9/25/87

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

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SEP 25 1987

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM:

Subject: Peer Review of Dichlorvos

From: Judith W. Hauswirth, Ph.D. JWH 7/24/87
Section Head, Section VI
Toxicology Branch/HED (TS-769C)

To: George La Rocca
Product Manager No. 15
Registration Division (TS-767C)

The Toxicology Branch Peer Review Committee met on July 1, 1987 to discuss and evaluate the weight of the evidence on dichlorvos (DDVP), with particular reference to its oncogenic potential.

A. Individuals in Attendance:

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

Theodore M. Farber — Theodore M. Farber

William Burnam — William Burnam

Reto Engler — Reto Engler

Richard Hill — Richard Hill

Robert Beliles — Robert Beliles

John A. Quest — John A. Quest

Esther Rinde — Esther Rinde

Judith W. Hauswirth — Judith W. Hauswirth

2. Scientific Reviewers: (Non-committee members responsible for presentation of data; signatures indicate technical accuracy of panel report.)

Joycelyn Stewart — Joycelyn Stewart

Albin Kocialski — Albin Kocialski

1

Bernice Fisher

C. J. Nelson

Irving Mauer

3. Peer Review Committee Members in Absentia: (Committee members who were not able to attend the discussion; signatures indicate concurrence with the overall conclusions of the Committee.)

Anne Barton

Diane Beal

Richard Levy

4. Other Attendees:

Carol Monroe

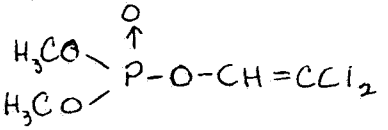
B. Material Reviewed:

The material available for review consisted of a package prepared by Dr. Stewart containing data evaluations of mouse and rat oncogenicity studies on DDVP, historical control data on relevant tumors in Fischer 344 rats and B6C3F1 mice conducted by NTP, and Toxicology Branch "One-liners".

C. Background Information:

Dichlorvos is an organophosphorous pesticide used to control a number of different species of insects affecting humans, animals, and plants. It is registered for use on greenhouse grown food crops including cucumbers, lettuce, mushrooms, radishes and tomatoes. It is also registered for use on non-perishable bulk raw agricultural commodities and for indirect use on dairy and beef cattle, poultry, goats, horses, sheep and swine. There are two technical formulations of DDVP: 93% technical and 100% technical.

The EPA conducted a Special Review of dichlorvos in 1980. Based on the data which were reviewed at that time, the Agency did not issue an RPAR for dichlorvos, but requested additional mutagenicity data from the registrants and decided to use information which was being generated from oncogenicity bioassays contracted by NTP to determine the oncogenic potential of dichlorvos.

Structure: 

Chemical Name: 2,2-dichlorovinyl dimethyl phosphate

D. Evaluation of Oncogenicity Studies:

1. Mouse Oncogenicity Study:

Reference: PWG Dichlorvos Two Year B6C3F1 Mouse Corn Oil Gavage Study. (Southern Research Institute; Study Numbers: 05049 Test 02. NTP C# 00113B, May 23, 1983; sponsored by National Toxicology Program) PWG May 14, 1986. The Final NTP report is still unpublished.

Dichlorvos (97.8-98.2% a.i.) was administered by gavage to B6C3F1 (60/sex/group) mice for 5 days/week for 103 weeks followed by a one week observation period. A 90-day range-finding study was conducted to determine appropriate dosages for this study. Dosages used for range-finding were 0, 5, 10, 20, 40, 80 and 160 mg/kg/day. All males and 9/10 females of the 160 mg/kg/day group died prior to study termination. Doses for the main study were set at 10 and 20 mg/kg/day for male mice and 20 and 40 mg/kg/day for females based upon expected cumulative cholinesterse inhibition. Corn oil was used as the vehicle.

The incidence of relevant tumors seen in the study are found in the following table.

Incidence of Pertinent Tumors in Mice (B6C3F1) Including Statistical Analysis[1]

	Males		
Dose	Vehicle Control	10.0 mg/kg	20.0 mg/kg
Stomach: Forestomach, papilloma squamous			
Tumor Rate: overall[2]	1/50(2%)	1/50(2%)	5/50(10%)
Statistical Tests: Cochran Armitage	p=0.049*		
Fisher Exact		p=0.753	p=0.102

	Females		
Dose	Vehicle Control	20.0 mg/kg	40.0 mg/kg
Stomach: Forestomach, papilloma squamous			
Tumor Rate: overall	5/49(10%)	6/49(12%)	18/50(36%)
Statistical Tests: Cochran Armitage	p<0.001**		
Fisher Exact		p=0.500	p=0.002**

Females (cont'd)

Forestomach: squamous cell carcinoma or papilloma squamous			
Tumor Rate: overall	5/49(10%)	6/49(12%)	19/50(38%)
Statistical Tests: Cochran-Armitage	p<0.001**		
Fisher Exact		p=0.500	p=0.001**

[1]Pertinent data taken directly from tables generated by the NTP.
[2]number of tumor-bearing animals/number of animals examined at site
*indicates significance at p<0.05 **indicates significance at p<0.01

A statistically significant increase in squamous cell forestomach papillomas and in combined squamous cell forestomach papillomas and carcinomas at the high dose was seen by pairwise comparison and a significant trend in female mice. In male mice, there was an increase in squamous cell forestomach papillomas which was not significant by pairwise comparison but was associated with a significant dose-related positive trend.

Administration of dichlorvos did not adversely affect survival and did not result in any changes in body weight in the test animals. Plasma cholinesterase values were significantly depressed in treated mice at all time intervals tested (except the last time interval after dosing had been terminated), indicating that the study had been performed at the maximum tolerated dose (MTD). RBC cholinesterase values were too variable to interpret any compound related effects.

Historical control data on the incidence of forestomach tumors in male and female B6C3F1 mice were made available to the Committee. The data were derived from the following reference: Haseman, et al. Toxicol. Path. 12: 126-135, 1984. These data can be summarized as follows.

Incidence of Forestomach Tumors in Untreated Control B6C3F1 Mice

Neoplasm	Male	%	Female	%
Squamous cell papilloma	6/2252	0.3	12/2336	0.5
Squamous cell carcinoma	0/2252	0	2/2336	0.1

2. Rat Oncogenicity Study:

Reference: Two Year Gavage Study of Dichlorvos in F344 Rats (Southern Research Institute; Study No. 05049; May 23, 1983 sponsored by the National Toxicology Program) PWG Report May 30, 1986.

Dichlorvos (97.8-98.2% a.i.) was administered by gavage to Fischer 344 rats (60/sex/group) once daily five days per week for 103 weeks. Dosages used were 0, 4 and 8 mg/kg/day. Corn oil was used as the vehicle. Dosages were selected for this study based upon findings from a 13 week study using dosages of 0, 2, 4, 8, 16, 32 and 64 mg/kg/day and from the published literature. All animals receiving either 32 or 64 mg/kg/day dichlorvos died prior to termination of the study. One out of 10 males and four out of ten females died at 16 mg/kg/day.

The incidence of relevant tumors seen in this study are found summarized in the following table.

Incidence of Pertinent Tumors in Rats (Fischer 344) Including Statistical Analysis[1]

Dose	Males Vehicle Control	4.0 mg/kg	8.0 mg/kg
Pancreas: Acinar adenoma			
Tumor Rates: overall[2]	16/50(32%)	25/49(51%)	30/50(60%)
Statistical Analysis: Cochran-Armitage	p<0.003**		
Fisher Exact		p=0.043*	p=0.004**
Lung: alveolar/bronchiolar adenoma			
Tumor Rates: overall	0/50(0%)	0/50(0%)	3/49(6%)
Statistical Analysis: Cochran-Armitage	p=0.041*		
Fisher Exact			p=0.117
All Organs: Leukemia: lymphocytic, monocytic, mononuclear, or undifferentiated			
Tumor Rates: overall	11/50(22%)	20/50(40%)	21/50(42%)
Statistical Analysis: Cochran-Armitage	p=0.022*		
Fisher Exact		p=0.041*	p=0.026*

Females			
Mammary Gland: Fibroadenoma			
Tumor Rates:			
overall	9/50(18%)	19/50(38%)	16/50(32%)
Statistical Analysis:			
Life Table	p=0.030*	p=0.007**	p=0.047*
Cochran-Armitage	p=0.070		
Fisher Exact		p=0.022*	p=0.083
Mammary Gland: fibroma, fibroadenoma, carcinoma, adenocarcinoma or adenoma			
Tumor Rates:			
overall	11/50(22%)	20/50(40%)	17/50(34%)
Statistical Analysis:			
Life Table	p=0.049*	p=0.015*	p=0.074
Cochran-Armitage	p=0.111		
Fisher Exact		p=0.041*	p=0.133

[1] Pertinent data taken directly from tables generated by the NTP.
[2] number of tumor-bearing animals/number of animals examined at site
* indicates significance at $p<0.05$ ** indicates significance at $p<0.01$

Administration of dichlorvos was associated with a statistically significant increase in the following tumor types:

a. an increase in pancreatic acinar adenomas in male rats which was statistically significant by pairwise comparison at both dosage levels tested and showed a significant positive dose-related trend;

b. an increase in alveolar/bronchiolar adenomas in male rats which showed a positive dose response trend but was not significant by pairwise comparison;

c. an increase in leukemia (lymphocytic, monocytic, mononuclear or undifferentialted) at all sites in male rats which was significant by pairwise comparison at both dosage levels and showed a significant positive dose-related trend;

d. an increase in fibroadenomas of the mammary gland in female rats which was significant by pairwise comparison at the low dose; and

e. an increase in combined mammary gland tumors (fibroma, fibroadenoma, carcinoma, adenocarcinoma or adenoma) in female rats which was significant at the low dose.

Historical control data were provided to the Committee on each of the above mentioned tumor types. The data were drawn from two publications both containing control data from NTP studies (Haseman et al. JNCI 75:975-984, 1985 and Eustis and Boorman JNCI 75:1067-1071, 1985) and are summarized below.

Historical Control Data on Pertinent Tumor Incidences in Untreated and Corn Oil Gavaged Fisher 344 Rats[1]

Neoplasm	Untreated	%	Range %	Corn Oil	%	Range%
Males						
lung adenoma	24/1723	1.4	0-6	28/1098	2.6	0-8
pancreatic acinar adenoma	3/1667	0.2	0-2	46/1086	4.2	0-28
multiple organ leukemia	458/1727	26.5	10-46	152/1100	13.8	2-28
Females						
Mammary gland adenoma	492/1772	27.8	10-49	280/1100	25.5	14-38

[1] Haseman et al. JNCI 75:975-984, 1985.

Incidence of Pancreatic Acinar Adenoma By Performing Laboratory[2]

Males - Untreated

Laboratory	A	B	C	D	E	F	G	H
Total	2/199	1/193	0/149	0/117	1/146	0/47	1/148	4/91
%	1	0.5	0	0	0.68	0	0.67	4.39
Range %	0-2	0-2	0	0	0-2	0	0-2	0-8

Males - Corn Oil Gavaged

Laboratory	A	B	C	D	E
Total	5/149	19/346	4/196	4/97	14/298
%	3.4	5.5	2.0	4.1	4.7
Range %	0-10	0-28	2	0-8	0-22

[2]Eustis and Boorman JNCI 75:1067-1071, 1985.

The following tumor types were outside the historical control range in the dichlorvos study: pancreatic acinar adenomas in males of both dose groups and multiple organ leukemia in males of both dose groups. It was noted by the Committee that corn oil gavage, in and of itself, is associated with an increase in pancreatic acinar adenomas as demonstrated by the historical control data of both untreated and corn oil-gavaged Fisher 344 male rats.

Administration of dichlorvos did not adversely affect survival or body weight gain in this study. Plasma cholinesterase values were significantly depressed in treated rats at all but the last testing interval. RBC cholinesterase values were also depressed but not as consistently nor to the same degree as plasma. The non-neoplastic lesions that were associated with treatment were pancreatic acinar hypertrophy, hepatocytic cytoplasmic vacuolation and adrenal cortical vacuolation. The Committee felt that the MTD had been reached in this study based upon plasma and RBC cholinesterase inhibition at both dosage levels.

3. Other Oncogenicity Studies:

Four other oncogenicity studies had been previously conducted on dichlorvos. An inhalation study was flawed due to poor survival in the control group, the large number of animals lost to autolysis and an inadequate number of tissues examined microscopically.

A feeding study conducted in CD rats was also considered to be inadequate due to poor survival (intercurrent infection in test animals).

In another rat (Osborne-Mendel) feeding study and a mouse (B6C3F1) feeding study conducted by NCI, the results were considered to be equivocal. In rats the only significant finding ($p=0.018$) was a departure from the Cochran-Armitage test for linear trend in high dose males with malignant fibrous histocytomas compared with pooled controls but not matched controls. In mice, gastric squamous epithelial hyperplasia was reported in three low dose males and one high dose female, esophageal papilloma in a high dose female, and squamous cell carcinoma in one low dose male and one high dose female. The Committee felt, however, that the esophageal lesions are relevant to the forestomach papillomas seen in the corn oil-gavage mouse study discussed above (Section D.1.).

E. Additional Toxicology Information:

1. Mutagenicity:

Dichlorvos was positive:

- for base pair reversions in Salmonella typhimurium strain TA 1535 and E. coli without metabolic activation;
- in the spot tests for differential toxicity at the polymerase A_2 locus of E. coli;
- for reversion at the histidine and leucine locus for two strains of Serratia marcescens;
- for reversion at the tryptophan locus of E. coli WP2 and CM881;
- for induction of second chromosome recessive lethals in Drosophila; and
- for mutations in mouse lymphoma cells both with and without metabolic activation.

In addition, dichlorvos methylates DNA at the N-7 position of guanine (Setervaick and Ehrenberg, Acta Pharmacol. Toxicol. 49:56-66, 1981).

Dichlorvos was negative in the mouse micronucleus test, in the sister chromatid exchange assay (mouse) and in the dominant lethal assay (mice) up to 10 mg/kg/day.

2. Reproduction and Teratology:

Dichlorvos was not teratogenic in mice or in rabbits when administered orally at 60 mg/kg to mice or at 5 mg/kg to rabbits. No teratogenic responses were observed when dichlorvos was administered by inhalation to mice or rabbits at 4 ug/L. When administered by inhalation at concentrations of up to 6.25 ug/L to rats, and of up to 4 ug/L to rabbits, no teratogenic responses were obtained.

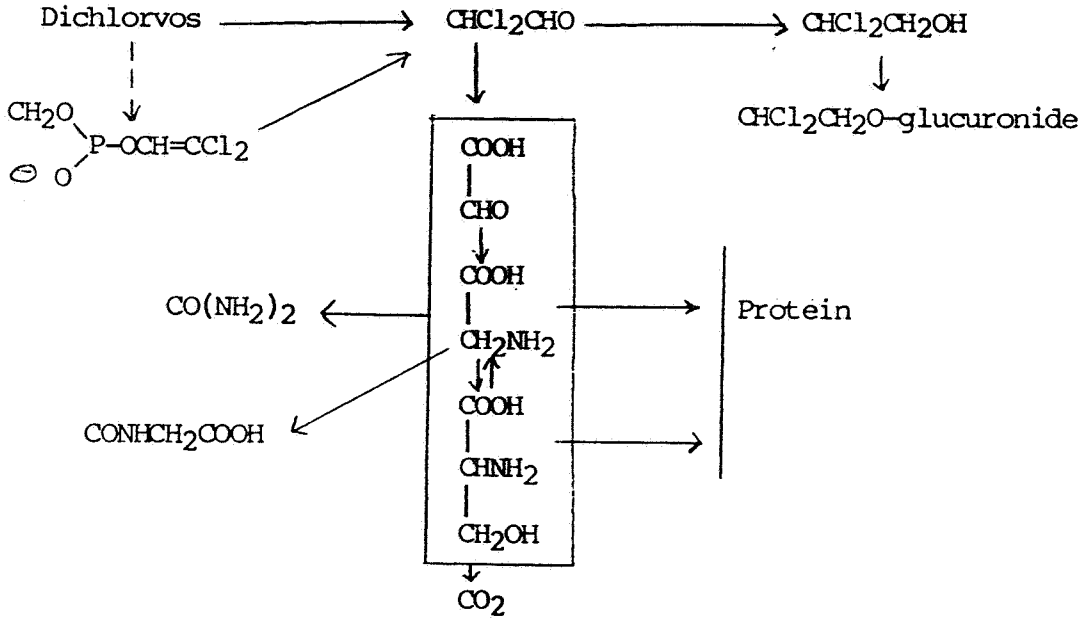
Dichlorvos did not demonstrate any adverse reproductive consequences when administered to rats at doses of up to 500 ppm for three generations.

4. Metabolism:

Metabolism studies indicate that dichlorvos is rapidly metabolized when administered orally to male and female rats. Within 4 days of oral administration to male and female rats, 16 to 23% of ^{14}C-dichlorvos was excreted in the urine and feces, while 36.8 to 38.8% was recovered as exhaled CO_2. Of the amount recovered in the excreta, 12.5 and 3.4% was found in the urine and feces of male rats, while 18.2 and 4.8% was found in the urine and feces of female rats, respectively.

Dichlorvos is metabolized either by hydrolysis to dichloroacetaldehyde (major pathway) or by O-demethylation to desmethyl dichlorvos. The proposed metabolic pathway is shown in Figure 1.

Figure 1.



Dichloroacetaldehyde has been reported to be positive in the Ames Salmonella assay with a potency greater than dichlorvos (Lofroth, A. Naturforsch, C:Biosci.:33C:783-5, 1978), positive in the mouse dominant lethal assay (Fischer, et al. Chem.-Biol. Interact. 19:205214, 1977) and negative for unscheduled DNA synthesis in human epithelial-like cell cultures (Aquilina, et al. Pestic. Sci. 15:439-442, 1984).

4. Structure Activity Relationship:

Dichlorvos is structurally related to naled and trichlorfon. Both of these chemicals can degrade to dichlorvos. Naled was negative when evaluated for oncogenic potential in Sprague-Dawley CD rats or in CD-1 mice. Two chronic studies indicate that trichlorfon is not oncogenic in rats, while another showed evidence of an increased incidence of mammary gland tumors in females, and a further study demonstrated malignant and benign tumors (not otherwise identified).

Dichlorvos is also structurally similar to other pesticidal compounds such as tetrachlorvinphos, dichloropropene, triallate, akton and phosphamidon in that they all contain a vinyl or vinylidene chloride moiety. Tetrachlorvinphos and triallate were reported to be oncogenic in the mouse, causing an increased incidence of liver tumors. No data were available on either of these chemicals in the rat. Dichlorpropene induces forestomach squamous cell tumors in both rats and mice, lung tumors in mice and hepatic neoplastic nodules in rats. Oncogenicity data is not available on the other pesticides listed above. Vinyl chloride is a human oncogen, causing angiosarcomas of the liver in workers exposed to this chemical.

F. Weight of Evidence Consideration:

The Committee considered the following facts regarding the toxicology data on dichlorvos to be of importance in a weight of the evidence determination of oncogenic potential.

1. Administration of dichlorvos to female B6C3F1 mice was associated with a statistically significant increase in squamous cell forestomach papillomas and in combined squamous cell forestomach papillomas and carcinomas. In male mice, there was an increase in squamous cell forestomach papillomas which was not significant by pairwise comparison but was associated with a significant dose-related positive trend.

2. Historical control data indicated that squamous cell forestomach tumors are relatively rare ($\leq$ 0.5%).

3. The MTD was reached in the mouse study based upon a significant depression in plasma cholinesterase values throughout the study in treated mice. RBC cholinesterase values were also depressed at some time points, but individual values were too variable to interpret any compound-related effects.

4. An increased incidence of pancreatic acinar adenomas and leukemia (all types and sites) was seen in male, Fishcer 344 rats. The increase was statistically significant by pairwise comparison at both dosage levels and was associated with a significant positive dose-related trend.

5. Dichlorvos administration was also associated with an increase in lung alveolar/bronchioalveolar adenomas in male rats (significant positive dose-related trend) and in mammary gland fibroadenomas and all mammary gland tumors in females (statistically significant at the low dose by pairwise comparison).

6. Historical control data indicated that the incidence of pancreatic acinar adenomas and leukemia was outside of the historical control range; however, the incidence of lung and mammary gland tumors was within the historical control range.

7. Dichlorvos was positive for mutagenicity in Salmonella typhimurium, E. coli, Serratia marcescens, Drosophila, and mouse lymphoma cells and negative in the mouse micronucleus test, sister chromatid exchange assay and in the mouse dominant lethal assay (up to 10 mg/kg). Dichlorvos has also been reported to alkylate DNA.

8. Metabolism studies indicate that dichlorvos is rapidly metabolized in rats. Within four days up to 23% of the radioactivity from ^{14}C-dichlorvos is eliminated in urine and feces and up to 39% in expired CO_2. The major pathway of metabolism is hydrolysis to dichloracetaldehyde.

9. Dichloroacetaldehyde has been reported in the open literature to be mutagenic giving positive results in the Ames Salmonella assay and the mouse dominant lethal assay.

10. Dichlorvos is not teratogenic toward rats, mice or rabbits and exhibited no reproductive toxicity in a 3-generation reproduction study.

11. Dichlorvos is structurally similar to dichlorpropene which causes forestomach squamous cell tumors in both rats and mice, lung tumors in mice and hepatic neoplastic nodules in rats.

G. Classification of Oncogenic Potential:

The Committee agreed, based upon the available information, that dichlorvos met the criteria of a B2 classification (probable human oncogen) since it induced an oncogenic response in two rodent species, satisfying (a) of this classification (in multiple species or strains), and induced a rare tumor type in mice, satisfying (c) of this classification (to an unusual degree in a single experiment with regard to high incidence, unusual site or type of tumor, or early age at onset).

In B6C3F1 mice, dichlorvos induced a statistically significant increase in forestomach squamous cell papillomas and combined forestomach squamous cell carcinomas and papillomas in females. This tumor type was also increased in male mice but was significant only for a positive dose-related trend.

In Fischer 344 male rats, administration of dichlorvos was associated with a statistically significant increase in leukemia (all sites and types) and in pancreatic acinar adenomas at both dosage levels tested. Supporting evidence included an increase in lung tumors in male rats at the high dose (significant positive dose-related trend) and an increase in mammary gland fibroadenomas and all mammary gland tumors at the low dose only (significant by pair-wise comparison) in female rats.

Further supporting evidence for the B2 classification is provided by positive mutagenicity data. Dichlorvos has been shown to be a mutagen in the Ames Salmonella assay and mouse lymphoma assay and has been reported to alkylate DNA. In addition, a major metabolite of dichlorvos, dichloroacetaldehyde

has been reported to be a more potent mutagen in the Ames Salmonella assay than dichlorvos, itself.

Positive SAR information is also available. Of particular relevance, is that dichloropropene, structurally similar to dichlorvos, induces forestomach squamous cell tumors in rats and mice.

The Committee also considered whether the stomach tumors in mice could be due to an irritant effect of dichlorvos on this organ. Since dichlorvos did not demonstrate either excessive dermal or eye irritation (Tox. Category IV and III, respectively), they concluded that the tumors were probably not due to irritancy of the material.

It was concluded that a quantitation of risk should be performed only on those tumors which showed positive dose-related trends and statistically significant increases by pairwise comparison and exceeded the historical control range.