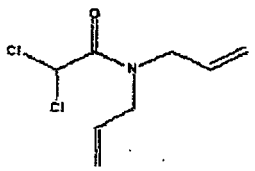
COMPLIANCE: Signed and dated Quality Assurance, Data Confidentiality, GLP and Flagging statements were not provided; the study was conducted before GLP guidelines were in effect (11/29/83).

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: Dichlormid (R-25788) (one lot: 99.5% a.i.; second lot 97.7 ± 2% a.i.)

   Description: none given
   Lot/Batch #: 1792-18; WRC-2012-9 (unclear if this is a lot number):
   Purity: lot # 1792-18: 99.5% pure; lot # WRC-2012-9 (unclear if this is a lot number): 97.7 ± 2%
   Stability of compound: not given
   CAS #: 37764-25-3
   Structure:



2. Vehicle and/or positive control

   The test material was given in the feed.

3. Test animals

   Species: dog
   Strain: beagle
   Age and weight at study initiation: age: "young adult"; weight of males: 8.5-13.0 kg; females: 6.4-16.9 kg
   Source: Hazleton Research Animals Inc., Richmond, VA and Antec Corp., Leesburg, VA.
   Housing: not described
   Diet: 200 g Dietrich and Gambrill dog meal plus 45 g canned beef daily (12 g dry weight + sufficient water for palatability)

Water: not specified
Environmental conditions:
Temperature: not specified
Humidity: not specified
Air changes: not specified
Photoperiod: not specified
Acclimation period: ≥ 3 weeks

B. STUDY DESIGN

1. In life dates

Not specified. The reviewer estimates that the start was roughly October or November, 1971 (test compound was received during this time), and the end was approximately March 1972 (the histopathology report was dated May 15, 1972).

2. Animal assignment

The method of assignment of the animals to the test groups, shown in Table 1, was not given.

TABLE 1: Study design

Test Group (as given in study)	Conc. in Diet (ppm)[1]	Mean Dose to Animal (mg/kg/day)[1]		Number of Animals	
		Male	Female	Male	Female
I	0 (Control)	0	0	4	4
IV	80	2	2	4	4
III	240	6	6	4	4
II	960	24	24	4	4

Data taken from page 2, MRID 225002430.
[1]Values calculated (by reviewer) by multiplying the nominal concentration of the test material in the diet by the dog food factor of 0.025.

3. Diet preparation and analysis

The test diets were prepared by dissolving the appropriate amounts of dichlormid in 100 mL acetone and mixing it with a small amount (not specified) of dog meal. The acetone was then permitted to evaporate. Additional dog meal was added to make 6000-g batches containing the desired concentration of test compound. The controls were given the dog meal, but it was not specified if any acetone was added to it. It was not stated how often the test diets were prepared or when animals were given fresh food.

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The stability, homogeneity, and concentration of dichlormid in the diets were not reported.

Results -

Homogeneity Analysis: not reported
Stability Analysis: not reported
Concentration Analysis: not reported

4. Statistics

No statistical analysis was conducted other than calculation of the group means for the four dogs/group.

C. METHODS

1. Observations

Animals were inspected once daily (weeks -3 through 13) for evidence of "untoward" clinical effects, changes in food consumption, behavior, stool consistency, urinary excretions, and emesis. Weekly detailed physical examinations were conducted: the dogs were weighed, and their body temperature, heart rate, respiration rate, skin and hair coat condition, mucous membrane appearance, and locomotor activity were assessed.

Blood pressure, electrocardiograms (lead II), and heart rates were measured at 0, 5, 8, and 13 weeks. Blood pressure was measured using an infant cuff and a condenser microphone taped to the underside of the tail. Electrocardiographic tracings and auscultation were used to measure the heart rates.

2. Body weight

All animals were weighed once a week at weeks -3, -2, -1, 0, and 1-13.

3. Food consumption and compound intake

It was not stated how the food consumption for each animal was determined, only that the animals were inspected daily for changes in food consumption. The total amount of food given to the animals daily was 245 g, which is quite a bit lower than the amount typically given to dogs, i.e. 450 g/day. The daily compound intake was not given but was calculated by the reviewer by multiplying the nominal concentration of the test material in the diet by the dog food factor of 0.025. Food efficiencies were not calculated.

4. Ophthalmoscopic examination

An ophthalmoscopic examination (direct and indirect) using the Keeler Pantoscope was conducted for all dogs initially and at weeks 5, 8, and 13. The intraocular tension was measured by the finger pressure technique.

5. Blood was collected from all animals twice prior to the beginning of treatment and at weeks 4, 8, and 13 for hematology and clinical chemistry analysis. The method of blood collection was not given. The CHECKED (X) parameters were examined.

a. Hematology

X		X	
x	Hematocrit (HCT)* (packed cell vol.)	x	Leukocyte differential count*
x	Hemoglobin (HGB)*		Mean corpuscular HGB (MCH)
x	Leukocyte count (WBC)*		Mean corpusc. HGB conc.(MCHC)
x	Erythrocyte count (RBC)*		Mean corpusc. volume (MCV)
x	Platelet count*		Reticulocyte count
x	Blood clotting measurements*	x	Erythrocyte sedimentation rate
	(Thromboplastin time)		
x	(Clotting time)		
x	(Prothrombin time)		

* Required for subchronic studies based on Subdivision F Guidelines.

b. Clinical chemistry

X	ELECTROLYTES	X	OTHER
	Calcium*		Albumin*
	Chloride*		Blood creatinine*
	Magnesium	x	Blood urea nitrogen*
	Phosphorus*		Total Cholesterol
	Potassium*		Globulins
	Sodium*	x	Glucose*
	_____		Total bilirubin
	ENZYMES		Total serum protein (TP)*
x	Alkaline phosphatase (ALK)		Triglycerides
	Cholinesterase (ChE)		Serum protein electrophores
	Creatine phosphokinase		
	Lactic acid dehydrogenase (LDH)		
x	Serum alanine amino-transferase (also SGPT)*		
x	Serum aspartate amino-transferase (also SGOT)*		
	Gamma glutamyl transferase (GGT)		
	Glutamate dehydrogenase		

* Required for subchronic studies based on Subdivision F Guidelines

6. Urinalysis

Urine was collected from all dogs prior to the beginning of treatment and at study weeks 4, 8, and 13. Urinalysis was conducted on "cage-collected samples"; further details of the collection procedure were not given. The CHECKED (X) parameters were examined.

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

7. Sacrifice and pathology

At the end of the 13-week treatment period, all the dogs were sacrificed by exsanguination under pentobarbital anesthesia and subjected to gross pathological examination. The CHECKED (X) tissues were examined histologically in the control and 960 ppm group animals. The (XX) organs, in addition, were weighed.

X	DIGESTIVE SYSTEM	X	CARDIOVASC./HEMAT.	X	NEUROLOGIC
	Tongue	x	Aorta*	xx	Brain*
x	Salivary glands*	xx	Heart*	x	Periph. nerve*
x	Esophagus*	x	Bone marrow*	x	Spinal cord (3 levels ??)
x	Stomach*	x	Lymph nodes*	xx	Pituitary*
x	Duodenum*	xx	Spleen*		Eyes (optic n.)
x	Jejunum*	x	Thymus*		
x	Ileum*				GLANDULAR
x	Cecum*		UROGENITAL	xx	Adrenal gland*
x	Colon*	xx	Kidneys*+		Lacrimal gland
	Rectum*	x	Urinary bladder*	x	Mammary gland
xx	Liver*+	xx	Testes*+		Parathyroids*++
x	Gall bladder*	xx	Ovaries	xx	Thyroids*++
x	Pancreas*		Epididymides		
		xx	Prostate		OTHER
	RESPIRATORY		Seminal vesicle		Bone
x	Trachea*	xx	Uterus*	x	Skeletal muscle
xx	Lung*			x	Skin
	Nose			??	All gross lesions
	Pharynx				and masses*
	Larynx				

* Required for subchronic studies based on Subdivision F Guidelines
\+ Organ weight required in subchronic and chronic studies.
++ Organ weight required for non-rodent studies.

II. RESULTS

A. OBSERVATIONS

1. Toxicity

The clinical signs for the animals were not given in the study report. It was stated that "No pharmacologic nor toxicologic signs were seen that could be ascribed with certainty to the administration of R-25788." Blood pressure, electrocardiograms, and heart rates were comparable in treated and control animals.

2. Mortality

No animals died during the study.

B. BODY WEIGHT AND WEIGHT GAIN

There were no consistent differences in the weekly body weights and total body weight gains (weeks 1-13) between compound-treated and control dogs. The mean body weights and body weight gains at selected time intervals are shown in Table 2.

TABLE 2. Group mean body weights (kg) and total body weight gains (kg) in dogs fed dichlormid for 13 weeks

Week of study	Exposure Concentration (ppm)			
	0	80	240	960
Males				
0	10.3	9.9	10.1	10.0
4	10.7	9.8	10.1	10.1
8	10.9	9.8	10.1	9.8
13	11.0	9.5	9.9	9.7
Week 1-13 weight gain (kg)[1]:	0.7	-0.4	-0.2	-0.3
Females				
0	9.4	8.9	8.5	8.7
4	9.1	8.9	8.5	9.0
8	9.1	9.0	8.3	8.8
13	9.3	9.1	8.3	8.8
Week 1-13 weight gain (kg)[1]:	-0.1	0.2	-0.2	0.1

Data taken from p. 5, MRID 225002430.
[1]Calculated by the reviewer.

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C. FOOD CONSUMPTION AND COMPOUND INTAKE

1. Food consumption

The amount of food consumed by the animals was not specifically stated. Because the animals were given a relatively small amount of food (~245 g), it is likely that they ate all of their daily food rations.

2. Compound consumption

Animals were given the test compound in the diet as shown in Table 1. The mean daily compound intake was not given in the study report, but was calculated (by the reviewer) by multiplying the nominal dietary concentration of the test material by the dog food factor of 0.025.

3. Food efficiency

Food efficiency was not given and could not be calculated (by the reviewer) because the amount of food consumed by the animals was not given.

D. OPHTHALMOSCOPIC EXAMINATION

There were no treatment-related ophthalmological findings.

E. BLOOD WORK

1. Hematology

Hematology measurements were comparable in control and treated dogs of both sexes.

2. Clinical chemistry

The most notable finding was an increase in serum alkaline phosphatase (AP) in high-dose animals: a 68-89% increase in males, and a 22-52% increase in females for weeks 4-13 compared to the screen II pre-treatment values (also elevated compared to control groups). The increased AP for the males and females was in each case largely due to one animal that had roughly 2-fold greater AP levels. AP levels were also elevated compared to pre-treatment levels at week 4 for mid-dose males and females (26-52% increase); in males the increase was primarily due to one animal. The AP levels of all the dose groups appeared to be moderating towards the end of the study. Values for the other clinical chemistry parameters were comparable to controls. The AP results are shown in Table 3.

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TABLE 3. Group mean alkaline phosphatase levels in dogs fed 0-960 ppm dichlormid for 13 weeks[1]

Week of study	Exposure Concentration (ppm)			
	0	80	240	960
Males				
0: screen I **screen II**	6.6 7.5	6.7 **9.1**	7.0 **8.9**	4.8 **7.4**
4	6.9	8.5 (93%)	13.5 (152%)	14.0 (189%)
8	4.4	8.8 (97%)	8.3 (93%)	13.1 (177%)
13	4.8	5.5 (60%)	9.1 (102%)	12.4 (168%)
Females				
0: screen I **screen II**	6.2 9.0	7.9 **10.2**	6.5 **9.0**	6.8 **9.1**
4	9.3	9.4 (92%)	11.3 (126%)	13.8 (152%)
8	8.0	7.5 (74%)	9.2 (102%)	12.5 (137%)
13	7.1	6.8 (67%)	7.4 (82%)	11.1 (122%)

Data taken from p. 6, MRID 225002430.
[1]Values in parentheses are the percent of the **screen II** pretreatment values for the same (dose) group of animals, calculated by the reviewer.

F. URINALYSIS

Urinalysis revealed no notable differences between treated and control dogs.

G. SACRIFICE AND PATHOLOGY

1. Organ weight

There were no treatment-related effects on absolute or relative (to body or brain (calculated by reviewer)) organ weights. The most notable difference from the controls was seen for the mean absolute weight of the lungs of the low-dose females: 113.08 g vs. 73.43 g for controls.

2. Gross pathology

The results of the gross pathology examinations were not given in the study report. It was stated (on p. 7 of MRID 225002430) that "Gross necropsy observations were negative with the exception of a small consolidated area on the lung of dog No. 6568 M receiving 960 ppm."

3. Microscopic pathology

There were no treatment-related microscopic findings.

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III. DISCUSSION

A. DISCUSSION

In a subchronic toxicity study (MRID 225002430) dichlormid (R-25788) was administered for 13 weeks to 4 beagle dogs/sex/dose in the diet at concentrations of 0, 80, 240, or 960 ppm (0, 2, 6, or 24 mg/kg/day, respectively, calculated using a dog food factor of 0.025).

No animals died during the study. Results of the daily clinical observations were not given in the study report, but it was stated that there were no treatment-related effects. Blood pressure, electrocardiograms, and heart rates were comparable in treated and control animals. Weekly body weights and the overall body weight gains were generally comparable in treated and control animals. The amount of food consumed by the animals was not given; it is likely that all of the daily food rations were eaten since the animals were given a relatively small amount of food (~245 g/day). The small gain (or loss) of weight by the control groups over the 13-week study period suggests that the animals were not adequately fed.

No treatment-related alterations were found during the assessment of ophthalmoscopic, hematologic, or urinalysis parameters. The only notable clinical chemistry finding was an increase in serum alkaline phosphatase (AP) in high-dose animals for weeks 4-13, and in mid-dose males and females at week 4. However, most of the increases were largely due to one animal with increased (roughly 2-fold) AP levels, and the differences between treated and untreated animals were becoming less pronounced towards the end of the study.

There were no treatment-related effects on absolute or relative (to body or brain) organ weights or microscopic pathology. The results of the gross pathology examinations were not given in the study report, but the study author indicated that there were no notable findings.

Based on the lack of treatment-related and toxicologically significant findings, a LOAEL cannot be established for either males or females. The NOAEL is ≥ 960 ppm (calculated as 24 mg/kg/day using the dog food factor of 0.025), the highest concentration tested. The maximum dose given to the animals was well below the limit intake of 1000 mg/kg/day currently recommended by EPA guidelines. There were a number of other serious study deficiencies, many of which likely occurred because the study was conducted before GLP guidelines were developed.

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B. STUDY DEFICIENCIES

The most serious deficiency of the study is the inadequate dosing of the animals: the high-dose animals received approximately 24 mg/kg/day dichlormid, which is well below the EPA recommended limit dose of 1000 mg/kg/day. The inadequate dosing is likely responsible for the lack of treatment-related adverse effects, including lack of an effect on body weight gain.

A substantial amount of information currently required by the EPA test guidelines for subchronic studies was missing in the study report. Missing information included clinical observations, gross pathology, parathyroid weight, and food consumption data, test compound stability, an analysis of the test diets for homogeneity, stability, and dichlormid concentration, a description of the animal housing and environmental conditions, statistical analysis of the data, the study in life dates, the method of assignment of the animals to the test groups (i.e randomization), and signed and dated Quality Assurance, Data Confidentiality, GLP, and Flagging statements. It was not stated if all gross lesions and masses were examined microscopically or if the spinal cord was examined at 3 levels. Also, the range of animal body weights at the beginning of the study appeared to be excessively large (males: 8.5-13.0 kg; females: 6.4-16.9 kg), particularly for the females. It is not clear whether this is due to the fact that the animals were obtained from two different laboratories. It is likely that many of these study deficiencies occurred because the study was conducted in 1971-1972, prior to development of the GLP guidelines.

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EPA Reviewer: Anna Bearden, Ph. D.
Registration Action Branch 3 (7509C)
EPA Secondary Reviewer: Ayaad Assaad, Ph. D.
Review Section _, Toxicology Branch _ (7509C)

Date 7/19/99

Date 7/19/99

DATA EVALUATION RECORD 014199

STUDY TYPE: Subchronic Inhalation Toxicity - Rat; OPPTS 870.3465 [§82-4]

DP BARCODE: D256314
P.C. CODE: 900497

SUBMISSION CODE: S546651

TEST MATERIAL (PURITY): R-25788; Dichlormid (97.6% a.i.)

SYNONYMS: N, N-diallyl dichloroacetamide

CITATION: Knapp, H.F. (1982). 14-Week inhalation study with R-25788 in rats. Stauffer Chemical Company. Farmington, Connecticut, 06032. Laboratory report number T-10773, August 9, 1983. MRID 00155678 Unpublished

SPONSOR: Stauffer Chemical Company, Environmental Health Center, 400 Farmington Avenue, Farmington, Connecticut, 06032.

EXECUTIVE SUMMARY:

In a subchronic inhalation toxicity study (MRID 00155678), R-25788 (97.6% a.i.) was administered to 18 Sprague-Dawley rats/sex/dose by whole body exposure at concentrations of 0, 2, 19.9, and 192.5 mg/m^3 (0, 2, 19.9, and 192.5 μg/L) for 6 hours per day, 5 days/week for a total of 65 days. The following parameters were measured: clinical signs, body weight, body weight change, food consumption, opthamology, organ weights, hematology, clinical chemistry, gross pathology, and histology.

Clinical chemistry and hematological parameters were not effected by treatment with R-25788. One male in the 19.9 mg/m^3 group died at day 17 of nontreatment related causes. The incidence of following clinical signs was significantly increased relative to the untreated control in both sexes in the 192.5 mg/m^3 group: salivation, dull behavior, aggressive behavior, stained integument, rough coat, and wet hair. Although a common observation in inhalation studies, as shown in 83% incidence in controls, the day of onset for chromorhinorrhea was significantly earlier in the19.9 and 192.5 mg/m^3 groups (day 15 and 1.5, respectively vs. day 54.5) relative to untreated control groups. Incidences of chromodacryrrhea (statistically significant) and closed eyes relative to the untreated control were observed in rats of the 19.9 and 192.5 mg/m^3 groups. The daily total number of seminal plugs observed in the afternoon following exposure increased 328% and 289% in the19.9 and 192.5 mg/m^3 groups, respectively compared to the control group. This increase in the daily total number of seminal plugs indicates that the rats were stressed from

the treatment.

Statistically significant body weights compared to the control were observed at pretest in the males and females making the interpretation of body weight data very difficult. Toxicologically and statistically significant decreases in body weight were observed in the 192.5 mg/m^3 groups of both sexes (-17% in males; -10% in females) compared to control. Animals in the 192.5 mg/m^3 group also consumed significantly less food relative to the control (-9.4%).

Both sexes exhibited toxicologically significant increases relative to the control in liver organ to body weight ratios in the 19.9 and 192.5 mg/m^3 groups (males +29% and +39%, respectively; females +7.4% and +23%, respectively). Females in the 192.5 mg/m^3 exhibited a 10% increase in absolute liver weight relative to the control. Both sexes exhibited toxicologically significant increases relative to the control kidney organ to body weight ratios in the 19.9 and 192.5 mg/m^3 groups (males +10% and +12%, respectively; females +35% and +47%). Differences in organ and organ to body weight ratios in heart, brain, lung, and adrenals relative to untreated control were the result of decreases in body weight and lack of feeding in the high dose group.

In both sexes increases in the following non-neoplastic histological changes were observed in the nasal passages of the 19.9 and 192.5 mg/m^3 groups : degeneration, necrosis, and/or sloughing of olfactory epithelium; attenuation of olfactory epithelium; intraepithelial cyst formation; and basal cell hyperplasia of olfactory epithelium. Rats exposed to 192.5 mg/m^3 R-25788 exhibited increased incidence of lymphoid aggregates in the submucosa of the larynx, the prostate, lungs, and salivary glands relative to untreated control. Female rats in the 192.5 mg/m^3 group exhibited increased lymphoid aggregates which were accompanied by cellular degeneration.

The LOAEL is 19.9 mg/m^3 (19.9 μg/L), based on clinical signs, gross pathology, opthamology, liver and kidney weights, and non-neoplastic histology. The NOAEL is 2 mg/m^3 (2 μg/L).

This study is considered **acceptable-guideline** and does satisfy the guideline requirement for a subchronic inhalation study (82-4; OPPTS 870.3465) in the rat.

COMPLIANCE: Signed and dated GLP, Data Confidentiality, and Flagging statements were not provided. Signed and dated Quality Assurance statement was provided. Study was performed before GLP regulations.

I. MATERIALS AND METHODS

A. MATERIALS:

1. Test Material: R-25788
   Lot/Batch #: EHC #0139-19
   Purity: 97.6% a.i.
   Stability of compound: Not given
   CAS #: 37764-25-3

2. Test animals: Species: rat
   Strain: Sprague-Dawley CD®
   Age and weight at study initiation: 49 days old; 255 g for males, 185 g for females
   Source: Charles River Breeding Laboratories, Kingston, NY
   Housing: Stainless steel wire mesh cages (5" wide x 8" long x 7.25" deep)
   Diet: Certified Purina Laboratory Rodent Chow #5002 ad libitum except during exposure period
   Water: purified ad libitum
   Environmental conditions: Temperature: 23± 2°C; during exposure periods temperature was lowered to maintain temperature inside exposure chambers.
   Humidity: 40-60%
   Air changes: Not given
   Photoperiod:12 hours dark/light
   Acclimation period: 7 days

B. STUDY DESIGN:

1. In life dates - start: August 27, 1981 end: November 30, 1981

2. Animal assignment: Animals were assigned by computer generated random numbers to the test groups in Table 1.

TABLE 1: STUDY DESIGN

Test group	Nominal Conc. (mg/m^3)	Analytical Conc. (mg/m^3)	MMAD μm	Rats/sex
Control	0 (clean air only)	0	not sampled	18
Low	2.0	2.0	no aerosol present	18
Mid	20	19.9	no aerosol present	18
High	200	192.5	3.0 ± 1.6 μm	18

Data extracted from MRID no.470117040 pp. 101-105, 107, 188-190

Rats were exposed to R-25788 for 6 hours a day, 5 days per week for a total of 65 days.

2. Generation of the test atmosphere and description of the chamber: Exposure chambers were 1.0 m^3 in size and constructed of stainless steel and glass according to Stauffer design by Hazelton, Inc. Total animal volume was < 2% of chamber volume. Air was drawn through the exposure chamber at 21 air exchanges/hour. Before discharging the chamber exhaust to the outside, the air passed through a roughing filter, prefilter, high-efficiency particulate filter, and an activated-charcoal element.

   The 2 and 19.9 mg/m^3 were produced by metering R-25788 onto a quartz heater contained inside a straight glass generation tube wrapped with heating tape. The 192.5 mg/m^3 concentration was produced using a Collison compressed air nebulizer which produced a fine mist which impacted the side of the nebulization flask. Dry, oil-free compressed air was supplied to both the generation flasks and the nebulization flask.

   Time to equilibrium was not given.

   Test atmosphere concentration. At the breathing zone of the rats, chamber air was drawn through resin adsorption tubes, extracted with acetone and analyzed by gas chromatography. The oxygen content was measured to be 20.5% at all exposure concentrations. Results are in Table 1 above.

   Particle size determination. Aerosol particle size analyses were performed using a high-volume cascade impactor (Sierra Cascade Impactor with type H glass fiber filters). The amount of test material on the filter was analyzed gravimetrically. Results are in Table 1 above.

3. Statistics: Body weights, hematology, clinical chemistry, and organ weights were analyzed by ANOVA, Bartlett's test for homogeneity of variance and Dunnett's t-test. Clinical observations and histopathology were analyzed by Mann-Whitney 2 Sample Rank Test or Fishers Test. A p value < 0.05 was used for the level significance.

C. METHODS:

1. Observations: Animals were observed twice daily for clinical signs and mortality.

2. Body weight: Body weight was determined at pretest and weekly during exposure.

3. Food consumption: Food consumption was determined weekly.

4. Ophthalmoscopic examination was performed at pretest and the end of the study.

5. Blood was collected from the orbital plexus of 6 rats/sex/dose at pretest and 6 weeks of exposure. At termination of the study, blood was collected from the abdominal aorta of all surviving animals. The anticoagulant utilized in histological analysis was not provided. The CHECKED (X) parameters were examined.

a. Hematology

X	Hematocrit (HCT)*	X	Leukocyte differential count*
X	Hemoglobin (HGB)*		Mean corpuscular HGB (MCH)
X	Leukocyte count (WBC)*		Mean corpusc. HGB conc.(MCHC)
X	Erythrocyte count (RBC)*		Mean corpusc. volume (MCV)
X	Platelet count*		Reticulocyte count
	Blood clotting measurements*		
	(Thromboplastin time)		
	(Thromboplastin time)		
	(Clotting time)		
	(Prothrombin time)		

* Required for subchronic studies based on Subdivision F Guidelines

b. Clinical Chemistry

	ELECTROLYTES		OTHER
X	Calcium*	X	Albumin*
X	Chloride*		Blood creatinine*
	Magnesium	X	Blood urea nitrogen*
	Phosphorus*	X	Total Cholesterol
X	Potassium*	X	Globulins
X	Sodium*	X	Glucose*
		X	Total bilirubin
	ENZYMES	X	Total serum protein (TP)*
X	Alkaline phosphatase (ALK)		Triglycerides
	Cholinesterase (ChE)		Serum protein electrophores
	Creatine phosphokinase		
X	Lactic acid dehydrogenase (LDH)		
X	Serum alanine amino-transferase (also SGPT)*		
X	Serum aspartate amino-transferase (also SGOT)*		
	Gamma glutamyl transferase (GGT)		
	Glutamate dehydrogenase		

* Required for subchronic studies based on Subdivision F Guidelines

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6. Sacrifice and Pathology: Animals were anesthetized with sodium pentobarbital administered intraperitoneally and exanguinated by severing the abdominal aorta and posterior vena cava. All animals that died and those sacrificed on schedule were subjected to gross pathological examination and the CHECKED (X) tissues were collected for histological examination and fixed in 10% neutral buffered formalin. The (XX) organs, in addition, were weighed.

X	DIGESTIVE SYSTEM	X	CARDIOVASC./HEMAT.	X	NEUROLOGIC
	Tongue	X	Aorta*	XX	Brain*Periph. nerve*
X	Salivary glands*	XX	Heart*		Spinal cord (3 levels)[T]
X	Esophagus*	X	Bone marrow*		Pituitary*
X	Stomach*	X	Lymph nodes*		Eyes (optic n.)[T]
X	Duodenum*	X	Spleen*		
X	Jejunum*	X	Thymus*		GLANDULAR
X	Ileum*			XX	Adrenal gland*
X	Cecum*		UROGENITAL		Lacrimal gland[T]
X	Colon*	XX	Kidneys*+	X	Mammary gland[T]
	Rectum*	X	Urinary bladder*	X	Parathyroids*++
XX	Liver*+	XX	Testes*+	X	Thyroids*++
	Gall bladder*		Epididymides		
X	Pancreas*	X	Prostate		OTHER
		X	Seminal vesicle	X	Bone
	RESPIRATORY	XX	Ovaries	X	Skeletal muscle
X	Trachea*	X	Uterus*	X	Skin
XX	Lung*				All gross lesions and masses*
X	Nose				
	Pharynx				
X	Larynx				

* Required for subchronic studies based on Subdivision F Guidelines.
+ Organ weight required in subchronic and chronic studies.
T = required only when toxicity or target organ.
++ Organ weight required for non-rodent studies.

II. RESULTS

A. Observations

1. Toxicity: The incidence of following clinical signs was significantly increased relative to the untreated control in both sexes in the 192.5 mg/m^3 group: salivation, dull behavior, aggressive behavior, stained integument, rough coat, and wet hair. Although a common observation in inhalation studies, as shown in 83% incidence in controls, the day of onset for chromorhinorrhea was significantly earlier in the19.9 and 192.5 mg/m^3 groups relative to untreated control groups. Incidences of chromodacryrrhea and closed eyes relative to the untreated control were observed in rats of the 19.9 and 192.5 mg/m^3 groups. The increase in the daily total number of seminal plugs relative control group indicates that the rats were stressed from the treatment.

Table 2. Clinical signs observed in rats exposed to R-25788 by inhalation. Data for clinical signs are combined for males and females.

Number exhibiting clinical sign	0 mg/m^3	2 mg/m^3	19.9 mg/m^3	192.5 mg/m^3
Chromorhinorrhea	Day 54.5 30	Day 31.5 30	Day 15* 36	Day 1.5* 36
Salivation	0	0	1	13*
Behavior-Dull	0	1	0	36*
Behavior-Hyperactive	0	0	0	2
Behavior-Aggressive	0	0	0	36*
Chromodacryrrhea of eye	9	9	18*	22*
Eye closed	0	0	1	2
Stained integument	3	5	1	36*
Rough coat	0	0	0	16*
Wet hair	0	0	0	21*
Presence of seminal plugs in P.M. postexposure (total daily #)	0.25± 0.55	0.22± 0.46	8.19± 3.89*	7.23± 3.21*
Swollen	0	0	0	2
Pale	0	0	0	2
Dead	0	0	1	0

Data extracted from MRID no.470117040 pp. 220-249

* $p < 0.05$

2. Mortality: One male in the 19.9 mg/m^3 was found dead at day 17. This animal was reported to be dehydrated, moderate chromodacryorrhea in both eyes, and diffuse bright red color in the lungs. This death was not considered treatment related.

B. Body weight and weight gain

1. Body weight: Statistically significant body weights relative to the control were observed at pretest in the males and females making the interpretation of this data very difficult. Toxicologically relevant body weight effects were observed in the 192.5 mg/m^3 groups of both sexes.

Table 3. Body weights (g) in rats exposed to R-25788 by inhalation. Mean, standard deviation, and percent different from control are given.

	0 mg/m^3	2 mg/m^3	19.9 mg/m^3	192.5 mg/m^3
Males				
Week 0	257 ± 13	282± 15* +9.7 %	243± 17* -5.4 %	238± 14* -7.4 %
Week 1	290± 19	301 ± 17 +3.8 %	276± 42 -4.8 %	251± 20* -13.4 %
Week 7	441± 40	438± 32 -0.7 %	415± 54 -5.9 %	375± 26* -15.0 %
Week 11	490± 44	480± 39 -2.0 %	467± 63 -4.7 %	408± 30* -16.7 %
Week 14	477± 47	470± 40 -1.5 %	452± 65 -5.2 %	396± 29* -17.0 %
Females				
Week 0	183± 13	194± 9* +6.0 %	183± 13 0 %	180± 10 -1.6 %
Week 1	200± 14	202± 11 +1.0 %	199± 15 -0.5 %	186± 11* -7.0 %
Week 7	258± 25	259± 19 +0.4 %	256± 29 -0.8 %	239± 20* -7.4 %
Week 11	276± 27	274± 20 -0.7 %	274± 34 -0.7 %	249± 21* -9.8 %
Week 14	262± 28	262± 20 0 %	260± 37 -0.8 %	236± 21* -9.9 %

Data extracted from MRID no.470117040 pp. 202-210

* $p < 0.05$

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2. Body weight change: Statistically significant body weights were observed at pretest in the males and females in dose groups relative to the untreated control making the interpretation of this data very difficult. Toxicologically relevant body weight effects were observed in the 192.5 mg/m^3 groups of both sexes compared to changes in control body weights. Statistically significant decreases in body weight change observed in the 2 mg/m^3 group of males is incidental since these animals at higher body weights at pretest.

Table 4. Body weight change (g) observed in rats exposed to R-25788 by inhalation. Mean, standard deviation, and percent different from control are given.

	0 mg/m^3	2 mg/m^3	19.9 mg/m^3	192.5 mg/m^3
Males				
Week 0-4	66.72± 11.63	43.94± 12.72* -34.1%	60.44± 32.78 -9.4%	43.89± 10.24* -34.2%
Week 0-7	184.28± 26.73	155.94± 19.50* -15.4%	172.29± 52.84 -6.5%	136.72± 25.35* -25.8%
Week 0-11	233.72± 31.95	197.78± 27.54* -15.4%	224.47± 58.94 -4.0%	170.33± 28.06* -27.1%
Week 0-14	248.28± 37.58	209.06± 24.11* -15.8%	239.12± 60.12 -3.7%	178.89± 27.93* -27.9%
Females				
Week 0-4	54.17± 12.40	49.56± 9.84 -8.5%	55.22 ± 17.16 +1.9%	34.56± 9.52* -36.2%
Week 0-7	74.33± 15.54	64.61± 14.40* -13.1%	73.89± 20.15 -0.6%	65.39± 19.37* -12.0%
Week 0-11	92.44 ± 18.79	79.44± 16.64 -14.1%	91.44± 24.55 -1.1%	69.28± 14.62 -25.1%
Week 0-14	98.22± 18.59	89.78± 15.83 -8.6%	102.44± 27.70 +4.3%	75.33± 15.68* -23.3%

Statistics calculated by reviewer. Raw data from MRID no.470117040 pp. 202-210
* $p < 0.05$

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C. Food consumption: Animals in the 192.5 mg/m^3 group consumed significantly less food for the duration of the study relative to the control.

Table 5. Mean food consumption (gram/rat/day) over 14 weeks in rats exposed to R-25788 by inhalation. (Mean, standard deviation, and percent different from control are given.)

	0 mg/m^3	2 mg/m^3	19.9 mg/m^3	192.5 mg/m^3
Week 0	24.0	23.9	23.2	23.4
Week 4	25.5	25.0	24.6	22.5
Week 7	24.1	25.1	25.0	21.7
Week 11	24.4	24.2	25.2	22.5
Mean food ± SD consumption	24.5± 0.6	24.5± 0.9	24.0 ± 1.8	22.2± 0.9* -9.4%

Raw data from MRID no.470117040 pp. 212-215
* p< 0.05

D. Ophthalmoscopic examination: There were no treatment related effects observed during the opthalmoscopic exam in rats exposed to R-25788 by inhalation.

E. Blood work

1. Hematology: There were no treatment related effects on hematological parameters measured in rats exposed to R-25788 by inhalation.

2. Clinical chemistry: There were no clinical chemistry parameters statistically or biologically significant.

H. Sacrifice and pathology

1. Organ weight: Male and female rats exposed to R-25788 by inhalation exhibited toxicologically significant increases relative to the control in liver and kidney organ to body weight ratios in the 19.9 and 192.5 mg/m^3 groups. Differences in organ and organ to body weight ratios in heart, brain, and adrenals relative to untreated control were the result of decreases in body weight and lack of feeding in the high dose group.

Table 6. Organ weights (g) in male rats exposed to R-25788 by inhalation. Mean, standard deviation, and percent different from control are given.

	0 mg/m^3	2 mg/m^3	19.9 mg/m^3	192.5 mg/m^3
Males				
Liver Organ Weight	11.835± 1.701	11.389± 1.342 -3.8%	12.135± 2.047 +2.5%	11.551± 1.378 -2.4%
Organ to BW Ratio	2.080± 0.148	2.420± 0.151 +16.4%	2.684± 0.184* +29.0%	2.898± 0.214* +39.3%
Brain Organ Weight	2.080± 0.073	2.045± 0.071 -1.7%	2.096± 0.132 +0.8%	2.063± 0.110 -0.8%
Organ to BW Ratio	0.440± 0.045	0.438± 0.041 0%	0.471± 0.062 +7.0%	0.524± 0.039* +19.1%
Heart Organ Weight	1.457± 0.025	1.431± 0.104 -1.8%	1.477± 0.250 +1.4%	1.358± 0.224 -6.8%
Organ to BW Ratio	0.305± 0.025	0.306± 0.025 0%	0.328± 0.043 +7.5%	0.344± 0.048* +12.8%
Kidney Organ Weight	2.991± 0.344	3.003± 0.321 0%	3.129± 0.488 +4.6%	2.761± 0.309 -7.7%
Organ to BW Ratio	0.628± 0.041	0.640± 0.053 +1.9%	0.693± 0.047* +10.4%	0.700± 0.074 +11.5%
Lung Organ Weight	1.499 0.137	1.600 0.210	1.619± 0.221 +8.0%	1.425± 0.171 -4.9%
Organ to BW Ratio	0.315 0.019	0.341 0.035	0.362± 0.048* +14.9%	0.359 ± 0.038* +14.0%
Adrenal Organ Weight	0.054± 0.007	0.055± 0.007 +1.9%	0.056± 0.011 +3.7%	0.053± 0.011 -1.9%
Organ to BW Ratio	0.011± 0.001	0.012± 0.001 +9.1%	0.013± 0.003 +18.2%	0.014± 0.003* +27.3%

Raw data from MRID no.470117040 pp. 284-309

* $p < 0.05$

Table 7 Organ weights (g) in female rats exposed to R-25788 by inhalation. Mean, standard deviation, and percent different from control are given.

	0 mg/m³	2 mg/m³	19.9 mg/m³	192.5 mg/m³
Females				
Liver Organ Weight	6.545± 0.869	6.593± 0.562 +0.7%	5.927± 0.979 -9.4%	7.201± 0.701* +10.0%
Organ to BW Ratio	2.489 ± 0.179	2.523± 0.190 +1.3%	2.672± 0.231* +7.4%	3.065± 0.246* +23.1%
Brain Organ Weight	1.909± 0.128	1.900± 0.087 0%	1.889± 0.084 -1.0%	1.881± 0.071 -1.5%
Organ to BW Ratio	0.736± 0.079	0.728± 0.051 -1.1%	0.738± 0.093 0%	0.504± 0.066* -31.5%
Lung Organ Weight	1.086± 0.091	1.186± 0.158 +9.2%	1.185± 0.173 +9.1%	1.038± 0.094 -4.4%
Organ to BW Ratio	0.418± 0.042	0.454± 0.062 +8.6%	0.467± 0.104 +11.7%	0.442± 0.043 +5.7%
Kidney Organ Weight	1.779± 0.201	1.768± 0.156 -0.6%	1.832± 0.179 +3.0%	1.819± 0.161 +2.2%
Organ to BW Ratio	0.528± 0.065	0.677± 0.067 +28.2%	0.713± 0.038 +35.0%	0.774± 0.057* +46.6%

Raw data from MRID no.470117040 pp. 284-309
* $p < 0.05$

2. Gross pathology : Facial soiling was observed in rats exposed to R-25788 by inhalation at 192.5 mg/m³

Table 8. Gross pathological findings in rats exposed to R-25788 by inhalation. Data for clinical signs are combined for males and females.

	0 mg/m³	2 mg/m³	19.9 mg/m³	192.5 mg/m³
Males **Facial soiling**	3	0	0	10
Females **Facial soiling**	0	0	0	6

Raw data from MRID no.470117040 pp. 268-282

3. Microscopic pathology
   a) Non-neoplastic: In both sexes increases in the following non-neoplastic histological changes were observed in the nasal passages of the 19.9 and 192.5 mg/m^3 groups : degeneration, necrosis, and/or sloughing of olfactory epithelium; attenuation of olfactory epithelium; intraepithelial cyst formation; and basal cell hyperplasia of olfactory epithelium. Rats exposed to 192.5 mg/m^3 R-25788 exhibited increased incidence of lymphoid aggregates in the submucosa of the larynx, the prostate, lungs, and salivary glands relative to untreated control.

Table 8. Non-neoplastic findings in male rats exposed to R-25788 by inhalation.

	0 mg/m^3	2 mg/m^3	19.9 mg/m^3	192.5 mg/m^3
Males				
Nasal passages--Acute inflammation of squamous epithelium, ventral nasal meatus	1	2	2	3
Nasal passages--Squamous metaplasia of epithelium	0	0	1	1
Nasal passages--Degeneration, necrosis and/or sloughing of olfactory epithelium	0	1	3	12*
Nasal passages--Attenuation of olfactory epithelium	0	2	7*	14*
Nasal passages--Basal cell hyperplasia of olfactory epithelium	0	0	7*	11*
Nasal passages--Intraepithelial cyst formation	0	0	2	9*
Lungs–Alveolar histiocytosis	0	4	3	3
Lungs–Subacute to chronic inflammation, subpleural and/or interstitial	1	3	4	3
Larynx–Subacute to chronic inflammation	2	--	--	5
Larynx–Lymphoid aggregates in submucosa	2	--	--	10*
Salivary glands–Lymphoid aggregates	2	--	--	5
Salivary glands–Subacute to chronic *inflammation*	0	--	--	4
Liver–hepatocellular necrosis	0	0	0	1
Prostate–Lymphoid aggregates	6	--	--	12

Raw data from MRID no.470117040 pp. 284-309
* $p < 0.05$

Table 9. Non-neoplastic findings in female rats exposed to R-25788 by inhalation.

	0 mg/m³	2 mg/m³	19.9 mg/m³	192.5 mg/m³
Females				
Nasal passages–Necrosis of respiratory epithelium	0	0	0	2
Nasal passages–Attenuation of respiratory epithelium–loss of cilia	0	0	0	2
Nasal passages–Degeneration, necrosis and/or sloughing of olfactory epithelium	2	3	5	13*
Nasal passages–Attenuation of olfactory epithelium	2	2	6	14*
Nasal passages–*Basal cell hyperplasia of* olfactory epithelium	2	0	4	10*
Nasal passages–Intraepithelial cyst formation	0	0	1	13*
Lungs–Subpleural lymphoid aggregates	1	2	5	2
Lungs–Acute inflammation subpleural and/or interstitial	0	0	0	2
Larynx–Lymphoid aggregates in submucosa	5	--	--	10
Liver–Lymphoid aggregates accompanied by cellular degeneration	8	11	10	15
Liver–Necrosis hepatocellular	0	0	0	2
Liver–Single cell, hepatocyte necrosis	0	2	1	1
Kidneys–Mineralization of pelvic epithelium and/or debris in pelvic lumen	2	5	8	10

Raw data from MRID no.470117040 pp. 284-309
* $p < 0.05$

b) Neoplastic: There were no treatment related effects on neoplastic histology in rats exposed to R-25788 by inhalation.

III. DISCUSSION

A. In this subchronic inhalation toxicity study, **the LOAEL for R-25788 is 19.9 mg/m^3, based on clinical signs, gross pathology, opthamology, liver and kidney weights, and non-neoplastic histology. The NOAEL is 2 mg/m^3.**

Clinical chemistry and hematology were not effected by treatment with R-25788. The incidence of following clinical signs was significantly increased relative to the untreated control in both sexes in the 192.5 mg/m^3 group: salivation, dull behavior, aggressive behavior, stained integument, rough coat, and wet hair. Although a common observation in inhalation studies, as shown in 83% incidence in controls, the day of onset for chromorhinorrhea was significantly earlier in the19.9 and 192.5 mg/m^3 groups (day 15 and 1.5, respectively vs. day 54.5) relative to untreated control groups. Incidences of chromodacryrrhea (statistically significant) and closed eyes relative to the untreated control were observed in rats of the 19.9 and 192.5 mg/m^3 groups. The daily total number of seminal plugs observed in the afternoon following exposure increased 328% and 289% in the19.9 and 192.5 mg/m^3 groups, respectively compared to the control group. This increase in the daily total number of seminal plugs indicates that the rats were stressed from the treatment.

Statistically significant body weights compared to the control were observed at pretest in the males and females making the interpretation of body weight data very difficult. Toxicologically and statistically significant decreases in body weight were observed in the 192.5 mg/m^3 groups of both sexes (-17% in males; -10% in females) compared to control. Animals in the 192.5 mg/m^3 group also consumed significantly less food relative to the control (-9.4%).

Both sexes exhibited toxicologically significant increases relative to the control in liver organ to body weight ratios in the 19.9 and 192.5 mg/m^3 groups (males +29% and +39%, respectively; females +7.4% and +23%, respectively). Females in the 192.5 mg/m^3 exhibited a 10% increase in absolute liver weight relative to the control. Both sexes exhibited toxicologically significant increases relative to the control kidney organ to body weight ratios in the 19.9 and 192.5 mg/m^3 groups (males +10% and +12%, respectively; females +35% and +47%). Differences in organ and organ to body weight ratios in heart, brain, lung, and adrenals relative to untreated control were the result of decreases in body weight and lack of feeding in the high dose group.

In both sexes increases in the following non-neoplastic histological changes were observed in the nasal passages of the 19.9 and 192.5 mg/m^3 groups : degeneration,

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necrosis, and/or sloughing of olfactory epithelium; attenuation of olfactory epithelium; intraepithelial cyst formation; and basal cell hyperplasia of olfactory epithelium. Rats exposed to 192.5 mg/m^3 R-25788 exhibited increased incidence of lymphoid aggregates in the submucosa of the larynx, the prostate, lungs, and salivary glands relative to untreated control. Female rats in the 192.5 mg/m^3 group exhibited increased lymphoid aggregates which were accompanied by cellular degeneration.

B. Study deficiencies: Signed and dated GLP, Data Confidentiality, and Flagging statements were not provided. Study was performed before GLP regulations. Although there are several deficiencies, they are not sufficient for study rejection.

Statistically significant body weights were observed at pretest in the males and females in dose groups relative to the untreated control making the interpretation of body weight data very difficult. The following required parameters were not analyzed: blood creatine, blood clotting measurement, blood phosphorus, histology of the rectum, and histology of the pituitary. The anticoagulant used was not given in the study report.

The collection of blood from the abdominal aorta at termination is considered a deficiency. The interim blood samples were taken from the orbital plexus thus reducing consistency of blood collection site. There is also potential contamination of the blood with abdominal fluids and possibly interstitial contents. Additionally different rats were used for interim blood collections.

Males of all dose groups, including control, exhibited "subacute to chronic inflammation" of the subpleural and/or interstitial region of the lung combined with alveolar histiocytosis. It is questionable whether or not the exposure period of 14 weeks is sufficient to induce chronic inflammation in the lung. It is possible that these rats contained infections prior to the study. The females in the study did not exhibit chronic inflammation or alveolar histiocytosis indicating these effects were not treatment related.

014199

DICHLORMID **SALMONELLA (84-2)**

Principal Reviewer: Nancy E. McCarroll
Toxicology Branch 1/HED (7509C)
Signature: Nancy E. McCarroll
Date: 3-15-99

Secondary Reviewer: Irving Mauer, Ph.D.
Toxicology Branch 2/HED (7509C)
Signature: Irving Mauer
Date: 3-16-99

DATA EVALUATION REPORT

STUDY TYPE: Mutagenicity: Salmonella typhimurium/mammalian microsome mutagenicity assay; OPPTS 870.5265 [§84-2]

DP BARCODE: D253247 SUBMISSION NO.: S546651

PC CODE: [Inert] TOX. CHEM. NO.: [Inert] MRID NO: 41561404

TEST MATERIAL (PURITY): R-25788 (97.2%)

COMPOSITION/SYNONYM(S): Dichlormid; EHC-0829-33

CITATION: J. B. Majeska (1987). Mutagenicity evaluation in Salmonella typhimurium R-25788 T-13178; ICI Americas Inc., Farmington, CT; Study No. T-13178; dated December 31, 1987. (Unpublished) MRID NO: 41561404.

SPONSOR: ICI Americas Inc., Wilmington, DE

EXECUTIVE SUMMARY: In independently performed microbial reverse gene mutation assays (MRID No. 41561404), Salmonella typhimurium strains TA1535, TA1537, TA98 and TA100 were exposed to Dichlormid as R-25788 (97.2%) at doses ranging from 188-3000 µg/plate without or without S9 fractions from two species (both trials). The S9 fractions were derived from Aroclor 1254-induced rat livers and mouse livers. The test material was delivered to the test system in dimethylsulfoxide.

Cytotoxicity and compound insolubility were evident at 5000 µg/plate -/+ rat or mouse S9 in the preliminary study; R-25788 was, therefore, tested to a high soluble and sub-cytotoxic dose in the main assay (both trials). All strains responded in the expected manner to the appropriate positive controls. **There was, however, no evidence that R-25788 induced a mutagenic effect under any test condition in either assay.**

This study is classified as **Acceptable** and satisfies the guideline requirements for a bacterial gene mutation assay (84-2).

COMPLIANCE: Signed and dated Data Confidentiality, GLP and Quality Assurance statements were provided.

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A. MATERIALS:

1. Test Material: R-25788

Description: Amber liquid
Identification number: WRC 4921-35-11 GGD-0101
Purity: 97.2%
Receipt date: 9/30/87
Stability: Unspecified; expiration date: 5/90
Contaminants: None listed
Solvent used: Dimethyl sulfoxide (DMSO)
Other provided information: The test material was stored in the dark at room temperature (~20°C) and at ambient humidity. The test material (50 µL) did not alter the pH or osmotic pressure of sodium phosphate buffer (2.5 mL) or sodium phosphate buffer (2.0 mL) containing 0.5 mL of an unspecified S9 mixture. Solutions of the test material were prepared immediately prior to use.

2. Control Materials:

Negative: Culture medium (Vogel-Bonner Minimal Medium supplemented with 2% glucose 0.5 mM biotin, and 0.5 mM histidine

Solvent/final concentration: DMSO/50 µL plate

Positive:

Nonactivation:

Sodium azide	10.0	µg/plate TA100, TA1535
2-Nitrofluorene	10.0	µg/plate TA98
ICR 191	5.0	µg/plate TA1537

Other:

Activation:

2-Aminoanthracene (2-AA)	4.0	µg/plate all strains with rat liver S9
	5.0	µg/plate all strains with mouse liver S9

Note: In the confirmatory test with strain TA1537, 5.0 µg/plate 2-AA was assayed with rat liver S9 activation.

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3. Activation: S9 derived from

x Aroclor 1254	x induced	x rat[a]	x liver
_____ phenobarbital	_____ noninduced	x mouse[b]	_____ lung
_____ none		_____ hamster	_____ other
_____ other		_____ other	

The rat liver S9 was prepared by the performing laboratory and was identified as Lot No. EHC-0475-26; the mouse liver S9 (Lot No. 0223) was purchased from Molecular Toxicology, College Park, MD.

S9 mix composition	Final concentration
Sodium phosphate buffer	100 mM
Glucose 6-phosphate	5 mM
NADP	4 mM
$MgCl_2$	8 mM
KCl	34 mM
S9 (rat or mouse)	100 µL/mL

4. Test Organism Used: S. typhimurium strains

_____ TA97	x TA98	x TA100	_____ TA102	_____ TA104
x TA1535	x TA1537	_____ TA1538		

list any others:

Test organisms were properly maintained: Storage condition not reported

Checked for appropriate genetic markers (rfa mutation, R factor): Yes

5. Test compound concentrations used:

(a) Preliminary cytotoxicity assay: Ten doses (20, 39, 78, 156, 313, 625, 1250, 2500, 5000, and 10,000 µg/plate) were evaluated with or without S9 activation in S. typhimurium strain TA100.

(b) Mutation assays:

(1) Initial: Five doses (188, 375, 750, 1500, and 3000 µg/ plate) were evaluated in the absence and presence of S9 activation derived from rat and mouse liver microsomes; all tester strains were used.

(2) Confirmatory: As above

[a]Male Sprague-Dawley rats

[b]Neither the strain nor the sex of the mice used to prepare the S9 fraction were reported.

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B. TEST PERFORMANCE:

1. Type of Salmonella assay: _x_ Standard plate test
_____ Pre-incubation (_____) minutes
_____ "Prival" modification
_____ Spot test
_____ Other (describe)

2. Protocol:

(a) Preliminary cytotoxicity/mutation assays: Similar procedures were used for the preliminary cytotoxicity and the mutation assays.

Approximately 10^8 cells from 12- to 16-hour broth cultures of the appropriate tester strain, 0.5 mL of phosphate buffer, and 50 µL of the appropriate test material dose, solvent, or positive controls were added to tubes containing 2-mL volumes of molten top agar. For the S9-activated tests, 0.5 mL of the appropriate S9-cofactor mix replaced the phosphate buffer; tester strains and test and control solutions were added as described. The contents of the tubes were mixed, poured over Vogel-Bonner minimal medium, and incubated at 37°C for ≈48–72 hours. At the end of incubation, plates were scored for revertant colonies using an automatic colony counter. Means and standard deviations were determined from the counts of triplicate plates per strain, per dose, per condition.

(b) Evaluation criteria:

(1) Assay validity: The assay was considered valid if the following criteria were met: (a) the medium, solvent, and positive control results were within the historical range of the reporting laboratory, (b) at least three doses were available for evaluation, and © the test material was assayed over a concentration range that included a cytotoxic level, a level that approached the solubility limit of the test material, or the maximum dose (from 3000 to 10,000 µg/plate) established by the performing laboratory for this assay system.

(2) Positive response: The test material was considered positive if it (a) caused a reproducible and dose-related (at least three concentrations) increase in mutant colonies of any tester strain and (b) at least one dose caused a 3-fold increase in mutant colonies of TA1535 or TA1537 or a 2.5-fold increase in mutant colonies of TA98 or TA100.

C. REPORTED RESULTS

1. Preliminary cytotoxicity assay: Ten doses of the test material ranging from 20 to 10,000 µg/plate were evaluated without and with rat liver S9 activation using strain TA100. Compound precipitation was apparent at 10,000 µg/plate +/- S9. The report stated that precipitation was also noted upon addition of the 5000-µg/plate

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concentration to the top agar. Mean revertant colony counts at ≥5000 µg/plate +/- S9 were reduced (≥42%) compared to the control. At lower concentrations, there was no clear indication of a cytotoxic effect. Based on these findings, the dose range selected for the nonactivated and S9-activated mutation assays was 188-3000 µg/plate.

2. Mutation assay: Representative results from the nonactivated and S9-activated initial and confirmatory mutation assays with R-25788 are presented in Tables 1 and 2, respectively. As shown, the slight reductions in revertant colonies of strains TA1535, TA1537, and/or TA100, which occurred under nonactivated or S9-activated (rat or mouse S9) conditions, were not reproducible or dose related. Our reviewers assume, therefore, that the reduced revertant colony counts probably resulted from normal plating variability. In general, revertant colony counts for strain TA98 were suppressed, compared to the control, at the majority of assayed levels, under all test conditions, and in both trials. At the highest assayed concentration (3000 µg/plate -/+ rat or mouse S9), there was a moderate reduction in mutant colonies of this strain in both the initial and repeat assays. However, R-25788 did not induce a mutagenic effect in any tester strain in either the absence or presence of rat or mouse liver S9 activation. In contrast, all strains responded to the appropriate nonactivated and S9-activated positive controls in both the initial and confirmatory assay. From the overall findings, the study author concluded that R-25788 was not mutagenic in this test system.

D. REVIEWER'S DISCUSSION/CONCLUSIONS: We assess that the study author's interpretation of the data was correct. Both in the absence and the presence of exogenous metabolic activation derived from rat and mouse liver microsomes, R-25788 was assayed to a subcytotoxic level but failed to induce a mutagenic effect. In addition, the response of all tester strains to the appropriate direct-acting or promutagenic positive control indicated that the assay had an adequate level of sensitivity to detect a mutagenic response. It was concluded, therefore, that R-25788 was not mutagenic in this microbial test system.

E. STUDY DEFICIENCIES: NONE

TABLE 1: Representative Results of the Initial Salmonella typhimurium/Mammalian Microsome Mutation Assay with R-25788

Substance	Dose/Plate	S9 Activation	Revertants per Plate of Bacterial Tester Strain[a]			
			TA1535	TA1537	TA98	TA100
Negative Control	--	-	17 ± 5	12 ± 4	40 ± 5	105 ± 29[d]
(Culture medium)	--	+[b]	21 ± 3	7 ± 5	56 ± 4[d]	108 ± 4[d]
	--	+[c]	15 ± 2	5 ± 4	37 ± 9	81 ± 12
Solvent Control						
(Dimethyl sulfoxide)	50 µL	-	15 ± 4	13 ± 6	28 ± 9	104 ± 19
	50 µL	+[b]	21 ± 3[d]	13 ± 5	46 ± 5	110 ± 9
	50 µL	+[c]	10 ± 1[d]	8 ± 2	37 ± 4	76 ± 3
Positive Controls						
Sodium azide	10 µg	-	1,012 ± 46	--	--	1,063 ± 43
ICR 191	5 µg	-	--	65 ± 20	--	--
2-Nitrofluorene	10 µg	-	--	--	916 ± 99	--
2-Aminoanthracene	4 µg	+[b]	272 ± 9	79 ± 3	2,368 ± 82	1,596 ± 436
	5 µg	+[c]	127 ± 15	278 ± 28	852 ± 200	1,148 ± 247
Test Material						
R-25788	1,500 µg[e]	-	23 ± 6	11 ± 3	22 ± 8	97 ± 18[d]
	3,000 µg	-	20 ± 2	11 ± 4	19 ± 5	105 ± 4
	1,500 µg[e]	+[b]	20 ± 4	7 ± 2	24 ± 6	112 ± 17
	3,000 µg	+[b]	20 ± 8	5 ± 3	23 ± 8	122 ± 7
	1,500 µg[e]	+[c]	25 ± 5	10 ± 5	17 ± 6	82 ± 7
	3,000 µg	+[c]	18 ± 2	4 ± 1	17 ± 2	70 ± 3

[a]Means and standard deviations of the counts from triplicate plates, except where indicated (see footnote d).
[b]Rat liver S9 activation
[c]Mouse liver S9 activation
[d]Means and standard deviations of the counts from duplicate plates; no explanation was provided for the reduction in replicates.
[e]Results for lower doses (188, 375, and 750 µg/plate -/+ rat or mouse S9) did not suggest a mutagenic effect.

NOTE: Phases of the assay (i.e., nonactivation, rat liver and/or mouse liver S9 activation) using various strains were conducted on different days.

TABLE 2: Representative Results of the Confirmatory Salmonella typhimurium/Mammalian Microsome Mutation Assay with R-25788

Substance	Dose/Plate	S9 Activation	Revertants per Plate of Bacterial Tester Strain[a] TA1535	TA1537	TA98	TA100
Negative Control	--	-	20 ± 3	10 ± 1	25 ± 1[d]	130 ± 31
(Culture medium)	--	+[b]	17 ± 6	10 ± 5	29 ± 2[d]	108 ± 15
	--	+[c]	9 ± 2	6 ± 0	27 ± 6	115 ± 9
Solvent Control						
(Dimethyl sulfoxide)	50 µL	-	11 ± 4	9 ± 5[d]	25 ± 5	90 ± 11
	50 µL	+[b]	20 ± 2	10 ± 2	30 ± 5	105 ± 3
	50 µL	+[c]	10 ± 3	7 ± 1	22 ± 5	88 ± 7
Positive Controls						
Sodium azide	10 µg	-	852 ± 83	--	--	706 ± 64
ICR 191	5 µg	-	--	69 ± 11	--	--
2-Nitrofluorene	10 µg	-	--	--	473 ± 172	--
2-Aminoanthracene	4 µg	+[b]	189 ± 33	--	926 ± 102	807 ± 141
	5 µg	+[b]	--	43 ± 9	--	--
	5 µg	+[c]	42 ± 4	204 ± 40	1,658 ± 149	538 ± 174
Test Material						
R-25788	1,500 µg	-	14 ± 1[d]	6 ± 4	13 ± 3[d]	86 ± 15[d]
	3,000 µg	-	15 ± 2	4 ± 2	8 ± 4[d]	84 ± 4
	1,500 µg	+[b]	11 ± 4[d]	8 ± 5	15 ± 0[d]	110 ± 3[d]
	3,000 µg	+[b]	12 ± 3	5 ± 2	9 ± 2	74 ± 13
	1,500 µg	+[c]	13 ± 5	5 ± 1	14 ± 1	91 ± 18
	3,000 µg	+[c]	17 ± 3	5 ± 1	12 ± 5	83 ± 11

[a]Means and standard deviations of the counts from triplicate plates, except where indicated (see footnote d).
[b]Rat liver S9 activation
[c]Mouse liver S9 activation
[d]Means and standard deviations of the counts from duplicate plates, no explanation was provided for the reduction in replicates.
[e]Results for lower doses (188, 375, and 750 µg/plate -/+ rat or mouse S9) did not suggest a mutagenic effect.

NOTE: Phases of the assay (i.e., nonactivation, rat liver and/or mouse liver S9 activation) using various strains were conducted on different days.

014199

DICHLORMID

GENE MUTATION IN CULTURED MAMMALIAN CELLS (84-2)

Principal Reviewer: Nancy E. McCarroll
Toxicology Branch 1/HED (7509C)

Signature: Nancy E. McCarroll
Date: 3/15-99

Secondary Reviewer: Irving Mauer, Ph.D.
Toxicology Branch 2/HED (7509C)

Signature: Irving Mauer
Date: 3-16-99

DATA EVALUATION REPORT

STUDY TYPE: Mutagenicity: Mammalian cells in culture gene mutation assay in mouse lymphoma cells; OPPTS 870.5300 [§84-2]

DP BARCODE: D253247 SUBMISSION NO.: S546651

PC CODE: [Inert] TOX. CHEM. NO.: [Inert] MRID NO: 41561405

TEST MATERIAL (PURITY): R-25788 (97.2%)

COMPOSITION/SYNONYM(S): Dichlormid; EHC-0829-33

CITATION: Tarca, J. P., and Majeska, J. B. (1987). Mutagenicity Evaluation in L5178Y Mouse Lymphoma Multiple Endpoint Test Forward Mutation Assay R-25788 T-13179; ICI Americas Inc., Farmington, CT; Study No. T-13179; date issued December 31, 1987. (Unpublished) MRID NO: 41561405.

SPONSOR: ICI Americas Inc., Wilmington, DE

EXECUTIVE SUMMARY: In independently *in vitro* mammalian cell gene mutation assays (MRID No. 41561405), L5178Y mouse lymphoma cells were exposed to Dichlormid as R-25788 (97.2%) at nonactivated doses ranging from 80-600 µg/mL (initial trial) or 200-600 µg/mL (repeat trial). Trials performed with S9 activation included S9 fractions from two species and investigated doses of 1.0-7.5 µg/mL + rat liver S9 or 2.5-40.0 µg/mL + mouse liver S9. The S9 fractions were derived from Aroclor 1254-induced rat livers or mouse livers, and the test material was delivered to the test system in dimethylsulfoxide.

Under nonactivated conditions, levels ≥600 µg/mL were severely cytotoxic. **The evidence of a mutagenic effect at 600 µg/mL in the first trial was confirmed in the repeat assay using a narrow range of doses (200, 300, 400, 550, and 600 µg/mL). Increased mutant colonies and mutation frequencies (MF) were seen at all assayed levels. However, the three highest concentrations induced clear dose-related increases in the MF; MFs were 2.1-, 2.6-, and 2.9-fold higher than the solvent control at 400, 550, and 600 µg/mL, respectively. Relative total growth (RTG) at these levels ranged from 25% at 400 to 9% at 600 µg/mL.**

In the presence of both S9 activation systems, R-25788 was more cytotoxic as indicated by the appreciably lower doses that were selected for these trials compared to the nonactivated phase of testing: 1.0, 2.5, 5.0, and 7.5 µg/mL + rat S9 and 2.5, 5.0, 7.5, 10.0, 25.0, and 40.0 µg/mL + mouse S9. **The results indicated that increased mutant colony counts and MFs accompanied exposure to 5.0 and 7.5 µg/mL + rat liver S9 and 25 and 40 µg/mL + mouse liver S9. At 5.0**

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and 7.5 µg/mL/+ rat S9, MFs were 1.8- and 2.8-fold higher than the solvent control; RTGs at these concentrations were ≥ 12%. In the presence of mouse liver S9 activation, the 25- and 40-µg/mL treatment levels induced 3.2- and 4.7-fold increases in the MF, respectively; RTG was 9% at 25.0 µg/mL and 5% at 40.0 µg/mL.

Although the study authors stated that the findings were inconclusive because of cytotoxicity, we assess that there was sufficient justification to include the cytotoxic levels in the interpretation of the results (see Section D--Reviewers Discussion and Interpretation of Results). **We assess, therefore, that R-25788 is mutagenic in L5178Y mouse lymphoma cells both with and without S9 activation at doses that extend into the cytotoxic range.**

The study is classified as **Acceptable** and satisfies the requirements for an in vitro mammalian forward gene mutation study (84-2).

COMPLIANCE: Signed and dated Data Confidentiality, GLP and Quality Assurance statements were provided.

I. MATERIALS AND METHODS

A. MATERIALS:

1. Test Material: R-25788

Description: Amber liquid
Identification No.: WRC 4921-35-11 GGD-0101
Purity: 97.2%
Receipt date: 9/30/87
Stability: Unspecified; expiration date: 5/90
Contaminants: None listed
Solvent used: Dimethyl sulfoxide (DMSO).
Other provide information: The test material was stored in the dark at room temperature (~20°C), and at ambient humidity. The report stated that test material concentrations ≤0.8 mg/mL -S9 and 0.06 mg/mL +S9 did not "substantially" alter the pH or osmolality of the treatment medium. Solutions of the test material were used within ≈2 hours of preparation.

2. Control Materials:

Negative: Fischer's medium supplemented with 10% horse serum, 1.9 mM glutamine, 210 µg/mL sodium pyruvate, 476 µg/mL plunonic, and antibiotics

Solvent/final concentration: DMSO/1%

Positive: Nonactivation (concentrations, solvent): Ethyl methanesulfonate (EMS) was prepared in an unspecified solvent to yield a final concentration of 0.5 µL/mL.

Activation (concentrations, solvent): N-nitrosodimethylamine (DMN) was prepared in an unspecified solvent to yield final concentrations of 0.04 and 0.05 µl/mL. The 0.04 µl/mL solution was assayed with rat liver S9; both concentrations were assayed with mouse liver S9.

3. Activation: S9 derived from

x Aroclor 1254	x induced	x rat[a]	x liver
_____ phenobarbital	_____ noninduced	x mouse[b]	_____ lung
_____ none		_____ hamster	_____ other
_____ other		_____ other	

[a]Male Sprague-Dawley rats

[b]Sex or strain not specified

The rat S9 liver homogenate was prepared by the performing laboratory and was assigned Lot No. EHC-0476-25; the mouse S9 liver homogenate was purchased from Molecular Toxicology, College Park, MD and was assigned Lot No. 0223.

S9 mix composition:

Component	Concentration/mL of Culture Medium
NADP	1.2 mg
Isocitrate	6.0 mg
S9 homogenate (rat or mouse)	0.04 mL

4. Test Cells: mammalian cells in culture

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

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

5. Locus Examined:

__x__ thymidine kinase (TK)
selection agent: _____ bromodeoxyuridine (BrdU)
(give concentration) _____ fluorodeoxyuridine (FdU)
4 µg/mL trifluorothymidine (TFT)

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

_____ Na^+/K^+ATP^{ase}
Selection agent: _____ ouabain
(give concentration)

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

6. Test Compound Concentrations Used:

(a) Preliminary cytotoxicity assay: Nine doses (40, 60, 80, 100, 200, 400, 800, 1400, and 1600 µg/mL) were evaluated with and without rat liver S9 activation. Single cultures were prepared/dose/condition.

(b) Mutation assay:

(1) Nonactivated conditions: Two nonactivated assays were performed; doses tested were as follows:

Initial trial: 80, 100, 200, 400, and 600 µg/mL (duplicate cultures/dose).

Repeat trial: 200, 300, 400, 550, and 600 µg/mL (duplicate cultures/dose).

(2) S9-activated conditions: One S9-activated assay was performed with each metabolic activation system; doses tested were as follows:

Rat liver S9 activation: 1.0, 2.5, 5.0, and 7.5 µg/mL (duplicate cultures/dose except the high dose; only one replicate was prepared).

Mouse liver S9 activation: 2.5, 5.0, 7.5, 10.0, 25.0 and 40.0 µg/mL (duplicate cultures/dose).

B. TEST PERFORMANCE:

1. Cell Treatments:

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

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

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

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

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

2. Statistical Methods: The data were not evaluated for statistical significance.

3. Evaluation Criteria:

(a) Assay validity: For the assay to be considered valid, the following criteria must be satisfied: 1) results for the negative and positive controls must be within the historical range of the reporting laboratory; 2) results for replicate solvent controls must be comparable; 3) the cloning efficiency (CE) of the solvent control should be ≥75%; and 4) the test material must be assayed to a dose causing ≈90% reduction in cell survival, to the limit of solubility, or to a maximum dose of 3 to 10 mg/mL.

b. Positive response: The test material was considered positive if it induced a dose-related increase in the mutation frequency (MF) that exceeded 2.5 times the MF of the solvent control.

C. REPORTED RESULTS:

1. Preliminary Cytotoxicity Assay: Nine doses of the test material (40 to 1600 µg/mL -/+ rat S9) were evaluated in the preliminary cytotoxicity assay. There was no indication in the report that the test material was insoluble at any dose. In the nonactivated assay, relative suspension growth (RSG) was reduced to ≤36% at levels ≥800 µg/mL. Below this concentration, RSG was ≥65%. In the presence of S9 activation, RSG was ≤19% for all doses except the low dose (40 µg/mL); at 40 µg/mL the RSG was 27%. By comparison to the results achieved under nonactivation, the findings suggest that the S9-activated test material was more cytotoxic.

2. Mutation Assays:

(a) Nonactivated conditions: Based on the evidence of cytotoxicity at ≥800 µg/mL -S9, the doses selected for the mutation assay without S9 activation ranged from 80 to 600 µg/mL. Average RSG ranged from >100% at levels ≤100 µg/mL to 9% at the high concentration (600 µg/mL). As shown in Table 1, there was a dose-related increase in mutant colonies and the MF at the three highest levels (200, 400, 600 µg/mL); fold increases in the MF at these concentrations ranged from 1.2 at 200 µg/mL to 4.5 at 600µg/mL. Results for the lower levels were comparable to the solvent control values.

Owing to the dose-dependent increase in MFs, the nonactivated assay was repeated with a narrower range of test material levels (200, 300, 400, 550, and 600 µg/mL). As the data presented in Table 2 show, the findings from the repeat nonactivated assay were in good agreement with the initial results and indicated that R-25788 induced a dose-related cytotoxic and mutagenic effect. Greater than 2-fold increases in the MF were obtained at the three

highest levels; the relative total growth (RTG) at these concentrations ranged from 25% at 400 µg/mL to 9% at 600 µg/mL.

(b) S9-activated conditions:

(1) Rat liver S9 activation: Results from the first trial conducted with a concentration range of 1 to 7.5 µg/mL in the presence of rat liver S9 activation are shown in Table 3. Cytotoxicity was dose related and the RSGs ranged from 81% at the low dose (1.0 µg/mL) to 10% at the highest assayed level (7.5 µg/mL); CE at all levels was >100%. The number of mutant colonies and the MFs increased with increasing doses of R-25788; MFs either approaching or >2-fold over control were obtained at the two highest levels (5.0 and 7.5 µg/mL). RTG at the mutagenic levels was ≥12%. Although mutant colony counts were increased at 1.0 and 2.0 µg/mL, the MFs were only slightly higher than the solvent control.

Mouse liver S9 activation: No explanation was provided for the use of a higher starting concentration in the mouse liver S9-activated trial; the six treatment levels that were assayed in the presence of mouse liver microsomes were 2.5, 5.0, 7.5, 10.0, 25.0, and 40.0 µg/mL. A dose-related decrease in cell survival with increasing concentrations of R-25788 was also noted in the presence of mouse S9 activation (Table 4). In contrast to the rat S9 activation results, the test material appeared less cytotoxic under mouse liver S9-activation conditions. At equivalent levels (2.5, 5.0, and 7.5 µg/mL/+ mouse S9), RSG were 98, 53, and 30%, respectively, as compared to 45, 25, and 10% RSG, respectively, in the presence of rat S9 activation. The data presented in Table 4 further show that marked increases in mutant colonies and MFs (≥3.2-fold) occurred at doses (25.0 and 40.0 µg/mL) that reduced RTG to ≤9%; however, CEs over the entire concentration range were ≥65%. No clear indication of a mutagenic effect was observed at lower levels.

Based on the overall results the study authors concluded that "R-25788 induced a mutagenic response to significant levels only at less than 20% survival. Mutagenic responses below the 20% range are difficult to interpret because chemically effected mutations can not be differentiated from possible non-specific effects also known to occur at this level.

For this reason the response observed with R-25788 appears not to be significant but can not be clearly interpreted and therefore must be considered inconclusive."

D. REVIEWERS' DISCUSSION AND INTERPRETATION OF RESULTS: We assess that the study was properly conducted; however, we disagree with the study authors statement that the results are inconclusive because "mutagenic responses below the 20% range are difficult to interpret."

Clive et al. (1983)[1] recommend that data from doses level that reduced the RTG to 10% be included in the evaluation of a mutagenic response. The more conservative evaluation criteria of Caspary et al. (1988)[2] allow the inclusion of data from severely cytotoxic levels if the RTG is between 1 and 5%, the CE is between 10 and 20%, and the increased MF is supported by an increased mutant colony count. As the data presented in Tables 1 through 4 show, the results from the cytotoxic levels satisfy all of the above criteria. We assess, therefore, that there is sufficient justification to include the cytotoxic doses in the interpretation of the results. Based on the above considerations, it was concluded that under nonactivated and S9-activated conditions, R-25788 induced a reproducible dose-related mutagenic response at levels that extend into the cytotoxic range.

E. STUDY DEFICIENCIES: NONE

[1]Clive, D., McCuen, R., Spector, J.F.S., Piper, C., Mavournin, K.H. (1983). Specific gene mutations in L5178Y cells in culture. A report of the U.S. Environmental Protection Agency Gene-Tox Program. Mutat. Res. 115:225-256.

[2]Caspary, W.J., Lee, Y.J., Poulton, S., Myhr, B.C., Mitchell, A.D., Rudd, C.J. (1988). Evaluation of the L5178Y mouse lymphoma cell mutagenesis assay: Quality-control guidelines and response categories. Environ. Mutagen. 12:19-36

TABLE 1. Representative Results of the Initial Nonactivated Mouse Lymphoma Forward Mutation Assay with R-25788

Substance	Dose	Average Percent Relative Growth[a]	Average Mutant Colonies[a]	Average Viable Colonies[a]	Average Percent Relative Cloning Efficiency[a]	Average Percent Relative Total Growth[a]	Average Mutation Frequency[a,b] x 10^{-6}	Fold Increase[c]
Negative Control								
Medium	--	116	60	377	101	117	31.5	--
Solvent Control								
Dimethyl sulfoxide	1%	100	64	373	100	100	34.0	--
Positive Control								
Ethylmethane sulfonate	0.5 µL/mL	56	784	183	49	27	857	27.2
Test Material								
R-25788	200 µg/mL[d]	90	75	365	98	88	41.0	1.2
	400 µg/mL	38	86	329	89	34	52.0	1.5
	600 µg/mL	9	141	183	50	5	154.0	4.5

[a]Average results from duplicate cultures except the positive control; a single replicate was used. Results were calculated by our reviewers.

[b]Mutation Frequency (MF) = $\frac{\text{Mutant Colonies}}{\text{Viable Colonies}} \times 2 \times 10^{-4}$.

[c]Fold increase = $\frac{\text{MF of Test Dose}}{\text{MF of Solvent Control}}$. Note: It was assumed that the positive control was diluted in culture medium.

[d]Results for lower doses (80 and 100 µg/mL) did not suggest a mutagenic effect.

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TABLE 2. Representative Results of the Repeat Nonactivated Mouse Lymphoma Forward Mutation Assay with R-25788

Substance	Dose	Average Percent Relative Growth[a]	Average Mutant Colonies[a]	Average Viable Colonies[a]	Average Percent Relative Cloning Efficiency[a]	Average Percent Relative Total Growth[a]	Average Mutation Frequency[a,b] x 10^{-6}	Fold Increase[c]
Negative Control								
Medium	--	101	34	409	106	106	16.5	--
Solvent Control								
Dimethyl sulfoxide	1%	100	39	387	100	100	19.5	--
Positive Control								
Ethylmethane sulfonate	0.5 µL/mL	70	658	178	46	32	739	44.8
Test Material								
R-25788	200 µg/mL	62	42	360	93	58	23.5	1.2
	300 µg/mL	44	51	340	86	39	30.0	1.5
	400 µg/mL	29	68	327	85	25	41.5	2.1
	550 µg/mL	14	74	287	74	11	51.0	2.6
	600 µg/mL	12	82	293	76	9	56.0	2.9

[a]Average results from duplicate cultures except the positive control; single replicate were used. Results were calculated by our reviewers.

[b]Mutation Frequency (MF) = $\frac{\text{Mutant Colonies}}{\text{Viable Colonies}} \times 2 \times 10^{-4}$.

[c]Fold increase = $\frac{\text{MF of Test Dose}}{\text{MF of Solvent Control}}$. Note: It was assumed that the positive control was diluted in culture medium.

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TABLE 3. Representative Results of the Rat Liver S9-Activated Mouse Lymphoma Forward Mutation Assay with R-25788

Substance	Dose	Average Percent Relative Growth[a]	Average Mutant Colonies[a]	Average Viable Colonies[a]	Average Percent Relative Cloning Efficiency[a]	Average Percent Relative Total Growth[a]	Average Mutation Frequency[a,b] x 10^{-6}	Fold Increase[c]
Negative Control								
Medium	--	98	89	342	116	114	52.0	--
Solvent Control								
Dimethyl sulfoxide	1%	100	85	296	100	100	57.5	--
Positive Control								
N-nitrosodimethylamine	0.04 µL/mL	75	276	211	71	53	262	5.0
Test Material								
R-25788	1.0 µg/mL	81	158	473	160	125	68.0	1.2
	2.5 µg/mL	45	132	336	113	51	77.5	1.3
	5.0 µg/mL	25	224	443	150	38	100.5	1.8
	7.5 µg/mL	10	283	358	121	12	158.1	2.8

[a]Average results from duplicate cultures except the positive control and the high dose; no explanation was provided for the use of a single replicate for the high dose. Results were calculated by our reviewers.

[b]Mutation Frequency (MF) = $\frac{\text{Mutant Colonies}}{\text{Viable Colonies}} \times 2 \times 10^{-4}$.

[c]Fold increase = $\frac{\text{MF of Test Dose}}{\text{MF of Solvent Control}}$. Note: It was assumed that the positive control was diluted in culture medium.

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TABLE 4. Representative Results of the Mouse Liver S9-Activated Mouse Lymphoma Forward Mutation Assay with R-25788

Substance	Dose	Average Percent Relative Growth[a]	Average Mutant Colonies[a]	Average Viable Colonies[a]	Average Percent Relative Cloning Efficiency[a]	Average Percent Relative Total Growth[a]	Average Mutation Frequency[a,b] x 10^{-6}	Fold Increase[c]
Negative Control								
Medium	--	121	44	374	86	104	23.5	--
Solvent Control								
Dimethyl sulfoxide	1%	100	67	437	100	100	30.5	--
Positive Control								
N-nitrosodimethylamine[d]	0.04 µL/mL	59	280	95	22	13	589	25.1
Test Material								
R-25788	2.5 µg/mL	98	77	408	94	91	37.5	1.2
	5.0 µg/mL	53	73	403	92	49	36.0	1.2
	7.5 µg/mL	30	64	410	94	28	31.0	1.0
	10.0 µg/mL	26	84	383	88	22	43.5	1.4
	25.0 µg/mL	13	144	297	68	9	97.5	3.2
	40.0 µg/mL	7	204	283	65	5	144.0	4.7

[a]Average results from duplicate cultures except the positive control; a single replicate was used. Results were calculated by our reviewers.

[b]Mutation Frequency (MF) = $\frac{\text{Mutant Colonies}}{\text{Viable Colonies}} \times 2 \times 10^{-4}$.

[c]Fold increase = $\frac{\text{MF of Test Dose}}{\text{MF of Solvent Control}}$. Note: It was assumed that the positive control was diluted in culture medium.

[d]Two levels of the positive control (0.04 and 0.05 µL/mL N-nitrosodimethylamine) were used; the lowest dose was selected as representative.

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DICHLORMID MICRONUCLEUS (84-2)

Principal Reviewer: Nancy E. McCarroll
Toxicology Branch 1/HED (7509C)
Signature: Nancy E McCarroll
Date: 2-24-99

Secondary Reviewer: Irving Mauer, Ph.D.
Toxicology Branch 2/HED (7509C)
Signature: Irving Mauer
Date: 02/24/99

DATA EVALUATION REPORT

STUDY TYPE: Mutagenicity: In vivo micronucleus assay in mice; OPPTS 870.5395 [§84-2]

DP BARCODE: D253247 SUBMISSION NO.: S546651

PC CODE: [Inert] TOX. CHEM. NO.: [Inert] MRID NO: 41561403

TEST MATERIAL (PURITY): R-25788 (97.2%)

COMPOSITION/SYNONYM(S): Dichlormid; EHC-0829-33

CITATION: J. B. Majeska (1987). Mutagenicity evaluation in bone marrow micronucleus R-25788 T-13182; ICI Americas Inc., Farmington, CT, Study No. T-13182; dated December 31, 1987. (Unpublished) MRID NO: 41561403.

SPONSOR: ICI Americas Inc., Wilmington, DE

EXECUTIVE SUMMARY: In an in vivo mouse micronucleus assay (MRID No. 41561403), groups of five male CD-1 mice received single oral gavage administrations of 1000, 1500 or 2000 mg/kg Dichlormid as R-25788 (97.2%) and five female per group received 500, 1000 or 1200 mg/kg. Bone marrow cells were collected 24, 48 or 72 hours after compound administration and were examined for micronucleated polychromatic erythrocytes (MPEs). The test material was delivered to the test animals in corn oil.

Deaths were observed in high-dose males and females, and a slight cytotoxic effect on the target organ (bone marrow cells) was seen in the high-dose groups at the 72-hour sacrifice. The positive control induced the expected high yield of MPEs. There was, however, no evidence that R-25788 was clastogenic or aneugenic at any dose or harvest time.

The study is classified as acceptable and satisfies the guideline requirement for a mouse micronucleus assay (84-2).

COMPLIANCE: Signed and dated Data Confidentiality, GLP and Quality Assurance statements were provided.

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A. MATERIALS:

1. Test Material: R-25788

Description: Amber liquid
Identification Number: WRC 4921-35-11 GGD-0101
Purity: 97.2%
Receipt date: 9/30/87
Stability: Unspecified; expiration date: 5/90
Contaminants: None listed
Solvent used: Corn oil
Other provided information: The test material was stored in the dark at room temperature (~20°C) and at ambient humidity. The report also indicated that analytical determinations were performed on the "test substance/vehicle mixtures" within 1 week of preparation and were found to be within 10% of the target concentrations.

2. Control Materials:

Negative/Route of administration: None

Vehicle/Final concentration/Route of administration: Corn oil (0.5 mL/animal) was administered by oral gavage.

Positive/Final concentration/Route of administration: Cyclophosphamide (CP) was dissolved in water and administered by an unspecified route at 100 and 150 mg/kg (males) and at 150 and 200 mg/kg (females).

Note: Only the 100-mg/kg and 150-mg/kg dose levels for males and females, respectively, were scored.

3. Test Compound:

Route of administration: Oral gavage

Dose levels used:

(a) Range-finding assay: 1000, 1500, 2000, 2500, and 3000 mg/kg (5 males and 5 females per dose)

(b) Micronucleus assay: 1000, 1500, and 2000 mg/kg (males: 5/dose/sacrifice time)
500, 1000, and 1200 mg/kg (females: 5/dose/sacrifice time)

(c) Secondary group: Additional animals (up to 10/sex) were randomly selected to receive the solvent or the low, mid, or high dose of the test material. Animals in the secondary group were used only to replace animals that died in the respective primary groups.

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4. Test Animals:

(a) Species mouse Strain CD1 Age 6-7 weeks
Weight range: At dosing 25.11 g (males), 21.29 g (females)
Source: Charles River Breeding Laboratories.

(b) No. animals used per dose:

(1) Range-finding assay: 5 males; 5 females
(2) Micronucleus assay: 15 males; 15 females

Note: Dosing was based on mean body weights (see Section 4a)

© Properly maintained? YES

B. TEST PERFORMANCE:

1. Treatment and Sampling Times:

(a) Test compound:
Dosing: x once _____ twice (24 hr apart)
N/A other (describe): _____
Sampling (after last dose): _____ 6 hr _____ 12 hr
x 24 hr x 48 hr x 72 hr

(b) Vehicle control:
Dosing: x once _____ twice (24 hr apart)
N/A other (describe): _____
Sampling (after last dose): x 24 hr x 48 hr
x 72 hr

© Positive control:
Dosing: x once _____ twice (24 hr apart)
N/A other (describe): _____
Sampling (after last dose): _____ 24 hr x 48 hr
_____ 72 hr

2. Tissues and Cells Examined:

x bone marrow N/A others (list):
Number of polychromatic erythrocytes (PCEs) examined per animal: 1000
Number of normochromatic erythrocytes (NCEs, more mature RBCs) examined per animal: 1000

3. Details of Slide Preparation: At 24, 48, and 72 hours after administration of the test material or the vehicle control, the appropriate groups of animals were sacrificed by cervical dislocation. Sacrifice time for the positive control group was 48 hours. Bone marrow cells were either aspirated or flushed from both tibiae, centrifuged, resuspended in fetal calf serum, and spread onto slides. Prepared slides were fixed in absolute methanol, stained with 2%

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Giemsa solution, coverslipped, and scored. The report did not indicate whether slides were coded prior to scoring.

4. Statistical Methods: The data were evaluated for statistical significance at $p<0.01$ using the Kastenbaum-Bowman tables.

5. Evaluation Criteria: The test material was considered positive for micronuclei induction if a significant ($p<0.01$) increase in micronucleated polychromatic erythrocytes (MPEs) compared to the solvent control was seen, and the response was dose- and/or time-related.

C. REPORTED RESULTS:

1. Range-Finding Assay: Groups of five male and five female mice received single oral gavage doses of 0, 1000, 1500, 2000, 2500, or 3000 mg/kg and were observed for signs of clinical toxicity and/or death for 3 days. Immediately after treatment, animals in all dose groups convulsed. Within 8 hours of treatment, one male and one female in the 2000-mg/kg group, three males and two females in the 2500-mg/kg group, and two males and three females in the 3000-mg/kg group died. Deaths observed at 24 hours were as follows: two females (1500 mg/kg); two males and one female (2500 mg/kg); and two males and two females (3000 mg/kg). At 48 hours, the remaining high-dose male died. Deaths reported in females at 48 hours were as follows: two at 1500 mg/kg, one at 2000 mg/kg, and one at 2500 mg/kg. One female administered 1500 mg/kg and another female administered 2000 mg/kg died at 72 hours. Overall, the results indicated that no males survived treatment with ≥2500 mg/kg and that 80% of the males receiving 2000 mg/kg survived. In females, ≥60% died within 3 days of administration of test material at levels of ≥1500 mg/kg; no deaths occurred in low-dose females. Based on these findings, treatment levels of 1000, 1500, and 2000 mg/kg (males) and 500, 1000, and 1200 mg/kg (females) were selected for the micronucleus assay.

2. Micronucleus Assay:

(a) Animal observations: The report stated that three high-dose males, one mid-dose female, and one high-dose female died prior to the scheduled sacrifice. Mice from the secondary group were used to replace the dead animals. No compound effects were reported for the low-dose group.

(b) Micronucleus assay: Representative results from the micronucleus assay conducted with R-25788 are presented in Table 1. At the 72-hour harvest, there was a decrease of ~54% and ~37% in the PCE/NCE ratio for high-dose males and females, respectively, compared to the corresponding vehicle control values. Although the PCE/NCE ratios for the control groups were relatively low at all harvest intervals, the

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values fell within the acceptable range (≤0.1).[1] Since control group PCE/NCE ratios tended to increase with time, the reduced PCE/NCE ratios noted in high-dose animals at the 72-hour sacrifice suggest a slight effect on hematopoiesis. As further shown in Table 1, the evaluated levels of R-25788 (1000, 1500, or 2000 mg/kg males; 500, 1000, or 1200 mg/kg females) did not cause a significant increase in the frequency of MPEs at any harvest time. By contrast, MPEs were significantly ($p<0.01$) increased in male and female mice administered the positive control (CP at 100 mg/kg). From the overall findings, the study author concluded that R-25788 was negative in the mouse micronucleus assay.

3. REVIEWER'S DISCUSSION/CONCLUSIONS: Our assessment is in agreement with the study author that R-25788 was not clastogenic in this in vivo assay. The evidence of overt compound toxicity in conjunction with a slight adverse effect on bone marrow stem cells indicated that the high doses (2000 mg/kg for males and 1200 mg/kg for females) selected for the study adequately demonstrated that the maximum tolerated dose was achieved.

Additionally, the sensitivity of the test system to detect a genotoxic response in male and female mouse bone marrow cells was shown by the significant results obtained with the positive control (100 mg/kg CP). We conclude, therefore, that the study provided acceptable evidence that R-25788 was negative in this test system.

E. STUDY DEFICIENCIES: Slides were not coded prior to analysis. However, it is doubtful this deficiency compromised the overall results.

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

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TABLE 1. Representative Results of the Micronucleus Assay in Mice with R-25788

Substance	Dose	Exposure Time[a] (hours)	Sex	Number of Animals Analyzed per Group	Number of PCEs Analyzed per Group[b]	Number of MPEs per Group[b]	Average Percent MPEs per Group[b]	Average Number PCEs/1,000 NCEs per Group[b]	PCE/NCE[b] Ratio
Vehicle Control									
Corn oil	0.5 mL	24	M	5	5,000	1	0.02	125.4	0.13
			F	5	5,000	10	0.20	191.8	0.19
		48	M	5	5,000	2	0.04	240.6	0.24
			F	5	5,000	3	0.06	167.2	0.17
		72	M	5	5,000	4	0.08	219.0	0.22
			F	5	5,000	3	0.06	300.2	0.30
Positive Control									
Cyclophosphamide	100 mg/kg	48	M	5	5,000	37[c]	0.74	149.2	0.15
	150 mg/kg		F	5	1,592	31[c]	1.95	32.8	0.03
Test Material									
R-25788	2,000 mg/kg[d,e]	24	M	5	5,000	2	0.04	195.6	0.20
	1,200 mg/kg[d,e]		F	5	5,000	2	0.04	302.2	0.30
	2,000 mg/kg	48	M	5	5,000	2	0.04	195.2	0.20
	1,200 mg/kg		F	5	5,000	2	0.04	169.2	0.17
	2,000 mg/kg	72	M	5	5,000	4	0.08	99.6	0.10
	1,200 mg/kg		F	5	5,000	1	0.02	193.0	0.19

[a]Time after compound administration
[b]Values were calculated by our reviewers.
[c]Significantly higher than the negative control ($p<0.01$) by Kastenbaum-Bowman tables
[d]Results for the low-dose (1,000 mg/kg, males; 500 mg/kg, females) and mid-dose (1,500 mg/kg, males; 1,000 mg/kg, females) groups were not significantly different from the vehicle control.
[e]Three high-dose males, one high-dose female, and one mid-dose female died prior to the scheduled sacrificed. Animals in the secondary group were used to replace dead animals.

PCE = Polychromatic erythrocytes
MPE = Micronucleated polychromatic erythrocytes
NCE = Normochromatic erythrocytes.

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014199

DICHLORMID

MAMMALIAN CELLS IN CULTURE CYTOGENETICS (84-2)

Principal Reviewer: Nancy E. McCarroll
Toxicology Branch 1/HED (7509C)

Signature: Nancy E. McCarroll
Date: 2-24-99

Secondary Reviewer: Irving Mauer, Ph.D.
Toxicology Branch 2/HED (7509C)

Signature: Irving Mauer
Date: 02/24/99

DATA EVALUATION REPORT

STUDY TYPE: Mutagenicity: Mammalian cells in culture cytogenetic assay in mouse lymphoma cells; OPPTS 870.5375 [§84-2]

DP BARCODE: D253247 SUBMISSION NO.: S546651

PC CODE: [Inert] TOX. CHEM. NO.: [Inert] MRID NO: 41561406

TEST MATERIAL (PURITY): R-25788 (Dichlormid EHC-0829-33) (97.2%)

CITATION: Majeska, J. B. (1987). Mutagenicity Evaluation in L5178Y Mouse Lymphoma Multiple Endpoint Test Cytogenetic Assay R-25788 T-13180; ICI Americas Inc., Farmington, CT, Study No.: T-13180; date issued December 31, 1987. (Unpublished) MRID NO: 41561406.

EXECUTIVE SUMMARY: In an *in vitro* chromosome aberration assay (MRID No. 41561406), mouse lymphoma L5178Y cells were exposed to Dichlormid as R-25788 (97.2%) at doses ranging from 200-500 μg/mL in the absence of S9 activation and doses of 8-60 μg/mL in the presence of S9 activation. Cells were harvested 12 hours post-treatment and metaphases were analyzed for structural chromosome aberrations. The S9 was derived from Aroclor 1254-induced Sprague Dawley male rat livers, and the test material was delivered to the test system in dimethylsulfoxide.

Under nonactivated conditions, R-25788 did not increase the frequency of structural or numerical chromosome aberrations in cells harvested 12 hours posttreatment. Cytotoxicity as indicated by a marked reduction in the mitotic index was apparent at the highest assayed level (500 μg/mL); higher concentrations were severely cytotoxic. **The findings indicate that nonactivated R-25788 was tested over an adequate range of test material concentrations but failed to induce a clastogenic effect.**

No conclusions can be reached; however, for the S9-activated phase of testing. No significant or dose-related increase in the percentage of cells with structural or numerical aberrations was seen. However, the presence of rare complex aberrations (i.e., triradials, quadriradials, and translocations) at the majority of the doses, although not sufficient to conclude that R-25788 is a

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clastogen, should have prompted the performance of a repeat test to resolve this issue. Similarly, the lack of a marked cytotoxic effect on high-dose cultures suggest that higher concentrations could have been evaluated. The study author claimed that the number of cells completing two cell cycles (M_2) was reduced at all S9-activated assayed levels in parallel cultures incubated with BrdU for 16 hours; no data were provided to support this statement. If cell cycle delay was suspected, the rationale for proceeding with the analysis of 12-hour posttreatment cultures, presumably with a high proportion of M_1 cells, is unclear. Based on the above considerations, we assess that conditions may not have been optimal to detect the potential, if any, of S9-activated R-25788 to induce clastogenesis.

STUDY CLASSIFICATION: The study is **Unacceptable** and does not satisfy the requirements for an *in vitro* mammalian cell cytogenetic assay (84-2)because definitive conclusions can not be reached It is recommended that the S9-activated assay be repeated using either higher test material levels and/or a prolonged cell harvest time.

COMPLIANCE: Signed and dated Data Confidentiality, GLP and Quality Assurance statements were provided.

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I. MATERIALS AND METHODS

1. Test Material: R-25788

Description: Amber liquid
Identification No.: WRC 4921-35-11 GGD-0101
Purity: 97.2%
Receipt date: 9/30/87
Stability: Unspecified; expiration date: 5/90
Contaminants: None listed
Solvent used: Dimethyl sulfoxide (DMSO).
Other provide information: The test material was stored in the dark at room temperature (~20°C) and at ambient humidity. The report stated that test material concentrations ≤0.8 mg/mL did not "substantially" alter the pH or osmolality of the treatment medium. Solutions of the test material were used within ≈2 hours of preparation.

2. Control Materials:

Negative: Fischer's medium supplemented with 10% horse serum, 1.9 mM glutamine, 210 µg/mL sodium pyruvate, 476 µg/mL pluronic, and antibiotics.

Solvent/final concentration: DMSO/1%

Positive: Nonactivation (concentrations, solvent): Ethyl methanesulfonate (EMS) was prepared in an unspecified solvent to yield a final concentration of 1.0 µL/mL.

Activation (concentrations, solvent): Cyclophosphamide (CP) was prepared in an unspecified solvent to yield a final concentration of 20 µg/mL.

3. Activation: S9 derived from male Sprague-Dawley

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

The rat S9 liver homogenate was prepared by the performing laboratory and was assigned Lot No. EHC-0476-25.

S9 mix composition:

Component	Concentration/mL of Culture Medium
NAD?	1.2 mg
Isocitrate	6.0 mg
S9	0.04 mL

4. Test Compound Concentration Used:

   a. Preliminary cytotoxicity assay: Two preliminary cytotoxicity assays were performed; doses evaluated were:

      (1) Initial trial: Nine doses (40, 60, 80, 100, 200, 400, 800, 1400, and 1600 µg/mL +/-S9).

      (2) Repeat trial: Seven nonactivated doses (200, 300, 350, 400, 500, 550, and 600 µg/mL) and five S9-activated doses (8, 10, 20, 40, and 60 µg/mL).

   Note: The repeat cytotoxicity assay was conducted concurrent with the cytogenetic assays.

   b. Cytogenetic assay:

      (1) Nonactivated conditions: Five doses (200, 300, 350, 400, and 500 µg/mL) with a 12-hour cell harvest.

      (2) S9-activated conditions: Five doses (8, 10, 20, 40, and 60 µg/mL) with a 12-hour cell harvest.

5. Test Cells: L5178Y ($TK^{+/-}$) mouse lymphoma cells, subclone 3.7.2 were obtained from Dr. Donald Clive, Borroughs Wellcome, Research Triangle Park, NC.

   Properly maintained? Yes.

   Cell line or strain periodically checked for mycoplasma contamination? Yes.

   Cell line or strain periodically check for karyotype stability? Not reported.

B. TEST PERFORMANCE:

1. Cell Treatment:

a. Cells exposed to test compound for:
__4__ hours (nonactivated) __4__ hours (activated)

b. Cells exposed to positive controls for:
__4__ hours (nonactivated) __4__ hours (activated)

c. Cells exposed to negative and/or solvent controls for:
__4__ hours (nonactivated) __4__ hours (activated)

2. Preliminary Cytotoxicity Assay: Prepared cells, seeded at a density of $6x10^5$ cells/mL, were exposed for 4 hours to the selected doses of the test material or the solvent (DMSO) with or without S9 activation. To terminate exposure, cells were washed, resuspended in growth medium, and incubated. After 24-hours, cells were counted to determine cytotoxicity (decrease in relative suspension growth). A second set of treated cultures was resuspended in growth medium containing 0.3 to $1x10^{-4}$ M BrdU and reincubated for 24 hours. Cultures were harvested and stained by the procedure of Perry and Wolff,[1] and 100 randomly selected metaphases were scored for the number of cells undergoing one (M_1), two (M_2), or three (M_3) cell cycles.

3. Cytogenetic Assay:

a. Dosing: Duplicate cultures containing $6x10^5$ cells/mL were exposed for 4 hours to the selected doses of the test material and the medium, solvent, or positive controls with or without S9 activation. At the end of exposure, cells were washed, resuspended at $3x10^5$ cells/mL, and incubated at 37°C in 5% CO_2.

b. Cytotoxicity assessment: At ≈10 hours postexposure, an aliquot of cells were counted to determine relative suspension growth. A second set of cultures, containing BrdU was harvested 24 hours after treatment to determine cell cycle kinetics.

c. Metaphase arrest/cell harvest: Approximately 10 hours post-exposure, cultures with ≥$1x10^5$ cells/mL were selected for harvest, centrifuged, resuspended in growth medium containing colcemid (0.08 µg/mL), and reincubated for an additional 2 hours. Cells were swollen with distilled water, incubated at room temperature

[1]Perry P., and Wolff, S. New Giemsa method for the differntial staining of sister chromatids. Nature (1974) 251:156-158.

for 10 minutes, and centrifuged. Harvested cells were fixed in Carnoy's fixative and incubated overnight at 4°C.

d. Slide preparation: Fixed overnight cells were placed onto slides, air dried, and stained with 10% Giemsa. All slides were coded prior to analysis.

e. Metaphase analysis: The five highest doses yielding sufficient scorable metaphases were selected for analysis. Fifty metaphases/culture were scored for structural and numerical chromosome aberrations. Chromatid and chromosome gaps were recorded in the raw data but were not reported. The mitotic index (MI) was determined for each dose by counting the number of mitoses/500 cells.

4. Statistical Analysis: Structural aberrations were analyzed on a per cell basis using Student's t-test (one-tailed) with no differentiation as to the type of aberration.

5. Evaluation Criteria:

a. Assay validity: The study was considered valid if (1) the results for the solvent and positive control were within the historical range of the reporting laboratory; (2) there was no significant differences between solvent control replicates; and (3) a sufficient number of cells was available from the solvent control and three doses of the test material.

b. Positive response: A test material was considered positive if it induced a dose-dependent response over three consecutive doses and the increase at the highest dose was signficantly higher ($p<0.01$) than the solvent control.

C. REPORTED RESULTS:

1. Preliminary Cytotoxicity Assay: The study author stated that the first trial of the cytotoxicity assay was conducted as part of the mouse lymphoma cell gene mutation assay (see Data Evaluation Record 91-5). As shown in Table 1, ≥36% of the cells survived exposure to nonactivated levels ≥800 µg/mL. Although the cell-cycle kinetics data were difficult to interpret, we assume that no metaphases were recovered from cultures treated with 1400 and 1600 µg/mL -S9. Results for 400 and 800 µg/mL-S9 suggested a slight delay in progression through the cell cycle. In the presence of S9 activation, ≤19% of the cells survived at doses ≥60 µg/mL. The lowest dose (40 µg/mL) reduced cell survival to 27% but had no effect on cell cycling. Based on these findings, a second cytotoxicity was performed concurrent with the cytogenetic assay; these results are discussed below in conjunction with the cytogenetic assay.

[illegible]:

[illegible] conditions: Initially seven doses (200 to 800 µg/mL [illegible]). Although cell survival for all doses was 37% [illegible] stated that an insufficient number of metaphases was from cultures treated with 550 and 600 µg/mL. Accordingly, metaphases harvested from exposure levels of 200, 300, 350, 400, and 500 µg/mL were scored for chromosome aberrations. Cytotoxicity, as indicated by the marked reduction in the MI, was clearly apparent at 500 µg/mL. MIs were suppressed for the majority of test doses compared to the solvent control; however, there was no significant increase in the percentage of cells with either structural or numerical aberrations (Table 2). By contrast, the nonactivated positive control (1.0 µl/mL EMS) induced a significant ($p<0.01$) increase in the percentage of cells with aberrations.

b. S9-activated conditions: The five doses investigated under S9-activated conditions ranged from 8 to 60 µg/mL. Cell survival over the entire dose range was ≥71% and there were no adverse effects on the MI. The study author claimed that the number of cells that completed two rounds of DNA synthesis following treatment with all S9-activated doses was reduced after an additional 16-hour incubation in the presence of BrdU; no data were presented to support this statement. However, the relative staining [illegible] for parallel cultures treated with the three highest doses and reincubated in culture medium containing BrdU for 22 hours (99.5% at 20 µg/mL; 98.5% at 40 µg/mL; and 96.5% at 60 µg/mL) did not indicate that M_2 or M_3 cells were reduced.

Numerical aberrations were not significantly higher than the control at any level. However, significant increases in the percentage of cells with structural aberrations were reported for single replicate cultures at 8, 10, and 20 µg/mL. We, therefore, combined the data from replicates and compared the average results to the pooled negative and solvent control values using the Student's t-test. As indicated in Table 2, when the data was combined for individual dose groups, no significant increase were seen. Similarly, neither the number of aberrations/cell nor the percentage of cells with aberrations indicated a dose-dependent effect. However, the occurrence of rare complex aberrations at the majority of test doses (1 translocation at 8 µg/mL; 1 triradial and 1 translocation at 20 µg/mL; and 1 quadriradial at 60 µg/mL) was an unusual finding that was ignored by the study author. It was, therefore, concluded that R-25788 was not clastogenic in this test system.

D. REVIEWER'S DISCUSSION/CONCLUSIONS: We assess that there was sufficient evidence to conclude that nonactivated R-25788 was tested up to a cytotoxic

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[illegible] induce a clastogenic response in L5178Y mouse lymphoma [illegible] conclusions can be reached regarding the S9-activated [illegible] appearance of rare complex aberrations at the majority of [illegible] levels should have prompted the performance of a repeat test [illegible] the reproducibility of this finding. Similarly, the 71% cell survival [illegible] g/mL and the lack of an adverse effect on the MI suggests that higher levels could have been tested. The study author claimed that M cells were reduced at all levels following a 16-hour incubation with BrdU. If cell cycle delay was suspected, it is unclear why the 12-hour harvest cytogenetic assay was continued. It would have been prudent to repeat the test using a prolonged cell harvest to ensure that conditions were optimal for the detection of a clastogen.

We assess, therefore, that the S9-activated cytogenetic assay with R-25788 should be repeated and that consideration should be given to either increasing the starting concentration and/or using a prolonged cell harvest.

E. STUDY DEFICIENCIES: See above

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DICHLORMID

MAMMALIAN CELLS IN CULTURE CYTOGENEITCS

[illegible] Test Results in the Initial Cytotoxicity Assay with [illegible]

Substance	[illegible]	% Aberration	Average Percent Relative Growth	[illegible] %
Negative Control/Solvent [illegible]				
Medium/Dimethyl sulfoxide			100	
			103	
Test Material				
R-25788	200		91	
	400		65	
	800		56	
	1400		34	
	1600		34	
	40		27	
	60		19	

Average Relative Staining Index = $\frac{\text{Total no. of cells in treatment groups that have completed two (M) or three (M) cycles in 48}}{\text{Total no. of M or M cells in the negative/solvent control group}}$

'Cell survival at lower concentrations (40, 60, 80, and 100 µg/mL) was >100%.

Cell survival at higher levels (80, 100, 200, 400, 800, 1400, and 1600 µg/mL) was <7%.

DICHLORMID

MAMMALIAN CELLS IN
CULTURE CYTOGENEITCS

[illegible] Chromosomal Aberration Assay in Mouse Lymphoma [illegible] Harvested 12 Hours After Treatment With R-25788

Substance	[illegible]	S9 Activation	Total No. of Cells Scored	Mitotic Index (%)	Total No. Aberrations	Number of Aberrations/Cell	% Cells with Aberrations	[illegible]
Negative Control								
Medium	-		100	7.0	2	0.02	2	[illegible]
		+	100	8.0	1	0.01	1	
Solvent Control								
Dimethyl sulfoxide	1%	-	100	6.5	1	0.01	1	[illegible]
	1%	+	100	8.4	0	0.00	0	
Positive Control								
Ethylmethane sulfonate 6 TR;	1.0 µl/mL	-	50	3.0	15	0.30	20	[illegible]
Cyclophosphamide	20.0 µg/mL	+	50	5.0	6	0.12	10	[illegible]
Test Material								
R-25788	500 µg/mL*	-	100	2.2	1	0.01	1	[illegible]
	8 µg/mL	+	100	6.5	3	0.03	3	[illegible]
	10 µg/mL	+	100	6.3	2	0.02	2	[illegible]
	20 µg/mL	+	100	5.9	5	0.05	5	[illegible]
	40 µg/mL	+	100	4.4	1	0.01	1	[illegible]
	60 µg/mL	+	100	6.3	4	0.04	4	[illegible]

Results from replicate cultures combined and recalculated by our reviewers.
*Significantly higher than the pooled negative and solvent control ($p \leq 0.05$) by Student's t test.
Significantly higher than the pooled negative and solvent control ($p \leq 0.01$) by Student's t test.
Results for lower nonactivated levels (200, 300, 350, and 400 µg/mL) did not [illegible] cytotoxic effect

Abbreviations used:
AF - Acentric fragment TB - Chromatid break [illegible]
F - Fragment TR - Triradial [illegible]

014199

DICHLORMID

MAMMALIAN CELLS IN CULTURE CYTOGENETICS (84-2)

Principal Reviewer: Nancy E. McCarroll
Toxicology Branch 1/HED (7509C)

Signature: Nancy E McCarroll
Date: 2/24/99

Secondary Reviewer: Irving Mauer, Ph.D.
Toxicology Branch 2/HED (7509C)

Signature: Irving Mauer
Date: 02/24/99

DATA EVALUATION REPORT

STUDY TYPE: Mutagenicity: Mammalian cells in culture cytogenetic assay in human lymphocytes; OPPTS 870.5375 [§84-2]

DP BARCODE: D253247 SUBMISSION NO.: S546651

PC CODE: [Inert] TOX. CHEM. NO.: [Inert] MRID NO: 41561407

TEST MATERIAL (PURITY): Dichlormid (R-25788) (97.2%)

COMPOSITION/SYNONYM(S): R-25788; EHC-0829-33

CITATION: Howard, C.A. (1989). Dichlormid: An Evaluation in the In Vitro Cytogenetic Assay in Human Lymphocytes; ICI Central Toxicology Laboratory, Macclesfield, Cheshire, UK; Study No.: SV0311; Report No. CTL/P/2470; date issued June 7, 1989. (Unpublished) MRID NO: 41561407.

SPONSOR: ICI Americas Inc., Wilmington, DE

EXECUTIVE SUMMARY: In an *in vitro* chromosome aberration assay (MRID No. 41561407), human lymphocytes obtained from one male and one female donor were exposed to Dichlormid (R-25788) (97.2%) at doses ranging from 2.5-1200 µg/mL in both the absence and presence of S9 activation. Cells were harvested and metaphases were analyzed from cells dosed with 75, 500 or 750 µg/mL +/-S9. The S9 was derived from Aroclor 1254-induced Alpk:APfSD male rat livers, and the test material was delivered to the test system in dimethylsulfoxide.

The high dose with or without S9 activation induced a ≥50% decrease in the mitotic index. The positive controls induced the expected high yield of cells with structural chromosome aberrations. There was, however, no evidence that Dichlormid induced a clastogenic response at any nonactivated or S9-activated dose.

The study is classified as **Acceptable** and satisfies the requirements for an in vitro mammalian cell cytogenetic assay (84-2).

COMPLIANCE: Signed and dated Data Confidentiality, GLP, Flagging and Quality Assurance statements were provided.

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I. MATERIALS AND METHODS

A. MATERIALS:

1. Test Material: Dichlormid (R-25788)
   Description: Amber liquid
   Identification No.: SC2/88
   Purity: 97.2%
   Receipt date: Not reported
   Stability: Unspecified for this study; however, R-25788 was listed as stable at <100°C in an additional report submitted by the sponsor (see Data Evaluation Record 91-8).
   Contaminants: None listed
   Solvent used: Dimethyl sulfoxide (DMSO)
   Other provide information: No information on test material storage conditions or the frequency of dose solution preparation were provided.

2. Control Materials:

   Negative: None

   Solvent/final concentration: DMSO/1µg/mL

   Positive: Nonactivation (concentrations, solvent): Mitomycin C (Mit C) was prepared in 0.85% physiological saline to yield a final concentration of 0.5 µg/mL.

   Activation (concentrations, solvent): Cyclophosphamide (CP) was prepared in 0.85% physiological saline to yield final concentrations of 50 and 100 µg/mL. Only cultures treated with 100 µg/mL were scored.

3. Activation: S9 derived from AlpK:APfSD male rats

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

The rat S9 liver homogenate was prepared by the performing laboratory.

S9 mix composition:

Component	Final Concentration in S9 Mix
Na_2HPO_4	75 mM
KCl	25 mM
NADP	3 mM
Glucose 6-Phosphate	4 mM
$MgCl_2$	6 mM
S9	50%

Note: 200 µl of the S9 mix were added to 10 mL of culture medium

4. Test Compound Concentration Used:

(a) Preliminary cytotoxicity assay: A preliminary cytotoxicity assay was not performed.

(b) Cytogenetic assay:

(1) Nonactivated conditions: A concentration range of 2.5 to 1200 µg/mL was initially used; cultures exposed to 75, 500, and 750 µg/mL were scored for structural aberrations.

(2) S9-activated conditions: As above.

5. Test Cells: Human lymphocytes were obtained from the blood of two healthy subjects (one male and one female) with an established history of low chromosome damage. Lymphocyte cultures were initiated and maintained in RPMI 1640 medium supplemented with 10% fetal bovine serum, 0.1 mg/mL phytohemagglutinin, and antibiotics.

Properly maintained? Yes.

Cell line or strain periodically checked for mycoplasma contamination? Not applicable.

Cell line or strain periodically check for karyotype stability? Not applicable.

B. TEST PERFORMANCE:

1. Cell Treatments:

(a) Cells exposed to test compound for:
2 hours and 40 minutes-3 hours and 55 minutes (nonactivated)
2 hours and 40 minutes-3 hours and 55 minutes (activated)

(b) Cells exposed to positive controls for:

2 hours and 40 minutes-3 hours and 55 minutes (nonactivated)
2 hours and 40 minutes-3 hours and 55 minutes (activated)

- Cells exposed to negative and/or solvent controls for:
  2 hours and 40 minutes-3 hours and 55 minutes (nonactivated)
  2 hours and 40 minutes-3 hours and 55 minutes (activated)

2. Cytogenetic assay:

(a) Treatment: At ≈44 hours after initiation, replicate cultures (two/sex), were exposed to the selected test material doses, the solvent control (DMSO), or the positive controls (Mit C or CP) in both the presence and absence of S9-activation. At the end of treatment, cells were centrifuged, refed culture medium, and reincubated. Colchicine (final concentration, 10 µg/mL) was added 2 hours before the cultures were harvested (72 hours postinitiation). Metaphase cells were collected, swollen in 0.075M KCl, and fixed in glacial acetic acid: methanol (1:3). Slides were stained with 10% Giemsa and coded.

(b) Metaphase analysis: Two hundred metaphase cells in treatment and solvent control groups (100 cells/culture/donor) were scored for chromosome aberrations; 25 cells/donor from one of the two replicate cultures for each positive control were also scored for aberrations. The mitotic index (MI) was determined for each treatment group.

- Statistical methods: The percentage of cells with chromosome aberrations (excluding gaps) was evaluated using the Fisher's exact test.

(d) Evaluation criteria: No criteria were provided to establish the validity of the assay or the biological significance of the results.

C. REPORTED RESULTS: The highest nonactivated and S9-activated dose (750 µg/mL) was selected for the evaluation of chromosomal aberration based on a ≥50% decrease in the MI, compared to solvent control value. As the results presented in Table 1 show, MIs for 750 µg/mL-S9 were ~70% lower than the solvent control; with S9-activation, MIs for the high-dose cultures were ~50 to 60% lower than the solvent control. However, dichlormid at 75, 500, or 750 µg/mL +/-S9 did not induce a clastogenic response in replicate cultures from the two donors. By contrast, significant ($p<0.01$) chromosome damage was seen in cultures exposed to the nonactivated (0.5 µg/mL Mit C) and the S9-activated (100 µg/mL CP) positive controls. The study author, therefore, concluded that dichlormid was not clastogenic in cultured human lymphocytes.

D. REVIEWERS' DISCUSSION/CONCLUSIONS: We assess that the study was properly conducted and that the study author's interpretation of the data was correct. Dichlormid was assayed to a cytotoxic level with no indication of a clastogenic effect either in the absence or presence of S9-activation. Additionally, the sensitivity of the test system to detect a clastogenic effect was adequately demonstrated by the significant results obtained in both donor cell cultures exposed to the nonactivated and S9-activated positive controls. The study, therefore, provides acceptable evidence of a negative response in this test system.

E. STUDY DEFICIENCIES: NONE

TABLE 1. Representative Results from the Human Lymphocyte In Vitro Cytogenetic Assay with Dichlormid

Substance	Dose/mL	S9-Activation	No. of Cells Scored	Mitotic Index (%)	Total No. Of Aberrations[a]	No of Cells with Aberrations[a]	Percent Cells with Aberrations[a]	Biologically Significant Aberrations[b] (No./Type)
Solvent Control								
Dimethyl Sulfoxide	1 µl	-	200[c]	15.0	0	0.00	0.00	--
	1 µl	-	200[d]	14.5	0	0.00	0.00	--
	1 µl	+	200[c]	15.0	0	0.00	0.00	--
	1 µl	+	200[d]	12.5	0	0.00	0.00	--
Positive Control								
Mitomycin C	0.5 µg	-	25[c]	5.0[e]	8	0.32	28.00[f]	2 B; 4 F-M; 2 Ot
	0.5 µg	-	25[d]	5.0[e]	5	0.20	20.00[f]	1 B; 2 F-M; 1 I; 1 Ot
Cyclophosphamide	100 µg	+	25[c]	4.0[e]	17	0.68	52.00[f]	9 B; 6 F-M; 2 Ot
	100 µg	+	25[d]	2.0[e]	9	0.36	36.00[f]	4 B; 4 F-M; 1 Ot
Test Material								
Dichlormid	750 µg[g]	-	200[c]	5.0[e]	0	0.00	0.00	--
	750 µg[g]	-	200[d]	4.5	0	0.00	0.00	--
	750 µg[g]	+	200[c]	7.5	0	0.00	0.00	--
	750 µg[g]	+	200[d]	5.0	0	0.00	0.00	--

[a]Gaps excluded.

[b]Abbreviations used:

B = Break
F-M = Fragments and minutes
I = Interchanges
Ot = other (not specified).

[c]Lymphocytes obtained from donor 1.

[d]Lymphocytes obtained from donor 2.

[e]Mitotic index was determined from a single culture.

[f]Significantly higher ($p<0.01$) than the solvent control by Fisher's exact test.

[g]Results for lower doses (75 and 500 µg/mL +/-S9) did not suggest a clastogenic effect.

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DICHLORMID

UNSCHEDULED DNA SYNTHESIS

EPA Reviewer: Irving Mauer, Ph.D
Toxicology Branch 2 (7509C)
EPA Secondary Reviewer: Nancy McCarroll
Toxicology Branch 1 (7509C)

Date: 3/15/99

Date: 3-16-99

DATA EVALUATION RECORD

STUDY TYPE: Other Genotoxicity: Unscheduled DNA Synthesis in Primary Rat Hepatocytes: OPPTS 870.5550 [84-2]

DP BARCODE: D252 242 (Sub-bean to D248305) SUBMISSION CODE: S546651

P.C. CODE: [Inert] TOX. CHEM. NO.: [Inert]

TEST MATERIAL (PURITY): R-25788 (Dichlormid, 97.2%)

SYNONYMS: [None]

CITATION: Tarca, J.P. and Majeska, J.B. (1988). Unscheduled DNA Synthesis in Rat Primary Hepatocytes: R-25788, performed at ICI America's Environmental Health Center (EHC), Final Report No. T-13181, dated 04/12/88. Unpublished. MRID No. 44606409.

SPONSOR: Imperial Chemical Industries (ICI) America, Wilmington, DE

EXECUTIVE SUMMARY: In an unscheduled DNA synthesis assay (MRID No. 44606409), primary rat hepatocyte cultures were exposed to R-25788 in DMSO at concentrations of 20-60 *u*g/mL for 19-20 hours. In addition to untreated (medium) and solvent (DMSO) controls, additional cultures were exposed to a known mutagen, dimethylnitrosamine (DMN) to serve as a positive control.

R-25788 was tested up to a cytotoxic concentration (60 *u*g/mL) . The positive controls induced the appropriate response. **There was no consistent, reproducible evidence that unscheduled DNA synthesis, as determined by radioactive tracer procedures [nuclear silver grain counts] was induced.**

This study is classified as Acceptable, and satisfies the requirement for FIFRA Test Guideline 84-2 for other genotoxic mutagenicity data.

COMPLIANCE: Signed and dated GLP, Quality Assurance and Data Confidentiality statements were provided.

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I. MATERIALS AND METHODS

A. MATERIALS

1. Test Material: R-25788 (dichlormid)
   Description: Amber liquid
   Lot/Batch No.: GGD 0101, WRC 4921-35-11
   Purity: 97.2% a.i.
   Stability of compound: Stated to be "unspecified: expires 5/90"
   Solvent used: Dimethylsulfoxide (DMSO)
   Other comments: Storage: Room temperature (approximately 20 degrees C), ambient humidity, in the dark.

2. Control Materials:
   Negative: Medium (untreated)
   Solvent/final concentration: DMSO/0.01 mL/mL
   Positive (concentrations, solvent): Dimethylnitrosamine (DMN), 10 mM [solvent not provided]

3. Test compound concentrations used (for preliminary cytotoxicity test, if performed, and main assay): 20-60 *u*g/mL.

4. Media: Complete Williams Medium E (WEC) supplemented with 10% fetal bovine serum (heat inactivated), glutamine (2mM), penicillin (100 units/mL) and streptomycin (100 *u*g/mL).

5. Test Cells: Primary rat hepatocytes prepared from adult male Sprague-Dawley rats.

6. Cell Preparation: According to referenced procedures.

   a. Perfusion Technique: According to referenced procedures.

   b. Hepatocyte Harvest/Culture Preparation: According to conventional (acceptable) cytological techniques.

B. TEST PERFORMANCE

1. Cytotoxicity Assay: [Not performed]

2. UDS Assay:

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a. Hepatocyte cultures attached to glass coverslips were treated for 19-20 hours with stated concentrations of test article in medium also containing 10 *u*Ci/mL tritrated thymidine (^{3}HTdR).

b. Preparation of Autoradiographs/Grain Development: After treatment, cells were fixed, autoradiographed [method and radiographic material not stated] and exposed at -20 degrees C to 4 degrees C for 4 to 7 days. After (photographic) development, cell cultures were stained with hematoxylin/eosin.

c. Grain Counting: Grains over nuclei of 50 cells/slide, 2 slides per treatment, were counted (with an Artek colony counter) connected to a Zeiss microscope linked to an Artek camera; gross nuclear counts were corrected for background (cytoplasmic counts).

d. Evaluation Criteria: Acceptable criteria for both assay acceptance and response were applied.

e. Statistical Analysis: Dunnett's t-test, $p < 0.01$.

II. REPORTED RESULTS

A. Preliminary Cytotoxicity Assay: [Not performed]

B. UDS Assay: R-25788 was tested over the dose range of 20-60 *u*g/mL; the highest concentration resulted in cell toxicity, as evidenced by reduced cell attachment and altered cell morphology. Increased nuclear grain counts were recorded in single replicates of 50 and 60 *u*g/mL (Report Table 1, p. 10, Attachment 1) but was not reproducible in companion cultures of the first trial, nor in a second trial using the same doses (Report Table 2, p. 11, Attachment 2).

The positive controls responded with large increases in net nuclear grain counts in both trials.

Hence, the authors concluded that the test article did not exhibit DNA damaging activity in rat primary hypatocyte cultures, as determined by thymidine incorporation (UDS) by treatment up to cytotoxic concentrations.

III. REVIEWER'S DISCUSSION/CONCLUSIONS

A. The reviewer agrees with the study authors that the test article was tested to reportedly toxic concentrations without inducing any increased repair (UDS), as measured by net nuclear grain counts.

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B. STUDY DEFICIENCIES

A number of minor deficiencies were noted (such as stability, characterization and homogenicity of the test article; reporting a preliminary cytotoxicity and/or solubility testing), but these did not impact the conclusions drawn by the authors, since cytotoxicity was evident at the highest dose of the test article and the positive controls were appropriately responsive.

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THE FOLLOWING ATTACHMENT IS NOT AVAILABLE ELECTRONICALLY -- SEE THE FILE COPY

ATTACHMENT 1

STUDY REPORT TABLE 1, p. 10
MRID NO. 44606409

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DICHLORMID TOXR 014199

Page 245 is not included in this copy.

Pages _____ through _____ are not included in this copy.

The material not included contains the following type of information:

_____ Identity of product inert ingredients.

_____ Identity of product impurities.

_____ Description of the product manufacturing process.

_____ Description of quality control procedures.

_____ Identity of the source of product ingredients.

_____ Sales or other commercial/financial information.

_____ A draft product label.

_____ The product confidential statement of formula.

_____ Information about a pending registration action.

X FIFRA registration data.

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

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ATTACHMENT 2

STUDY REPORT TABLE 2, p. 11
MRID NO. 44606409

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DICHLORMID TOXR 014199

Page 247 is not included in this copy.

Pages _____ through _____ are not included in this copy.

The material not included contains the following type of information:

_____ Identity of product inert ingredients.

_____ Identity of product impurities.

_____ Description of the product manufacturing process.

_____ Description of quality control procedures.

_____ Identity of the source of product ingredients.

_____ Sales or other commercial/financial information.

_____ A draft product label.

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X FIFRA registration data.

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

_____ The document is not responsive to the request.

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

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DICHLORMID

IN VIVO/IN VITRO UDS (84-2)

Principal Reviewer: Nancy E. McCarroll
Toxicology Branch 1/HED (7509C)

Signature: Nancy E. McCarroll
Date: 3-15-99

Secondary Reviewer: Irving Mauer, Ph.D.
Toxicology Branch 2/HED (7509C)

Signature:
Date: 03-16-99

DATA EVALUATION REPORT

STUDY TYPE: Mutagenicity: In vivo/in vitro unscheduled DNA synthesis assay in primary rat hepatocytes following in vivo dosage [§84-2]

DP BARCODE: D253247 SUBMISSION NO.: S546651

PC CODE: [Inert] TOX. CHEM. NO.: [Inert] MRID NO: 41561408

TEST MATERIAL (PURITY): R-25788 (97.2%)

COMPOSITION/SYNONYM(S): Dichlormid; EHC-0829-33

CITATION: Manley, A., Hamilton, C., Steinmetz, K., Mirsalis, J.C., and Sutherland, R. (1989). Evaluation of the Potential of Dichlormid to Induce Unscheduled DNA Synthesis in the In Vivo-In Vitro Hepatocyte DNA Repair Assay in the F-344 Rat; SRI International, Menlo Park, CA; Study No. ICI Study No. SV0350; SRI Project No. LSC-7257; Study Issued August 18, 1989 (Unpublished) MRID NUMBER: 41561408.

SPONSOR: Imperial Chemical Industries, Macclesfield, Cheshire, UK

EXECUTIVE SUMMARY: In independently conducted *in vivo/in vitro* unscheduled DNA synthesis (UDS) assay (MRID No. 41561408), groups of male Fischer-344 rats were administered single oral gavage doses of 350, 700 or 1400 mg/kg Dichlormid (97.2%) prepared in corn oil. Animals were sacrificed at 2 hours (2 males/dose in the first trial; 3 males/dose in the second trial) or 16 hours (3 males/dose in the first trial; 2 males/ dose in the second trial) posttreatment, and hepatocytes were analyzed for UDS.

Clinical signs of toxicity (e.g., weakness, loss of balance, and low body temperature), adverse effects on the target organ (i.e., mottled livers), and cytotoxic effects on the hepatocyte cultures were apparent in the high-and/or mid-dose groups. The results obtained with the positive controls confirmed the sensitivity of the test system to detect UDS. **There was no evidence that Dichlormid at the selected doses increased the frequency of UDS in treated hepatocytes at either sacrifice interval.**

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The study is classified as Acceptable (Nonguideline) and does not satisfy the requirements for FIFRA Test Guideline 84-2 for UDS mutagenicity data. It can, however, be considered supplemental information.

COMPLIANCE: Signed and dated GLP, Quality Assurance , Flagging and Data Confidentiality statements were provided.

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A. MATERIALS

1. Test Material: Dichlormid (R-25788)
Description: Amber liquid
Identification Number: WRC 4921-35-11
Purity: 97.2%
Receipt date: 1/9/89
Stability: Stable at <100°C
Contaminants: None listed
Solvent used: Corn oil
Other provided information: The test material was stored at room temperature. Doses of the test material suspended in corn oil were prepared immediately prior to use.

2. Control Materials

Negative/route of administration: None

Vehicle/final concentration/route of administration: Corn oil at a 5 mL/kg was administered by oral gavage.

Positive/final concentration/Route of administration:
- Dimethylnitrosamine (DMN) was administered in water at 10 mg/kg by oral gavage.
- Dinitrotoluene (DNT) was administered in corn oil at 125 or 150 mg/kg by oral gavage.
- 2-Acetylaminofluorene (2-AAF) was administered in corn oil at 50 mg/kg by oral gavage.

3. Test Compound

Route of administration: Oral gavage

Dose levels used:

(a) Range-finding test: 300, 600, 1200, 2500, 5000 mg/kg
(3 males/group)

(b) UDS assay: 350, 700, or 1400 mg/kg

- Trial 1--2 males/group (2-hour sacrifice)
3 males/group (16-hour sacrifice)

- Trial 2--3 males/group (2 hour-sacrifice)
2 males/group (16 hour-sacrifice)

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4. Test Animals

a. Species rat ; Strain Fischer-344 ; Age >9 weeks (at dosing); Sex males ; Weight range 155.3 to 282.9 g (at dosing)

Source: Harlan Sprague-Dawley Laboratories, Inc., Frederick, MD

b. Number of animals used per dose: See below

Substance	Dose (mg/kg)	Number of Male Rats/Trial/Sacrifice Interval			
		Trial 1		Trial 2	
		2 hours	16 hours	2 hours	16 hours
Vehicle Control					
Corn oil	--	1	1	1	1
Positive Control					
Dimethylnitrosamine	10	1	-	2	1
Dinitrotoluene	125	-	-	-	1
	150	-	1	-	-
2-Acetylaminofluorene	50	-	-	-	1
Test Material					
Dichlormid	350	2	3	3	2
	700	2	3	3	2
	1400	2	3	3	2

c. Properly maintained? Yes

B. TEST PERFORMANCE

1. UDS Assay

(a) Perfusion techniques/hepatocyte harvest: At 2 and 16 hours post-treatment, animals in the appropriate groups were anesthesized with sodium pentobarbital and livers were perfused with an undefined collagenase solution for an unspecified time. Hepatocytes were released with a comb and inoculated into six-well cultures dishes. Each well contained a coverslip and Williams' Medium E (WME), supplemented with 2 mM L-glutamine, 10% fetal bovine serum, and antibiotics. Cultures were placed in a humidified incubator at 37°C and 5% CO_2 for a 1.5- to 2-hour attachment period. Unattached cells were removed; viable cells were refed serum-free WME containing 10 µCi/mL [^{3}H]-thymidine for 4 hours, washed, and reincubated for 14-18 hours in WME containing 0.25 mM unlabeled thymidine.

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(b) Slide preparation: Hepatocytes attached to coverslips were washed, swollen with 1% sodium citrate, fixed in glacial acetic acid:ethanol (1:3), washed, and mounted.

© Preparation of autoradiographs/grain development: Slides were coated with Kodak NTB-2 emulsion, stored for 7 days at -20°C, stained with 1% methyl-green Pyronin Y, and coded.

(d) Grain counting: Hepatocytes harvested from animals that were sacrificed at 2 and 16 hours were used to determine UDS. The nuclear grains of at least 30 morphologically normal cells/slide/animal/dose group were counted with an automatic colony counter. Net nuclear grain counts were determined by subtracting the highest cytoplasmic grain count of two nuclear-sized areas adjacent to each nucleus from the nuclear grain counts of each cell. Mean net nuclear grain counts, standard error of the mean, and the percentage of cells in repair were calculated.

(e) Statistical methods: Data for the UDS were not analyzed statistically.

2. Evaluation Criteria

(a) Assay validity: The assay was considered acceptable if (1) the vehicle control data were within historical ranges and (2) if the positive controls showed an increase in net nuclear grain counts and the percentage of cells undergoing repair.

(b) Positive response: The test material was considered positive if the mean net nuclear grain count for any dose was >5 and the percentage of cells in repair was >20%. If mean net nuclear grain counts fell between 0 and 5, the data were assessed relative to dose response, increases in percentage of cells in repair, reproducibility of the results among animals, and the frequency of the cellular responses.

C. REPORTED RESULTS

1. Preliminary Range-Finding Assay: Groups of 3 male rats were administered single oral gavage doses of 300, 600, 1200, 2500, or 5000 mg/kg of the test material and were sacrificed 7 days post-treatment. The report indicated that all rats in the high-dose group and 1 rat in the 2500-mg/kg dose group died following dosing on day 0. The remaining animals administered 2500 mg/kg dichlormid died on day 1; no further deaths occurred over the 7-day observation period. Clinical signs seen in the 1200-mg/kg group included uncoordinated motor control and rough fur. No treatment-related effects were reported from the gross examination of rats in the three lowest dose groups (300, 600, 1200 mg/kg). Based on these findings, the median lethal dose was calculated as 1734 mg/kg. The doses selected for the UDS assay, 350, 700, and 1400 mg/kg, therefore, represented 20%, 40%, and 80% of the $LD_{50/7}$, respectively.

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2. UDS Assay

(a) Clinical observations: No deaths occurred in the treatment groups. Rats in the high-dose group showed signs of weakness, drowsiness, loss of balance, low body temperature, and rough fur at 2 and 16 hours posttreatment. Drowsiness and rough fur were also reported for the mid-dose animals at 16 hours. No clinical signs of compound toxicity were noted in animals receiving 350 mg/kg of the test material. At the time of perfusion, the livers of mid- and high-dose rats were reported to be mottled.

(b) UDS results: Two independent UDS assays were performed with the selected range of dichlormid doses. Pyknotic cells, indicative of cytotoxicity, were reported in monolayers established from all treatment groups; the effect was most pronounced in the high-dose cultures harvested at 16 hours. Hepatocytes recovered from a total of 5 male rats per test material group sacrificed at 2 hours (2 in the first trial; 3 in the second trial) or 16 hours (3 in the first trial; 2 in the second trial) were scored for UDS activity. Because of the small sample sizes used in both trials, the data were combined and are presented in Table 1. As shown, the total number of animals in the control groups was low; however, the net nuclear grain counts (NG) and the percentage of cells in repair (%IR) were within the historical range published by the reporting laboratory for male Fischer-344 rats administered corn oil (-5.1±0.5 NG; 1±0 %IR).[1] Similarly, the responses induced by the positive controls (10 mg/kg DMN, 125 mg/kg DNT, and 50 mg/kg 2-AAF) were comparable to the results published for these gentoxicants.[2] Our reviewers, therefore, used the historical data to evaluate the activity of the test material. The combined results indicated that the single oral gavage administration of 350, 700, or 1400 mg/kg dichlormid did not increase the frequency of UDS in hepatocytes recovered from male rats 2 or 16 hours following treatment.

Based on the overall results, the study authors concluded that dichlormid was not genotoxic in this in vivo/in vitro UDS assay.

C. REVIEWER'S DISCUSSION AND INTERPRETATION OF STUDY RESULTS: We assess, in agreement with the study authors, that dichlormid did not increase the frequency of net nuclear grains in hepatocytes harvested from male rats 2 and 16 hours following dosing. The evidence of overt clinical signs (e.g., weakness, loss of balance, and low body temperature), adverse effects on the target organ (mottled livers), for the 700- or 1400-mg/kg treatment groups, and cytotoxic effects on the hepatocyte cultures (presence of pyknotic cells) indicated that an appropriate range of test material doses was evaluated.

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[1]Mirsalis, J. C.; Tyson, C. K.; and Butterworth, B. E. Detection of genotoxic carcinogens in the in vivo-in vitro hepatocyte DNA repair assay. Environ. Mutagen. 4(1982):553-562.

[2]Ibid.

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While the number of animals at each time point (2-3) in the treatment and control groups was lower than required by FIFRA Mutagenicity Guidelines for whole animal studies, the group sizes satisfy the OECD Draft Guidelines for *in vivo* USD assays (486, Feburary 1997). We conclude, therefore, that the study provides acceptable evidence that Dichlormid was negative in this test system.

E. STUDY DEFICIENCIES : FIFRA Guidelines do not exist for the *in vivo/in vitro* UDS assay; however, the findings can be considered supplemental and acceptable information.

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TABLE 1. Combined Representative Results of the In Vivo/In Vitro Unscheduled DNA Synthesis (UDS) Assays in Male Rats Dosed with Dichlormid

Substance	Dose (mg/kg)	Exposure Time[a] (hours)	No. of Animals per Group	No. of Hepatocytes Scored	Mean Nuclear Grain Count ± S.E.[b]	Mean Cytoplasmic Grain Count ± S.E.[b]	Net Nuclear Grain Counts ± S.E.[b]	Mean Percent Cells in Repair ± S.E.[b] (Cells with >5 Net Nuclear Grains)
Vehicle Control								
Corn oil	--	2	2	180	6.4	13.0	-6.6	0
		16	2	180	6.7	13.7	-7.0	2
Positive Control								
Dimethylnitrosamine[c]	10	2	3	270	37.2±4.7	10.7±0.4	26.5±4.4	93±2
Dinitrotoluene[d]	125	16	1	90	16.4	8.0	8.4	77
2-Acetylaminofluorene	50	16	1	90	29.2	14.7	14.4	88
Test Material								
Dichlormid	1400[e,f]	2	5	450	8.5±0.3	16.0±0.5	-7.5±0.5	2±1
		16	5	450	8.4±0.6	11.6±0.7	-3.2±0.4	3±(5±2)[g]

[a]Time after compound administration
[b]Means and standard error of the counts from 90 cells/animal (30 cells/slide)
[c]Two sacrifice times (2 and 16 hours) were conducted with dimethylnitrosamine; results from the 2-hour cell harvest were selected as representative.
[d]Dinitrotoluene was assayed at two concentrations (125 and 150 mg/kg); results from the lower level were selected as representative.
[e]Results for lower doses (350 and 700 mg/kg) did not suggest a genotoxic effect.
[f]Clinical signs of compound toxicity and mottling of the liver were reported for mid- and high-dose animals.
[g]Recalculated by our reviewers; presented mean was in error; S.E. value was not legible.

014199

[Dichlormid]

Acute Oral Study (§81-1)

EPA Reviewer: Jessica Kidwell, M.S.
RAB1, Health Effects Division(7509C)
Jessica Kidwell 2/23/99
[Signature and Date]
EPA Secondary Reviewer: Melba Morrow, D.V.M.
RAB1, Health Effects Division(7509C)
M S Morrow 2/26/99
[Signature and Date]

DATA EVALUATION RECORD

STUDY TYPE: Acute Oral Toxicity - [rat]
OPPTS 870.1100 [§81-1]

DP BARCODE: D248305
P.C. CODE: N/A
SUBMISSION CODE: S546651
TOX. CHEM. NO.: N/A

TEST MATERIAL (PURITY): Dichlormid (97.2%)

SYNONYMS: R25788; N, N-diallyl dichloroacetamide; 2,2-dichloro-N,N-di-2-propenylacetamide; N,N-diallyl-2,2-dichloroacetamide

CITATION: Robinson, P.(1990) Dichlormid: Acute Oral Toxicity to the Rat. ICI Central Toxicology Laboratory, Cheshire, UK. Report no. CTL/P/2197. November 16, 1990. MRID 44606401

SPONSOR: Zeneca Ag Products

EXECUTIVE SUMMARY: In an acute oral toxicity study (MRID 44606401), groups of fasted, young adult Wistar-derived albino rats/sex (5/sex) were given a single oral dose of dichlormid (97.2 % a.i.) in corn oil at doses of 500, 1,000, 2,000, 3,000, or 4,000 mg/kg and observed for 15 days.

Oral LD_{50} Males = 2,816 mg/kg (95% C.I. 2,143, 3,664 mg/kg)
Females = 2,146 mg/kg (95% C.I. 1,478, 2,910 mg/kg)

Dichlormid is classified as **TOXICITY CATEGORY III** based on the LD_{50} in both sexes.

No compound-related mortality occurred at 500 or 1,000 mg/kg. However, one female rat dosed at 500 mg/kg was killed *in extremis* on Day 5, but this was not thought to be compound related. Compound-related mortality occurred in 19/30 animals tested at ≥2,000 mg/kg within 5 days of administration. Clinical signs of toxicity seen in all dose groups included piloerection, upward curvature of the spine, decreased breathing rate, decreased activity, bizarre behavior and excessive grooming(500 and 1,000 mg/kg only), chromodacryorrhea, lachrymation, and salivation. Most animals dosed at 500 mg/kg and 1,000 mg/kg recovered by day 5. Animals from the 2,000, 3,000 or 4,000 mg/kg dose groups also exhibited tip toe gait, dehydration, reduced stability, and breathing abnormalities. All surviving animals in the ≥2,000 mg/kg dose groups recovered in 9 or 10 days after dosing. No

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treatment-related effects on body weight were observed in surviving animals. Gross necropsy of decedent animals revealed abnormal livers in 3 animals at 2,000 mg/kg, mottled liver and dark red intestines in one female at 3,000 mg/kg, and pale livers in 2 females at 4,000 mg/kg. Necropsy of animals sacrificed on day 15 revealed no abnormalities.

This acute oral study is classified **ACCEPTABLE (§81-1)**. This study does satisfy the guideline requirement for an acute oral study (§81-1) in the rat.

COMPLIANCE: Signed and dated GLP, Quality Assurance, Data Confidentiality, and Flagging statements were provided.

I. MATERIALS AND METHODS

A. MATERIALS:

1. Test Material: Dichlormid
   Description: Amber liquid
   Lot/Batch #: CTL Reference #Y06015/002/007
   Purity: 97.2% a.i.
   CAS #: N/A
   Verification of concentration/homogeneity as necessary

2. Vehicle: Corn Oil

3. Test animals: Species: Rat
   Strain: Alpk:APfSD, albino
   Age and weight at dosing: 7.5 weeks old, 213-304 g (males); 9 weeks old, 160-268 g (females)
   Source: Animal Breeding Unit, ICI Pharmaceuticals, Cheshire, UK
   Acclimation period: 6 days (minimum)
   Diet: Porton Combined Diet, Special Diet Services Ltd., ad libitum
   Water: Automatic system, ad libitum
   Housing: 5 rats/sex/cage
   Environmental conditions:
   Temperature: 15-24°C
   Humidity: 50±10%
   Air changes: 20-30 ACH
   Photoperiod: 12-hr light/12-hr dark

B. STUDY DESIGN and METHODS:

1. In life dates - Approximately July 20-August 3, 1988 (based on QA inspection/audit dates). Specific dates

were not reported.

2. Animal assignment and treatment - Animals were assigned to the test groups noted in Table 1. Following an overnight fast (16-24 hours), rats were given a single dose of dichlormid by gavage then observed between 30 and 90 minutes after dosing, between 4 and 6 hours after dosing, and once daily, up to 15 days. The animals were weighed on the day before dosing (Day -1), the day of dosing (Day 1), and on Days 3, 5, or 6, 8 and 15. Survivors were sacrificed and a necropsy was performed.

TABLE 1. Doses, mortality/animals treated

Dose (mg/kg)	males	Females	Combined
500	0/5	1/5*	1/10
1,000	0/5	0/5	0/10
2,000	1/5	3/5	4/10
3,000	2/5	3/5	5/10
4,000	5/5	5/5	10/10

*One female rat dosed at 500 mg/kg was killed *in extremis* on Day 5, but this was not thought to be compound related.

3. Statistics - The oral LD_{50} was calculated using logistic regression. Confidence limits were calculated using a likelihood ratio interval.

II. **RESULTS AND DISCUSSION:**

A. Mortality is given in table 1. There was no compound-related mortality at 500 or 1,000 mg/kg. However, one female rat dosed at 500 mg/kg was killed *in extremis* on Day 5. Necropsy findings for this animal included enlarged bladder and large mass above it which hemorrhaged. This finding was not thought to be compound related. Compound-related mortality occurred in 19/30 animals tested at ≥2,000 mg/kg within 5 days of administration. Females were more sensitive to the test material.

Oral LD_{50} (C.I.) for males = 2,816 mg/kg (95% confidence limits (2,143, 3,664 mg/kg))
females = 2,146 mg/kg (95% confidence limits (1,478, 2,910 mg/kg))

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B. Clinical observations - Clinical signs of toxicity seen in all dose groups included piloerection, upward curvature of the spine, decreased breathing rate, decreased activity, bizarre behavior and excessive grooming(500 and 1,000 mg/kg only), chromodacryorrhea, lachrymation, and salivation. Most animals dosed at 500 mg/kg and 1,000 mg/kg recovered by day 5. Animals from the 2,000, 3,000 or 4,000 mg/kg dose groups also exhibited tip toe gait, dehydration, reduced stability, and breathing abnormalities. All surviving animals in the ≥2,000 mg/kg dose groups recovered in 9 or 10 days after dosing.

C. Body Weight - No treatment-related effects on body weight were observed in surviving animals, which exhibited overall (day 1-day 15) increases of 30% for males and 31% for females.

D. Necropsy - Gross necropsy of decedent animals revealed abnormal livers in 3 animals (1 male [pale liver], 2 females [no description reported]) at 2,000 mg/kg, mottled liver and dark red intestines in one female at 3,000 mg/kg, and pale livers in 2 females at 4,000 mg/kg.

E. Deficiencies - There were no deficiencies that affected the validity of the study results.

SignOff Date:
DP Barcode:
HED DOC Number:
Toxicology Branch:

[Dichlormid] Acute Dermal Study (§81-2)

EPA Reviewer: Jessica Kidwell, M.S.
RAB1, Health Effects Division (7509C)
Jessica Kidwell 2/23/99
[Signature and Date]
EPA Secondary Reviewer: Melba Morrow, D.V.M.
RAB1, Health Effects Division (7509C)
MMorrow 2/26/99
[Signature and Date]

DATA EVALUATION RECORD

STUDY TYPE: Acute Dermal Toxicity - rat
OPPTS 870.1200 [§81-2]

DP BARCODE: D248305
P.C. CODE: N/A
SUBMISSION CODE: S546651
TOX. CHEM. NO.: N/A

TEST MATERIAL (PURITY): Dichlormid (R25788)(97.2%)

SYNONYMS: N, N-diallyl dichloroacetamide; 2,2-dichloro-N,N-di-2-propenylacetamide; N,N-diallyl-2,2-dichloroacetamide

CITATION: Robinson, P.(1990) Dichlormid (R25788): Acute Dermal Toxicity to the Rat. ICI Central Toxicology Laboratory, Cheshire, UK. Report no. CTL/P/2187. Study No. CR2445. November 16,1990. MRID 44606402.

SPONSOR: Zeneca Ag Products

EXECUTIVE SUMMARY: In an acute dermal toxicity study (MRID 44606402), five Wistar-derived albino rats/sex were dermally exposed to dichlormid (97.2% a.i.) at 2,000 mg/kg (limit dose) for 24 hours. The test material was applied as received to approximately 10% of the total body surface area. Animals then were observed for clinical signs of toxicity and mortality for up to 14 days following administration.

Dermal LD_{50} Males >2,000 mg/kg (observed)
Females >2,000 mg/kg (observed)

Dichlormid is classified as **TOXICITY CATEGORY III** based on the observed LD_{50} values for both sexes.

All animals survived the 14-day observation period. No clinical signs of toxicity or skin irritation were seen in any of the animals. Initially (between Days 1 and 3), nine of ten animals showed a decrease in body weight. By Day 8, however, all animals showed an increased body weight compared to their initial weight (Day 1) and continued to gain weight until the end of the study. No macroscopic abnormalities were seen in any of the animals at gross necropsy.

This acute dermal study is classified as **ACCEPTABLE (§81-2)**. It does satisfy the guideline requirement for an acute dermal study (§81-2) in the rat.

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COMPLIANCE: Signed and dated GLP, Quality Assurance, Data Confidentiality, and Flagging statements were provided.

I. MATERIALS AND METHODS

A. MATERIALS:

1. Test Material: Dichlormid (R25788)
Description: Amber liquid
Lot/Batch #: Y06015/002/007 (CTL Reference No.)
Purity: 97.2% a.i.
CAS #: Not provided

2. Vehicle: None employed

3. Test animals: Species: Rat
Strain: Wistar-derived albino (Alpk:APfSD strain)
Weight at dosing: 254-297 g for males; 183-216 g for females.
Age: Not reported.
Source: Animal Breeding Unit, ICI Pharmaceuticals, Alderly Park, Cheshire, UK.
Acclimation period: Six days prior to the study.
Diet: Porton Combined Diet (Special Services Ltd.) ad libitum
Water: Automatic system, ad libitum
Environmental conditions: Temperature: 15°C-24°C
Humidity: 50±10%
Air changes/hour (ACH):20-30 ACH
Photoperiod:12 light/12 dark

B. STUDY DESIGN and METHODS:

1. In life dates - April 13-27, 1988.

2. Animal assignment and treatment - Fur from the dorsal-lumbar areas (approximately 10 cm x 5 cm)of five animals/sex was clipped 16-32 hours prior to dermal administration of dichlormid at 2,000 mg/kg (limit dose). The test material was evenly spread, as received, over the clipped region using a 1 ml sterile disposable plastic syringe. A volume of 2 ml/kg was applied to each animal. The test substance was kept in contact with the skin for approximately 24 hours using occlusive dressings. A gauze patch was used to cover the test site; the patches were covered by a patch of plastic film, held in position with an adhesive bandage, and secured by two pieces of PVC tape wrapped around the animal. The coverings were removed 24 hours following application, and the test sites were cleaned using swabs of absorbent cotton wool soaked in warm water and dried with tissue paper.

The rats were observed for signs of systemic toxicity once between one and four hours after application and then once daily for systemic toxicity and skin irritation up to Day 15. Body weights were recorded on Days 1 (immediately prior to dosing), 3, 6, 8, and 15. At the end of the study, the surviving animals were sacrificed, necropsied, and examined for gross pathological changes.

3. Statistics: Not applicable to this study.

II. RESULTS AND DISCUSSION:

A. Mortality: All animals survived the 14-day observation period.

Dermal LD_{50} males >2,000 mg/kg (observed)
females >2,000 mg/kg (observed)

B. Clinical observations: No signs of skin irritation or toxicity were seen in any of the animals.

C. Body Weight: Nine of ten animals showed a decrease in body weight between Days 1 and 3, with an average decrease of 7% for males and 2% for females. By Day 8 all animals showed an increased body weight compared to their initial weight (Day 1). Overall (Days 1-15), all animals exhibited weight gains ranging from 13 to 35% (mean weight gain = 20% for both sexes).

D. Necropsy: No macroscopic abnormalities were seen in any of the animals at gross necropsy.

E. Deficiencies: There were no deficiencies that affected the validity of the study results.

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SignOff Date:
DP Barcode:
HED DOC Number:
Toxicology Branch:

[Dichlormid] Acute Inhalation Study (§81-3)

EPA Reviewer: Jessica Kidwell, M.S.
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EPA Secondary Reviewer: Melba Morrow, D.V.M.
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[Signature and Date]

DATA EVALUATION RECORD

STUDY TYPE: Acute Inhalation Toxicity - *rat*
OPPTS 870.1300 [§81-3]

DP BARCODE: D248305
P.C. CODE: N/A
SUBMISSION CODE: S546651
TOX. CHEM. NO.: N/A

TEST MATERIAL (PURITY): Dichlormid (97.2%)

SYNONYMS: R-25788; N,N-diallyldichloroacetamide; 2,2-dichloro-N,N-di-2-propenylacetamide; N,N-diallyl-2,2-dichloro-acetamide

CITATION: Hext, P.M. 1991. Dichlormid: 4-Hour Acute Inhalation Toxicity Study in the Rat. ICI Central Toxicology Laboratory (Cheshire, UK). Report number CTL/P/2305, January 10, 1991. MRID 44606403. Unpublished.

SPONSOR: Zeneca Ag Products

EXECUTIVE SUMMARY: In an acute inhalation toxicity study (MRID 44606403, groups of young adult Wistar-derived (Alpk:APfSD) albino rats (5/sex) were exposed by inhalation route to dichlormid, 97.2% a.i.,for 4 hours to a nose only exposure concentration of 5.5 mg/L with a particle size of 4.35 (GSD = 2.10). Animals were then observed for 14 days.

LC_{50} for males and females is >5.5 mg/L (limit test).

Dichlormid is classified as TOXICITY CATEGORY IV based on males and females.

None of the animals died during inhalation exposure or during the two week post-exposure period. Treatment-related clinical signs noted in test animals during exposure included salivation, lachrymation, and reduced response to sound. Immediately after exposure until Day 2, clinical signs indicative of neurological effects were seen and included head flicking, paw flicking, and salivation. During the 14-day observation period, abnormal respiratory noise (indicative of upper respiratory tract irritation) was noted in all males on Day 2 and continued in most males until Day 15, however, it was only present in 1-2 females from Days 2-5. Treated males and females had reductions in body weight and body weight gain initially after exposure on Day 1, however, by Day 8, weight gain was similar to or greater than

that of controls. There were no treatment related necropsy findings.

This acute inhalation study is classified as **ACCEPTABLE (§81-3)**, and satisfies the guideline requirement for an acute inhalation study (§81-3) in the rat.

COMPLIANCE: Signed and dated GLP, Quality Assurance, Data Confidentiality, and Flagging statements were provided.

I. MATERIALS AND METHODS

A. MATERIALS:

1. Test Material: Dichlormid
   Description: Amber liquid
   Lot/Batch #: Y06015/002/008
   Purity: 97.2% a.i.
   CAS #: N/A

2. Vehicle: None

3. Test animals: Species: rat
   Strain: Alpk:APfSD (Wistar-derived), albino
   Age: Approximately 7 weeks
   Weight at dosing: 225-246 g (males); 201-222 g (females)
   Source: Alderly Park, Cheshire, UK
   Acclimation period:
   Diet: Porton Combined Diet (Special Diets Services Ltd., Witham, Essex, UK), ad libitum
   Water: Automatic water system, ad libitum (except during exposure)

B. STUDY DESIGN and METHODS:

1. In life dates - start: June 22, 1988 end: July 6, 1988

2. Animal assignment and treatment - Animals were assigned to the groups noted in Table 1. Rats were exposed to dichlormid by nose only exposure for 4 hours. They were observed frequently during exposure, at the end of the 4-hour exposure, and daily for 14 days. Body weights were recorded on Day -1 , Day 1 (prior to exposure), and on Days 2, 3, 8, and 15. Survivors were sacrificed and a necropsy was performed.

TABLE 1. Concentrations, Exposure Conditions, Mortality/Animals Treated

Test Group	Nominal Conc. (mg/L)	Analytical Conc. (mg/L)	MMAD μm	GSD μm	Mortality/Animals treated		
					Males	Females	Both
Vehicle Control	0	0	--	--	0/5	0/5	0/10
Test Agent	33.26[a]	5.5(±0.49)	4.35	2.10	0/5	0/5	0/10

[a]The difference between the nominal and analyzed concentrations of dichlormid can be accounted for by chamber wall losses.

3. **Exposure chamber:** The animals were exposed nose-only in restraining tubes (Battelle) which were inserted into an ICI-designed PERSPEX exposure chamber (approximately 9.2 liter volume, however, in this study, two sections were connected giving an approximate volume of 27.6 liters) once the target concentration had been achieved and shown to be stable over 30 minutes. Chamber temperature and humidity (Vaisala HMI 31 portable, digital temperature and humidity monitor) were measured during exposure.

Atmosphere generation: The test atmosphere was generated using a concentric-jet glass atomizer. Dichlormid (neat) was pumped into the atomizer and air was passed through the atomizer at a flow rate of 14 liter/minute (at normal temperature and pressure), which carried the atmosphere to the exposure chamber. The control chamber was also supplied with air at a flow rate of 15 liter/minute. Air flow rates were altered as necessary to maintain the target concentrations. The test atmosphere was controlled on the basis of the total particulate concentration.

Particle Size Analysis: Particle size samples were obtained from the animals' breathing zone at half-hourly intervals during exposure by pulling chamber atmosphere through a 25 mm diameter Vinyl Metricel (VM-1) filter. The filter was weighed before and after the sample was taken. The aerodynamic particle size of the test atmosphere was measured using a Marple Cascade Impactor (supplied by Shaeffer Instruments Ltd.) which aerodynamically separates airborne particles into pre-determined size ranges.

Analytical Agent Sampling: Atmospheric sampling for analytical concentrations was taken by extracting the material deposited on the VM-1 filters and the stages of the Cascade Impactor in acetone and then analyzing the resultant solutions by gas chromatography/flame ionization detector.

5. Statistics - Test and control data were compared statistically using a two-sided Student's t-test.

II. RESULTS AND DISCUSSION:

A. Chamber conditions - Test atmosphere concentrations and particle size determinations are given in Table 1.

B. Mortality is given in Table 1. There were no deaths.

The LC_{50} for males and female rats is >5.5 mg/L.

B. Clinical observations - During exposure, both test and control animals had stains around the snout, wet fur, and chromodacryorrhea which, according to the study report, are effects frequently seen in animals when restrained. Treatment-related clinical signs noted in test animals during exposure included salivation, lachrymation, and reduced response to sound.

Immediately after exposure, both test and control animals had stains around the nose, wet fur, hunched posture and piloerection which, according to the study report, are effects frequently seen in animals when restrained. Piloerection persisted throughout the 14-day observation period in treated males and females and control males and in female controls through Day 8. Treatment-related neurological signs noted in the test animals immediately after exposure included head flicking, paw flicking, and salivation. Treated animals recovered by day 2.

During the 14-day observation period, abnormal respiratory noise (indicative of upper respiratory tract irritation) was noted in all males on Day 2 and continued in most males until Day 15, however, it was only present in 1-2 females from Days 2-5. Upward curvature of the spine was noted in 1/5 males from Days 3-7 and in 2-4 females from days 2-5.

C. Body Weight - Treated males and females had reductions in body weight and body weight gain after exposure on Day 1. Statistically significant differences, compared to controls, in body weight (bw) (<10% decrease in both sexes) and body weight gain were seen on Days 3 ($p<0.01$ for bw and bw gain)) and/or 8 ($p<0.05$ for bw gain) for males, and on days 2 ($p<0.05$ for bw gain, $p<0.01$ for bw) and 3 ($p<0.01$ bw gain) for females. Thereafter, weight gain was similar to or greater than that of controls and final group mean body weights were comparable.

D. Organ Weights - There were no significant differences in absolute or relative lung and liver weights between treated and

control animals.

E. Necropsy -No treatment-related findings were seen at gross necropsy.

E. Deficiencies - No deficiencies were noted.

SignOff Date:
DP Barcode:
HED DOC Number:
Toxicology Branch:

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[R-25788 (DICHLORMID)] Primary Eye Irritation Study (§81-4)

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DATA EVALUATION RECORD

STUDY TYPE: Primary Eye Irritation - Rabbit
OPPTS 870.2400 [§81-4]

DP BARCODE: D248305
P.C. CODE: N/A
SUBMISSION CODE: S546651
TOX. CHEM. NO.: N/A

TEST MATERIAL (PURITY): R-25788 (97.2%)

SYNONYMS: Dichlormid; N,N-diallyldichloroacetamide; 2,2-dichloro-N,N-di-2-propenylacetamide; N,N-diallyl-2,2-dichloro-acetamide

CITATION: Morgan, R.L. (1989) Ocular Irritation Test for R-25788. ICI Americas Inc., WRC Toxicology Laboratory, Richmond, CA. Laboratory report number T-13361, July 7, 1989. MRID 44606404.

SPONSOR: Zeneca Ag Products

EXECUTIVE SUMMARY: In a primary eye irritation study (MRID 44606404), 0.1 mL of R-25778 (dichlormid) (97.2% purity, Lot# WRC-4921-35-11) was instilled into the conjunctival sac of the left eye of each of nine Stauffland white rabbits. The treated eyes of three female rabbits were washed with water 20-30 seconds after exposure. The treated eyes of the remaining 6 rabbits (5 males, 1 female) were left unwashed. The right eye of each animal served as an untreated control. The animals were observed for ocular irritation at 1, 24, 48, 72, and 96 hours after treatment and eye irritation was scored by the Draize scheme.

One hour following instillation, 5/6 rabbits whose eyes were left unwashed had mild to moderate conjunctival redness (scores of 1-2) and 3/6 rabbits had mild conjunctival chemosis (score of 1). In all three rabbits whose eyes were washed 20-30 seconds after treatment, mild conjunctival redness and chemosis (score of 1) were observed one hour following instillation. All irritation subsided by 24 hours.

In this study, **R-25778 (dichlormid) is a mild ocular irritant**, and is classified as **TOXICITY CATEGORY IV** based on the mild to moderate ocular effects in 8/9 rabbits which subsided by 24 hours.

This study is classified **ACCEPTABLE (§81-4)**, and satisfies the

guideline requirement for a primary eye irritation study in the rabbit.

COMPLIANCE: Signed and dated GLP, Quality Assurance, Data Confidentiality, and Flagging statements were provided.

I. MATERIALS AND METHODS

A. MATERIALS:

1. Test Material: R-25788
   Description: Amber liquid
   Lot/Batch #: WRC-4291-35-11
   Purity: 97.2% a.i.
   CAS #: N/A

2. Vehicle: None

3. Test animals: Species: Rabbit
   Strain: Stauffland White
   Age: Young adult
   Weight at dosing: Not reported
   Source: Nitabell Rabbitry, Hayward, CA
   Acclimation period:
   Diet: Purina Certified Chow, ad libitum
   Water: ad libitum
   Environmental conditions: Temperature: 65-75°F
   Humidity: Not reported
   Air changes: Not reported
   Photoperiod: Not reported

B. STUDY DESIGN and METHODS:

1. In life dates: March 14-18, 1988

2. Animal assignment and treatment: The eyes of nine rabbits (5 males, 4 female) were examined at least 24 hours before application for ocular and periocular abnormalities. A fluorescein dye exam was also performed to detect corneal epithelial abnormalities. A 0.1-mL aliquot of R-25788 was instilled into the conjunctival sac of the left eye of each of nine rabbits. The treated eye was washed with water 20-30 seconds after exposure in three female rabbits. The eyes were left unwashed in the remaining 6 rabbits (5 males, 1 female). The right eye of each animal served as an untreated control. The animals were observed for ocular irritation at 1, 24, 48, 72, and 96 hours after treatment. Observations with fluorescein staining were

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made 24 hours post application until there was no staining for three consecutive observations. Fluorescein staining was graded using a modified procedure outlined by the National Research Council[1]. Eye irritation was scored by the Draize[2] scheme (scale not provided). Body weights were not reported.

II. RESULTS AND DISCUSSION:

A. Clinical observations: In six rabbits whose eyes were left unwashed, mild to moderate conjunctival redness (scores of 1-2) (5 rabbits) and mild conjunctival chemosis (score of 1) (3 rabbits) were observed one hour following instillation. In all three rabbits whose eyes were washed 20-30 seconds after treatment, mild conjunctival redness and chemosis (score of 1) were observed one hour following instillation. All irritation subsided by 24 hours. Based on the results of this study, R-25788 is a mild ocular irritant.

B. Deficiencies: There were no deficiencies that affected the validity of the study results.

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[1]National Research Council. (1977) Principals and Procedures for Evaluating the Toxicity of Household Substances. National Academy of Sciences, Washington, DC. Pp. 50-51.

[2]Draize, J.H. (1965) Appraisal of the Safety of Chemicals in Foods, Drugs, and Cosmetics - Dermal Toxicity. Association of Food and Drug Officials of the U.S. Topeka, Kansas, pp. 49-52.

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SignOff Date:
DP Barcode:
HED DOC Number:
Toxicology Branch:

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DATA EVALUATION RECORD

STUDY TYPE: Primary Dermal Irritation - (rabbit)
OPPTS 870.2500 [§81-5]

DP BARCODE: D252248
P.C. CODE: N/A
SUBMISSION CODE:
TOX. CHEM. NO.: N/A

TEST MATERIAL (PURITY): Dichlormid

SYNONYMS: R25788; N, N-diallyl dichloroacetamide; 2,2-dichloro-N,N-di-2-propenylacetamide; N,N-diallyl-2,2-dichloroacetamide

CITATION: Robinson, P. 1990. Dichlormid: Skin Irritation to the Rabbit. ICI Central Toxicology Laboratory, Cheshire, UK. Laboratory Report No. CTL/P/2188, Study No. EB3495. November 16, 1990. MRID 42807902. Unpublished.

SPONSOR: Not reported.

EXECUTIVE SUMMARY: In a primary dermal irritation study (MRID 42807902) six male New Zealand white albino rabbits were dermally exposed to 0.5 mL of dichlormid for 4 hours. Animals were then observed for twelve days. Irritation was scored by the Draize scale.

By 72 hours, 4/6 animals had well defined erythema. Very slight (2/6) or severe edema (3/6) was seen at 72 hours. Dermal irritation was reversible after day 5. In this study **dichlormid is a severe dermal irritant.** Dichlormid is classified as **TOXICITY CATEGORY II** for primary dermal irritation based on the degree of dermal effects observed at 72 hours.

This study is classified as **ACCEPTABLE** and satisfies the guideline requirement for a primary dermal irritation study (§81-5) in the rabbit.

COMPLIANCE: Signed and dated GLP, Quality Assurance, Data Confidentiality, and Flagging statements were provided.

I. MATERIALS AND METHODS

A. MATERIALS:

1. Test Material: Dichlormid

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Description: Amber liquid
Lot/Batch #: Y06015/002/007
Purity: 97.2% a.i.
CAS #: N/A

2. Vehicle: None.

3. Test animals
Species: Rabbit
Strain: New Zealand albino
Age: Not reported
Weight: 3,032-3,624 g (males)
Source: Mellor Rabbits, Greater Manchester, UK
Acclimation period: Six days
Housing: Single
Diet: Labsure CRB Rabbit diet, ad libitum
Water: Automatic system, ad libitum

B. STUDY DESIGN and METHODS:

1. In life dates - Approximately April 1988.

2. Animal assignment and treatment - Six male rabbits were given a single dermal dose of dichlormid (0.5 mL). Twenty-four hours prior to application, the hair was removed from the left flank of each animal. Dichlormid (0.5 mL) was dermally applied to the left flank of each rabbit. The test site was approximately 2.5 cm x 2.5 cm. The treated area was covered with gauze (2.5 x 2.5 cm), secured by surgical tape, covered by a piece of impermeable rubber sheeting wrapped once around the trunk of the animal and secured with polyethylene tape. All dressings were left in place for 4 hours then carefully removed. The application sites were gently cleaned using cotton wool soaked in warm water and then dried with tissue paper. The Draize scale[1] was used to grade the degree of erythema and edema at the application sites at 30-60 minutes, 1, 2, 3, 5 or 6, 8 or 9, and 11 or 12 days after removal of the dressings.

II. RESULTS AND DISCUSSION:

A. Thirty to sixty minutes after removal of the dressings, very slight or well defined erythema was seen in 5/6 and 1/6 animals, respectively, and very slight or slight edema was seen in all animals. By 72 hours, 4/6 animals had

[1]Draize, JH. 1959. Appraisal of the Safety of Chemicals in Foods, Drugs, and Cosmetics. Association of Food and Drug Officials of the US, 49.

well defined erythema and 2/6 animals had no erythema. Very slight (2/6) or severe edema (3/6) was seen at 72 hours. Dermal irritation was reversible after day 5. Mean erythema and edema scores were 1.4 and 2.5, respectively. Slight thickening on the application site was seen in one animal on day 5 and slight desquamation was seen in another animal on day 8.

B. Deficiencies - There were no deficiencies that affected the validity of the study results.

[DICHLORMID] Dermal Sensitization Study (§81-6)

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RAB1, Health Effects Division(7509C) [Signature and Date]
EPA Secondary Reviewer: Melba Morrow, D.V.M. MS Morrow 2/23/99
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DATA EVALUATION RECORD

STUDY TYPE: Dermal Sensitization - guinea pig
OPPTS 870.2600 [§81-6]

DP BARCODE: D248305 SUBMISSION CODE: S546651
P.C. CODE: N/A TOX. CHEM. NO.: N/A

TEST MATERIAL (PURITY): Dichlormid (97.2%)

SYNONYMS: R-25788; N,N-diallyldichloroacetamide; 2,2-dichloro-N,N-di-2-propenylacetamide; N,N-diallyl-2,2-dichloro-acetamide

CITATION: Robinson, P. (1990) Dichlormid: Skin Sensitization to the Guinea Pig. ICI Central Toxicology Laboratory (Cheshire, UK) Laboratory Report No. CTL/P/2220, Study No. GG4288. November 16, 1990. MRID 44606405. Unpublished.

SPONSOR: Zeneca Ag Products

EXECUTIVE SUMMARY: In a dermal sensitization study (MRID 446064-05) with dichlormid (97.2% a.i., Lot # Y06015/002/007)) in corn oil, twenty female Dunkin Hartley albino guinea pigs were tested using the maximization test method of Magnusson and Kligman. Ten females were used for induction and challenge controls and 10 males were used as controls for rechallenge. Data from a positive control study using formaldehyde (40% aqueous solution) were provided.

Following rechallenge with a 10% w/v preparation of dichlormid in corn oil, 4/20 (20%) test animals had scattered mild redness at 24 hours. No dermal irritation was observed in the control animals. The net percentage response was 20% indicating that rechallenge with a 10% w/v preparation of dichlormid in corn oil elicited a mild skin sensitization response (with no irritant response)in previously induced guinea pigs. Acceptable positive control data were provided to validate the test species and methods employed.

In this study, dichlormid (10% w/v in corn oil) is a mild dermal sensitizer.

This study is classified as **ACCEPTABLE (§81-6)**, and does satisfy

the guideline requirement for a dermal sensitization study (§81-6) in the guinea pig.

COMPLIANCE: Signed and dated GLP, Quality Assurance, Data Confidentiality, and Flagging statements were provided.

I. MATERIALS AND METHODS

A. MATERIALS:

1. Test Material: Dichlormid
Description: Amber liquid
Lot/Batch #: Y06015/002/007
Purity: 97.2% ai.
CAS #: Not available
Verification of concentration/homogeneity as necessary

2. Vehicle: Corn oil
Positive control: Formaldehyde (40% aqueous solution)

The most recent positive control data for formaldehyde (40% aqueous solution) were provided. A 1% (w/v) aqueous dilution of a 40% aqueous solution of formaldehyde was used for the intradermal injections. A 30% (w/v) dilution of a 40% aqueous solution of formaldehyde was applied both for the inductions and the challenges.

3. Test animals: Species: guinea pig
Strain: Alpk:Dunkin Hartley, albino
Age: Not reported
Weight at start of treatment: 241-353 g (females); 340-446 g females(weight at beginning of positive control study); males used for rechallenge controls were not weighed at the beginning of the study.
Source: Animal Breeding Unit (ICI Pharmaceuticals, Cheshire, UK)
Acclimation period: 6 days (minimum)
Diet: Labsure RGP Guinea Pig Diet, ad libitum
Water: Automatic system, ad libitum

B. STUDY DESIGN and METHODS:

1. In life dates - March 29-April 22, 1988

2. Animal assignment and treatment - The study was conducted according to the guinea pig maximization test method of Magnusson and Kligman [Allergic Contact Dermatitis in the Guinea Pig, Pub Thomas, USA (1970)]. Dose selection for the main study was based on the

results of a sighting study in which groups of two or more guinea pigs were used and up to four dose levels were tested on each group of animals. Intradermal injections of the test material in corn oil were tested to determine the highest concentration , up to 10% (w/v), that could be well tolerated locally and systemically (first induction). Topical applications of the test material in corn oil were tested to determine the highest concentration which did not cause greater than a mild to moderate irritation response in animals that had been injected with Freund's Complete Adjuvant at least 14 days previously (second induction). Topical application of the test material in corn oil was tested to determine the highest concentration which did not produce irritation in animals that had been injected with Freund's Complete Adjuvant at least 14 days previously (challenge).

Main study: For the first induction treatment, hair on the scapular region (5cm x 5cm) of 20 young adult female guinea pigs was clipped (time not specified) prior to the following row of three 0.5-mL injections made on each side of the mid-line. The injections were: 1) *Top*: Freund's Complete Adjuvant:corn oil (1:1); 2) *Middle*: dichlormid (10% w/v in corn oil); 3) *Bottom*: dichlormid (10% w/v in 1:1 preparation of Freund's Complete Adjuvant:corn oil. The injections were checked for adverse effects for up to 48 hours.

For the second induction treatment, 7 days following the intradermal treatment, the scapular area was clipped again and a 2- x 4-cm piece of filter paper was saturated with undiluted dichlormid and affixed over the injection sites of each animal in the treatment group. The filter pater was secured with surgical tape, covered by a strip of adhesive bandage (20-30 cm x 5 cm), and secured by self-adhesive PVC tape for an exposure period of 48 hours.

To serve as controls, an additional ten female animals received intradermal injections using an identical procedure to that used for the test animals, except that the injections were: 1) *Top*: Freund's Complete Adjuvant:corn oil (1:1); 2) *Middle*: corn oil; 3) *Bottom*: Freund's Complete Adjuvant:corn oil (1:1). The injections were checked for adverse effects for up to 48 hours. For the topical application, animals in the control group were similarly treated with filter paper

patches with nothing applied.

For the challenge treatment, 2 weeks following the topical induction treatment, both flanks (15 cm x 5 cm) of all the test and control animals were clipped free of hair and an occlusive dressing was prepared which consisted of two pieces of filter paper (1 cm x 1-2 cm) stitched to a piece of rubber sheeting (12 cm x 5 cm). Dichlormid (30% w/v in corn oil) was applied to one of the pieces of filter paper (affixed to the left shorn flank) and undiluted dichlormid was applied to the second piece of filter paper (affixed to the right shorn flank). The occlusive dressing was covered with a strip of adhesive bandage (25-40 cm x 7.5 cm) and secured with self-adhesive PVC tape. The occlusive dressing was removed after 24 hours.

Twenty-four and 48 hours following patch removal for the challenge application, dermal reactions were scored for erythema according to the following scale:

0 No reaction
1 Scattered mild redness
2 Moderate diffuse redness
3 Intense redness and swelling

The net sensitization response was determined by subtracting the percentage of control animals that responded from the percentage of test animals that responded. The net sensitization response was then compared with the following scheme:

% Net Response	Description
0	Not a sensitizer
>0-8	Weak sensitizer
>8-28	Mild sensitizer
>28-64	Moderate sensitizer
>64-80	Strong sensitizer
>80-100	Extreme sensitizer

Seven days following the initial challenge, the animals were rechallenged using two concentrations of dichlormid: 30% and 10% w/v in corn oil. The test sample was applied to different sites than those used for the initial challenge. A group of ten male control guinea pigs, which had been injected with Freund's Complete Adjuvant ten days previously, was used as the control group for the rechallenge.

Body weights of the females only were recorded at the

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beginning and termination of the experiment, on study days 1, 25 (controls), and 32 (test animals).

The positive control experiments were conducted with twenty test animals/study and ten control animals/study in the same manner as described above.

II. RESULTS AND DISCUSSION:

A. Induction reactions and duration - Observations following intradermal or epidermal induction treatments were not reported.

B. Challenge reactions and duration - Following challenge with undiluted dichlormid, scattered mild redness to moderate diffuse redness was observed in 13/20 (65%) test animals at 24 hours following patch removal. Scattered mild redness was also seen in 4/10 (40%) control animals at 24 hours. The net percentage response was 25% (mild sensitizer), however, dichlormid also elicited an irritant response.

Following challenge with a 30% w/v preparation of dichlormid in corn oil, 14/20 (70%) test animals showed scattered mild redness to moderate diffuse redness at 24 hours following patch removal. Five out of ten (50%) control animals showed scattered mild redness at 24 hours. The net percentage response was 20% (mild sensitizer), however, dichlormid also elicited an irritant response.

Following rechallenge with a 10% w/v preparation of dichlormid in corn oil, 4/20 (20%) test animals had scattered mild redness at 24 hours. No dermal irritation was observed in the control animals. The net percentage response was 20% indicating that rechallenge with a 10% w/v preparation of dichlormid in corn oil elicited a mild skin sensitization response (with no irritant response) in previously induced guinea pigs.

The irritant response was confirmed following a second challenge with a 30% w/v preparation of dichlormid in corn oil. Six of 20 test animals and 3/10 controls animals had scattered mild redness.

No effects on overall body weight were observed during the study, with average increases of 26% for the test group and 28% for the control group.

C. Positive control - In the formaldehyde study, 20/20 (100%)

of the induced animals exhibited a sensitization response (scattered mild redness to intense redness and swelling) following challenge with a 30% w/v dilution of a 40% w/v aqueous solution of formaldehyde at 24 hours. No response was seen in the controls. The net percentage was 100%, therefore, a 30% w/v dilution of 40% w/v aqueous solution formaldehyde elicited an extreme skin sensitization response in previously induced guinea pigs. These data validate the test species and methods employed.

D. Deficiencies - None.

[DICHLORMID] Dermal Sensitization Study (§81-6)

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