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

JUN 24 1992

MEMORANDUM

SUBJECT: Carcinogenicity Peer Review of Dicofol

FROM: Whang Phang, Ph.D. 5/20/92
Tox. Branch II
Health Effects Division (H7509c)

and
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Science Analysis and Coordination Branch
Health Effects Division (H7509c)

TO: D. Edwards/ M. Coombs
Product Manager #19
Insecticide-Rodenticide Branch
Reregistration Division (H7505c)

The Health Effects Division Carcinogenicity Peer Review Committee met on April 15, 1992 to discuss and evaluate the weight-of-the-evidence on dicofol with particular reference to its carcinogenic potential.

The Peer Review Committee agreed that dicofol should be classified as Group C- possible human carcinogen and recommended that for the purpose of risk characterization the Reference Dose (RfD) approach should be used for quantification of human risk.

A. Individuals in Attendance:

1. Peer Review Committee: (Signatures indicate concurrence with the peer review unless otherwise stated.)

Karl Baetcke

Marcia Van Gemert

Reto Engler

Robert Beliles

Lucas Brennecke

Marion Copley

George Ghali

Jean Parker

Hugh Pettigrew

William Sette

Yin-Tak Woo

2. Reviewers: (Individuals responsible for data presentation; signatures indicate technical accuracy of panel report.)

Whang Phang[1]

Jim Rowe

3. Peer Review Members in Absentia: (Committee members who were unable to attend the discussion; signatures indicate concurrence with the overall conclusions of the Committee.)

Penelope Fenner-Crisp

William L. Burnam

Julie Du

Kerry Dearfield

Richard Hill

John Quest

Esther Rinde

4. Other Attendees:

Eve Andersen (Clement)
Ann Clevenger and Linnea Hansen (HED)

[1]Also a member of the PRC for this chemical; signature indicates concurrence with the peer review unless otherwise stated.

B. Material Reviewed:

The material available for review consisted of DER's and other data summaries prepared by Whang Phang. The material reviewed is attached to the file copy of this report. The data reviewed are based on studies submitted to the Agency by Rohm and Haas Co.

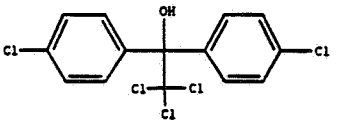
C. Background Information:

Dicofol (Kelthane) has been the subject of four cancer peer reviews including the FIFRA Scientific Advisory Panel (SAP), the OPP Final Special Review Position Document (PD4), the Carcinogen Assessment Group (CAG) and CRAVE. The conclusion of OPP in its PD4 (1986) and the SAP was that dicofol has limited evidence of carcinogenicity (Class C carcinogen) and quantitation of risk should not be performed. The CAG concluded in 1985 (final report 1986) that since dicofol is structurally similar to DDT, it should be elevated from a Class C to a B2 carcinogen classification. This report was used by the CRAVE group in their evaluation which was reflected in the Integrated Risk Information System (12/01/88) as a Class C carcinogen with quantitation (q_1*).

The Office of Pesticides Program is presently considering the revocation of the food additive regulation for residues of dicofol in or on dried tea due to considerations of the Delaney clause of Section 409 of the Federal Food, Drug and Cosmetic Act. In 1991, the registrant requested that HED perform a cancer peer review for dicofol, including the most recent scientific data. However, the CRAVE evaluation and the IRIS carcinogenicity classification is an Agency consensus position which includes OPP representation. At that time, it was thought that the most efficient approach was to provide the additional materials to the CRAVE workgroup for their reevaluation. In December 1991, CRAVE evaluated the additional data and maintained the original classification (Class C carcinogen) with quantitation (q1*). The registrant has responded to the CRAVE's decision and requested that the Agency conduct a new "weight of the evidence analysis ... to determine whether quantitative risk assessment is warranted for dicofol".

The Caswell (or Tox Chem) Number of dicofol is 93
The Chemical Abstracts Registry Number (CAS No.) is 115-32-2

The structure of dicofol is



D. Evaluation of Carcinogenicity Evidence:

1. Mouse Carcinogenicity Study

Reference: NCI. 1978. Bioassay of dicofol for possible carcinogenicity. CAS No. 115-32-2. Carcinogenesis Report Series 90-1978

a. Experimental Design

Groups of B6C3F1 mice (50/sex) were fed dicofol at 150-300 ppm (low dose males, 300-600 ppm (high dose males), 55-150 ppm (low dose females), and 110-300 ppm (high dose females) for up to 45 weeks, terminating with an untreated period of 14-15 weeks. The time-weighted average concentrations were 264 and 528 ppm for males and 122 and 243 ppm for females. The control groups consisted of 20 mice/sex.

b. Discussion of Tumor Data

A statistically significant increase in the incidence of liver tumors, mainly carcinomas, was originally reported in high dose male mice (NCI-CG-TR-90). At the request of EPA, Dr. Maronpot of the National Toxicology Program re-evaluated the liver pathology slides (letter from Maronpot to JA Moore, April 5, 1985). The major difference was the reclassification of most carcinomas to adenomas which reflected a change in the conventions of the pathology community for classifying mouse liver tumors. The incidence of mouse liver tumors from the two evaluations is presented below.

THE INCIDENCE OF LIVER TUMORS IN DICOFOL-TREATED MALE MICE[+]

	Control	Low Dose	High Dose
1978 NCI			
Hepatocellular adenomas	0/18 (0%)	1/50 (2%)	1/47 (2%)
Hepatocellular carcinomas	3/18 (17%)**	22/50 (44%)*	35/47 (74%)**
Combined tumors	3/18 (17%)**	23/50 (46%)*	36/47 (77%)**
1985 NTP (Maronpot)			
Hepatocellular adenomas	0/18 (0%)**	13/48 (27%)**	23/47 (49%)**
Hepatocellular carcinomas	2/18 (11%)	12/48 (25%)	9/47 (19%)
Combined tumors	2/18 (11%)**	25/48 (52%)**	32/47 (68%)**

[+] Number of tumor bearing animals/Number of animals examined.

Significance of trend denoted at Control.
Significance of pairwise comparison with control denoted at Dose level.
*$p<0.05$ **$p<0.01$

Based on the Maronpot re-evaluation, the increase in hepatocellular adenomas and combined adenomas/carcinomas was statistically significant by pairwise comparisons at both doses. There was also a statistically significant positive trend for adenomas and combined adenomas/carcinomas. The incidence of hepatocellular carcinomas was increased at both doses as compared to controls, but there was no pair-wise or trend significance.

It was noted that the background incidence of hepatocellular adenomas in male B6C3F1 mice historically is generally high[2]. However in this particular study, there were no hepatocellular adenomas in the control animals.

c. Non-neoplastic Lesions

The compound did not affect the survival rate of the treated animals relative to controls. It produced no effect on the body weights of the treated males. There was a decrease in the body weights of high dose females.

d. Adequacy of Dosing for Assessment of Carcinogenic Potential

The NCI report states that "a dose-related mean body weight depression was apparent in females from approximately week 40 until the bioassay was terminated".

EPA originally considered the NCI study to be invalid due to the reported decomposition of the test material during the test period. Subsequent chemical analysis of the archived test material showed that it was representative of technical dicofol (approximately 90%), and that there was no evidence of decomposition.

2. Rat Carcinogenicity Study

Reference: NCI. 1978. Bioassay of dicofol for possible carcinogenicity. CAS No. 115-32-2. Carcinogenesis Report Series 90-1978

a. Experimental Design

Groups of Osborne-Mendel rats (50/sex/dose) received dicofol for 78 weeks followed by 34 weeks of untreated diet. Dicofol concentrations were 380-500 ppm for the low dose males (TWA = 471 ppm), 760-1000 ppm for the high dose males (TWA = 942 ppm), 380 ppm for the low dose females, and 760 ppm for the high dose females.

b. Discussion of Tumor Data

The results provided no evidence that dicofol induced an increase in tumor incidence in Osborne-Mendel rats at any site.

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[2]Haseman JK, J Huff, GA Borman. (1984) The use of historical control data in carcinogenicity studies in rodents. Toxicol. Pathol. 12:126-135.

c. Non-neoplastic Lesions

A "dose-related mean body weight depression in males and females" was reported by the NCI.

d. Adequacy of Dosing for Assessment of Carcinogenic Potential

A "dose-related mean body weight depression in males and females" was reported by the NCI for these rats.

3. Rat Carcinogenicity Study

Reference: Hazleton GA, Harris DC, 1989. Dicofol (Kelthane® MF miticide) Twenty-four month dietary chronic oncogenicity test in rats. Rohm and Haas, #86R-190. March 29, 1989. MRID No. 411500-01.

a. Experimental Design

Groups of CRL:CD^R rats (60/sex/dose) received dicofol (93.3% pure) at dietary concentrations of 0, 5, 50, and 250 ppm for 24 months (corresponding to 0, 0.22, 2.23 and 11.34 mg/kg/day for the males and 0, 0.27, 2.69, and 14.26 mg/kg/day for the females). Interim sacrifices were carried out at 3, 12, and 18 months with additional groups of 10 rats/sex/dose.

b. Discussion of Tumor Data

No increase in the tumor incidence of any tissue was found in the 3- or 12-month interim sacrifices. No treatment-related increases in tumor incidence (any tissues) were seen in any group of the treated animals relative to the controls. The incidence of hepatocellular adenomas and carcinomas for the 18-month interim and terminal sacrifices is presented below.

THE INCIDENCE OF LIVER TUMORS IN DICOFOL TREATED RATS
* = number of tissues examined

Liver		Control	Low dose	Mid dose	High dose
18-Month Interim:					
Adenomas	M	0/8*	0/8	0/7	1/9
	F	0/8	0/8	0/9	0/10
Carcinomas	M	0/8	1/8	0/7	0/9
	F	0/8	0/8	0/9	0/10
24-Month Terminal:					
Adenomas	M	0/58	1/57	1/60	0/58
	F	0/59	1/60	2/60	2/59
Carcinomas	M	1/58	0/57	0/60	2/58
	F	1/59	0/60	0/60	1/59

c. Non-neoplastic Lesions

Dicofol produced a marked decrease in body weights throughout the treatment period at 250 ppm in both sexes (approximately 15% and 28% by the end of the study in males and females, respectively) and a slight reduction in food consumption in female rats. There was a decrease in triglyceride levels in high dose animals. Hepatic mixed function oxidase activity was increased in 50 and 250 ppm animals (both sexes) but an increase in liver microsomal protein concentration was not noted, consistent with the lack of absolute liver weight changes.

At the terminal sacrifice, an increase in the incidence of focal discoloration of the liver was seen in the 250 ppm females. Histopathological changes in the liver of 50 and 250 ppm males and females and in the adrenal glands of 250 ppm females were consistently seen at all interim sacrifices and at the termination of the study. The microscopic changes in the liver were characterized by minimal to moderate hypertrophy of centrilobular hepatocytes which also was accompanied by an increased amount of centrilobular, diffuse, or midzonal hepatocellular vacuolation. In some rats, necrosis of single hepatocytes or multiple foci of hepatocytes were seen. In the adrenal glands, diffuse vacuolation of cortical cells was seen often.

d. Adequacy of Dosing for Assessment of Carcinogenic Potential

Based upon the significant depression in body weights of both sexes at the high dose and the histopathologic changes observed in the liver and adrenal gland, this study employed dosing adequate to characterize carcinogenic potential.

E. Additional Toxicology Data on Dicofol:

1. Metabolism

In a 1987 metabolism study, groups of rats (4/sex) received a single oral dose of [^{14}C]-dicofol (50 mg/kg). The pattern of tissue distribution, excretion and metabolite profiles were examined at 48 and 168 hours post-dosing. The major route of elimination was through feces for all groups (62% and 32% of the dose in males and females, respectively) after 168 hours. Approximately 15% of the dose was excreted in the urine of both sexes after 168 hours. The highest residue levels of [^{14}C]-dicofol equivalents were observed in adipose tissue at 48 hours (31% of the dose in males and 81% of the dose in females). The majority of the radioactivity in the fat tissue was the parent compound which accounted for 83% and 89% of the radioactivity in the fat of males and females, respectively. [^{14}C] residues in fat, liver, and blood decreased with time for both sexes, but the decrease was faster in males than in females.

Urine, feces, fat, liver, and plasma were analyzed for dicofol metabolites. Dicofol was metabolized primarily by a mechanism involving replacement of a nonring chlorine atom with hydrogen (reductive dehalogenation) and subsequent oxidation to form dichlorobenzophenone (DCBP) and dichlorobenzoic acid (DCBA).

Further metabolism yields dichlorobenzil (DCBH) and various hydroxy or conjugated secondary metabolites. The results indicated that the major metabolite of dicofol was FW-152 (DCD, dechlorodicofol) in feces, urine, as well as in fat. Chromatographic analysis of the radioactive residues in the feces, and liver indicated a zone which accounted for a maximum of 0.5% of the administered radioactivity and which cochromagraphed with standard DDE. After a single purification of this zone, 85% of this zone's radioactivity did not cochromatograph with standard DDE. Based upon these results, DDE maximally accounted for less than 0.1% of the administered radioactivity. The presence of the DDE in the feces and liver tissue samples could have been due to the DDT (0.2%) and DDE (0.01%) in the test article. These results support the belief that the hydroxy group of the trichlo-roethanol moiety of dicofol is not cleaved and, therefore, the metabolism of dicofol is different from that of DDT which metabolized to DDE. Information made available after the PRC meeting indicates that in mice, dicofol is also metabolized to dechlorodicofol (DCD), DCBP and DCBH, but not to DDE[3].

There are two other metabolism studies which compared the distribution and elimination profiles of ^{14}C-dicofol and ^{14}C-DDT in rats. One study used a single oral administration; the other study used repeated oral dosing (16 doses). The results indicated that, with single dosing, ^{14}C-dicofol was eliminated faster than ^{14}C-DDT. Essentially all administered ^{14}C-dicofol (approximately 99%) was eliminated by 192 hrs post-dosing; in contrast 15 to 25% of the administered ^{14}C-DDT still remained in the rats by 192 hrs after dosing. The major route of elimination was via feces for both dicofol and DDT. Urinary elimination of dicofol was markedly greater than that of DDT.

Both dicofol and DDT were distributed to all tissues, and at 24 to 48 hr post-dosing, the maximum tissue levels were reached. Fat contained the highest levels of the radiolabeled residue from either dicofol or DDT treatment. The tissue radioactivity levels in dicofol treated rats dropped faster than that in DDT treated rats as indicated by the values of the $t_{1/2}$ for tissue elimination for dicofol (male, 31.5 hr; female, 30.0 hr) and DDT treated rats (male, 94.9 hr; female, 54.9 hr). (Accession No. 256328).

With repeated dosing (16 daily doses), the radioactivity level in whole blood of ^{14}C-dicofol treated female rats was substantially higher than that of ^{14}C-DDT treated females, whereas radioactivity levels in fat, adrenals, gonads, and liver were lower in ^{14}C-dicofol treated females relative to those of ^{14}C-DDT treated females. The radioactivity level in fat, adrenals, liver, and whole blood persisted longer in DDT treated female than in dicofol treated ones. The major route of elimination was via feces, and the radioactivity in ^{14}C-dicofol treated females was consistently eliminated twice as fast as that in ^{14}C-DDT treated ones. The levels of radioactivity recovered in this study were poor; however, many of the results were consistent with those seen in the single dose studies. (Accession No. 256328).

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[3]Brown MA, Casida JE. (1987) Metabolism of a dicofol impurity alpha-chloro-DDT, but not dicofol or dechlorodicofol, to DDE in mice and a liver microsomal system. Xenobiotica. 17(10):1169-1174.

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2. Mutagenicity

Study type	Results	Comments
In vitro forward mutation(CHO/HGPRT) MRID 40042049	-(+/-S9)	Acceptable
In vitro cyto-genetics(CHO) MRID 40042051	-(+/-S9)	Acceptable
Salmonella assay MRID 40042048	-(+/-S9)	Provisionally unacceptable pending submission of suitable positive control data
In vivo cyto-genetics MRID 40042050	-	Unacceptable: no toxicity; no data for females
Unscheduled DNA synthesis MRID 40042052	-	Unacceptable: unable to verify cytotoxicity data

The two acceptable studies satisfy two of the three categories of mutagenicity testing: gene mutation and chromosomal aberrations. The other genotoxic effect category remains as a data gap and a study needs to be performed to satisfy this category. The NTP studies have negative results for the Salmonella assay and for gene mutations and aberrations in CHO cells; this is consistent with submitted results.

3. Developmental Toxicity

a) Two generation reproduction study in rats

Crl:CD^R BR rats were exposed to Dicofol over two consecutive generations at dietary levels of 5, 25, 125, and 250 ppm. The Systemic NOEL = 5 ppm and the Systemic LOEL = 25 ppm, based upon histopathological changes in the liver and ovaries of parental animals. There were no effects on reproductive performance and/or offspring growth and development. The reproductive NOEL = 5 ppm based upon vacuolation in the ovaries of P2 females, an observation which is compatible with enhanced steroidogenic activity; the reproductive toxicity LOEL = 25 ppm. The incidence of vacuolation in the ovaries was seen also in the high dose P1 females.

b) Developmental toxicity study in rats

Dicofol was administered by oral gavage to Crl:COBS^{R}CD^R(SD)BR female rats at doses of 0.25, 2.5 or 25 mg/kg/day on gestation Days 6-15. Dose- and treatment-related incidences of salivation occurred in the mid- and high-dose groups during the time of dosing. At the high dose, decrements in maternal

body weight and food consumption were noted for the period of dosing. At necropsy, high-dose maternal absolute and relative (to body weight) liver weights were increased, and histopathological evaluation revealed a treatment-related increase in the incidence of centrilobular hepatocyte hypertrophy. The maternal NOEL = 0.25 mg/kg/day, and the maternal LOEL = 2.5 mg/kg/day. There was no evidence of developmental toxicity resulting from administration of the test material; therefore, the developmental toxicity NOEL and LOEL > 25 mg/kg/day.

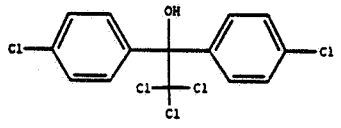
c) Developmental toxicity in rabbits

Dicofol was administered to NZW rabbits by oral gavage from day 7-19 of gestation at doses of 0.4, 4.0, or 40.0 mg/kg/day. Signs of maternal toxicity in the high-dose group consisted of abnormal feces, decreased food consumption and body weight gain during dosing, a significant increase in the liver-to-terminal-body weight ratios at necropsy, and an increase in the incidence of cytoplasmic hyalinization and diffuse vacuolation of hepatocytes at histopathological evaluation. The maternal NOEL = 4.0 mg/kg/day, and the maternal LOEL = 40.0 mg/kg/day. Although there was no evidence of fetal teratogenicity, there was an increased incidence of dams aborting in the high-dose group, thus the developmental LOEL = 40.0 mg/kg/day, with a developmental NOEL = 4.0 mg/kg/day.

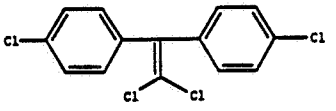
4. Structure-Activity Correlations

Structurally, dicofol is closely related to DDT which differs from dicofol by only a hydroxy group the non-ring carbon atom. Based upon the available metabolism data, this difference substantially influences the major metabolites formed and the rate of elimination.

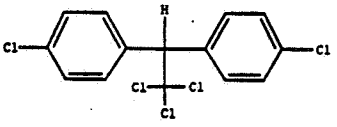
Dicofol



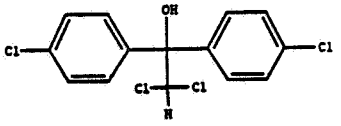
DDE



DDT



DCD



F. Weight of Evidence Considerations:

The Committee considered the following facts regarding the toxicology data on dicofol in a weight-of-the-evidence determination of carcinogenic potential:

1) The NCI mouse bioassay showed that dietary administration of dicofol to B6C3F1 mice produced an increase in the incidence of liver tumors in male mice only. The TWA doses to the males were 0, 264 and 528 ppm in the diet. The increase in adenomas and combined adenomas/carcinomas was significant by pairwise comparison ($p<0.01$) at both doses, and there was a statistically significant positive trend ($p<0.01$) for adenomas and combined tumors.

2) Male B6C3F1 mice historically have had a high spontaneous incidence of hepatocellular adenomas.

3) Two bioassays with different strains of rats showed no evidence of a carcinogenic response.

4) There is no evidence from available mutagenicity studies that dicofol has genotoxic activity.

5) Dicofol is not metabolized to DDE, which is the putative carcinogenic metabolite of DDT. Dicofol is stored in fat depots in both male and female rats as the parent molecule. It is eliminated from the body faster than DDT.

6) Dicofol has an apparent hormonal action supported by an increase in vacuolation of the ovaries of P1 and P2 females as observed in a two-generation reproduction study. It is known that DDT has estrogenic activity. No specific developmental toxicity was observed in rats and rabbits.

G. Classification of Carcinogenic Potential:

The Peer Review Committee considered the criteria contained in the EPA's "Guidelines for Carcinogen Risk Assessment" [FR51: 33992-34003, 1986] for classifying the weight of evidence for carcinogenicity.

The Peer Review Committee agreed that the classification for dicofol should be Group C - possible human carcinogen and recommended that, for the purpose of risk characterization, the Reference Dose approach should be used for quantification of human risk (RfD).

This decision was based on the findings of a statistically significant increase in liver tumors (adenomas and combined adenomas/carcinomas) in one sex (male) of one species (mouse). Historically, liver adenomas have been shown to have a high spontaneous background rate in male B6C3F1 mice. In the present study, the adenomas were not shown to progress to carcinomas. The study was conducted using adequate doses for the determination of carcinogenic activity.

Although dicofol is structurally related to DDT, it is metabolized in a different manner. It does not form DDE, which is a putative carcinogenic metabolite of DDT. Genotoxicity testing does not indicate that dicofol has mutagenic activity.