CASWELL FILE

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

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

007223

PC 031101

MAY 31 1989

MEMORANDUM:

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

SUBJECT: Dicloran: Oncogenicity Study in Mice

TO: Schnaubelt/Mitchell PM 21
Registration Division (H7505C)

FROM: K. Clark Swentzel K. Clark Swentzel 5/24/89
Acting Section Head
Toxicology Branch II (HFAS)
HED (H7509C)

THRU: Marcia van Gemert, Ph.D. Marcia van Gemert 5/23/89
Acting Branch Chief
Toxicology Branch II (HFAS)
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EPA ID No.: 45639-128,110,115,109,106,114,112,113,121
MRID/Acc. No.: 40977101
Project No.: 9-0767
Caswell No.: 311
Registrant: NOR-AM Chemical Co.

Requested action:

Review subject study.

Response:

This study was reviewed by Dynamac Corp. (final signature: May 16, 1989) and the Data Evaluation Report is attached. Dicloran was administered to male and female CD-1 mice (50/sex/group) at dietary levels of 50, 175 or 600 ppm for 80 weeks. The data generated in this study did not show an oncogenic response from ingestion of the test material. No adverse effects were indicated by mortality, clinical signs, body weight, palpable masses, food consumption or conversion, hematology, macroscopic pathology or tumor incidence. Histopathologic changes in the liver, which were noted at the high dietary level (600 ppm), included centrilobular hepatocyte enlargement, centrilobular hemosiderosis, focal necrosis (males), acute inflammatory cell infiltration (males), single cell necrosis (males) and vacuolation of centrilobular hepatocytes (females). Other organs, in which histopathologic changes were seen in animals administered the high dietary level, were spleen (increased incidence of erythropoiesis in males) and uterus (increase in the severity and incidence of cystic endometrial hyperplasia). Also, liver weight in females and kidney weight in males were elevated in high-dose animals. Since no treatment-related changes were evident at the lower dosages, the NOEL in this study was 175 ppm (30.0 mg/kg/day) and the LEL was 600 ppm (102.7 mg/kg/day).

Core-classification (by Dynamac): guideline

Tox Chem No. 311 File Last Updated _____ Current Date 5/22/89

Study/Lab/Study #/Date	Material	EPA MRID/Acc. No.	Results: LD_{50}, LC_{50}, PIS, NOEL, LEL	TOX Category	CORE Grade/ Doc. No.
Oncogenicity-mouse/ Schering Agrochemicals/ TOX/86006/ January 6, 1989	Dicloran tech 96.2-97.2%	40977101	Dietary levels: 50, 175 & 600 ppm Effects at 600 ppm: Liver:centrilobular hep. enlarg., centrilobular hemosiderosis, focal necrosis (males), acute inflamm. cell infiltra.(males), single cell necrosis (males), vacuol. of centrilob. hepatocytes (females), increased wt.(females) Spleen: increased incidence of erythropoiesis in males Uterus: increase in severity & incidence of endometrial hyperplasia. Kidney: increased wt. in males NOEL = 175 ppm(30.0 mg/kg/day) LOEL = 600 ppm (102.7 mg/kg/day) Oncogenicity : negative		guideline

EPA No.: 68D80056
DYNAMAC No.:163-A
TASK No.: 1-63A
May 15, 1989

DATA EVALUATION RECORD

DICLORAN

T104 Technical Dicloran: Oncogenicity Study in the Mouse

APPROVED BY:

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

Signature: Robert J. Weir

Date: 5/16/89

CASWELL FILE

EPA No.: 68D80056
DYNAMAC No. 163-A
TASK No.: 1-63A
May 15, 1989

DATA EVALUATION RECORD

DICLORAN

T104 Technical Dicloran: Oncogenicity Study in the Mouse

REVIEWED BY:

Claire Kruger-McDermott, Ph.D.
Principal Reviewer
Dynamac Corporation

Signature: Claire Kruger
Date: May 15, 1989

William McLellan, Ph.D.
Independent Reviewer
Dynamac Corporation

Signature: William L. McLellan
Date: May 15, 1989

APPROVED BY:

Roman J. Pienta, Ph.D.
Department Manager
Dynamac Corporation

Signature: Roman J Pienta
Date: may 15 1989

K. C~~-Swe~~ntzel, Ph.D.
EPA Reviewer, Section II
Toxicology Branch II
(TS-769C)

Signature: K. Clark Swentzel
Date: May 19, 1989

Marcia Van Gemert, Ph.D.
EPA Acting Section Head,
Section II
Toxicology Branch II
(TS-769C)

Signature: Marcia Van Gemert
Date: May 23/1989

DATA EVALUATION RECORD

GUIDELINE § 83-2

STUDY TYPE: Oncogenicity feeding study in mice.

ACCESSION/MRID NUMBER: 409771-01.

TEST MATERIAL: Technical dicloran.

SYNONYM(S): Botran; DCNA; 2,6-dichloro-4-nitroaniline.

STUDY NUMBER(S): TOX/86006.

SPONSOR: NOR-AM Chemical Company.

TESTING FACILITY: Schering Agrochemicals Ltd., Chesterford Park Research Station, Essex, England.

TITLE OF REPORT: T104 Technical Dicloran: Oncogenicity Study in the Mouse.

AUTHOR(S): B. A. Mallyon, and L. P. Markham.

REPORT ISSUED: January 6, 1989.

CONCLUSIONS: Dicloran was not oncogenic in male or female CD-1 mice when fed at dietary levels of 50, 175, or 600 ppm for 80 weeks. No adverse effects were observed on mortality, clinical signs, body weight, palpable masses, food consumption, food conversion, hematology, macroscopic pathology, or tumor incidence. Histopathologic examination showed the liver to be the principal target organ with changes noted at the high dose. Treatment resulted in centrilobular hepatocyte enlargement, centrilobular hemosiderosis, focal necrosis (males), acute inflammatory cell infiltration (males), single-cell necrosis (males), and vacuolation of centrilobular hepatocytes (females). Additional histopathologic alteration at 600 ppm were seen in the spleen (higher incidence of erythropoiesis) of males and the uterus (increase in severity and incidence of cystic endometrial hyperplasia) of females. An elevated liver weight in females and an increase in kidney weight of males was seen at 600 ppm. No treatment-related effects were seen at 50 or 175 ppm. The NOEL is 175 ppm (30.0 mg/kg/day).

Classification: CORE guideline.

A. MATERIALS:

1. Test Compound: Technical dicloran; description: a fine yellow powder; batch No.: CR 20642/3; purity: 96.2 to 97.2%.

2. Test Animals: Species: mice; strain: CD-1(ICR)BR; age: 4 weeks; weight: 15 to 20 g for males and 15 to 18 g for females; source: Charles River U.K. Ltd., Kent, England.

B. STUDY DESIGN:

1. Animal Assignment: Animals were assigned randomly to obtain similar group mean body weights and weight distribution to the following test groups:

Test group	Dose in diet (ppm)	Main study[a] (80 weeks) Males	Females
1 Control	0	50	50
2 Low (LDT)	50	50	50
3 Mid (MDT)	175	50	50
4 High (HDT)	600	50	50

[a]Additional groups of five/sex were used for microbiological screening and baseline histopathology.

Dose levels were reported to be based on an earlier range-finding study (60 days) in which mice receiving 600 ppm dicloran in the diet had increased methemoglobin, splenic pigment change, increased hematopoiesis, centrilobular hepatocyte enlargement and fatty degeneration of hepatocytes.

Mice were housed five/cage in a temperature and humidity controlled room with a 12-hour light/dark cycle.

2. Diet Preparation: Diets were prepared weekly. Optimum mixing procedures were determined in pretest trials that analyzed homogeneity of distribution and stability. Samples of diet at each level were collected weekly for analysis.

   Results: Analytical data on diets are presented in a separate report and were not available for review.

3. Food and Water Consumption: Animals received food (Modified SQC Expanded Ground Rodent Diet, Special Diets Service, Ltd.) and water ad libitum.

4. Statistics: The following procedures were utilized in analyzing the numerical data. Body weight, hematology, and organ weight data were subjected to Bartlett's test to determine homogeneity of variance. If data were heterogeneous, it was log transformed and reanalyzed. Parametric tests were performed on homogeneous data; one-way analysis of variance was used and the Student "t" test compared each group with controls. The Kruskal-Wallis rank test was used for group comparison of heterogeneous data. The Peto methodology was used for survival analysis and analysis of neoplastic and nonneoplastic histology data.

5. Quality Assurance: A quality assurance statement was signed and dated January 6, 1989.

C. METHODS AND RESULTS:

1. Observations: Animals were observed twice daily for signs of toxicity and behavioral effects. Mice were also individually examined in detail weekly and palpated for tissue masses. Time of onset, severity, and duration of abnormal signs were recorded as well as the size and location of masses.

Results: Table 1 summarizes mortality and percent survival at selected intervals. There were no dose-related effects on survival. It was reported that there were no dose-related effects on behavior, condition, or toxicity. Table 2 reports the incidence of palpable masses and median time of onset. There were no reported treatment-related increases or earlier onsets of palpable masses.

2. Body Weight: Body weight was recorded at receipt, start of treatment, weekly during the first 13 weeks, every 2 weeks thereafter, and at the terminal kill.

   Results: Table 3 reports the mean body weight of mice at selected intervals. No treatment-related effects were reported at any dose level for mean body weight.

3. Food Consumption and Compound Intake: The total weekly food consumption for each group was measured throughout the study and the mean as g/animal/day was calculated. Group mean food conversion and mean compound intake were calculated.

   Results: Food consumption and food conversion were similar among groups of males and females. Calculated mean compound intake for the entire study was 7.4, 24.5, or 86.5 mg/kg/day for males receiving dietary levels of 50, 175, or 600 ppm and 10.1, 35.4, or 118.8 mg/kg/day for females receiving the same dietary levels of dicloran.

4. Hematology and Clinical Chemistry: Blood samples for differential white cell counts were collected from the retro-orbital sinus at 12 months and at termination (18 months) from 10 mice/sex/group. Other parameters were not examined. Clinical chemistry and urinalysis were not performed.

   Results: No compound-related changes were seen. There was a significant increase ($p<0.05$) in the number of eosinophils in males receiving 50 ppm at 12 months and a significant decrease ($p<0.05$) in the number of eosinophils in females receiving 50 ppm at 12 months. The authors considered these changes to be sporadic, within the normal control range, and not of toxicologic importance. Variations of values for individual animals could not be correlated with any other findings.

TABLE 1. Cumulative Mortality and Percent Survival in Mice Fed Dicloran for 80 Weeks[a]

Dietary level (ppm)	No. of mortalities and (percent survival) at week: 24	52	80
		Males	
0	1 (98)	5 (90)	25 (50)
50	2 (96)	9 (82)	25 (50)
175	0 (100)	3 (94)	18 (64)
600	1 (98)	8 (84)	22 (56)
		Females	
0	0 (100)	4 (92)	8 (84)
50	0 (100)	2 (96)	12 (76)
175	1 (98)	4 (92)	12 (76)
600	0 (100)	2 (96)	11 (78)

[a]Fifty animals per group per sex at initiation; interim sacrifices were not performed.

TABLE 2. Incidence of Palpable Masses and Median Time of Onset in Mice Fed Dicloran

	Dietary level (ppm)			
	0	50	175	600
		Males		
Incidence	18/50	19/50	14/50	21/50
Median time of onset (weeks)	63	61	54	55
		Females		
Incidence	3/50	1/50	0/50	3/50
Median time of onset (weeks)	75	68	-	61

TABLE 3. Representative Results of Mean Body Weights of Mice Fed Dicloran for 80 Weeks

Dietary group (ppm)	Mean body weights (g ± S.D.) at week 0	4	13	27	51	79
			Males			
0	28 ± 2.0	34 ± 3.0	39 ± 3.6	42 ± 4.0	43 ± 3.7	43 ± 3.9
50	28 ± 2.3	34 ± 2.3	40 ± 3.1	43 ± 3.2	44 ± 3.1	43 ± 2.9
175	28 ± 2.3	34 ± 3.2	39 ± 3.9	43 ± 4.7	45 ± 5.6*	45 ± 5.3
600	28 ± 2.1	35 ± 2.7	40 ± 3.5	43 ± 4.1	45 ± 5.4	43 ± 5.4
			Females			
0	21 ± 1.4	26 ± 1.8	29 ± 2.7	32 ± 4.1	35 ± 5.8	38 ± 6.0
50	22 ± 1.4	26 ± 2.2	29 ± 2.3	32 ± 3.2	35 ± 3.5	37 ± 4.4
175	21 ± 1.5	26 ± 2.0	29 ± 2.7	32 ± 3.9	34 ± 5.2	36 ± 5.7
600	22 ± 1.2	26 ± 1.6	29 ± 2.1	32 ± 3.5	35 ± 4.9	37 ± 5.9

*Significantly different from control values ($p \leq 0.05$).

5. Sacrifice and Pathology: All animals that died and were sacrificed moribund or on schedule were subject to gross pathological examination and the CHECKED (X) tissues were collected for histological examination from the control and high-dose groups. In addition, the (XX) organs were weighed for all groups. Tissues from the low- and intermediate-dose groups that were examined at terminal sacrifice were any tissue showing macroscopic abnormalities, spleen, uterus, liver, kidney, and lungs.

	Digestive System		Cardiovasc./Hemat.		Neurologic
X	Tongue	X	Aorta*	XX	Brain (3 levels)*
X	Salivary glands*	XX	Heart*	X	Peripheral nerve (sciatic nerve)*
X	Esophagus*	X	Bone marrow*	X	Spinal cord (3 levels)*
X	Stomach*	X	Lymph nodes*	X	Pituitary*
X	Duodenum*	XX	Spleen*	X	Eyes (optic nerve)*
X	Jejunum*	X	Thymus*		
X	Ileum*				
X	Cecum*				
X	Colon*				
X	Rectum*		Urogenital		Glandular
XX	Liver*	XX	Kidneys*	XX	Adrenals*
X	Gallbladder*	X	Urinary bladder*	X	Lacrimal gland
X	Pancreas*	XX	Testes*	X	Mammary gland*
		X	Epididymides*	X	Thyroids*
		X	Prostate*		Parathyroids*
		X	Seminal vesicle*	X	Harderian glands
	Respiratory	XX	Ovaries*	X	Preputial gland
X	Trachea*	X	Uterus*		
X	Lung*	X	Vagina*		
					Other
				X	Bone (sternum and femur)*
				X	Skeletal muscle*
				X	Skin*
				X	All gross lesions and masses*
				X	Femur

*Recommended by Subdivision F (October 1982) Guidelines.

Results:

a. Organ Weights: The absolute liver weight ($p<0.05$) and relative liver weight ($p<0.01$) were significantly increased in females receiving 600 ppm. Liver weights in high-dose males were slightly but nonsignificantly increased. Absolute kidney weight was slightly but significantly increased ($p<0.05$) in males at 600 ppm; the slight increase in kidney-to-body weight ratio was not statistically significant. No treatment-related effects were observed at 50 or 175 ppm. Table 4 presents data for liver and kidney weights. There were no other effects on organ weights that the authors considered to be compound related. Absolute and relative weights of heart and adrenals were increased ($p<0.05$) in males and females receiving 50 ppm dicloran, respectively, but there were no effects at higher doses.

b. Gross Pathology: There were no remarkable gross necropsy findings.

c. Microscopic Pathology:

1) Nonneoplastic: Table 5 summarizes the frequently occurring nonneoplastic findings. The authors reported an increased incidence of centrilobular hepatocyte enlargement and centrilobular hemosiderocytes in both males and females at 600 ppm. The incidence was significant ($p<0.001$ and $p<0.01$, respectively) in males but not females. Males at 600 ppm also exhibited a higher incidence, compared to control, of focal necrosis, acute inflammatory cell infiltration, and single-cell necrosis in the liver; females at 600 ppm had a higher incidence, compared to control, of vacuolation of centrilobular hepatocytes. Significant, positive, dose-related trends were noted for centrilobular hepatocyte enlargement and centrilobular hemosiderocytes in the liver of both sexes. Additional findings noted at 600 ppm were a slightly higher incidence of increased erythropoiesis in the spleen of males and an increase in the severity and incidence of cystic endometrial hyperplasia of the uterus. A significant, positive, dose-related trend was observed for the increased erythropoiesis in the spleen of males. No treatment-related findings were noted at 50 or 175 ppm in either sex.

TABLE 4. Mean Liver and Kidney Weights and Liver or Kidney-to-Body Weight Ratios in Mice Fed Dicloran for 80 Weeks[a]

Dose	Males		Females	
	g	%	g	%
		Liver		
0	2.44 ± 0.70	5.66 ± 1.55	1.76 ± 0.32	4.76 ± 0.72
50	2.42 ± 0.67	5.63 ± 1.36	1.73 ± 0.22	4.83 ± 0.55
175	2.35 ± 0.34	5.27 ± 0.80	1.65 ± 0.35	4.74 ± 0.55
600	2.74 ± 0.83	6.34 ± 1.68	1.93 ± 0.38*	5.32 ± 0.80**
		Kidney		
0	0.75 ± 0.17	1.74 ± 0.42	0.46 ± 0.07	1.26 ± 0.23
50	0.78 ± 0.11	1.82 ± 0.25	0.45 ± 0.05	1.27 ± 0.17
175	0.80 ± 0.11	1.79 ± 0.23	0.43 ± 0.06	1.26 ± 0.14
600	0.83 ± 0.11*	1.93 ± 0.27	0.48 ± 0.08	1.36 ± 0.30

[a]Values are means ± standard deviations for all mice surviving to terminal sacrifice.
*Significantly different from control values ($p \leq 0.05$).
**Significantly different from control values ($p \leq 0.01$).

TABLE 5. Selected Nonneoplastic Lesions in Mice Fed Dicloran for 80 Weeks

Organ/findings	Dietary level (ppm)							
	Males				Females			
	0	50	175	600	0	50	175	600
Kidneys	(50)[a]	(50)	(50)	(50)	(50)	(50)	(50)	(50)
Mononuclear cells under the pelvic epithelium	24	34	29	35	36	39	37	34
Perivascular lymphoid foci	42	43	45	41	38	39	35	42
Pyelonephritis	0	2	1	5	1	0	0	0
Hydronephrosis	4	1	3	5	1	1	0	0
Interstitial lymphoid infiltration	13	15	21	14	19	17	9	17
Liver	(50)	(50)	(50)	(50)	(50)	(50)	(50)	(50)
Centrilobular hepatocyte enlargement	8^{+++}	8	7	26***	1^{++}	0	2	5
Focal necrosis	4	2	5	10	5	4	3	8
Acute inflammatory infiltration	2	4	9	9*	2	3	0	5
Single-cell necrosis	1	1	0	6	1	0	0	0
Centrilobular hemosiderocytes	1^{+++}	2	4	12**	7^{+}	5	3	13
Vacuolation of centrilobular hepatocytes	6	4	8	4	4	3	3	12*
Spleen	(49)	(49)	(50)	(50)	(50)	(50)	(50)	(50)
Increased erythropoiesis	7^{+}	7	8	15	4	3	3	7
Hemosiderosis	8	5	5	11	10	16	4	14
Uterus	-	-	-	-	(50)	(50)	(50)	(50)
Cystic endometrial hyperplasia	-	-	-	-	17	21	24	31

[a]The numbers in parentheses are the numbers of animals with tissues examined; includes animals that were sacrificed at study termination and those found dead or moribund.

*Significantly different from control values, $p < 0.05$; **, $p \leq 0.01$; *** 0.001 by pairwise comparison.

+Significant trend, $p < 0.05$; ++ = $p \leq 0.01$; +++ $p \leq 0.001$.

2) Neoplastic: There was no oncogenic response related to compound administration. The tumors recorded were considered by the authors to be within the normal range for this strain and age of mouse. Table 6 summarizes the neoplastic findings.

D. STUDY AUTHORS' CONCLUSIONS:

Dicloran was not oncogenic when fed to groups of 50 male and 50 female CD-1 mice for up to 80 weeks at levels of 50, 175, or 600 ppm (equivalent to 8.8, 30.0, or 102.7 mg/kg/day). There were no effects of dosing on mortality, clinical signs, palpable masses, food consumption, differential white blood counts, macroscopic pathology, or tumor incidence. Histopathological examination showed the liver to be the principal target organ. Treatment at 600 ppm resulted in centrilobular hepatocyte enlargement and centrilobular hemosiderosis in both males and females. Males at 600 ppm also exhibited a higher incidence than controls of focal necrosis, acute inflammatory cell infiltration, and single-cell necrosis of the liver; females at 600 ppm had a higher incidence of vacuolation of centrilobular hepatocytes. Liver weight was significantly elevated in females at 600 ppm. In addition, a higher incidence of increased erythropoiesis in the spleen of males and an increase in the severity and incidence of cystic endometrial hyperplasia of the uterus was found at 600 ppm. The kidney weight was increased in males at 600 ppm. No treatment-related findings were noted at 50 or 175 ppm in either sex. The NOEL was 175 ppm (30.0 mg dicloran/kg/day).

E. REVIEWERS' DISCUSSION AND INTERPRETATION OF RESULTS:

The conduct and reporting of the study were, in general, adequate. We agree with the study authors' conclusion that dicloran was not oncogenic in CD-1 mice under the conditions of the study. The tumors recorded were within the normal range for this strain and age of mouse. Survival was acceptable in all groups; at 80 weeks, female group survival ranged from 76 to 84 percent and male group survival from 50 to 64 percent.

There were no effects of dosing on mortality, clinical signs, palpable masses, food consumption, differential white blood cell counts, macroscopic pathology, or tumor incidence. The liver was the principal target organ, exhibiting histopathologic changes at 600 ppm, which were more pronounced in males than females (with the exception of centrilobular hepatocyte vacuolation). Liver weight was significantly increased in females at 600 ppm.

TABLE 6. Summary Incidences of Neoplastic Findings in Mice Fed Dicloran for 80 Weeks[a]

Organ/findings	Dietary level (ppm)							
	Males				Females			
	0	50	175	600	0	50	175	600
Liver	(50)[b]	(50)	(50)	(50)	(50)	(50)	(50)	(50)
Hepatocellular adenomas	11	11	12	13	0	0	0	0
Hepatocellular carcinomas	3	4	5	3	0	0	0	0
Hemangiosarcoma	0	0	1	0	0	0	0	0
Lung	(50)	(50)	(50)	(50)	(50)	(50)	(50)	(50)
Adenoma	11	8	10	8	10	4	7	11
Adenocarcinoma	3	1	3	1	1	0	2	1
Mammary Gland	-	-	-	-	(50)	(12)	(12)	(50)
Adenocanthoma	-	-	-	-	1	0	0	0
Adenocarcinoma	-	-	-	-	1	0	0	1
Uterus	-	-	-	-	(50)	(22)	(22)	(50)
Endometrial fibroma	-	-	-	-	1	1	0	1
Endometrial sarcoma	-	-	-	-	0	0	0	1
Kidney	(50)	(50)	(50)	(50)	(50)	(50)	(50)	(50)
Adenoma	0	0	0	0	1	0	0	0
Lymphoreticular Neoplasms	(49)	(49)	(47)	(49)	(44)	(44)	(45)	(45)
Lymphoma, malignant	1	1	3	1	6	6	5	5

[a]Includes animals at terminal sacrifice and those that died or were sacrificed moribund.

[b]The numbers in parentheses are the number of animals with a specific organ/tissue examined histologically.

There were also histopathological changes noted in the spleen and uterus at 600 ppm in males and females, respectively. No treatment-related findings were noted at 50 or 175 ppm. Not all tissues were examined microscopically at the low and intermediate dose levels; however, the spleen, uterus, liver, kidney, and lungs were examined.

We assess that the LOEL is 600 ppm (102.7 mg/kg/day) based on effects on the liver and the NOEL is 175 ppm (30.0 mg/kg/day).

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Chemical:	**Dicloran**
PC Code:	031301
HED File Code	13000 Tox Reviews
Memo Date:	05/31/89
File ID:	TX007223
Accession Number:	412-02-0007

HED Records Reference Center
12/26/2001