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

OFFICE OF PREVENTION, PESTICIDES, AND TOXIC SUBSTANCES

TXR No.: 0054321

MEMORANDUM

DATE: September 5, 2006

SUBJECT: DICLORAN: Report of the Cancer Assessment Review Committee
PC Code: 031301

FROM: Jessica Kidwell, Executive Secretary
Cancer Assessment Review Committee
Health Effects Division (7509C)

Jessica Kidwell

TO: Byong-Han Chin, Toxicologist (RRB1)
Toiya Goodlow, Risk Assessor (RRB1)
Health Effects Division (7509P)

Nathan Mottle, Chemical Review Manager
SRRD (7508P)

The Cancer Assessment Review Committee met on June 9, 2005 to evaluate the carcinogenic potential of Dicloran. Attached please find the final Cancer Assessment Document.

cc: J. Pletcher
Y. Woo

CANCER ASSESSMENT DOCUMENT

EVALUATION OF THE CARCINOGENIC POTENTIAL OF *DICLORAN*

PC CODE 031301

August 29, 2006
FINAL

CANCER ASSESSMENT REVIEW COMMITTEE
HEALTH EFFECTS DIVISION
OFFICE OF PESTICIDE PROGRAMS

DATA PRESENTATION: Byong-Han Chin, Toxicologist

DOCUMENT PREPARATION: Jessica Kidwell, Executive Secretary

CARC MEMBERS WHO COMMENTED AND VOTED:

Karl Baetcke — retired

William Burnam (Chair)

Vicki Dellarco

Kit Farwell

Abdallah Khasawinah

Nancy McCarroll

Tim McMahon

Esther Rinde

Linda Taylor

NON-COMMITTEE MEMBERS IN ATTENDANCE: (Signature indicates concurrence with the pathology report)

John Pletcher, Consulting Pathologist

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EXECUTIVE SUMMARY

The CARC evaluated the carcinogenic potential of dicloran via an electronic meeting on June 9, 2005. This is the first time that the CARC has evaluated the carcinogenic potential of dicloran.

Byong-Han Chin of Reregistration Branch 1 provided information on the chronic toxicity/carcinogenicity studies in rats and mice. 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 Wistar (HsdCpb:WU) rats at concentrations of 0, 60, 240 or 1200 ppm for the first 105 days. The dietary concentration was raised from 1200 ppm to 1440 ppm on treatment day 106 because the effects on body weight gains in animals, especially females was less than expected from the 90-day range finding study. Therefore, the calculated time-weighted average dietary concentration for the high dose main group was 1405 ppm. The dietary concentrations were equivalent to 0, 2.8, 11.3, and 71.0 mg/kg/day, respectively, for males and 0, 3.7, 15.0, and 94.1 mg/kg /day, respectively, for females. Additional groups of 10 male and 10 female rats were administered the same diets for 12 months for interim evaluations. In a carcinogenicity study (MRID 40977101), dicloran technical (96.2-97.4% a.i.; Batch No. CR 20642/3) was administered in the diet to Crl:CD-1(ICR)BR mice (50/sex/dose) at concentrations of 0, 50, 175, or 600 ppm (equivalent to 0/0, 7.4/10.1, 24.5/35.4, and 86.5/118.8 mg/kg/day in males/females) for up to 18 months.

The CARC concluded the following:

Carcinogenicity

Rats

• In male rats the incidence of benign testicular leydig cell tumors was 0/50, 1/50 (2%), 1/50 (2%), and 5/50 (10%) for the control, 60, 240, and 1405 ppm dose groups, respectively. There was a significant increasing trend, at $p \leq 0.01$, and a significant difference in the pair-wise comparison of the 1405 ppm dose group with the control, at $p \leq 0.05$, for benign testicular Leydig cell tumors. The incidence of the Leydig cell tumors was outside the historical control range of 0-8% for the testing laboratory. Accompanying these tumors was a statistically significant increase in leydig cell hyperplasia at the high dose (34%, high dose vs. 8%, control). The CARC considered the Leydig cell tumors to be treatment-related at the top dose.

• In female rats the incidence of malignant uterine endometrial adenocarcinoma was 3/50 (6%), 7/29 (24%), 7/21 (33%), and 9/50 (18%) in control, low-, mid-, and high-dose females, respectively. Although there were significant differences in the pair-wise comparisons of the 60 ppm dose group, at $p \leq 0.05$, and the 240 ppm dose group, at $p \leq 0.01$, with the controls for endometrial adenocarcinomas, not all animals were

examined for endometrial tumors in these dose groups. Therefore, the significant findings at 60 and 240 ppm are not considered to be biologically relevant. There was no significant increase in the pair-wise comparison of the high dose group (18%) with the controls (6%) ($p=0.06$). In addition, the incidence at the high dose (18%) was within the historical control range of the testing laboratory (0-22%). 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, but was not statistically significant. Therefore, the CARC did not consider the endometrial adenocarcinomas to be treatment-related.

• Dosing was considered adequate and not excessive for evaluating the carcinogenic potential of dicloran based on decreased body weight, decreased body weight gain, and histopathologic lesions in the brain and spinal cord of both sexes, optic nerve in females and Leydig cell hyperplasia in the testes in males.

Mice

• No treatment-related tumors were seen in male or female mice.

• Dosing is considered adequate to assess the carcinogenic potential of dicloran in mice based upon histopathologic changes in organs (liver and uterus) and increased liver and kidney weights seen at 600 ppm (86.5 mg/kg/day for males, 118.8 mg/kg/day for females).

Mutagenicity

Dicloran was a confirmed positive in the Ames assay, and was negative up to precipitating concentrations (10-20 ug/ml) in both the *in vitro* chromosome aberration assay in human lymphocytes and primary rat hepatocyte unscheduled DNA synthesis assay. The CARC recommended a confirmation study of gene mutations in mammalian cells.

Structure Activity Relationship

The metabolite DCPD (4-amino-2,6-dichloroaniline) is not a rat metabolite but it is found in the plant residues. DCPD was tested by the NTP (1982, TR-219). It was negative in rats but was positive for combined liver adenomas and carcinomas in mice of both sexes when tested at doses of 1000 or 3000 ppm in the diet for 103 weeks.

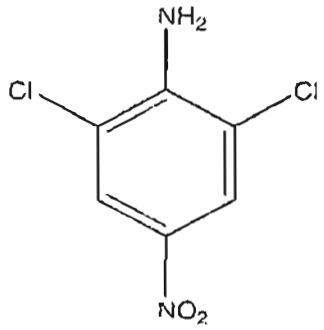
Classification and Quantification

In accordance with the EPA Final Guidelines for Carcinogen Risk Assessment (March 29, 2005), the CARC classified Dicloran as **"Suggestive Evidence of Carcinogenic Potential"** based on benign testicular Leydig cell tumors in male rats (1 sex, 1 species) at the high dose, which was considered adequate but not excessive, as well as a positive Ames test. In addition, there is some evidence that a plant metabolite, but not an animal metabolite, had some carcinogenic activity. No evidence of carcinogenicity was seen in mice at doses that were considered to be adequate for the assessment of carcinogenicity of dicloran. Quantification is not required.

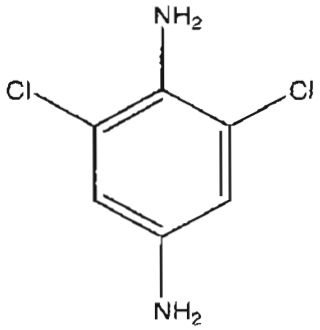
I. INTRODUCTION

The CARC evaluated the carcinogenic potential of dicloran via an electronic meeting on June 9, 2005. The data on dicloran was sent out by email to the members on June 9, 2005 and the email voting ended on June 16, 2005. This was the first time that the CARC evaluated the carcinogenic potential of dicloran.

II. BACKGROUND INFORMATION



dichloran



DCPD

The major commercial use of dicloran (2,6-dichloro-4-nitroaniline (DCNA)) is as a fungicide and as an intermediate in manufacture of dye. The fungicide is registered for treatment of many crops. It delays germination and causes a severe check to hyphal growth. It is suggested that dicloran is a structurally non-specific toxicant exerting its effect by disorganizing cell growth and division in particular plant pathogens. Dicloran has low acute toxicity. The target organs include the liver, kidney, spleen and hematopoietic system (anemia) particularly destruction of red blood cells.

The metabolite DCPD (4-amino-2,6-dichloroaniline) is not a rat metabolite but it is found in the plant residues. DCPD was tested by the NTP (1982).

III. EVALUATION OF CARCINOGENICITY STUDIES

1. Combined Chronic Toxicity and Carcinogenicity Study with Dicloran in Wistar Rats

Reference: 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.

A. Experimental Design

Dicloran (94.9% a.i.; batch/lot # 000313) was administered in the diet to groups of 50 male and 50 female Wistar (HsdCpb:WU) rats at concentrations of 0, 60, 240 or 1200 ppm for the first 105 days. The dietary concentration was raised from 1200 ppm to 1440 ppm on treatment day 106 because the effects on body weight gains in animals, especially females was less than expected from the 90-day range finding study. Therefore, the calculated time-weighted average dietary concentration for the high dose main group was 1405 ppm. The dietary concentrations were equivalent to 0, 2.8, 11.3, and 71.0 mg/kg/day, respectively, for males and 0, 3.7, 15.0, and 94.1 mg/kg /day, respectively, for females. Additional groups of 10 male and 10 female rats were administered the same diets for 12 months for interim evaluations.

B. Discussion of Survival and Tumor Data

Survival

Survival was not affected by treatment with the test material.

Table 1. Survival (%) at terminal sacrifice in rats treated with dicloran in the diet for up to 2 years

Parameter	Dose (ppm)			
	0	60	240	1405
Males, Terminal Sacrifice	72	66	74	88
Females, Terminal Sacrifice	78	66	70	74

Data taken from survival table in DER.

Tumor Data

At the doses tested, the incidence of benign Leydig cell tumors was 0/50, 1/50, 1/50, and 5/50 in control, low-, mid-, and high-dose males rats, respectively. All Leydig tumors were found in animals sacrificed at study termination. There was a significant increasing trend, at $p \leq 0.01$, and a significant difference in the pair-wise comparison of the 1405 ppm dose group with the control, at $p \leq 0.05$, for testicular benign Leydig cell tumors (Table 2). High-dose male rats also had a significantly increased incidence (34%) of Leydig cell hyperplasia compared with that of controls (8%). The historical control incidences for leydig cell tumors are listed in Table 4.

Table 2. Dicloran - Wistar Rat Study (MRID 46360701)
Male Testicular Leydig Cell Tumor Rates and *ad hoc*[a] Fisher's Exact Test and Trend Tests and Non-Neoplastic Findings

Tissue and observation	Dietary concentration (ppm)			
	0	60	240	1405
Benign Testicular Leydig cell tumor (%)	0/50 (0)	1/50 (2)	1/50 (2)	5/50 (10)
p =	0.004543**	0.50000	0.500000	0.02814*
Leydig cell hyperplasia (%)	4/50 (8)	5/50 (10)	5/50 (10)	17/50** (34)

** - $p \leq 0.01$; * - $p \leq 0.05$

[a]*ad hoc* statistical analysis performed by L. Brunsman, 6/1/05, from data taken from Table 58, MRID 46360701.

[b]2/5 rats (Nos. 5112 and 5116) had both Leydig cell tumor and Leydig cell hyperplasia. Three other rats with Leydig cell tumor did not have Leydig cell hyperplasia.

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. Although there were significant differences in the pair-wise comparisons of the 60 ppm dose group, at p≤0.05, and the 240 ppm dose group, at p≤0.01, with the controls for endometrial adenocarcinomas, not all animals were examined for endometrial tumors in these dose groups (Table 3). Therefore, the significant findings at 60 and 240 ppm are not considered to be biologically relevant. 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. The historical control incidences for endometrial adenocarcinomas is listed in Table 4.

Table 3. Dicloran - Wistar Rat Study (MRID 46360701)
Female Endometrial Tumor Rates and *ad hoc*[a] Fisher's Exact Test and Trend Tests and Non-Neoplastic Findings

Tissue Observation	Dietary Concentration (ppm)			
	0	60[b]	240[b]	1405
Adenocarcinoma (%) p	3/50 (6) 0.3298	7/29 (24) 0.02524*	7/21 (33) 0.00551**	9/50 (18) 0.06062
Endometrial hyperplasia (%) p	4/50 (8)	2/29 (7)	1/21 (5)	9/50 (18) 0.12

[a]*ad hoc* statistical analysis performed by L. Brunsman, 6/1/05, from data taken from Table 58, MRID 46360701.
[b]Not all animals were examined in these dose groups.
* = p≤0.05; ** = p≤0.01

Table 4. The Ranges for the Historical controls of Leydig cell tumor (Benign) and Endometrial adenocarcinoma (Malignant)

Tissue and observation	Rallis Research Center (the testing laboratory: n=11)	Bomhard and Rinke, 1994[a]	Charles River Laboratories, 2003[b]	MRID 46360701 Highest dose tested
Testes Leydig cell tumor (Benign)	0-8% (average =1.7%)	2.1-16.3% (average =7%)	1.67-10.91% (average= 2.16%)	10%
Uterus Endometrial adenocarcinoma (Malignant)	0-22% (average =10%)	0-16.3% (average =7.8%)	1.67-5.45% (average =2.3%)	18%

[a]Bomhard, E. and Rinke M. 1994. Frequency of spontaneous tumours in Wistar rats in 2-year studies. *Exp Toxic Pathol* 46 (1994):17-29
[b]Giknis, M and Clifford, C. 2003. Spontaneous Neoplasms and Survival in Wistar Han Rats:Compilation of Control Group Data. Charles River Laboratories, March 2004.

C. Non-Neoplastic Lesions

The incidences of microscopic non-neoplastic lesions are summarized in Table 5a and 5b. **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. One male rat in the mid-dose group had vacuolation in the cerebral cortex. 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 severity 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% ($p \leq 0.05$-0.01), respectively, of male rats in the mid-dose group and 30% and 76% ($p \leq 0.01$), 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. 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% ($p \leq 0.05$-0.01) of males at all doses compared with only 18% in the control group and in 80% ($p \leq 0.01$) of high-dose females compared with 36% in the control group. 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 a significantly increased incidence (34%) of Leydig cell hyperplasia compared with that of controls (8%) (Table 5b). 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 5).

TABLE 5a. 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 (10)	4	12* (24)[b]	15** (30)	5	1	3	6
Hepatocellular hypertrophy	3 (6)	7	15** (30)	38** (76)	28	27	35	36
Necrobiotic focus(1)	10	2	10	18	5	5	7	15**
Spleen [# animals examined]	[50]	[50]	[50]	[50]	[50]	[50]	[50]	[50]
Increased hemosiderosis	9 (18)	23* (46)	35** (70)	42** (84)	18 (36)	21	26	40** (80)
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** (96)	0	0	0	49** (98)
Optic chiasma, vacuolation	0	0	0	14** (28)	0	0	0	17** (34)
Cerebellar cortex, vacuolation	2	0	0	31** (62)	1	0	0	42** (84)
Medulla/pons, vacuolation	1	0	0	48** (96)	0	0	0	46** (92)
Spinal cord [# examined]	[50]	[50]	[50]	[50]	[50]	[50]	[50]	[50]
Cervical, vacuolation	0	0	0	43** (86)	0	0	0	43** (86)
Thoracic, vacuolation	0	0	0	42** (84)	1	0	0	40** (80)
Lumbar, vacuolation	0	0	0	28** (56)	0	0	0	23** (46)
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

[b]Numbers in parentheses are percent of control calculated by the reviewer.

*p≤0.05, **p≤0.01 statistically significant, treated group compared with the control group, calculated by the reviewer using Fisher's exact test

Table 5b. Summary of Histopathological (Non-neoplastic and Neoplastic) Findings of Combined Fates

Tissue and observation	Dietary concentration (ppm)			
	0	**60**	**240**	**1405**
Testes [# animals examined] Leydig cell tumor (Benign)	50 0 (0) [a]	50 1 (2)	50 1 (2)	50 5 (10) [b]
Leydig cell hyperplasia	50 4 (8)	50 5 (10)	50 5 (10)	50 17 (34)
Uterus [# animals examined] Endometrial adenocarcinoma (Malignant)	50 3 (6)	29 7 (24)	21 7 (33)	50 9 (18)
Endometrial hyperplasia	50 4 (8)	29 2 (7)	21 1 (5)	50 9 (18)

Data taken from Table 58, MRID 46360701.

[a] Numbers in parentheses: Percentage value

[b] 2 rats (Nos. 5112 and 5116) had both Leydig cell tumor and Leydig cell hyperplasia. Three other rats with Leydig cell tumor did not have Leydig cell hyperplasia.

D. Adequacy of the Dosing for Assessment of Carcinogenicity

Dosing was considered adequate and not excessive for evaluating the carcinogenic potential of dicloran based on decreased body weight, decreased body weight gain, and histopathologic lesions in the brain and spinal cord of both sexes, optic nerve in females and Leydig cell hyperplasia in the testes in males.

2. Carcinogenicity Study in Mice

Reference: Mallyon, B.A. and L.P. Markham (1989) T104 Technical Dicloran: oncogenicity study in the mouse (final report) part 1. Schering Agrochemicals Limited, Essex. England. Laboratory Project Id.: Tox/86006, January 6, 1989. MRID 40977101. Unpublished.

A. Experimental Design

Dicloran technical (96.2-97.4% a.i.; Batch No. CR 20642/3) was administered in the diet to Crl:CD-1(ICR)BR mice (50/sex/dose) at concentrations of 0, 50, 175, or 600 ppm (equivalent to 0/0, 7.4/10.1, 24.5/35.4, and 86.5/118.8 mg/kg/day in males/females) for up to 18 months.

B. Discussion of Tumor Data

No treatment-related increase in tumor incidence was reported.

C. Non-Neoplastic Lesions

Treatment-related non-neoplastic lesions were observed (Table 6). Hepatotoxicity was indicated in the 600 ppm males. A positive trend ($p \leq 0.05$) in the incidence of focal necrosis with dose was observed, and the incidence of minimal to moderate focal necrosis was increased ($p > 0.05$) in the 600 ppm males (10/50) vs minimal to slight in controls (4/50). Increased ($p \leq 0.05$-0.01) incidences (# affected/50) in the following hepatic lesions were also observed in the 600 ppm males: (i) minimal to moderate single cell necrosis in treated (6) vs moderate in controls (1); (ii) slight to severe centrilobular hepatocyte enlargement in treated (26) vs minimal to moderate in controls (8); (iii) minimal to slight acute inflammatory infiltration in treated (9) vs minimal to slight in controls (2); and (iv) centrilobular hemosiderocytes present in treated (12) vs controls (1).

An increased ($p \leq 0.05$) incidence of minimal to severe vacuolation of centrilobular hepatocytes was observed in the 600 ppm females (12/50) vs slight to moderate in controls (4/50). Minimal to severe cystic endometrial hyperplasia in the uterus was increased ($p \leq 0.05$) at $\geq$175 ppm (24-31/50) vs minimal to severe in controls (17/50). A positive trend ($p \leq 0.01$) in the incidence of

each lesion (vacuolation and hyperplasia) with dose was found. The incidence of all other lesions in the treated groups were similar to the controls.

Table 6. Incidence (# affected/50) of selected non-neoplastic microscopic lesions in mice treated with Dicloran in the diet for up to 80 weeks.[a]

Microscopic lesion		Dose (ppm)			
		0	50	175	600
Males					
Liver	Focal necrosis (total)	4*	2	5	10
	Minimal	3	0	3	4
	Slight	1	2	1	5
	Moderate	0	0	0	1
	Severe	0	0	1	0
	Single cell necrosis (total)	1**	1	0	6*
	Minimal	0	0	0	1
	Slight	0	1	0	4
	Moderate	1	0	0	1
	Centrilobular hepatocyte enlargement (total)	8***	8	7	26***
	Minimal	1	2	0	0
	Slight	5	5	5	10
	Moderate	2	1	1	15
	Severe	0	0	1	1
	Acute inflammatory infiltration (total)	2*	4	9	9*
	Minimal	1	2	4	3
	Slight	1	1	3	6
	Moderate	0	1	1	0
	Severe	0	0	1	0
	Centrilobular hemosiderocytes present	1***	2	4	12**
Females					
Liver	Vacuolation of centrilobular hepatocytes (total)	4***	3	3	12*
	Minimal	0	0	0	1
	Slight	3	2	2	8
	Moderate	1	1	1	2

Microscopic lesion		Dose (ppm)			
		0	50	175	600
	Severe	0	0	0	1
Uterus Cystic endometrial hyperplasia		17**	21	24	31*
	Minimal	5	5	1	1
	Slight	9	9	13	15
	Moderate	2	7	7	12
	Severe	1	0	3	3

a Data were obtained from Table 11 on pages 95, 121, and 132 and Appendix VIII on pages 1073-1080 of MRID 40977101.

* Treatment group significantly differs from controls or a positive trend of response with dose; $p \leq 0.05$

** Treatment group significantly differs from controls or a positive trend of response with dose; $p \leq 0.01$

** Treatment group significantly differs from controls or a positive trend of response with dose; $p \leq 0.001$

D. Adequacy of the Dosing for Assessment of Carcinogenicity

Dosing is considered adequate to assess the carcinogenic potential of dicloran in mice based upon histopathologic changes in organs (liver and uterus) and increased liver and kidney weights seen at 600 ppm (86.5 mg/kg/day for males, 118.8 mg/kg/day for females).

IV. TOXICOLOGY

1. Metabolism

Dicloran is rapidly absorbed and metabolized in rats following oral administration (MRID 44061001). Approximately 96% of the administered dose was excreted in 24 hours. The urine was the major route of excretion (86.3% of the administered dose), and smaller amounts in feces (8.7% of the administered dose). Dicloran does not appear to accumulate in tissues. The major urine metabolites were DCHA-sulfate (4-amino-3,5-diclorophenol), and DCHA-glucuronide which accounted for 45.5% to 79% of the total dose. Other metabolites detected were DCHA (3.3 to 22.8%), DCAP (4, amino-2,6-diclorophenol, 0.3% to 8.5%), and DCNAP (3,5-dichloro-4-hydroxyacetanilide, <0.1% to 1.0%). A small amount of dicloran was detected in feces.

2. Mutagenicity

Dicloran was a confirmed positive in the Ames assay (40508801), and was negative up to precipitating concentrations (10-20 ug/ml) in both the *in vitro* chromosome aberration assay in human lymphocytes (40508802) and primary rat hepatocyte unscheduled DNA synthesis assay (40619001).

There is reproducible evidence of a positive response both in the presence and the absence of S9 activation in *Salmonella typhimurium* TA1538 and TA98 at 500, 1500 and 5000 ug/plate +/-S9. The response appears to be slightly greater in the presence of S9 (TA1538 - 5.1X at 5000 ug/plate+S9 vs 3 X at 5000 ug/plate -S9; TA98 - 3.7X at 5000 ug/plate+S9 vs 2.2 X at 5000 ug/plate -S9). The response is valid because: 1) the effect was reproducible and 2) the findings with strain TA1538 were confirmed by TA98 (almost always, + with strain TA1538 is followed by a + with TA98, that's why TA1538 is no longer necessary in a guideline Ames test). There is also some residual activity with TA100.

The CARC recommended a confirmation study of gene mutations in mammalian cells.

No mutagenicity studies were found in the open literature.

3. Structure-Activity Relationship

The metabolite DCPD (4-amino-2,6-dichloroaniline) is not a rat metabolite but it is found in the plant residues. DCPD was tested by the NTP (1982, TR-219). It was negative in rats but was positive for combined liver adenomas and carcinomas in mice of both sexes when tested at doses of 1000 or 3000 ppm in the diet for 103 weeks.

4. Subchronic and Chronic Toxicity

a) Subchronic Toxicity

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 Wistar (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/kg/day, respectively, for males and 0, 25.4, 72.4, 133.6, and 264.6 mg/kg/day, respectively, for females. The following parameters were examined: clinical signs, body weight, food consumption, clinical pathology (hematological 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 than controls, respectively, and females weighed 6-13% and 8-19% less than controls, 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 56% 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. However, over the entire study, the 1000 ppm group gained 11% (N.S.) less weight than controls. 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 reduced by 10-19% and 15-36% ($p \leq 0.05$) less food than 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 16-21%, 20-28%, and 21-37% ($p \leq 0.05$) less food than controls for the remaining weeks of the study.

In male and female rats at 2000 and 4000 ppm, the RBC counts were lower and MCV and MCH were higher than controls. 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).

b) Chronic Toxicity

Rat

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 Wistar (HsdCpb:WU) rats at concentrations of 0, 60, 240 or 1200 ppm for the first 105 days. The dietary concentration was raised from 1200 ppm to 1440 ppm on treatment day 106 because the effects on body weight gains in animals, especially females was less than expected from the 90-day range finding study. Therefore, the calculated time-weighted average dietary concentration for the high dose main group was 1405 ppm. The dietary concentrations were equivalent to 0, 2.8, 11.3, and 71.0 mg/kg/day, respectively, for males and 0, 3.7, 15.0, and 94.1 mg/kg /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 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 10-14% ($p \leq 0.05$) 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 hematological 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 hematological 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. Significant changes in clinical chemistry parameters in high-dose rats were transient and 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. 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, reduced body weight gain, and histopathologic lesions in the brain and spinal cord of both sexes, optic nerve in females and testes in males. In addition, treatment-related increase in the relative weights in the liver, brain and testes in males and relative liver weight in

females were observed. The no-observed-adverse-effect level (NOAEL) is 240 ppm (M//F:11.3/15.0 mg/kg bw/day.

Mouse

EXECUTIVE SUMMARY: In a carcinogenicity study (MRID 40977101), dicloran technical (96.2-97.4% a.i.; Batch No. CR 20642/3) was administered in the diet to Crl:CD-1(ICR)BR mice (50/sex/dose) at concentrations of 0, 50, 175, or 600 ppm (equivalent to 0/0, 7.4/10.1, 24.5/35.4, and 86.5/118.8 mg/kg/day in males/females) for up to 18 months.

No treatment-related effects were observed on mortality, clinical signs, body weights, body weight gains, food consumption, food conversion ratios, or differential leukocyte counts.

Hepatotoxicity was indicated in the 600 ppm males due to increased incidences (# affected/50; $p \leq 0.05$-0.01) in the following findings: (i) minimal to moderate focal necrosis in treated (10) vs minimal to slight in controls (4); (ii) minimal to moderate single cell necrosis in treated (6) vs moderate in controls (1); (iii) slight to severe centrilobular hepatocyte enlargement in treated (26) vs minimal to moderate in controls (8); (iv) minimal to slight acute inflammatory infiltration in treated (9) vs minimal to slight in controls (2); and (vi) centrilobular hemosiderocytes present in treated (12) vs controls (1).

In the 600 ppm females, absolute and relative (body) liver weights were slightly increased ($p \leq 0.05$-0.01; 810-12%), and an increased ($p \leq 0.05$) incidence of minimal to severe vacuolation of centrilobular hepatocytes was observed (12/50) vs slight to moderate in controls (4/50). Also in the 600 ppm females, the uterus was distended and enlarged (8/50 treated, each lesion vs 2-3/50 controls). Minimal to severe cystic endometrial hyperplasia in the uterus was increased ($p \leq 0.05$) at $\geq$175 ppm (24-31/50) vs minimal to severe controls (17/50). As there were no further effects observed at 175 ppm, the endometrial hyperplasia at this dose was not considered adverse.

No effects were observed at the lower doses.

The LOAEL is 600 ppm (equivalent to 86.5/118.8 mg/kg/day in males/females), based on an increased incidence of microscopic hepatic lesions in males, centrilobular hepatocyte vacuolation in females, and distended, enlarged uterus with cystic endometrial hyperplasia. The NOAEL is 175 ppm (24.5/35.4 mg/kg/day in males/females).

5. Mode of Action Studies

No mode of action data were submitted.

V. COMMITTEE'S ASSESSMENT OF THE WEIGHT-OF-THE-EVIDENCE

1. Carcinogenicity

Rats

• In male rats the incidence of benign testicular leydig cell tumors was 0/50, 1/50 (2%), 1/50 (2%), and 5/50 (10%) for the control, 60, 240, and 1405 ppm dose groups, respectively. There was a significant increasing trend, at $p<0.01$, and a significant difference in the pair-wise comparison of the 1405 ppm dose group with the control, at $p<0.05$, for benign testicular Leydig cell tumors. The incidence of the Leydig cell tumors was outside the historical control range of 0-8% for the testing laboratory. Accompanying these tumors was a statistically significant increase in leydig cell hyperplasia at the high dose (34%, high dose vs. 8%, control). The CARC considered the Leydig cell tumors to be treatment-related at the top dose.

• In female rats the incidence of malignant uterine endometrial adenocarcinoma was 3/50 (6%), 7/29 (24%), 7/21 (33%), and 9/50 (18%) in control, low-, mid-, and high-dose females, respectively. Although there were significant differences in the pair-wise comparisons of the 60 ppm dose group, at $p\leq0.05$, and the 240 ppm dose group, at $p\leq0.01$, with the controls for endometrial adenocarcinomas, not all animals were examined for endometrial tumors in these dose groups. Therefore, the significant findings at 60 and 240 ppm are not considered to be biologically relevant. There was no significant increase in the pair-wise comparison of the high dose group (18%) with the controls (6%) ($p=0.06$). In addition, the incidence at the high dose (18%) was within the historical control range of the testing laboratory (0-22%). 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, but was not statistically significant. Therefore, the CARC did not consider the endometrial adenocarcinomas to be treatment-related.

• Dosing was considered adequate and not excessive for evaluating the carcinogenic potential of dicloran based on decreased body weight, decreased body weight gain, and histopathologic lesions in the brain and spinal cord of both sexes, optic nerve in females and Leydig cell hyperplasia in the testes in males.

Mice

• No treatment-related tumors were seen in male or female mice.

• Dosing is considered adequate to assess the carcinogenic potential of dicloran in mice based upon histopathologic changes in organs (liver and uterus) and increased liver and kidney weights seen at 600 ppm.

2. Mutagenicity

Dicloran was a confirmed positive in the Ames assay, and was negative up to precipitating concentrations (10-20 ug/ml) in both the *in vitro* chromosome aberration assay in human lymphocytes and primary rat hepatocyte unscheduled DNA synthesis assay. The CARC recommended a confirmation study of gene mutations in mammalian cells.

3. Structure Activity Relationship

The metabolite DCPD (4-amino-2,6-dichloroaniline) is not a rat metabolite but it is found in the plant residues. DCPD was tested by the NTP (1982, TR-219). It was negative in rats but was positive for combined liver adenomas and carcinomas in mice of both sexes when tested at doses of 1000 or 3000 ppm in the diet for 103 weeks.

4. Mode of Action

No mode of action data were submitted.

VI. CLASSIFICATION OF CARCINOGENIC POTENTIAL

In accordance with the EPA Final Guidelines for Carcinogen Risk Assessment (March 29, 2005), the CARC classified Dicloran as **"Suggestive Evidence of Carcinogenic Potential"** based on benign testicular Leydig cell tumors in male rats (1 sex, 1 species) at the high dose, which was considered adequate but not excessive, as well as a positive Ames test. In addition, there is some evidence that a plant metabolite, but not an animal metabolite, had some carcinogenic activity. No evidence of carcinogenicity was seen in mice at doses that were considered to be adequate for the assessment of carcinogenicity of dicloran.

VII. QUANTIFICATION OF CARCINOGENIC POTENTIAL

Quantification is not required.

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