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MEMORANDUM

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

Subject: Fifth Carcinogenicity Peer Review of Dichlorvos
Tox Chem # 328 PC Code 084001

To: Dennis Utterback PM# 61
Special Review Branch
Special Review and Reregistration Division (7508W)

From: Joycelyn E. Stewart, Ph.D., Head, *JS*
Section 2, Toxicology Branch I *8/5/96*
Health Effects Division (7509C)

and

William Burnam, Chief, *WB*
Scientific Analysis Branch,
Health Effects Division (7509C)

Thru: Stephanie Irene, Ph.D., *Stephanie R. Irene*
Acting Division Director, *8/28/96*
Health Effects Division (7509C)

On March 27, 1996 the HED Carcinogenicity Peer Review Committee (CPRC) convened to consider new information provided by the registrant to support their request for reclassification of DDVP with respect to its carcinogenic potential.

The CPRC concluded that the new information on mononuclear cell leukemia (MLC) staging did not refute but reinforced the original conclusion that the mononuclear cell leukemia observed in the male rats was related to administration of DDVP. The Committee also concluded that the new data on the effects of DDVP on the mouse forestomach was not convincing in terms of showing a mechanism of action. The mouse forestomach tumors were still considered treatment related but their relevance to human health risks was questioned by some members of the Committee and thus they were not included in the quantitation. Consequently, the Committee concluded that DDVP should remain classified as a C carcinogen with a linear low dose risk extrapolation based on the mononuclear cell leukemia and not on the geometric mean of the mononuclear cell leukemia and the mouse forestomach tumors as was previously done. The quantification was retained because mononuclear cell leukemias are malignant tumors. In addition, HED currently calculates risk



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estimates using the most significant tumors observed in the carcinogenicity bioassay. The Committee also recommended a risk extrapolation using the LED 10.

A. Individuals in Attendance at the meeting:

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

Stephanie Irene

Stephanie Irene

William Burnam

William Burnam

Karl Baetcke

Karl Baetcke

for Mike Ioannou

James N. Rowe

Marion Copley

Marion Copley

Hugh Pettigrew

Hugh Pettigrew

Richard Hill

Richard Hill

Kerry Dearfield

Kerry Dearfield

Yin Tak Woo

Yin Tak Woo

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

Joycelyn Stewart¹

Joycelyn Stewart

Bernice Fisher

Bernice Fisher

Lucas Brenneke²
(PAI/ORNL)

Lucas Brenneke

3. Other attendees: William Hazel, Yung Yang

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

2. Signature indicates concurrence with pathology report

B. New information submitted by the registrant

1. Leukemia staging in Fisher 344 rats

Slides of the spleens and livers of all male rats, and lungs of some male rats diagnosed with mononuclear cell leukemia were examined by an Expert Panel of board certified pathologists convened by the registrant to determine the progression of the disease. When the data were analyzed by RIDIT and Chi Square analysis, there was no statistical significant increase in the severity when the treated animals were compared with the controls. There is no historical control data for comparison of severity of mononuclear cell leukemia in male Fisher rats.

2. Comparison of unscheduled DNA synthesis, replicative DNA synthesis, and histopathological changes in the mouse forestomach in response to DDVP, MNNG, and BHA.

The registrant submitted several studies in which the effects of dichlorvos on the mouse forestomach tissue were compared with those of two other forestomach carcinogens: 1-methyl-3-nitro-1-nitrosoguanidine (MNNG) which is an alkylating agent inducing carcinomas in both forestomach and glandular stomach of the mouse (IARC, 1987), and butylated hydroxyanisole (BHA), which is considered to be a nongenotoxic carcinogen agent acting via prolonged stimulation of cellular proliferation. The hypothesis is that genotoxic agents would induce unscheduled DNA synthesis (UDS), while non genotoxic agents would induce replicative DNA synthesis (RDS), and/or histopathological changes, including hyperplasia.

In most of the studies, the data showed wide inter and intra-animal variations, therefore no firm conclusions could be drawn from them. The data displayed large standard deviations and the method development study showed inconsistent results and was not validated using repeated trials.

3. An in vivo cytogenetics assay in bone marrow and spermatogonia cells after administration of DDVP at doses of 0, 12.5, 25, or 50 mg/kg/day for 5 days showed no increases in frequency of aberrations in either tissue.

The Cancer Peer Review Committee concluded that the new data did not lessen the concern for the carcinogenic potential of DDVP and reaffirmed the previous conclusion that DDVP was a class C carcinogen.

C. Data Considered

The material available for review consisted of:

DER's, information from the literature and other data summaries prepared and/or summarized by Dr. Joycelyn Stewart, and tables and statistical analyses prepared by Bernice Fisher. The material reviewed is attached to the file copy of this report.

D. Evaluation of Data on Mononuclear Cell Leukemia Severity

Reference: Talmage T. Brown. Staging of Mononuclear Cell Leukemia in male rats from Toxicology and Carcinogenesis Study of Dichlorvos in F344/N Rats. Pathology Working Group Review. Jan 31, 1995. (MRID

A Panel of Experts re-evaluated slides of the spleens and livers from all male rats, and lungs from some male rats diagnosed with mononuclear cell leukemia. All members of the panel were board certified veterinary pathologists. Since mononuclear cell leukemia is known to be a rapidly fatal disease, the emphasis of this re-evaluation was to determine the progression of the disease in the dichlorvos treated rats. The criteria used for the re-evaluation were developed by the NTP and were as follows:

Stage 1-the spleen was not enlarged or only slightly enlarged with small numbers of mononuclear cells in the red pulp; no or very few mononuclear cells in the liver sinusoids and no identifiable neoplastic cells in other organs. Rarely a few mononuclear cells were identified in other tissues but still only a very minimal splenic involvement was present.

Stage 2-the spleen was moderately enlarged with moderate to large numbers of mononuclear cells in the red pulp: architectural features including lymphoid follicles and periarteriolar lymphocytic sheaths remain intact. There was minimal to moderate involvement of the liver. Mononuclear cells may be evident in blood vessels in other organs but aggregates/masses of neoplastic cells generally are limited to spleen and liver.

Stage 3- Advanced disease with multiple organ involvement. the spleen is usually markedly enlarged with effacement of normal of normal architectural features by accumulated cells. The liver is moderately to markedly enlarged and nodular: the hepatic parenchyma shows variable degenerative changes associated with the accumulation of neoplastic cells. There is accumulation of neoplastic mononuclear cells in other organs including lungs, lymph nodes, kidney, brain, adrenal gland, and others.

These data were analyzed by Science Analysis Branch (SAB). The

statistical analysis is shown in Tables 1 and 2, extracted from SAB's memorandum: Fisher to Stewart dated 8/3/95.

Table 1. Distribution of Severity -Male Rat's MCL (DDVP)

Severity Codes	Doses, vehicle control	4 mg/kg	8mg/kg
	no.of animals		
stage 0	39	30	29
stage 1	4	5	2
stage 2	2	5	9
stage 3	5	10	10
total animals examined	50	50	50

Table 2. DDVP- Male Rat MCL Severity- RIDIT scores & Chi Square results

Mean RIDIT of MCL	vehicle control	4mg/kg	8mg/kg
	0.4329	0.5264	0.5408
Chi Square (2df)		3.89	5.08
associated p value		0.1430	0.0789
Chi Square (2,05)	5.991		

SAB concluded that administration of dichlorvos at the stated doses did not result in any statistically significant difference in the severity of mononuclear cell leukemia when the treated animals at both the low and high doses were compared with the controls.

Discussion of the Data

It is known that mononuclear cell leukemia is a common tumor in Fisher 344 rats, and does not occur in other strains. It is often referred to as "Fisher rat leukemia." (Goodman, D.G., Boorman, G.A., and Strandberg, Selection and Use of the B6C3F1 Mouse and F344 rat in long term assays. in Handbook of Carcinogenicity Testing. 282-323, 1987). The tumor incidence increases with age, being most commonly observed after 18 months of age (Davey, F. and Moloney, W. Post mortem observations on Fisher rats with leukemia and other disorders. Lab. Investigations 23: 327-334, 1970). It occurs spontaneously, with a high rate of variability, and is considered a lethal neoplasm. It generally causes death in from two to six weeks of onset (Stromberg, P. and Vogtsberger. Pathology of the mononuclear cell leukemia of Fisher rats.1. Morphologic studies. Vet. Path. 20: 698-708, 1983). It is a diffuse tumor which makes it difficult to establish quantitative criteria from histological sections, especially in the early stages.

The Committee considered the data in the light of the registrants' reference to the Office of Science and Technology policy reported in the Federal Register March 14 1985 and agreed

with the registrants claim that dichlorvos did not decrease the latency of tumor appearance, and that the presence of these tumors did not alter the animals lifespan. However, based on the doubling of number of animals with Stage 2 and Stage 3 mononuclear cell leukemia the Committee concluded that these tumors were related to administration of DDVP, notwithstanding the increased tumor incidence was not statistically significant.

E. Evaluation of DNA synthesis and histopathology in the mouse forestomach after chemical treatment.

Reference: D.J. Benford. Final Report No: R188/0408. Project Identification. Robens Institute of Health and Safety, 8/17/1988. MRID 42855602

a. Groups of twelve male and female non-fasted B6C3F1 mice were administered the following chemicals in a single oral gavage dose, and 3/sex/dose were sacrificed at 2, 4, 24, or 48 hours later: MNNG 100 and 200 mg/kg; BHA, 150, 300, 500, and 1000 mg/kg. Eight additional mice were fasted four hours, administered 200 mg/kg MNNG in 2% gum tragacanth or 2% gum tragacanth and fasted an additional four hours prior to sacrifice. Control animals were administered corn oil only. Stomachs were excised and cut into forestomach strips. One strip was used for histopathological examination, and the others were incubated with ³H-thymidine with and without hydroxyurea to determine unscheduled DNA or replicative DNA synthesis.

All B6C3F1 mice administered BHA at doses of 500 and 1000 mg/kg died prior to study termination. In the BHA mice treated with 150 and 300 mg/kg and sacrificed two hours later, grossly there was redness and thickening of the stomach mucosa, but no histopathological changes. However, the stomach mucosa was hyperplastic in animals sacrificed at 24 and 48 hours. Male and female mice treated with 200 mg/kg of MNNG demonstrated cellular damage including nuclear enlargement, hyperemia, cellular necrosis and pyknosis.

BHA did not seem to induce unscheduled DNA synthesis at either dose level when compared to the controls although there were wide interanimal variations. For all dosage groups, the proportion of cells in repair were lower in the presence of hydroxyurea. In fasted animals, the percent of cells in repair in both the control and MNNG treated groups were lower than in the nonfasted groups, also with marked variations between animals. There was no increase in cells in S-phase or mitosis in mice treated with MNNG or BHA and sacrificed after 24 or 48 hours i.e. did not induce replicative DNA synthesis.

Reference: Benford, D.J. Effects of BHA on Mouse Forestomach. Robens Institute of Health and Safety, 12/7/1990. MRID 42855603.

b. Groups of five male and female C57B16 mice administered BHA 300 mg/kg in corn oil in a single oral dose and sacrificed after eight or twelve hours. Control animals received corn oil only. Stomach were excised and cut into strips, one of which was used for histopathological examination, and the others used for assessment of DNA synthesis.

BHA treated rats appeared to have an increased number of forestomach cells in S-phase demonstrating increased replicative DNA synthesis when the animals were sacrificed at 12 but not 8 hours post compound administration. They also appeared to demonstrate edema of the submucosa and lamina propria and focal hyperplasia at 8 and at 12 hours. These data provide supplementary information but this study by itself does not provide information on the mechanism of DDVP carcinogenicity.

Reference: Benford, D.J. Investigation of the genotoxic and/or irritant effects of dichlorvos on mouse forestomach. Robens Institute of Health and Safety. 9/25/91. MRID 42880101

c. Groups of five male and female B6C3F1 mice were administered dichlorvos in corn oil at doses of 10, 20, 40, or BHA 100 mg/kg in corn oil or MNNG 200 mg/kg in 2% gum tragacanth in a single gavage dose and sacrificed at 2, 4, 12 and 48 hours post dosing. Control animals received corn oil only. Stomachs were opened, contents removed and the forestomach were dissected into four longitudinal strips. One strip was immediately fixed in neutral buffered formalin for histopathological examination and three strips were incubated with ³H-thymidine for assessment of unscheduled DNA synthesis or replicative DNA synthesis.

In the dichlorvos treated mice, moribundity and mortality occurred at the high dose only. No clinical signs were reported at other dose levels. Histologically, hyperplasia was observed at 12 and 24 hours in all dosage groups. Significant increases in thymidine incorporation into cells in S-phase were reported in mice treated with 10 mg/kg and 40 mg/kg of dichlorvos.

In the MNNG treated male and female mice there was minimal hyperplasia, significant increases unscheduled DNA synthesis, and minimal increases in replicative DNA synthesis in the forestomach.

BHA treatment resulted in the death of one male mouse. Hyperplasia accompanied by edema of the lamina propria was observed in the forestomach of these mice at 12 and 48 hours post treatment. BHA did not induce unscheduled DNA synthesis in the mouse forestomach.

Reference: Benford, D.J. Detection of Hyperplasia in forestomach of B6C3F1 mice following treatment with butylated hydroxyanisole. Robens Institute of Health and Safety, 10/1/91. MRID 42855604

d. Groups of five male and female B6C3F1 mice were administered a single oral dose of 300 mg/kg of butylated hydroxyanisole (BHA) or of corn oil. After periods of 6, 8, 10, and 12 hours the forestomachs were removed for assessment of replicative DNA synthesis by incorporation of ³H-thymidine into DNA, which was measured by autoradiography and by scintillation counting.

Under the study conditions, administration of 300 mg/kg of BHA to male and female B6C3F1 mice did not result in an increased incidence of hyperplasia in the forestomach, although cellular damage was inferred because hydropic degeneration was noted in forestomachs of one male mouse 10 hours after treatment, and in two mice 12 hours post treatment. Although there seemed to be an increase in the percent of cells in S-phase in the treated animals at 10 and 12 hours when compared to the controls in the autoradiography studies, this was not corroborated by the ³H-thymidine incorporation liquid scintillation studies. In addition, interanimal variation and the consequent large standard deviations render the results unreliable. Furthermore, the study was reported too sketchily to determine whether the protocol was adequate to measure the endpoints reported. These data do not add significantly to the understanding of the mechanism of the forestomach tumorigenicity of BHA.

Reference: Benford, D.J. Investigation of the irritant effects of dichlorvos on the mouse forestomach. Robens Institute, 11/16/92. MRID 42881101.

e. Groups of five male or five female B6C3F1 mice were administered a single oral dose of dichlorvos at doses of 10, 20, 40 or 100 mg/kg, and sacrificed at 8 or 10 hours post dosing. Stomachs were excised and the forestomachs cut into strips and examined for histopathology, or incubated with ³H-thymidine for unscheduled DNA synthesis or replicative DNA synthesis.

Microscopic examination of forestomachs of dichlorvos treated mice sacrificed 48 hours post dosing demonstrated focal hyperplasia accompanied by cellular hypertrophy at all dose levels. This lesion was also seen in BHA treated mice. MNNG treatment caused severe cellular damage including pyknosis, karyolysis and necrosis.

There were significant increases in thymidine incorporation at 10 but not at 8 hours; the increases occurred at 10 and 40 mg/kg in males, and at 20 and 100 mg/kg in females and was not dose dependent. BHA treated mice had significant increases in ³H-thymidine incorporation at 8 hours. MNNG was reported not to cause an increase in ³H-thymidine incorporation in the mouse forestomach.

Discussion of the Data

The effects of dichlorvos on the mouse forestomach tissue were compared with those of two other forestomach carcinogens: 1-methyl-3-nitro-1-nitrosoguanidine (MNNG) which is an alkylating agent inducing carcinomas in both forestomach and glandular stomach of the mouse (IARC, 1987), and butylated hydroxyanisole (BHA), which is considered to be a nongenotoxic carcinogen agent acting via prolonged stimulation of cellular proliferation. The hypothesis is that genotoxic agents would induce unscheduled DNA synthesis (UDS), while non genotoxic agents would induce replicative DNA synthesis (RDS), and/or histopathological changes, including hyperplasia.

In this group of studies, the chemicals were administered to male and female B6C3F1 mice in a single oral dose following which the animals were sacrificed at intervals of 2, 4, 24, or 48 hours. Stomachs were excised, cut into strips, and the forestomachs processed either for histopathology or for ³-H thymidine incorporation by autoradiography or scintillation counting. The criteria used for these determinations were: nuclei containing large numbers of overlapping grains were considered to be in S-phase (replicative DNA synthesis, while those containing fewer than 25 discrete grains were considered to be in repair. UDS was assessed by the mean number of grains per nucleus, and also by the percentage of cells in repair (3-25 grains). Slides were scored blind.

The preliminary studies to define the protocol for the definitive studies used only three animals/sex/dose and resulted in wide interanimal variations and large standard deviations in the data. Most of the definitive studies used only five animals per dose level, thereby decreasing the statistical power of the determinations. Based on review of the preliminary study, Toxicology Branch did not accept and the Committee concurred that data from the definitive studies were reliable. Data from the preliminary studies are attached.

The above data were reviewed with the expectation that information provided to the U.S. Food and Drug Administration by an Expert Panel on the Carcinogenicity of Butylated Hydroxyanisole (BHA) would provide guidance on the relevance of mouse forestomach tumors to human health risks. However, although the Panel considered that BHA was a carcinogen with a demonstrable association between dose level and cellular proliferation, followed by a carcinogenic response, they did not provide any guidance with respect to the relevance of rodent forestomach tumors to human health following gavage administration of a chemical (FASEB:1994. Evaluation of the Evidence for the Carcinogenicity of Butylated Hydroxyanisole). The Cancer Peer Review Committee was unable to reach a conclusion regarding the relevance of these tumors.

Table 1

Assessment of Unscheduled DNA Synthesis (Preliminary Study A)

Dose Group	% Cells in Repair (≥ 3 ng)			
	2 hours		4 hours	
	Males	Females	Males	Females
+ hydroxyurea				
Control	0.6 \pm 1.1	0.1 \pm 0.2	0.3 \pm 0.5	0.1 \pm 0.2
100 mg/kg MNG	0.6 \pm 0.2	0.1 \pm 0.1	0.7 \pm 0.9	0.3 \pm 0.3
200 mg/kg MNG	6.0 \pm 5.0	0.1 \pm 0.1	3.0 \pm 2.4	0.1 \pm 0.1
150 mg/kg BHA	0.1 \pm 0.2	0.0 \pm 0.0	1.2 \pm 1.0	0.1 \pm 0.1
300 mg/kg BHA	0.1 \pm 0.2	1.3 \pm 1.4	0.0 \pm 0.1	0.4 \pm 0.6
- hydroxyurea				
Control	0.6 \pm 1.0	0.7 \pm 0.6 ^a	0.6 \pm 0.5	1.0 \pm 1.4 ^a
100 mg/kg MNG	1.3 \pm 1.9	4.8 \pm 4.0	4.0 \pm 0.6*	1.8 \pm 1.8
200 mg/kg MNG	8.7 \pm 6.1*	2.8 \pm 2.6	6.9 \pm 3.3*	7.2 \pm 8.1
150 mg/kg BHA	0.6 \pm 0.4	0.7 \pm 1.0	1.5 \pm 1.0	1.1 \pm 1.1
300 mg/kg BHA	0.8 \pm 1.0	2.2 \pm 1.7 ^a	0.4 \pm 0.2	0.7 \pm 0.5 ^a

Results are Mean \pm S.D. for three animals

- a. Increased levels of S-phase are seen in one or more animals
 * Significantly different from controls

Table 2

Assessment of UDS following withdrawal of food before dosing (Preliminary Study B)

Dose Group	% Cells in Repair (≥ 3 ng)
Control (2% gum tragacanth)	0.11 \pm 0.06
200 mg/kg MNNG	1.46 \pm 1.51*

Results are means \pm S.D. for four male rats.

* significantly different from controls $p < 0.05$

Table 3

Induction of Replicative DNA Synthesis in Mouse Forestomach after administration of BHA (Method Development for Definitive Study)

Dose Group	No of animals with increased S-phase in forestomach*			
	24 hours		48 hours	
	M	F	M	M
Control	1	0	0	1
100 mg/kg MNNG	0	0	0	0
200 mg/kg MNNG	0	0	0	0
150 mg/kg BHA	0	0	NT	NT
300 mg/kg BHA	2	0	NT	NT
500 mg/kg BHA	NT	NT	0	1

* > 10 S-phase cells per section

NT = Not Tested

F. Evaluation of in vivo cytogenetics Assay

Reference: Putnam, D.L. and Shadly, E.H. In vivo Cytogenetics Assay: Analysis of Chromosomal Aberrations in Bone Marrow and Spermatogonial Cells Following repeated dose Administration. Microbiological Associates, Bethesda Md., 1992 (MRID 42619901)

ICR mice were treated with daily doses of DDVP of 0, 12.5, 25, or 50 mg/kg/day in distilled water for five days. The animals were adequately dosed based on data from a preliminary range-finding study which demonstrated an 80% mortality at 70 mg/kg/day. Cyclophosphamide was used as the positive control and was administered at 40 or 150 mg/kg by oral gavage. Colchicine 1.6 mg/kg i.p. was used as a spindle inhibitor and was administered 2 hours prior to sacrifice. Animals were sacrificed 24 hours following administration of the final dose of the test material, vehicle or the positive control. Cells from one femur, and the testes of each animal were examined for structural aberrations. Fifty metaphase cells per animal were examined for the bone marrow and spermatogonia determination. The mitotic index (MI) was determined for each animal per 500 scored cells.

There were no increases in the frequency of aberrations in the bone marrow cells or spermatogonia of any treated animals or dose groups. The positive control induced a significant increase in the frequency of structural aberrations in both cell types.

Discussion of the Data

This study was requested because reports in the literature suggested possible heritable mutagenic effects (Dearfield, 1988). Based on the results of this study, there was no longer a concern for heritable effects of dichlorvos. However, it should still be noted that dichlorvos still has mutagenic effects, and that it alkylates DNA, albeit weakly.

G. Previous Carcinogenicity Evidence

Rat Chronic/Carcinogenicity Studies

1. Reference(s): Pathology Working Group Report for Dichlorvos. Unpublished data received from NTP January 1987.

Toxicology and Carcinogenicity Studies of Dichlorvos in F344/N Rats and B6C3F1 Mice (Gavage Studies). National Toxicology Program. Technical Report Series 342: U.S. Dept Health and Human Services, Public Health Service. National Institutes of Health. September 1989.

a. Experimental Design

Dichlorvos (technical grade Vapona, 99% pure) was administered by gavage to 60 Fisher 344 rats per sex at doses of 0, 4, or 8 mg/kg/day in corn oil, 5 days per week for 103 weeks. The control animals were administered corn oil only. The gavage solutions were administered at 0.5 ml/100 gram of body weight. After treatment ceased, the animals were kept under observation for an additional week. Five animals/sex/group were used for plasma and RBC cholinesterase activity at 6 weeks and at 3, 6, 9, 12, and 24 months, and five animals/sex/group were used for brain and sciatic nerve histology. Rats were approximately 48 days of age and weighed 92-120 (F) grams and 111 to 148 grams (M) at study start. Animals were observed twice daily throughout the study for mortality and clinical signs, including palpation for masses beginning on day 270. Body weights were reported weekly for the first 14 weeks, then monthly thereafter. Moribund animals were sacrificed and examined grossly. At study termination all surviving animals were sacrificed, examined grossly, and tissues preserved for histopathological examination.

b. Discussion of Tumor Data

Administration of dichlorvos was associated with an increased incidence of mononuclear leukemia in male rats at all sites (11/50, 20/50, and 21/50 for the control, low and high dose groups), which was significant by pairwise comparison at both dosage levels, with a significant positive trend. The incidence of this tumor type in the treated animals was outside the historical control range of the NTP studies (mean 15.2%, range 2-22%). Administration of DDVP was also associated with an increased incidence of total pancreatic acinar adenomas in both dosage groups. The incidences were as follows: 25/50 (50%), 30/49 (61%), and 33/50 (66%) in the control, low, and high dose groups respectively. The pancreatic adenoma incidences for both control and treated animals were outside the limited available historical control incidence (mean 37.4%, range 30-40%). The incidences of hyperplasia were 37/50 (74%), 45/50 (90%) and 39/50 (74%). Analysis of the pancreatic acinar tumor incidence by the Office of Pesticide Programs using the Cochran-Armitage and Fisher Exact tests demonstrated no significant differences between the control and treated animals. Tumor incidences are presented in Table 4. Historical control values for the incidences of these tumor types are shown in Table 5.

TABLE 4: Pertinent Tumors Observed in Rats Administered Dichlorvos by Corn Oil Gavage for 103 Weeks including Statistical Analysis¹

<u>Tumor Site</u>	<u>Males Dose (mg/kg)</u>		
	<u>Vehicle Controls</u>	<u>4</u>	<u>8</u>
All organs: Leukemia; lymphocytic, monocytic mononuclear, or undifferentiated			
Tumor Rates: overall	11/50 ² (22%)	20/50(40%)	21/50(42%)
Statistical analysis: ³ Cochran-Armitage Fisher Exact	p=0.022*	p=0.041*	p=0.026*
Pancreas: (Acinar adenoma/longitudinal sections) single multiple total	16/50 ² 9/50 25/50(50%)	8/49 22/49 30/59(61%)	14/50 19/50 33/50(66%)
Statistical analysis: ⁴ Cochran-Armitage Fisher Exact	n/s	p=0.178	p=0.078
¹ pertinent data taken directly from tables generated by the NTP ² Number of tumor bearing animals/number of animals examined at site. ³ Statistical analysis performed by NTP statisticians ⁴ Statistical analysis performed by HED statisticians *Indicates significance at p < 0.05			

Table 5

Historical Control Incidence in Male Fisher 344 Rats of Tumors Observed in the Dichlorvos Study

	Mean%	Range%
Mononuclear Cell Leukemia at SRI	8.8	2-22
Mononuclear Cell Leukemia ¹	15.2	2-44
Pancreatic Acinar Adenoma ²	37.4	30-40

Source: 1. Haseman et.al. JNCI 75: 975-984, 1985
 2. Boorman, G.A., D.A. Banas, S.L. Eustis, and J.K. Haseman. Proliferative Exocrine Pancreatic Lesions in Rats. The Effect of Sample Size on the incidence of lesions. Tox. Path 15: 451-456, 1987.

c. Non-neoplastic Lesions

Survival was not adversely affected by administration of dichlorvos. Toxic signs reported in male rats were: brown fur around mouth and nose and anal area, leaning head, cloudy and/or opaque eyes and diarrhea. In female rats, the toxic signs were: cloudy or clear-fluid vaginal discharge, diarrhea, and wet fur in peri-anal or pelvic area. Mean body weights were similar in control and both groups of treated animals. Plasma cholinesterase was significantly decreased at all reporting periods in male and female rats. In high dose rats, the decreases were 72, 55, 53, 65, 71, 66 and 18 percent and 88, 84, 82, 85, 87, 87, and 8 percent below control at 6, 12, 24, 36, 52, 78, and 104 weeks in male and female rats, respectively. In low dose animals, the decreases were 58, 52, 53, 58, 61, 72, and 6 percent and 79, 78, 75, 80, 83, 85, and 4 percent below control in male and female rats at the same time intervals. Erythrocyte cholinesterase was significantly lower in dosed rats than in controls at 9, 12, and 18 months; however, the investigators indicated that these data were variable because of technical difficulties encountered during the aspiration/decantation steps and hemolysis during the washing steps. No gross lesions were reported. Male rats demonstrated a significantly increased incidence of adrenocortical vacuolation at both dosage levels, and a significantly increased incidence of hepatocyte vacuolation at the high dose. Female rats demonstrated a significantly increased incidence of pancreatic acinar atrophy at the high dose only. Selected lesions are shown in Table 3.

A NOEL for systemic toxicity was not demonstrated in this study, based on significant cholinesterase activity depression in all dosed male and female rats. The LOEL was 4 mg/kg/day.

d. Adequacy of dosing for Assessment of Carcinogenic Potential.

Doses were chosen from a 90 day NTP study in which Fisher 344 rats were gavaged at doses of 0, 2, 4, 8, 16, 32, and 64 mg/kg/day. Mortality occurred at doses of 16 through 64 mg/kg/day. Clinical signs of trembling, diarrhea, wet fur around the mouth, and convulsions immediately preceding death were observed in the animals which died on study. Based on these observations, doses used in the study were selected to be 0, 4 and 8 mg/kg/day.

Although there were no survival problems, body weight differences, or gross pathology relating to this study, it is apparent that the study was conducted at adequate dosage as indicated by the significant decrease in plasma and RBC cholinesterase activity in both sexes at all reporting periods. Dose related clinical cholinergic signs were reported in both sexes, indicated by brown fur around the mouth and anus, cloudy and opaque eyes, leaning head and diarrhea.

2. Reference: Enomoto, M. An assessment of the carcinogenic potential of dichlorvos administered in drinking water to male and female F344 rats for two years. Unpublished data submitted to EPA under MRID 410418-02, March 12, 1989.

Fisher 344 rats were exposed to dichlorvos in drinking water at doses of 0, 140, 280 ppm equivalent to 0, 8.5, and 17.5 mg/kg/day for the males and 0, 10.4, and 21.8 mg/kg/day for the females. The following tumor incidences were reported: there appeared to be a marginal increase in combined leukemias in treated males and a slight increase in mammary gland fibroadenomas in females.

a. Discussion of the Data

The data were of limited value because tumor incidences appeared to have been obtained by grouping the animals which received a histopathological examination with those which received only a gross necropsy.

3. Reference: Blair, D., K.M. Dix, and P.F. Hunt. Two Year inhalation exposure of rats to dichlorvos vapour. Unpublished data submitted to E.P.A. under an unknown Acc. No. on October 2, 1974 by Shell Chemical Company. Additional information submitted July 7, 1988.

Reference: Blair, D., Dix, K.M., Hunt, P.F., Thorpe, E., Stevenson, D.E., and Walker, A. A Two Year Carcinogenesis study in Rats. Arch. Toxicol. 25: 281-294, 1976.

a. Experimental Design

Groups of 50 CFE rats/sex were exposed to nominal concentrations of 0, 0.05, 0.5, and 5.0 mg/m³ of dichlorvos for up to 2 years. The

actual concentrations were 0, 0.043, 0.47, and 4.9 mg/m³. The dichlorvos was stated to be greater than 97% pure. The animals were exposed to the dichlorvos vapor for 23 hours/day. Concentrations of DDVP vapor in the chamber were determined daily, and the weekly average concentrations were summarized. Food and water were available ad libitum.

b. Non-neoplastic Lesions

In male rats, survival was 22, 32, 30 and 64 percent and in females, survival was 50, 60, 58, and 76 percent in the control, low, mid, and high dose groups, respectively at 99 weeks. The remaining treated males were sacrificed over the next three weeks so that blood samples could be taken and complete necropsies could be performed.

Body weights were significantly decreased in mid and high dose males up to week 76 of the study, in high dose males up to study termination, and in high dose females, throughout the study when compared to the controls (22% and 16% in high dose male and female rats respectively). Food consumption was comparable among all groups. At terminal sacrifice, SGOT and SGPT were statistically significantly elevated in high dose males. The biological significance of this is not clear, since only one determination was done. Under the study conditions, this investigation did not demonstrate any increases in tumor incidences when compared to the control animals.

c. Adequacy of dosing for Assessment of Carcinogenic Potential

The study was conducted at adequate dosage as indicated by the reduced body weights and significantly reduced cholinesterase activity in plasma, RBC and brain cholinesterase in the mid and high dose groups (76, 72, and 90 and 83, 68, and 90 percent of control in mid dose males and females, and to 38, 4, and 21, and 22, 5, and 16 percent of control in the high dose male and female groups, respectively). In addition, RBC cholinesterase was reduced to 88 percent of control in the low dose females. Body weight was significantly reduced in high dose male and female rats.

d. Discussion of the Data

Subdivision F Guidelines recommend survival of 50% of the animals in long term rodent studies at 78 weeks, and survival of 25% at 24 months. It should be noted that survival of control males in this study at 78 weeks was 41/50 or 82 percent, which is within the suggested guidelines. It should also be noted that survival in control males at Week 99 of the study was 22 percent, which is a total of one animal less than is recommended in the guidelines. In response to questions raised by ORD about survival rates in this study, survival to termination was 22, 32, 30, and 64 percent in the control, low mid, and high does males, respectively.

Additionally, the duration of exposure of the animals in this study was 23 hours/day, significantly greater (approximately 4 times) than the six hours/day recommended by the guidelines. The rat strain used in this study was Carworth Farms, which, according to the Institute of Laboratory Resources, National Academy of Sciences, is a Sprague Dawley derived strain.

Mouse Carcinogenicity Studies

1. Reference(s): NTP Pathology Working Group Report for Dichlorvos(1986). Two Year Gavage Study of Dichlorvos in F344 Rats and B6C3F1 Mice. (Unpublished data received from NTP on 1/15/1987).

Technical Report: Toxicology and Carcinogenicity of Dichlorvos in F344 rats and B6C3F1 mice (gavage studies). Technical Report Series 342. U.S. Dept. HHS, 1989.

a. Experimental Design

Dichlorvos was administered by gavage to 60 animals/sex/group of B6C3F1 mice at doses of 0, 10, and 20 mg/kg/day in corn oil in males and 0, 20, and 40 mg/kg/day in females. Control animals received corn oil only. Animals received the test compound 5 days/week for 103 weeks. Dosing was followed by a one week observation period. Dosage volume was 10 ml/kg body weight. Five animals/sex/ group were used for plasma and RBC cholinesterase activity determinations at 6, 12, 24, 36, 52, 78, and 104 weeks and 5 animals/sex/group were used for brain and sciatic nerve histology at study termination.

b. Discussion of the Tumor Data

An increased incidence of forestomach squamous cell papilloma and papilloma/carcinoma combined was observed in high dose male and female mice, which was significant in females but not in males (5/49, 6/49, 19 /50 and 1/50, 1/50, 5/50 for female and male mice, respectively). All of these tumor incidences, including those in the controls, were outside the NTP's historical control range (mean 1.92%, range 0-8%). The tumor incidences observed in this study are shown in Table 6.

TABLE 6: Incidence of Pertinent Tumors in Mice Including Statistical Analysis ¹			
	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 Fisher Exact	p<0.001**	p=0.500	p=0.002**
Forestomach, squamous cell carcinoma or papilloma squamous			
Tumor rate: overall	5/49 (10%)	6/49 (12%)	19/50 (38%) ³
Statistical Tests: Cochran-Armitage Fisher exact	p=0.001**	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 ³ One animal had both a papilloma and carcinoma *Indicates significance at p<0.05 **Indicates significance at p<0.01			

c. Non-neoplastic Lesions

Survival was unaffected by administration of dichlorvos. Animals surviving the study were: 35, 27, and 29 males and 26, 29, and 34 females of the control, low and high dose groups, respectively. Body weights were comparable in control and treated animals. Clinical signs noted were: left pelvic masses in high dose males and distended abdomens in treated females. Plasma cholinesterase was inhibited greater than 20% in all treated groups at all reporting periods. RBC cholinesterase was reduced in male mice 20 percent or more at 3 and at 6 months. No additional RBC cholinesterase determinations were made in male mice. In female mice, cholinesterase activity was also reduced. However, it must be noted that the investigators reported technical difficulties with respect to measurements of RBC cholinesterase activity. No

gross lesions and no non-neoplastic microscopic lesions attributable to dichlorvos administration were identified. No malignant squamous cell tumors were found in the historical controls.

d. Adequacy of dosing for Assessment of Carcinogenic Potential

Doses for this study were determined from a 13-week gavage study in which the chemical was administered to 10 animals/sex/group at doses of 0, 5, 10, 20, 40, 80 or 160 mg/kg/day. All males and 9/10 females receiving the high dose and 5/10 males receiving 80 mg/kg died prior to study termination. Final mean body weights were similar in control and treated animals. No clinical signs attributable to administration of dichlorvos were reported. There were no compound related gross or microscopic lesions. Based on the results of this range finding study, doses of 10 and 20 mg/kg/day were chosen for male mice, and doses of 20 and 40 mg/kg/day were chosen for the female mice. The study was conducted at adequate dosage as demonstrated by significant depression of plasma cholinesterase in low dose male mice.

2. Konishi, Y; J.H. Mennear, and B.K. Bernard. An assessment of the carcinogenic potential of Dichlorvos administered in drinking water to male and female B6C3F1 mice for two years. Unpublished data submitted to EPA, under MRID 410418-01, March 12, 1989.

Male and female B6C3F1 mice were administered DDVP in drinking water study at doses of 0, 400 and 800 ppm. The equivalent doses were 0, 58, and 94.8 mg/kg/day for males, and 0, 56.2, and 102.3 mg/kg/day for females. There were increased numbers of males in the treated groups with malignant fibrous histiocytomas and an increased number of males in the low dose group with thymomas. The data demonstrated gross trends only, and were not amenable to statistical analysis.

H. Additional Toxicology Data on DDVP

1. (Subchronic, chronic)

Reference: J.M. Kleeman: Thirteen Week Gavage Toxicity Study with DDVP in Rats: Hazleton Laboratories, America; Madison Wisconsin, 1988.

When DDVP was administered orally by gavage to male and female Crl:CD(SD) BR rats at doses of 0.1, 1.5, and 15 mg/kg day for thirteen weeks, salivation and urine staining were observed in high dose male and female rats 30 to 60 minutes post dosing during weeks 6-12 in males and weeks 8-12 in females. There were no other clinical signs attributable to compound administration. Plasma and RBC cholinesterase activity were significantly reduced at dosage levels of 1.5 mg/kg and above. Brain cholinesterase activity was reduced in high dose male and female rats. The NOEL and LOEL are

based on cholinesterase inhibition and are 0.1 and 1.5 mg/kg/day respectively.

Reference: Tyl, R.W., Marr, M.C., and Myers, C.B. Developmental Toxicity Evaluation of DDVP Administered by Gavage in New Zealand White Rabbits. Research Triangle Institute, 1991.

Dichlorvos was not a developmental toxicant when administered to pregnant New Zealand White rabbits on gestation days 7 through 19, at doses of 0, 0.1, 2.5, and 7 mg/kg/day by gavage. Maternal toxicity was observed at 2.5 mg/kg/day and above, manifested as increased mortality, clinical cholinergic signs, and decreased body weight gain during the dosing period (MRID 41802401)

Reference: Markiewicz, Victoria R. A 52 Week Chronic Toxicity Study on DDVP in Dogs. Hazleton Laboratories America. August 6, 1990.

Male and female beagle dogs were administered Dichlorvos 97.3 percent in capsules at doses of 0.1, 1.0, and 3.0 mg/kg/day for one year. The low dose was reduced to 0.05 mg/kg/day on day 22 due to reduced cholinesterase activity. No mortality occurred in the study. Mean cumulative body weight gain was reduced in high dose males from Week 1 through Week 7. Thereafter, body weight gains were equal to, and in some instances higher in the treated animals as compared to the controls. Cholinesterase activity was significantly reduced in plasma and RBC from Week 13 in males and females in the 1.0 and 3.0 mg/kg/day groups. The inhibition ranged from 39.1 to 59.2 percent in males and 41.0 to 56.7 percent in the 1.0 mg/kg/day group; from 65.1 to 74.3 percent in males and from 61.1 to 74.1 percent in females in the 3.0 mg/kg/day group. Administration of DDVP had no apparent effect on organ weights, or gross or microscopic pathology. At study termination brain cholinesterase activity was inhibited approximately 22 percent in males in the 1.0 mg/kg/day group, and 63 and 31 percent in males and females respectively in the 3.0 mg/kg/day group. The NOEL and LOEL were 0.05 and 1 mg/kg/day, based on the cholinesterase activity depression observed in the study.

2. Mutagenicity

Dichlorvos gave negative responses in the following Acceptable mutagenicity studies:

a. mouse micronucleus test at doses of up to 40 mg/kg; clinical signs were observed at this dose (MRID 00152240).

b. in vivo sister chromatid exchange assay in CD-1 mice at doses of up to 30 mg/kg. Clinical signs of lethargy and tremors were reported at the high dose (Acc No 259602, MRID 00152681).

c. dominant lethality in CD-1 mice when males were treated by i.p. injection with levels of 1, 3, and 10 mg/kg/day for 5 days prior to

being mated sequentially with virgin females for 8 weeks (MRID 00152682).

d. a second dominant lethal assay in CD-1 mice treated up to levels of severe clinical toxicity. Doses administered to males in this study were 8, 16, and 32 mg/kg/day by i.p injection for five days prior to being mated sequentially with virgin females for 8-weeks (MRID 40166901).

Dichlorvos was positive in

e. a mouse lymphoma forward mutation assay without metabolic activation at cytotoxic levels. The study did not include metabolic activation. Concentrations used in this study were 0.0, 6.25, 12.6, 25, 50, 100, 200, and 250 mg/mL. No cells survived the three highest doses. In the first trial, the mutation frequency was similar to controls at the two lowest doses, while in the second trial, the mutation frequencies were 1.0x, 1.7x, and 6.9x that of the ETOH controls at the three lowest doses (MRID 00151414).

In addition a survey of the literature was performed by Kerry Dearfield, Ph.D., and the information presented to the Cancer Peer Review Committee in the following reference: Dearfield, K. Review of the in vivo studies concerning Dichlorvos. Memorandum to J. Hauswirth, Health Effects Division, OPP, August 10, 1988

In this survey Dichlorvos generally gave negative responses in vivo studies, such as: host mediated assays using Salmonella and S. marcescens and in S. cerevisiae; sex linked recessive lethal mutation assay with Drosophila; chromosomal aberrations in bone marrow of exposed mice and hamsters, and in increased mitotic or replicative indices or SCE in cultured mouse B lymphocytes in up to lethal doses.

Dichlorvos gave positive responses in in vitro mutagenicity studies, such as Salmonella typhimurium TA 100, E. coli WP2, E. coli CM 881, Serratia marcescens, Saccharomyces cerevisiae, mammalian cells in culture, chromosomal aberrations and SCE exchanges in V79 cells without activation, and in Chinese hamster ovary cells with and without metabolic activation, in UDS in cultured EUE cells, and in the Syrian hamster embryo transformation assay.

For the in vivo activity of dichlorvos to be fully understood, aspects of its metabolites should be highlighted. It appears that the metabolism may play an important role in the magnitude of the potential mutagenic activity. Dichlorvos is metabolized through two possible pathways. It can be demethylated, which would lead to its alkylating activity. Dichlorvos can also be hydrolyzed via esterase-catalyzed hydrolysis. It is suggested that the esterase reactions predominate in in vivo situations with the demethylation reaction being a minor one or one that comes into play more

prominently when the esterases are overwhelmed. It should be noted that one of the products of the esterase-catalyzed hydrolysis is dichloroacetaldehyde, which is known to be mutagenic. It is suggested, however, that dichloroacetaldehyde is a short-lived intermediate and may not reach amounts that would be of concern (Dearfield)

3. Metabolism

Reference: Hutson, D.H., Hoadley, E.C.; and Pickering, B.A. The metabolic fate of [Vinyl-1-¹⁴C] dichlorvos in the rat after oral and inhalation exposure. *Xenobiotica* 1: 593-611, 1971.

Reference: Cheng, T. Metabolism of ¹⁴C-DDVP in rats (preliminary and definitive phases): Unpublished study No. HLA 6274-105 performed by Hazleton Laboratories America. Madison, Wisconsin August 30, 1989. MRID 41228701

Reference: Cheng, T. Supplement to Metabolism of ¹⁴C-DDVP in rats (preliminary and definitive phases) Study No HLA 6274-10501, MRID 41833901, 3/28/91.

When ¹⁴C-DDVP was administered to Sprague-Dawley rats in a single oral dose of 1 or 20 mg/kg, a single intravenous dose of 1 mg/kg, or a single oral dose of unlabelled DDVP for 15 days followed by a single oral dose of ¹⁴C DDVP, approximately 43 to 57 percent of the dose was eliminated in the urine, feces or expired air within 24 hours. Within 7 days, approximately 84 to 93 percent of the administered radioactivity was recovered. The majority of the [¹⁴C] eliminated was recovered as [¹⁴C] CO₂ (about 40 to 58% of the dose); smaller amounts were excreted into the urine (14 to 17 percent) and feces (4 to 7 percent). There were no sex or dose related differences in the excretion or distribution of ¹⁴C-DDVP. Excretion patterns were similar in iv- and orally dosed animals.

Among five radioactive components detected in the urine, urea and hippuric acid identified by mass spectrometric analysis. Hippuric acid concentration ranged from 6.8 to 10.5 percent (low dose group) and 4.2 to 5.6 percent (high dose group) of the urinary radioactivity. Urea comprised 19.6 to 33.1 percent (low dose group) and 41.5 to 51.1 percent (high dose group) of the urinary radioactivity. Both urea and hippuric acid were also found in the feces. Three other metabolites were not identified. They were assumed to be dehalogenated metabolites. Enzyme hydrolysis of the urine revealed the possible presence of glucuronide conjugates. These conjugates were not identified.

The large amount of radioactivity eliminated in the expired air (as CO₂), the two identified metabolites (urea and hippuric acid), and the two dehalogenated metabolites suggest that the metabolic pathways involve the one-carbon pool biosynthesis mechanism pathway for the natural products and conjugates. The proposed metabolic pathway is attached.

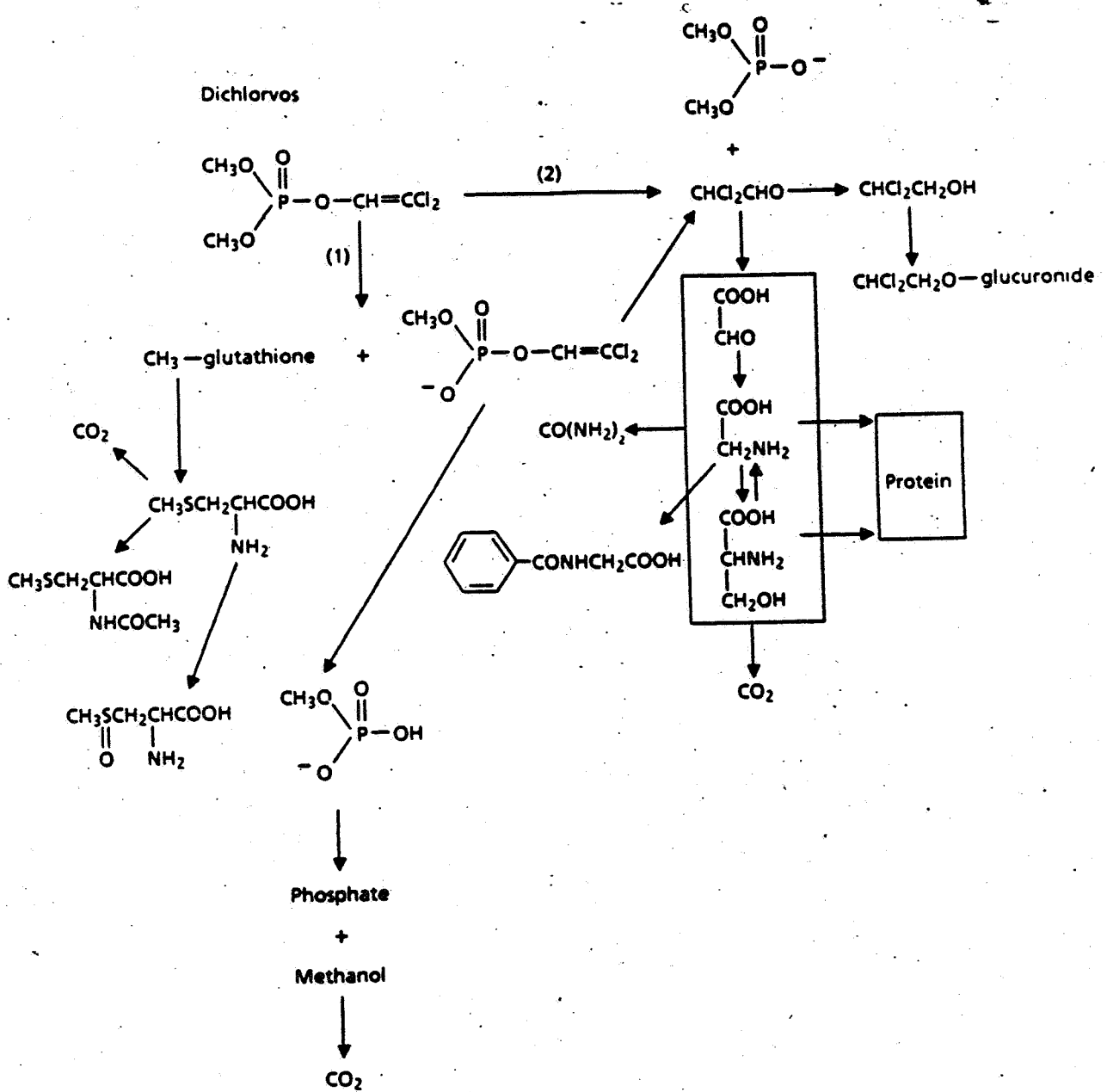


FIGURE 1. METABOLIC PATHWAYS OF DICHLORVOS
(Wright et al., 1979)

24A

I. Structure-Activity Relationships

DDVP is structurally related to trichlorfon, naled, tetrachlorvinphos, and phosphamidon. Naled and trichlorfon are both negative for carcinogenicity. The HED Peer Review Committee classified tetrachlorvinphos a Group C carcinogen (October 22, 1987), and phosphamidon a Group C carcinogen (January 9, 1989). Dichloropropene has previously been considered a structural analog for dichlorvos. New information obtained by HED indicates that it is not appropriate to consider this chemical as an analog for dichlorvos.

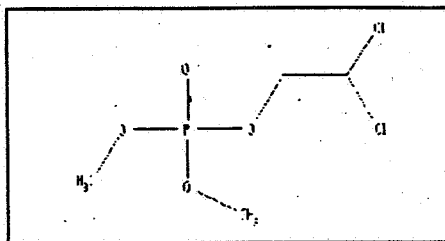
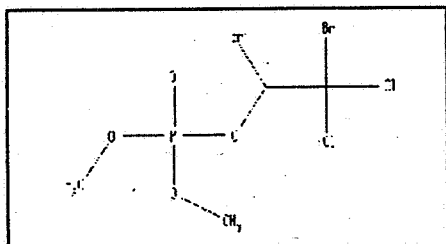
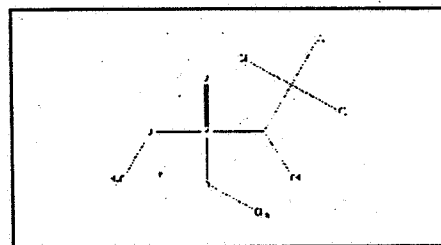


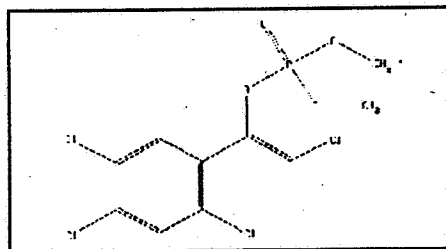
Figure 1 DDVP



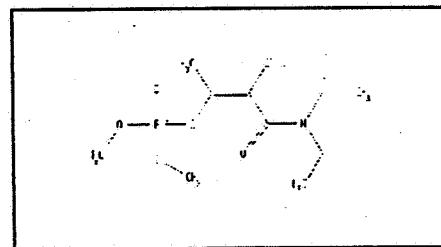
Naled



Trichlorfon



Tetrachlorvinphos



Phosphamidon

I. Background Information

Dichlorvos (2,2-dichlorovinyl dimethyl phosphate) is an organophosphorus pesticide. It is registered for use as an insecticide to be used to control flies, mosquitoes, gnats, cockroaches, and other insect pests in agricultural, commercial, institutional, industrial and domestic sites.

Dichlorvos is a colorless to amber liquid with a mild aromatic odor with a density of 1.415 g/ml at 25° C, a boiling point of 35° C, a vapor pressure of 0.012 mm Hg at 20°, and a refractive index of 1.452° at 25°.

The Caswell No is 328, the PC code is 084001, and the Chemical Abstracts Registry Number (CAS No) is 62-73-7

The Reference Dose (RfD) for Dichlorvos is 0.0005 mg/kg/day, based on a "no-observable effect" of 0.05 mg/kg/day for plasma and RBC cholinesterase inhibition in males and females demonstrated in a chronic feeding study in dogs. Brain cholinesterase activity was inhibited in males at 1 mg/kg/day. An uncertainty factor of 100 was used to account for intra- and inter-species differences.

A Registration Standard was issued for Dichlorvos in September 1987, a PD 1 in February 1988 and a PD2/3 in September 1995.

The chemical structure is:

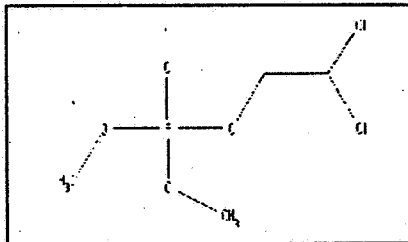


Figure 6 DDVP

Dichlorvos has been the subject of four reviews by the HED Cancer Peer Review Group, has been considered by the Scientific Advisory Panel on two occasions, and has been discussed in the CRAVE workgroup..

In the first Cancer Peer Review meeting on July 1, 1987, the Committee considered a report from the Pathology Working Group (PWG) for dichlorvos, prior to publication of the NTP Technical Report. The PWG evaluated the study slides and prepared the report. The data indicated the following tumor incidences: increased

pancreatic acinar adenomas in male rats, which was significant at both dose levels accompanied by a significant positive dose-related trend; an increased incidence of alveolar/bronchiolar adenomas in high dose male rats which showed not significant by pairwise comparison with the controls, but showed a trend; an increased incidence of leukemia at all sites in male rats which was significant at both dose levels when compared with the controls, and showed a significant trend; an increase in fibroadenomas and in combined mammary tumors (fibromas, fibroadenomas, carcinomas, adenomas) which was significant at the low dose only; increased incidences of forestomach squamous cell papillomas and forestomach cell papillomas/carcinomas combined in the high dose females. The Cancer Peer Review Committee classified dichlorvos a Group B2 carcinogen based on these data. The Peer Review Committee determined 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 (Peer Review Document 1). Accordingly the Q_1^* was calculated to be 2.9×10^{-1} using the geometric mean of the male rat pancreatic adenoma, male rat mononuclear cell leukemia and the female forestomach tumors.

The Peer Review's classification was discussed by the FIFRA Scientific Advisory Panel on September 25, 1987. They classified dichlorvos a Group C carcinogen based on (1) the lack of a dose response for pancreatic acinar adenomas which are benign tumors, for which there is also a causal relationship with corn oil gavage in male rats, (2) absence of a dose response for leukemia, along with the high degree of variability in controls for this endpoint in male rats, (3) lack of a dose response for the mammary tumor incidence and study to study variability of this tumor type and the tumor incidence even in the controls was outside of the historical control range, (4) the relevance of the forestomach tumors (the majority of which were benign and appeared only in high dose females, and (5) all mammalian in vivo mutagenicity studies were negative, which suggests that detoxification plays a key role (FIFRA Scientific Advisory Panel Report 1987).

The Cancer Peer Review Committee met on September 29, 1987 to consider the comments of the SAP. After the discussion the Committee still classified dichlorvos a B2 carcinogen based on the following reasons: (1) a dose response relationship of statistical significance was seen for pancreatic adenomas (which have the potential to progress to malignancy) and mononuclear cell leukemia was seen in male rats (2) a dose response relationship of statistical significance was seen in the female mouse for forestomach squamous cell papillomas which have the potential to progress to carcinomas (3) some forestomach carcinomas which are rare were seen in the female mouse (4) a significant positive trend was seen for forestomach papillomas in male mice at a dose which did not achieve the MTD (5) supporting evidence was provided by a statistically significant increase in mammary tumors at the low

dose in the female rat and an increase in lung tumors in the male rat which was associated with a significant trend and (6) mutagenicity data were available indicating that DDVP is positive in vitro in bacterial and mammalian cells with and without metabolic activation.

The Cancer Peer Review Committee met for a third time on June 2, 1988 to consider the carcinogenicity classification of DDVP in the light of the April 18, 1988 meeting of the NTP Panel of Experts with respect to a recut of the slides into longitudinal sections which demonstrated a significantly higher incidence of adenomas in the control male rats than was previously reported. This reclassification resulted in the NTP downgrading the evidence of carcinogenicity in male rats from clear evidence to some evidence. The Peer Review Committee continued to classify dichlorvos a B2 carcinogen because (1) the results of the transplantable rat mononuclear cell leukemia model indicate that the increased incidence of mononuclear cell leukemia in DDVP treated rats was treatment related (2) although the results of longitudinal sectioning of the pancreas diminished the significance of the pancreatic acinar adenomas in the male rats, the incidence of animals with multiple tumors was still increased with DDVP treatment (3) DDVP is a direct acting mutagen and alkylates DNA. This was considered an interim classification pending review of a Japanese drinking water study of DDVP in rats and in vivo mutagenicity studies in the literature.

The HED Cancer Peer Review concluded on June 1, 1989 that based on the available evidence that Dichlorvos was a Group C carcinogen and recommended quantification for all routes of exposure except inhalation (Ghali, 1989). This decision was based on the increased incidence of mononuclear cell leukemia in treated male rats (11/50, 20/50, and 21/50 for the control, low and high dose groups) which was significant by pairwise comparison with the controls, with a significant positive trend. The tumor incidence was within the NTP historical control range. It was also based on the increased incidence of forestomach squamous cell papilloma and papilloma/carcinoma combined in high dose female mice when compared to the control incidence (5/49, 6/49, 19/50 for control, low dose and high dose groups respectively). These tumor incidences, including those of the controls were outside of the NTP historical control range. The quantification was based on the geometric mean of these two tumor types. A revised Q_1^* was calculated to be 2.0×10^{-1} . The Q_1^* was subsequently revised to 1.2×10^{-1} using the 3/4 interspecies scaling factor.

On September 28, 1989 the FIFRA Scientific Advisory Committee again discussed the "weight of the evidence" and classification of DDVP as a Class C carcinogen. They determined that the C classification was clearly justified based on (1) the marginal increase in pancreatic acinar adenomas in an animal model that is so sensitive to the vehicle alone i.e., corn oil, (2) the lack of a carcinogenic

response in the rat inhalation study (3) the variability in the incidence of mononuclear cell leukemia in the Fisher 344 rat where the spontaneous level is very high, and (4) the difficulties associated with interpreting forestomach papillomas in rats that receive the test agent by gavage. It was the opinion of the SAP that forestomach papillomas after gavage of the test substance may represent an injury (mechanical or chemical), and that this type of lesion is often reversible. However, carcinomas in the forestomach as opposed to papillomas would represent good evidence for the oncogenic capabilities of the test substance.

On January 16, 1992 the C classification and oral quantitation were verified by a vote of the CRAVE workgroup members.

On March 31, 1992 the issue of quantification of the cancer risk by the inhalation route of exposure was discussed in the CRAVE Workgroup. It was decided to affirm the HED Peer Review Group's recommendation to quantitate the cancer risk by routes other than by inhalation based on the following uncertainties: the quality of the oral cancer data, the route specificity of the target organs, and the reliability and accuracy in estimating the target organ dose.

On April 8, 1993 the HED RfD Committee determined that it was inappropriate to extrapolate carcinogenicity data generated by oral gavage administration of this chemical for the purpose of quantifying human risk resulting from dermal exposure. One major reason for this decision is the fact in a dermal absorption study in rats only ten percent of topically applied ¹⁴-C labelled dichlorvos was absorbed after 120 hours, and the maximum blood level did not exceed 0.2 percent of the dose at different intervals regardless of the amount applied. Thus, after 120 hours, very little or no dichlorvos penetrated the skin and entered the blood circulation.