US EPA ARCHIVE DOCUMENT

DATA EVALUATION RECORD

DICLORAN/031301

STUDY TYPE: CHRONIC TOXICITY/CARCINOGENICITY FEEDING - RAT [OPPTS 870.4300 (OECD 453)]
MRID 46360701 (main study) & 46360702 (range-finding)

Prepared for

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

Prepared by

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Task Order 98-2005

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Oak Ridge National Laboratory, managed by UT-Battelle, LLC, for the U.S. Dept. of Energy under contract DE-AC05-00OR22725

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DATA EVALUATION RECORD

STUDY TYPE: Combined chronic toxicity/carcinogenicity feeding - rat
OPPTS 870.4300 [§83-5]; OECD 453.

PC CODE: 031301 **DP BARCODE**: D294474
SUBMISSION NO.: none

TEST MATERIAL (PURITY): Dichloran (94.9% a.i.)

SYNONYMS: 2,6-dichloro-4-nitroaniline

CITATION: Ramesh, E. 2004. Combined chronic toxicity and carcinogenicity study with dicloran in Wistar rats. Toxicology Department, Rallis Research Centre, Rallis India Ltd., Peenya II Phase, Bangalore - 560 058, India, Laboratory project ID 3080/00, August 10, 2004. MRID 46360701. Unpublished.

Ramesh, E. 2001. Dicloran: 90-day dietary dose range finding study in Wistar rats. Toxicology Department, Rallis Research Centre, Rallis India Ltd., Peenya II Phase, Bangalore - 560 058, India, Laboratory project ID 3080/00, December 10, 2001. MRID 46360702. Unpublished.

SPONSOR: Gowan Company, 370 South Main St., Yuma, AZ 85364

EXECUTIVE SUMMARY: In a combined chronic toxicity/carcinogenicity study (MRID 46360701), dicloran (94.9% a.i.; batch/lot # 000313) was administered in the diet to groups of 50 male and 50 female HsdCpb:WU rats at concentrations of 0, 60, 240 or 1405 (1200 ppm raised to 1440 ppm on day 106 of the study) for 2 years (main group). The dietary concentrations were equivalent to 0, 2.8, 11.3, and 71.0 mg dicloran/kg bw/day, respectively, for males and 0, 3.7, 15.0, and 94.1 mg dicloran/kg bw/day, respectively, for females. Additional groups of 10 male and 10 female rats were administered the same diets for 12 months for interim evaluations.

No treatment-related signs of toxicity were observed during daily observations, weekly physical examinations, or monthly veterinarian examinations. No adverse neurological effects were observed as assessed by the functional observational battery (FOB) conducted at 12 months. Survival was not affected by treatment with the test material, and no eye abnormalities were observed during ophthalmoscopic examinations. High-dose male and female rats gained 48% and 31% less weight, respectively, than controls during the first week of treatment resulting in body weights 16% and 8% (both $p \leq 0.05$) less than that of controls. Mean body weight of high-

dose males remained 8-13% ($p \leq 0.05$) less than that of controls for the remainder of the study, and mean body weight of high-dose females was 9-14% ($p \leq 0.05$) less than that of controls during the second year of the study. High-dose males and females gained 13% ($p \leq 0.05$) and 20% ($p \leq 0.05$) less weight, respectively, than controls for the entire study. High-dose rats consumed significantly less food than controls during the first 17 weeks (males) and 73 weeks (females); total food consumption was not affected and food efficiency for the entire study was similar for high-dose and control rats. Body weight, weight gain, and food consumption were not significantly and adversely affected in low- and mid-dose rats of either sex.

Analysis of hematologic parameters showed very mild transient changes in red blood cell (RBC) count, mean cell volume, and mean cell hemoglobin in high-dose male and female rats and was indicative of a mild hyperchromatic macrocytic anemia. These changes are not considered adverse. Other hematologic changes (total white blood cell (WBC), neutrophil, and lymphocyte counts in male and female rats and prothrombin time and platelet count in females) were not considered treatment-related. Statistically significant changes in clinical chemistry parameters in high-dose rats were too small to be considered adverse, were transient, or were not correlated with histopathologic findings.

Statistically significant changes in absolute organ weights and organ:body weight ratios were due to decreased terminal body weight. Postmortem examination showed no treatment-related gross findings in male or female rats receiving any dose of the test material. The primary target for microscopic lesions appeared to be the brain and spinal cord. Vacuolation was observed in the cerebral cortex including the optic chiasma, cerebellar cortex, and medulla/pons regions of the brain and in the cervical, thoracic, and lumbar segments of the spinal cord of high-dose males and females at 12 and 24 months (except the lumbar segment in males at 12 months). In the main group, vacuolation was observed in the brain of 62-96% of males and 84-98% of females and in the spinal cord of 56-86% of males and 46-86% of females compared with 0-4% of male controls and 0-2% of female controls. In addition, vacuolation in the optic chiasma in the cerebral cortex occurred in 28% of high-dose males and 34% of high-dose females compared with none of the controls. Vacuolar changes in the optic nerve were observed in 8% ($p=0.059$) of high-dose females compared with none of the controls, and the incidence of Leydig cell hyperplasia in the testes was 34% ($p \leq 0.05$) in high-dose males compared with 8% of controls.

The lowest-observed-adverse-effect level (LOAEL) for dicloran in rats is 1405 ppm (71.0 and 94.1 mg/kg bw/day for males and females, respectively) based on reduced body weight and weight gain and histopathologic lesions in the brain and spinal cord of both sexes, optic nerve in females and testes in males. The no-observed-adverse-effect level (NOAEL) is 240 ppm (11.3 and 15.0 mg/kg bw/day for males and females, respectively).

At the doses tested, the incidence of benign Leydig cell tumors was 0/50, 1/50, 1/50, and 5/50 ($p \leq 0.05$) in control, low-, mid-, and high-dose males rats, respectively. All Leydig tumors were found in animals sacrificed at study termination. The incidence of malignant endometrial adenocarcinoma was 3/50, 7/29, 7/21, and 9/50 ($p=0.061$) in control, low-, mid-, and high-dose females, respectively. Both tumor types occurred in hormone-responsive tissues, but there was no other evidence suggesting that dicloran is an endocrine disruptor. The increased incidences of benign Leydig cell tumors and endometrial adenocarcinoma is considered some evidence of

carcinogenicity of dicloran in rats. Dosing was considered adequate based on decreased body weight and weight gain in male and female rats.

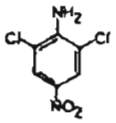
This chronic toxicity/carcinogenicity study in the rat is **Acceptable/Guideline** and satisfies the guideline requirement for a chronic toxicity/carcinogenicity study in the rat [OPPTS 870.4300); OECD 453].

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

I. MATERIALS AND METHODS:

A. MATERIALS:

1. **Test material:** Dicloran

Description:	Yellow powder
Lot/Batch #:	000313
Purity:	94.9% a.i.
Compound Stability:	For the duration of the study
CAS # for TGAI:	99-30-9
Structure:	

2. **Vehicle and/or positive control:** The test material was administered in feed (Ssniff rat/mice powdered food). A positive control was not included in this study.

3. **Test animals:**

Species:	Rat	
Strain:	HsdCpb:WU (Wistar)	
Age/weight at study initiation:	5 Weeks old; males: 71-110 g; females; 71-100 g	
Source:	In-house bred at Rallis Research Centre	
Housing:	Individually housed in suspended polypropylene cages with stainless steel tops	
Diet:	Ssniff rat/mice powdered feed, *ad libitum*	
Water:	Deep borewell water passed through activated charcoal filter and radiated with UV light in Aquaguard on-line water filter cum-purifier	
Environmental conditions:	**Temperature:**	19-25°C
	Humidity:	30-70%
	Air changes:	12-15/hour
	Photoperiod:	12 hours dark/12 hours light
Acclimation period:	5 days	

B. STUDY DESIGN:

1. **In life dates:** Start: April 25, 2001; End: May 2, 2003

2. **Animal Assignment/Dose Levels:** Animals were assigned randomly to the test groups noted in Table 1 based on body weight so that the group mean weights varied by no more than 20%.

TABLE 1: Study design

Test Group	Conc. in Diet (ppm)	Dose to animal (mg/kg/day)		Main Study 24 months		Interim Sacrifice 12 months	
		Male	Female	Male	Female	Male	Female
Control	0	0	0	50	50	10	10
Low (LDT)	60	2.8	3.7	50	50	10	10
Mid (MDT)	240	11.3	15.0	50	50	10	10
High (HDT)	1405[a]	71.0	94.1	50	50	10	10

Data taken from pages 65 and 214, MRID 46360701.
[a]Time-weighted average concentration; dietary concentration was raised to 1440 ppm on treatment day 104.

3. **Dose selection**: The dose levels were selected based on the results from a 90-day feeding study in Wistar rats administered the test material at concentrations of 0, 300, 1000, 2000, or 4000 ppm. No effects were observed at 300 ppm. Body weight gain was reduced by 11, 29, and 56% for males and 24, 29, and 34% for females at 1000, 2000, and 4000, respectively. Food consumption was within 7% of control for all groups of male rats and 14, 21, and 22% less, respectively, for the 1000-, 2000-, and 4000-ppm female groups compared with the control groups. A very mild macrocytic, hyperchromatic anemia was observed in males and females at 1000, 2000, and 4000 ppm. Blood urea nitrogen was elevated in males at 4000 ppm and in females at 2000 and 4000 ppm. Liver hypertrophy was observed in all females at 4000 ppm and in almost all males at 2000 and 4000 ppm compared with none of the controls. In addition, males at 4000 ppm had hyaline droplet formation in the renal tubular epithelium and the incidence of increased hemosiderin in the spleen was elevated in males at 2000 and 4000 ppm and in females at all doses. Therefore, the doses selected for the 2-year study were 60, 240, and 1200 ppm. The details of this study are described in the Appendix.

4. **Diet preparation and analysis:** Diet was prepared at 8-day intervals by mixing appropriate amounts of test substance with Ssniff rat/mouse powdered diet in a grinder mixer to prepare a premix. The premix was added to additional diet and mixed manually in a stainless steel drum for 2 minutes followed by the addition of the remaining diet to attain the target concentration and mixed in a stainless steel ribbon mixer for 20 minutes. The control diet containing no test substance was mixed in the stainless steel ribbon mixer for 20 minutes. The diet was stored in the refrigerator at +2°C to +8°C in polyethylene bags until used. Fresh food was offered four times a week. Homogeneity was determined on samples taken from the top, middle, and bottom of the mixer of each dietary concentration at study initiation and at months 12 and 24. Stability was tested on 100-ppm samples stored at room temperature for 2 or 5 days or refrigerated for 4-10 days and on 4000-ppm samples stored at room temperature for up to 21 days. Stability was also tested on a 60-ppm sample stored at room temperature for 2 days or refrigerated for 2-8 days. The concentration of test material in the diet was verified on samples taken at study initiation, and at months 3, 6, 12, 18, and 24 during the study.

Results:

Homogeneity analysis: The concentration in samples taken from the top, middle, and bottom of the mixer of each concentration did not vary from each other by more than 6%.

Stability analysis: 100-ppm samples: 8.7-9.2% and 19.0-25.3% of the test material was lost after storage for 2 or 5 days, respectively, at room temperature; 4.4-6.8% was lost after being refrigerated for 4-8 days, and 11.2% was lost after being refrigerated for 10 days. **4000-ppm samples**: ≤8.9% of the test material was lost after storage for up to 21 days at room temperature; **60-ppm samples**: 8.5% of the test material was lost after storage for 2 day at room temperature and ≤9.6% after being refrigerated for 2-8 days.

Concentration analysis: All samples were within ±10% of the target concentration.

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:** Functional observational battery (FOB), body weight, weight gain, food consumption, hematology and clinical chemistry parameters, and organ weight were analyzed by Bartlett's test for homogeneity. The data were transformed if heterogeneous, and homogeneous data were subjected to one-way analysis of variance (ANOVA) followed by Dunnett's test for pairwise comparison if ANOVA was significant.

   Incidences of gross and microscopic lesions were analyzed using the Z-test where applicable/necessary. The incidence of neoplastic lesions were analyzed using Cochran-Armitage trend test, the Life Table test for fatal neoplasms, and Peto's test for incidental neoplasms. The reviewer also analyzed incidence data using Fisher's exact test.

   Statistical significance was evaluated at the 5% level ($p \leq 05$).

   The reviewer considers the statistical tests appropriate for the data with the exception of the Z-test for incidence data.

C. **METHODS:**

1. **Observations:**

   1a. **Cageside observations**: Animals were inspected daily for signs of toxicity (appearance and behaviorial, clinical, and neurological signs) and twice daily for morbidity and mortality.

   1b. **Clinical examinations:** Clinical examinations with palpation for tumors and masses were conducted before start of treatment and weekly during treatment. A veterinary physical examination was conducted before grouping, after grouping, and monthly during the treatment period.

1c. **Neurological evaluations**: A functional observational battery (FOB) was conducted after treatment with the test material for 12 months. The following groups of parameters were evaluated: home cage observations, handling observations, open-field observations, sensory observations, neuromuscular observations, and physiological observations. The CHECKED (X) parameters were examined. Respiratory character included the following parameters: normal respiratory character, rales, retching, dyspnea, and gasping.

X	HOME CAGE OBSERVATIONS	X	HANDLING OBSERVATIONS	X	OPEN FIELD OBSERVATIONS
	Posture*		Reactivity*	X	Mobility
	Biting	X	Lacrimation* / chromodacryorrhea		Rearing+
X	Convulsions*	X	Salivation*	X	Arousal/ gereral activity level*
X	Tremors*	X	Piloerection*	X	Convulsions*
	Abnormal Movements*		Fur appearance	X	Tremors*
X	Palpebral closure*	X	Palpebral closure*		Abnormal movements*
	Faeces consistency		Respiratory character		Urination / defecation*
	Impairment of gait		Red/crusty deposits*	X	Grooming
	SENSORY OBSERVATIONS		Mucous membranes /eye /skin colour	X	Gait abnormalities / posture*
	Approach response+	X	Eye prominence*		Gait score*
	Touch response+	X	Muscle tone*		Bizarre / stereotypic behaviour
X	Startle response*		Pupil size and response	X	Backing
	Pain response*	X	Ease of removal from cage		Time to first step
X	Pupil response	X	Ease of handling animal in hand		Piloerection
	Eyeblink response		**PHYSIOLOGICAL OBSERVATIONS**		**NEUROMUSCULAR OBSERVATIONS**
	Forelimb extension		Body weight*		Hindlimb extensor strength
	Hindlimb extension	X	Body temperature+	X	Forelimb grip strength*
X	Air righting reflex+		Catalepsy	X	Hindlimb grip strength*
	Olfactory orientation		**OTHER OBSERVATIONS**	X	Landing foot splay
			Vocalization		Rotarod performance
				X	Motor activity

*Required parameters; +recommended parameters

2. **Body weight**: Animals were weighed before initiation of treatment, weekly (end of the week) for the first 13 weeks, at 4-week intervals thereafter (the last measurement took place 3 weeks after the previous measurement), and at necropsy.

3. **Food consumption and compound intake**: Food consumption for each animal was measured during the last 4 days of each week for the first 13 weeks and at 4-week intervals thereafter except the last measurement took place 3 weeks after the previous measurement. The mean daily diet consumption was calculated as g food/animal/day and g food/kg bw/day. The average food efficiency for the first 12 months and the 2 years of the study was calculated by the reviewer as (total g body weight gain/total g food consumed). Compound intake (mg/kg bw/day) values were calculated from the consumption and body weight gain data.

4. **Ophthalmoscopic examination**: The eyes of all animals were examined with an ophthalmoscope before initiation of treatment and at 6-month intervals during treatment. The pupils were dilated with a 1% tropicamide solution before the examination.

5. **Hematology & clinical chemistry**: Blood was collected from the orbital sinus of 20 males and 20 females per dose level in the main group after overnight fasting (water allowed) at months 3, 6, 12, 18, and 24. Blood was collected in sodium citrate, heparinized tubes, or EDTA tubes for analysis of prothrombin time, clinical chemistry parameters, and hematologic parameters. Hematologic parameters were analyzed in all 20 animals/sex/group at all time points; clinical chemistry parameters were analyzed in 10 animals/sex/group at months 6, 12, 18, and 24. Blood smears were prepared from all 20 animals/sex/group at each time point for differential leukocyte count, but only the slides from the control and high-dose groups were examined. The CHECKED (X) parameters were examined.

a. Hematology:

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

* Recommended for combined chronic/carcinogenicity studies based on Guideline 870.4300.

b. Clinical Chemistry

	ELECTROLYTES		OTHER
	Calcium*	X	Albumin*
	Chloride*	X	Creatinine*
	Magnesium*	X	Urea nitrogen*
	Phosphorus*	X	Total Cholesterol*
X	Potassium*		Globulins*
X	Sodium*	X	Glucose (fasting)*
	ENZYMES (more than 2 hepatic enzymes)*	X	Total bilirubin
X	Alkaline phosphatase (ALK)*	X	Total protein (TP)*
	Cholinesterase (ChE)		Triglycerides
	Creatine phosphokinase		Serum protein electrophoresis
	Lactic acid dehydrogenase (LDH)		
X	Alanine aminotransferase (ALT/ SGPT)*		
X	Aspartate aminotransferase (AST/ SGOT)*		
X	Gamma glutamyl transferase (GGT)*		
	Sorbitol dehydrogenase		
	Glutamate dehydrogenase*		

* Recommended for combined chronic and carcinogenicity studies based on Guideline 870.4300.

6. **Urinalysis:** Urine was collected overnight from 10 fasted (water allowed) animals/sex/group at months 3, 6, 12, 18, and 24 before blood collection. The CHECKED (X) parameters were examined.

X	Appearance*	X	Glucose*
X	Volume*	X	Ketones*
X	Specific gravity / osmolality*	X	Bilirubin*
X	pH*	X	Blood/ red blood cells* & leukocytes
X	Sediment (microscopic)	X	Nitrite
X	Protein*	x	Urobilinogen

* Recommended for combined chronic and carcinogenicity studies based on Guideline 870.4300.

7. **Sacrifice and pathology** - All animals that died and those sacrificed moribund or on schedule were subjected to gross pathological examination. The animals surviving to scheduled sacrifice at 12 and 24 months were fasted overnight before being subjected to ether anesthesia and exsanguination. The CHECKED (X) tissues were collected, preserved in 10% buffered neutral formalin (eyes were preserved in Davidson's fluid), and processed for microscopic examination. All tissues, gross lesions, and masses from rats in the control and high-dose group were examined microscopically, and gross lesions, masses, liver, spleen, testes (males), and brain from low- and mid-dose groups also were examined microscopically. The (XX) organs were weighed.

	DIGESTIVE SYSTEM		CARDIOVASC./HEMAT.		NEUROLOGIC
	Tongue	X	Aorta, thoracic*	XX	Brain (multiple sections)*+
X	Salivary glands*	XX	Heart*	X	Peripheral nerve*
X	Esophagus*	X	Bone marrow* (femur & sternum)	X	Spinal cord (3 levels)*
X	Stomach*	X	Lymph nodes*	X	Pituitary*
X	Duodenum*	XX	Spleen*+	X	Eyes (retina, optic nerve)*
X	Jejunum*	X	Thymus		GLANDULAR
X	Ileum*			XX	Adrenal gland*+
X	Cecum*		UROGENITAL		Lacrimal gland
X	Colon*	XX	Kidneys*+		Harderian gland
X	Rectum*	X	Urinary bladder*	XX	Parathyroids*
XX	Liver*+	XX	Testes*+	XX	Thyroids*
	Gall bladder* (not rat)	XX	Epididymides*+	X	Mammary gland*
X	Bile duct (rat)	X	Prostate*		
X	Pancreas*	X	Seminal vesicle* + coagulating gland		
	RESPIRATORY	XX	Ovaries*+		OTHER
X	Trachea*	XX	Uterus*+	X	Bone (femur + joint)
X	Lung*			X	Skeletal muscle
X	Nose*			X	Skin*
X	Pharynx*			X	All gross lesions, tumors, & masses*
X	Larynx*				

* Required for combined chronic/carcinogenicity studies based on Guideline 870.4300.
+Organ weight required in combined chronic/carcinogenicity studies.

II. RESULTS:

A. OBSERVATIONS:

1. **Clinical signs of toxicity**: No treatment-related clinical signs were observed during daily observations, monthly physical examinations, or weekly physical examinations of interim or main groups.

2. **Mortality**: Only one male and one female rat in the high-dose group died during the first year of the study. Survival percentage at the end of 730 days was 72, 66, 74, and 88% for males and 78, 66, 70, and 74% for females in the control, low-, mid-, and high-dose groups, respectively.

3. **Neurological evaluations**: Body temperature was slightly, but significantly lower in low- and high-dose males and mid-dose females than in controls. Body temperature was slightly higher in low-dose females than in the controls. The hind limb foot splay was 16% ($p \leq 0.01$) less in high-dose males and 10% ($p \leq 0.05$) less in high-dose females. Fore limb grip strength was 5-6% ($p \leq 0.05$) less in high-dose males and females and low-dose males than in the controls, and hind limb grip strength was 10%, 7%, and 11% less ($p \leq 0.05$) in low-, mid-, and high-dose males, respectively, than in the control; hind limb grip strength was similar in treated and control rats.

B. BODY WEIGHT:

Body weight and weight gain data are summarized in Table 2. After treatment for 1 week, high-dose male rats weighed 16% ($p \leq 0.05$) less than controls, and weighed 8-13% ($p \leq 0.05$) less than controls for the remainder of the study. Mid-dose male rats weighed 3-5% ($p \leq 0.05$) less than controls during the first year starting at week 4, and body weight of low-dose males was similar to that of controls throughout the study. High-dose males gained 48% ($p \leq 0.05$) less weight than controls during the first week of treatment, 11-13% ($p \leq 0.05$) less during the first 13 weeks, the first year, and the entire study. No apparent impact on weight gain was observed in high-dose group male rats after the dietary concentration was raised to 1440 ppm at day 106 relative to weight gain before the dietary concentration was raised.

High-dose female rats weighed 8% ($p \leq 0.05$) less than controls after the first week of treatment, 4-5% ($p \leq 0.05$) less than controls during weeks 2-3 and 7-9, and 17-29, and 6-9% ($p \leq 0.05$) for the remainder of the first year. During the second year of treatment, high-dose female rats weighed 10-14% ($p \leq 0.05$) less than controls. Body weights of low- and mid-dose females were similar to that of controls throughout the study. High-dose females gained 31% ($p \leq 0.05$) less weight than controls during the first week of treatment, 13% ($p \leq 0.05$) less during the first year, and 61% less during the second, resulting in a 20% ($p \leq 0.05$) lower weight gain for the entire study compared with that of control rats.

TABLE 2: Selected body weights and weight gain in male and female rats fed								
Week of Study	Dietary Concentration (ppm)							
	0	60	240	1405	0	60	240	1405
	Males				Females			
Mean body weight (g)								
0	98 ± 12.5[a]	100 ± 13.2	98 ± 11.8	98 ± 11.3	91 ± 9.6	90 ± 9.7	89 ± 9.4	90 ± 9.4
1	144 ± 13.9	147 ± 15.7	142 ± 14.5	121 ± 14.0* (84)[b]	117 ± 11.7	119 ± 9.6	118 ± 10.4	108 ± 10.4* (92)
13	412 ± 29.3	417 ± 30.4	399 ± 29.8* (97)	374 ± 31.8* (91)	215 ± 19.6	222 ± 19.9	219 ± 20.0	208 ± 15.4
25	475 ± 32.7	477 ± 33.5	454 ± 31.2* (96)	436 ± 35.2* (92)	241 ± 20.8	247 ± 20.3	246 ± 22.1	231 ± 16.9* (96)
53	535 ± 37.0	534 ± 37.6	517 ± 33.8* (97)	484 ± 34.5* (90)	277 ± 28.1	282 ± 27.2	280 ± 31.5	252 ± 20.2* (91)
77	555 ± 43.1	561 ± 46.6	543 ± 42.7	498 ± 36.9* (90)	305 ± 33.5	313 ± 31.3	309 ± 42.9	266 ± 25.6* (87)
104	515 ± 57.8	518 ± 47.1	510 ± 48.9	460 ± 44.0* (89)	305 ± 41.8	315 ± 36.3	297 ± 47.0	263 ± 27.2* (86)
Body weight gain (g)								
38352	46 ± 5.1	47 ± 5.6	45 ± 6.0	24 ± 8.0* (52)	26 ± 6.2	29 ± 6.6	29 ± 5.9	18 ± 4.5* (69)
38364	314 ± 31.3	317 ± 30.5	301 ± 28.8	277 ± 34.7* (88)	124 ± 19.0	132 ± 20.7*	129 ± 18.5	118 ± 15.4* (95)
13-25[c]	63	60	55	62	26	25	27	23
25-53[c]	60	57	63	48	36	35	44	21
0-53	437 ± 39.3	435 ± 39.3	419 ± 33.3* (96)	387 ± 39.2* (89)	186 ± 26.9	192 ± 27.7	191 ± 29.6	162 ± 19.6* (87)
53-104[c]	-20	-16	-7	-24	28	33	17	11
0-104	419 ± 60.6	418 ± 48.8	412 ± 46.3	364 ± 47.0* (87)	215 ± 41.9	223 ± 36.6	208 ± 42.6	173 ± 25.0* (80)

Data obtained from Tables 8-11 (pages 199-206), MRID 46360701.
[a]Mean ± standard deviation
[b]Numbers in parentheses are percent of control calculated by the reviewer.
[c]Weight gain calculated by the reviewer using mean body weight.
*$p<0.05$, statistically significant, treated group compared with controls.

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C. FOOD CONSUMPTION AND COMPOUND INTAKE:

1. **Food consumption:** Food consumption data are summarized in Table 3. High-dose male rats consumed 18% ($p \leq 0.05$) less food than controls during the first week of treatment, 5-12% ($p \leq 0.05$) less from weeks 2-17, and 6-9% ($p \leq 0.05$) less at sporadic time points for the remainder of the study. Mid-dose males consumed 4-7% ($p \leq 0.05$) less food than controls at sporadic time points during the study. In general, food consumption by low-dose males was similar to that of controls. High-dose females consumed 14% ($p \leq 0.05$) less food than controls during the first week of the study; the weekly values fluctuated considerably up to week 73 ranging from 6-16% ($p \leq 0.05$) less than that of controls. Low and mid-dose females consumed 9-12% ($p \leq 0.05$) less food than controls from week 4-11 and, except for a few sporadic time points, consumed amounts similar to those of controls for the remainder of the study. The total amount of food consumed by high-dose male rats was similar to that of controls and was decreased by only 8-9% in high-dose females.

2. **Compound consumption:** Compound consumption values are summarized in Table 1.

3. **Food efficiency:** Food efficiency values for the entire study are summarized in Table 3. No treatment-related effect was observed on food efficiency. The values for high-dose males and females were similar to those of controls for the first year and the entire study indicating that the decreased weight gain was likely due to decreased food consumption.

TABLE 3. Selected food consumption data and food efficiency values for male and female rats fed Dicloran for up to 24 months

	Dietary concentration (ppm)							
	0	60	240	1405	0	60	240	1405
	Males				Females			
Food consumption (g/animal/day)								
1	18.9 ± 1.63[a]	18.7 ± 2.05	18.2 ± 1.74	15.5 ± 2.44*	15.0 ± 1.88	14.6 ± 1.46	14.6 ± 1.52	12.9 ± 1.69*
53	21.8 ± 1.98	21.8 ± 1.66	21.0 ± 1.71	21.1 ± 1.85	15.9 ± 1.85	15.9 ± 1.91	15.3 ± 1.61	15.0 ± 1.74*
104	22.0 ± 2.56	21.3 ± 3.25	20.6 ± 2.76	20.5 ± 2.49	18.2 ± 2.27	18.4 ± 2.44	16.4 ± 3.05*	17.4 ± 2.83*
Food consumption (g/animal)								
0-53	8600.9	8741.7	8176.6	8424.4	6174.1	5717.9	6048.1	5616.6
0-104	17129.1	16811.2	16287.3	16240.9	12681.4	12376	12127.7	11724
Food efficiency (g body weight gain/g food consumed)[b]								
0-53	0.051	0.05	0.051	0.046	0.03	0.034	0.032	0.029
0-104	0.024	0.025	0.025	0.022	0.017	0.018	0.017	0.015

Data taken from Table 12-15 (pages 207-214, MRID 46360701.
[a]Mean ± standard deviation
[b]Food efficiency calculated by the reviewer from body weight gain and total food consumption data for the intervals indicated.
*$p<0.05$, statistically significant, treated group compared with the control group.

D. OPHTHALMOSCOPIC EXAMINATION:

The ophthalmoscopic examinations revealed no treatment-related eye abnormalities in either sex at any time during the study.

E. BLOOD ANALYSES:

1. **Hematology:** Transient changes in hematologic parameters were observed in high-dose group male and female rats. The decrease in the red blood cell (RBC) count, hematocrit, and hemoglobin did not exceed 8%, the increase in mean cell volume (MCV) and mean cell hemoglobin (MCH) did not exceed 3% in either sex compared with those of controls at any time during the study . The decrease in the neutrophil and increase in lymphocyte count were not accompanied by significant changes in the total white blood cell (WBC) count in males and only a 19-24% increase in WBC in females compared with that of controls. High-dose male rats also had a 12% ($p \leq 0.05$) increase in prothrombin time (+2 seconds) at the 6th month, and high-dose females had an 8% increase at the 12th month (+1.2 seconds) and 24th month (+2.2 seconds) and a 6% decrease (-1.2 seconds) at the 18th month. Prothrombin time also was increased in mid-dose females by 2.1 seconds compared with controls at the 24th month. High-dose female rats had a 17% ($p \leq 0.05$) increase in platelet count at the 12th month. Low- and mid-dose group rats had small sporadic changes in some hematologic parameters, but none showed clear dose-response relationships.

2. **Clinical chemistry:** Small transient statistically significant ($p \leq 0.05$) changes in clinical chemistry parameters were observed in high-dose male and female rats. Total protein concentration was significantly ($p \leq 0.05$) elevated by 5% and 9% in high-dose male and female rats, respectively, compared with that of controls at the 6th month and by 7% in high-dose females at the 12th month accompanied by non-statistically significant increases in albumin concentration. Male rats had slightly elevated serum sodium levels (1-2%) at all dose levels at the 6th month, elevated (18%) creatinine at the high-dose level, decreased (9-10%) potassium level at the mid- and high-dose levels at the 12th month, and decreased (35-37%) aspartate aminotransferase (AST) at the 12th and 18th months compared with control values. Female rats had elevated (31%) blood urea nitrogen (BUN) at the 6th month at the high-dose level, elevated (30-42%) cholesterol at the 6th and 12th month at the high-dose level, elevated (3%) serum sodium at the high-dose level at the 12th month, and decreased (46%) alanine aminotransferase (ALT) activity at the 18th month.

F. URINALYSIS: No treatment-related changes in urinalysis parameters were observed in male or female rats receiving any dose of the test material.

G. SACRIFICE AND PATHOLOGY:

1. **Organ weight:** In high-dose male rats, the terminal body weight was 9% less ($p \leq 0.05$) than that of controls at 12 months (interim sacrifice group) and 24 months (main study group). The epididymis in high-dose males weighed 9% ($p \leq 0.05$) less than that of controls, but the organ:body weight ratio was similar to that of controls at 12 months. The organ: body weight ratios of the testes, kidneys, liver, heart, brain, and spleen of high-dose males were significantly ($p \leq 0.05$) greater than those of controls (15, 11, 22, 12, 9, and 15%, respectively) at 12 months due to small non-statistically significant increases in absolute organ weights and/or the significant decrease in terminal body weight. At 24 months, the absolute heart weight of high-dose males was 12% ($p \leq 0.05$) less than that of controls, but the organ:body weight ratio was similar to that of controls. The organ:body weight ratios of the testes, liver, and brain were significantly greater (30, 17, and 13%, respectively) than those of the control

group due to non-statistically significant increases in absolute organ weight and/or the significant decrease in the terminal body weight.

In high-dose group female rats, the terminal body weight at 12 months was similar to that of the control group, and the absolute liver and spleen weights were 16% and 12% ($p \leq 0.05$) greater and the organ:body weight ratios were 19% and 15% greater than those of the control group. At 24 months, the terminal body weight of high-dose female rats was 15% ($p \leq 0.05$) less than that of controls, and the organ:body weight ratios of the kidneys and liver were 20% and 33% ($p \leq 0.05$) greater than those of the controls due to the significant decrease in terminal body weight and the non-statistically significant increase in absolute liver weight.

2. **Gross pathology:** No treatment-related gross lesions were observed in male or female rats sacrificed at 12 months or in rats in the main group.

3. **Microscopic pathology:**

a. **Non-neoplastic:** The incidences of microscopic non-neoplastic lesions are summarized in Table 4. At interim sacrifice (12 months), treatment-related microscopic lesions were observed in the liver (males only), spleen, brain, and spinal cord of high-dose male and female rats. The incidences of hepatocellular hypertrophy was increased in mid- and high-dose males and the incidence of increased hemosiderosis in the spleen was increased in mid- and high-dose males and at all doses in females. The incidence of vacuolation in the three regions of brain and spinal cord were increased in high-dose males and females and one male rat in the mid-dose group had vacuolation in the cerebral cortex. Vacuolation occurred in the white matter. Cerebral vacuolation occurred bilaterally in the corpus callosum, cerebral peduncles, substantia nigra and the anterior commissures with minimal to moderate severity in males and mild to moderate in females. Cerebellar vacuolation was minimal to mild in both sexes, and medullar/pons vacuolation was minimal to moderate in both sexes. Spinal cord vacuolation was minimal to moderate.

The liver, spleen, brain and spinal cord also were affected in male and female rats in the main study group along with the testes in male rats and optic nerve in females. Eosinophilic foci and hepatocellular hypertrophy were observed in the liver of 24% and 30%, respectively, of male rats in the mid-dose group and 30% and 76%, respectively, in the high-dose group compared with only 10% and 6%, respectively, of controls. The incidences of eosinophilic foci and hepatocellular hypertrophy were not significantly increased in female rats, but the incidence of necrobiotic foci was marginally increased in high-dose males and was significantly increased in high-dose females compared with incidences in the controls. Increased hemosiderosis in the spleen was observed in 46-84% of males at all doses compared with only 18% in the control group and in 80% of high-dose females compared with 36% in the control group. Vacuolation was observed in the three regions of the brain and spinal cord, and the incidence approached 100% in at least one region in high-dose male and female rats. In addition, vacuolation in the optic chiasma was also observed in both sexes at the high dose. The incidence of vacuolation in the three regions of the brain and spinal cord ranged from 62-96% and 56-86%, respectively, of high-dose males and 84-98% and 46-86%, respectively, of high-dose females compared with no more than 4% of male controls and 2% of female controls. High-dose females also had a marginally significant

increased incidence of vacuolar changes in the optic nerve (8% vs 0% for controls). Vacuolation in the optic chiasma was observed in 28% of high-dose males and 34% of high-dose females. The severity was minimal to severe in the cerebral cortex and medulla/pons, minimal to moderate in the cerebellum, and minimal to severe in the spinal cord. High-dose male rats also had an significantly increased incidence (34%) of Leydig cell hyperplasia compared with that of controls (8%).

The incidence of endometrial hyperplasia was increased in high-dose females (9/50 vs 4/50 for controls, p=0.12) compared with that of controls, and the incidence of thyroid c-cell hyperplasia was increased in high-dose males (11/50 vs 5/50 for controls, p=0.086) (not listed in Table 4).

b. **Neoplastic**: Benign Leydig cell tumors were observed in the testes of 0/50, 1/50, 1/50, and 5/50 ($p \leq 0.05$) control, low-, mid-, and high-dose male rats, respectively. These tumors were found in animals sacrificed at study termination. Malignant endometrial adenocarcinoma was observed in 3/50, 7/29, 7/21, and 9/50 (p=0.061) females in the control, low-, mid-, and high-dose groups, respectively. There was no notable increase in the total number of treated rats of either sex with neoplasms, benign neoplasms, or malignant neoplasms. However, fewer high-dose female rats had benign neoplasms (48%) compared with the controls (68%, $p \leq 0.05$); this finding is considered incidental.

TABLE 4. Histopathological findings in male and female								
Organ/Lesion	Dietary concentration (ppm)							
	0	60	240	1405	0	60	240	1405
	Males - 12 month interim group				Females - 12 month interim group			
Liver [# animals examined]	[10]	[10]	[10]	[10]	[10]	[10]	[10]	[10]
Hepatocellular hypertrophy	0	0	4	6	3	5	6	7
Spleen [# animals examined]	[10]	[10]	[10]	[10]	[10]	[10]	[10]	[10]
Increased hemosiderosis	2	3	8	7	2	7	7	9
Brain [# animals examined]	[10]	[10]	[10]	[10]	[10]	[10]	[10]	[10]
Cerebral cortex, vacuolation	0	0	1	10	0	0	0	10
Cerebellar cortex, vacuolation	0	0	0	2	0	0	0	8
Medulla/pons, vacuolation	0	0	0	9	0	0	0	10
Spinal cord [# examined]	[10]	[0]	[0]	[10]	[10]	[0]	[0]	[10]
Cervical, vacuolation	0			2	0			9
Thoracic, vacuolation	0			2	0			6
Lumbar, vacuolation	0			0	0			3
	Males - main group				Females - main group			
Liver [# animals examined]	[50]	[50]	[50]	[50]	[50]	[50]	[50]	[50]
Eosinophilic focus(i)	5	4	12*	15**	5	1	3	6
Hepatocellular hypertrophy	3	7	15**	38**	28	27	35	36
Necrobiotic focus(f)	10	2	10	18†	5	5	7	15**
Spleen [# animals examined]	[50]	[50]	[50]	[50]	[50]	[50]	[50]	[50]
Increased hemosiderosis	9	23*	35**	42**	18	21	26	40**
Testes [# animals examined]	[50]	[50]	[50]	[50]	NA	NA	NA	NA
Leydig cell hyperplasia	4	5	5	17**				
Brain [# animals examined]	[50]	[50]	[50]	[50]	[50]	[50]	[50]	[50]
Cerebral cortex, vacuolation	1	0	1	48**	0	0	0	49**
Optic chiasma, vacuolation	0	0	0	14**	0	0	0	17**
Cerebellar cortex, vacuolation	2	0	0	31**	1	0	0	42**
Medulla/pons, vacuolation	1	0	0	48**	0	0	0	46**
Spinal cord [# examined]	[50]	[50]	[50]	[50]	[50]	[50]	[50]	[50]
Cervical, vacuolation	0	0	0	43**	0	0	0	43**
Thoracic, vacuolation	0	0	0	42**	1	0	0	40**
Lumbar, vacuolation	0	0	0	28**	0	0	0	23**
Eyes, optic nerve [# examined]	[50]	[24]	[16]	[50]	[50]	[17]	[17]	[50]
Vacuolar changes	0	0	0	1	0	0	0	4†

Data taken from Table 51 (pages 259-274) and 58 (pages 379-432) MRID 46360701.
†p= 0.059, *p≤0.05, **p<0.01, statistically significant, treated group compared with the control group, calculated by the reviewer using Fisher's exact test.

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III. DISCUSSION and CONCLUSIONS:

A. INVESTIGATORS' CONCLUSIONS: The investigator concluded that treatment with dicloran had no effect on survival or the average lifespan, clinical signs observed during daily inspections, observations during clinical and physical examinations, ophthalmoscopic findings, abnormalities observed during conduct of the FOB, clinical chemistry parameters, urinalysis parameters, of gross findings in either sex. Body weight and food consumption were reduced in high-dose male and female rats, but food consumption was not reduced enough to affect food efficiency. The increases or decreases of erythrocyte parameters (RBC, hematocrit, and hemoglobin) represented threshold treatment-related effects in light of the increased incidence of splenic hemosiderosis. Treatment-related histopathological changes included hepatocellular hypertrophy in mid- and high-dose males, eosinophilic foci in the liver of high-dose males, and necrobiotic foci in the liver of high-dose females, splenic hemosiderosis at all doses in males and high-dose females, and Leydig cell hyperplasia in the testes of high-dose males. The investigators considered vacuolation in the brain and spinal cord in high-dose males and females to be treatment related, but not adverse because there were no associated clinical signs or effect on survival. The investigator concluded that the no-observed-adverse-effect level (NOAEL) was 60 ppm for both sexes. The investigators noted that there was a treatment-related increase in the incidence of benign Leydig cell tumors in high-dose males and concluded that the test substance was not carcinogenic under the testing conditions, because the tumors were correlated with increased incidence of Leydig cell hyperplasia and the Cochran-Armitage test, Peto's test for incidental tumors, and Life Table analysis of fatal tumors showed no treatment-related differences between treated and control groups.

B. REVIEWER COMMENTS: Treatment with dicloran at concentrations up to 1405 ppm had no effect on signs of toxicity observed during daily observations, monthly physical examinations, or weekly physical examinations of male or female rats. The statistically significant changes in FOB parameters (hind and fore limb grip strength and hind limb foot splay) were very small in magnitude and/or showed no clear dose-related trend. Survival, eye abnormalities, urinalysis parameters, and the incidences of gross lesions were not affected by treatment with the test material in either sex. Mean body weight of high-dose males rats was significantly less than that of controls throughout the study due primarily to a marked decrease in weight gain during the first week of treatment. Weight gain by high-dose females was also reduced during the first week of treatment but to a lesser degree than male rats, prompting the investigators to raise the dietary concentration from 1200 ppm to 1440 ppm at day 106. The effect on mean body weight and weight gain in high-dose females during the second year of the study was much greater than that of the first year. The significant changes on mean body weight in mid-dose males did not achieve a level considered adverse. Food consumption by high-dose male and female rats was reduced during the first week of treatment; the total amount of food consumed by high-dose rats was within 10% of that of control and did not result in an overall decrease in food efficiency.

Erythrocyte parameters (RBC, hematocrit, hemoglobin, MCV, and/or MCH) in high-dose male and female rats were significantly increased or decreased at various times during the study. The magnitude of the difference between the treated and control groups did not exceed 10%. The decrease/increase in neutrophil/lymphocyte counts in high-dose rats was not accompanied by a statistically significant change in WBC in males and only a 19-24% increase in females. Because similar changes were observed in the 90-day feeding study

(MRID 46360702), the changes in erythrocyte parameters in the 24-month study are considered treatment related but not adverse. The changes in other hematologic parameters (prothrombin time, platelet count) in high-dose rats were very small and transient and are not considered treatment related.

No treatment-related and adverse changes were observed in clinical chemistry parameters. The increase in total serum protein due to increased albumin in high-dose rats was transient, the changes in serum sodium, creatinine, potassium, BUN, and cholesterol were too small to be considered adverse, were transient, or were not correlated with histopathologic findings.

The increased epididymis weight in high-dose males at 12 months, the increased heart weight in high-dose males at 24 months, and the increased absolute weight and the organ-body weight ratios of the liver and spleen in females at 12 months were not accompanied by histopathological findings and appear to be due decreased body weight. The increases in organ:body weight ratios of the other organs in high-dose males 12 and/or 24 months and the liver and kidney in high-dose females at 24 months were due primarily to significantly decreased terminal body weight.

No treatment-related gross findings were observed in male or female rats receiving any dose of the test material. Treatment-related histopathologic findings included liver lesions (hepatocyte hypertrophy, eosinophilic foci, and necrobiotic foci), increased splenic hemosiderosis, and vacuolation in the cerebral cortex including the optic chiasma, cerebellar cortex, and medulla/pons in the brain and cervical, thoracic, and lumbar regions of the spinal cord in high-dose male and female rats. Hepatocyte hypertrophy and increased hemosiderosis in the spleen also were observed at increased incidences in mid-dose male rats. Hepatocyte hypertrophy observed in mid- and high-dose males is an adaptive lesion and that is not considered adverse. The eosinophilic foci in male rats did not progress to neoplasms and are not considered toxicologically significant. The necrobiotic foci in female rats are treatment-related, but the toxicologic significance is uncertain. Increased hemosiderosis in the spleen is indicative of increased turnover of erythrocytes; however, analysis of hematology parameters provided no evidence of increased turnover.

The lesions in the brain and spinal cord occurred in almost all rats of both sexes in at least one region of the brain or spinal cord and the severity of the lesions ranged from minimal to moderate or minimal to severe for most regions in both sexes. According to the investigator, the vacuolar changes in the optic nerve was an extension of the vacuolation in the optic chiasma. The investigator did not consider vacuolation in the brain or spinal cord to be adverse, since no clinical signs indicative of neurotoxicity were observed in high-dose male or female rats. The reviewer considers the lesions to be serious because they involved morphological changes in the brain and spinal cord that were induced during the first year of the study and were seen in almost all high-dose animals in the main group. Although vacuolation could be caused by indirect effects, the presence of these lesions also suggests that the test material crossed the blood-brain barrier, which should be considered a serious event.

The lowest-observed-adverse-effect level (LOAEL) for dicloran in rats is 1405 ppm (71.0 and 94.1 mg/kg bw/day for males and females, respectively) based on based on reduced body weight and weight gain and histopathologic lesions in the brain and spinal cord of both sexes, optic nerve in females and testes in males. The no-observed-adverse-effect level (NOAEL) is 240 ppm (11.3 and 15.0 mg/kg bw/day for males and females, respectively).

The investigator concluded that the NOAEL for dicloran was 60 ppm. The investigator did not state explicitly the basis for the NOAEL; however, it appears that splenic hemosiderosis and hepatocyte hypertrophy observed in mid-dose animals resulted in the investigator establishing the NOAEL at 60 ppm. The reviewer concludes that neither lesion is considered adverse in this study. Hepatocyte hypertrophy is an adaptive response and splenic hemosiderosis was not accompanied by adverse hematologic changes suggesting an increased turnover of erythrocytes.

The incidence of benign Leydig cell tumors was significantly increased in high-dose male rats compared with the control incidence and the incidence of endometrial adenocarcinoma was marginally increased in high-dose female rats. The Leydig cell tumors did not progress to malignancy and the incidence of endometrial adenocarcinoma did not reach statistical significance at the 5% level compared with control incidence. It should be noted that both tissues are hormone responsive; however, there was no other evidence indicating that dicloran is an endocrine disruptor. The increased incidences of these two neoplasms is considered some evidence of carcinogenicity in rats fed dicloran for 24 months. The study investigator acknowledged the treatment-related increase in the incidence of Leydig cell tumors in high-dose male rats, but concluded that the test material was non-carcinogenic as evaluated by Cochran Armitage trend test for total tumor incidence and Peto's incidental tumor analysis and the Life table analysis for fatal tumors. The reviewer used Fisher's exact test for statistical analysis of the incidences of Leydig cell tumors and endometrial adenocarcinoma. The animals were adequately dosed based on decreases in body weight and weight gain in both sexes at the high-dose level.

C. **STUDY DEFICIENCIES:** No major deficiencies were noted for this study.

DATA FOR ENTRY INTO ISIS

Chronic/Carcinogenicity Study - rodents (870.4300)

PC code	MRID	Study	Species	Duration	Route	Admin	Dose range (mg/kg/day)	Doses (mg/kg/day)	NOAEL (mg/kg/day)	LOAEL (mg/kg/day)	Target organ	Comments
31301	446360701	chronic tox/ oncogenicity	rat	24 months	oral	feed	M: 2.8 -71.0 F: 3.7 - 94.1	M: 0, 2.8, 11.3, 71.0 F: 0, 3.7, 15.0, 94.1	M: 11.3 F: 15.0	M: 71.0 F: 94.1	Brain, spinal cord, and testes	Toxicity, some evidence of carcinogenicity

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APPENDIX

TXR#: 0053066

DATA EVALUATION RECORD

STUDY TYPE: Subchronic toxicity feeding - rat
OPPTS 870.3100 [§82-1]; OECD 408.

PC CODE: 031301 **DP BARCODE:** D294474
SUBMISSION NO.: none

TEST MATERIAL (PURITY): Dichloran (94.6% a.i.)

SYNONYMS: 2,6-dichloro-4-nitroaniline

CITATION: Ramesh, E. 2001. Dicloran: 90-day dietary dose range finding study in Wistar rats. Toxicology Department, Rallis Research Centre, Rallis India Ltd., Peenya II Phase, Bangalore - 560 058, India, Laboratory project ID 3080/00, December 10, 2001. MRID 46360702. Unpublished.

SPONSOR: Gowan Company, 370 South Main St., Yuma, AZ 85364

EXECUTIVE SUMMARY: In a 90-day ranging-finding study (MRID 46360702), dicloran (94.6% a.i.; batch/lot # 000313) was administered in the diet to groups of 10 male and 10 female HsdCpb:WU rats at concentrations of 0, 300, 1000, 2000, or 4000 ppm for 90 days. The dietary concentrations were equivalent to 0, 19.4, 61.5, 121.2, and 246.8 mg dicloran/kg bw/day, respectively, for males and 0, 25.4, 72.4, 133.6, and 264.6 mg dicloran/kg bw/day, respectively, for females. The following parameters were examined: clinical signs, body weight, food consumption, clinical pathology (hematologic and clinical chemistry parameters), gross lesions, selected organ weights, and histopathology of selected tissues and organs.

All animals survived to study termination and no treatment-related clinical signs of toxicity were observed at any time during treatment. Body weight, weight gain, and food consumption were significantly ($p \leq 0.05$) decreased throughout the study in males and females at 2000 and 4000 ppm. At 2000 and 4000 ppm, males weighed 10-14% and 19-29% less, respectively, than controls and females weighed 6-13% and 8-19% less, respectively. Males and females in the 2000- and 4000-ppm groups lost weight during the first week of the study. The males gained 29% and 54% less weight, respectively, and the females gained 29% and 44% less weight, respectively, over the entire study. Male rats in the 1000 ppm group weighed 5% ($p \leq 0.05$) less and gained 55% less weight than controls after the first week and only 11% (N.S.) less weight than controls over the entire study. Females in the 1000-ppm group weighed up to 7% ($p \leq 0.05$) less than controls from weeks 9-13 and gained 24% ($p \leq 0.05$) less weight over the entire study. Females in the 1000-ppm group had weekly weight gains similar to the controls after week 1; therefore, the cumulative weight gain is not indicative of an adverse effect. Body weight and weight gain were not affected at 300 ppm. Weekly food consumption was markedly reduced by

42% and 71% in 2000- and 4000-ppm male groups, respectively, during week 1 and was significantly less ($p \leq 0.05$) than that of controls for the remaining weeks. Males in the 1000-ppm group consumed 7-16% ($p \leq 0.05$) less food than controls. Females in the 1000-, 2000-, and 4000-ppm groups consumed 24%, 54%, and 66% less food than controls during week 1 and significantly less ($p \leq 0.05$) for the remaining weeks of the study.

A mild hyperchromatic macrocytic anemia was observed in male and female rats at 2000 and 4000 ppm and in females at 1000 ppm. Blood urea nitrogen (BUN) was elevated in males and females at 2000 and 4000 ppm, total protein and albumin levels were elevated in males at 4000 ppm, and total cholesterol was elevated in females at 2000 and 4000 ppm. Except for hepatocyte hypertrophy (see below), which may be associated with increased protein and BUN, clinical chemistry findings were not associated with pathologic findings.

At necropsy, terminal body weight was significantly decreased in males at 2000 and 4000 ppm and absolute epididymis weight was significantly decreased at 4000 ppm. Organ:body weight ratios of the adrenals, testes, kidneys, liver, brain, and spleen were increased at 4000 ppm, kidney, liver and brain at 2000 ppm, and liver at 1000 ppm. Terminal body weight and absolute adrenal weight were significantly decreased and liver weight was significantly increased in females at 2000 and 4000 ppm; spleen weight was significantly increased in females at 4000 ppm. Organ:body weight ratios of liver and kidneys were significantly increased at 1000-4000 ppm, and the ratios of brain and spleen weights were significantly increased in females at 4000 ppm. Except for increased liver weight, changes in absolute organ weights and organ:body weight ratios were due primarily to decreases in terminal body weight. No treatment-related gross lesions were observed in male or female rats. Microscopic examination showed significantly increased incidences of hepatocellular hypertrophy in males at 2000 and males and females at 4000 ppm, increased hemosiderosis in the spleen of males at 2000 and 4000 ppm and in females at all doses, and hyaline droplets in the renal tubular epithelium of males at 2000 and 4000 ppm. Hepatocellular hypertrophy and hemosiderosis in the spleen are not considered adverse effects and hyaline droplet formation in the kidney tubules is not relevant to humans.

Based on the range-finding study, the high dose selected for the 24-month study was 1200 ppm.

The LOAEL for dicloran in the 90-day feeding study in the rat is 2000 ppm (121.2 mg/kg bw/day for males and 133.6 mg/kg/day for females) based on decreased body weight, weight gain, and food consumption. The corresponding NOAEL is 1000 ppm (61.5 mg/kg bw/day for males and 72.4 mg/kg bw/day for females).

This subchronic toxicity study in the rat is **Acceptable/Guideline** and satisfies the guideline requirement for a subchronic toxicity study in the rat [OPPTS 870.3100); OECD 408].

13544

R154818

Chemical:

PC Code:

HED File Code: 258 Contractor DER TOX Warning: This Document (review) was completed by a contractor and has not undergone secondary review. This document may not reflect Agency policies

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