



FILE COPY

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

SEP 18 1989

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Fourth Peer Review of Dichlorvos (DDVP)

FROM: George Z. Ghali, Ph.D. *G. Ghali*
Science Analysis and Coordination Branch
Health Effects Division (H7509C)

TO: George LaRocca, PM 15
Insecticide-Rodenticide Branch
Registration Division (H7505C)

The Health Effects Division (HED) Peer Review Committee met on July 19, 1989 to discuss and evaluate the weight of the evidence on dichlorvos (DDVP), with particular reference to its oncogenic potential. The Committee concluded that the chemical should be downgraded from Category B2 oncogen (probable human carcinogen) to Category C (possible human carcinogen). A recommendation was also made for the quantification of human risk for all routes of exposure except inhalation.

A. Individuals in Attendance

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

Penelope A. Fenner-Crisp

William L. Burnam

Reto Engler

Karl Baetcke

Edwin Budd

Esther Rinde

Marion Copley

Penelope A. Fenner-Crisp
W. L. Burnam
Reto Engler
Karl Baetcke
Edwin Budd
Esther Rinde
Marion Copley

Lynnard Slaughter

Kerry Dearfield

William Sette

Richard Levy

George Z. Ghali

Kerry Dearfield
William Sette
Richard A. Levy
G. Ghali

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

Marcia van Gemert

Robert Beliles

John Quest

Richard Hill

Julie Du

Marcia van Gemert
Robert Beliles
John A. Quest
Richard Hill
Julie Du

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

Joycelyn Stewart

Joycelyn Stewart

4. Scientific Observers:

Bernice Fisher, SACB/HED

Hugh Pettigrew, SACB/HED

Yinnakis Ioannou, HFAB/HED

B. Material Reviewed

1. NTP Panel of Experts report.
2. New oncogenicity data including:
 - a. Two-year oncogenicity study in the CFE rats by inhalation, (summary).
 - b. Two-year oncogenicity studies in Fischer 344 rats and B6C3F1 mice with DDVP administered in drinking water, (DER's).
3. A summary of relevant toxicology information prepared by Dr. J. Stewart, HED/OPP.
4. Two NTP publications:
 - a. Neoplasms observed in untreated and corn oil gavage control groups of F344/N rats and (C57BL/6N X C3H/HeN)F1 (B6C3F1) mice. (1985). JNCI, 75 No 5, 975 - 984.
 - b. Proliferative exocrine pancreatic lesion in rats. The effect of sample size on the incidence of lesions. (1987). Toxicol. Pathology, 15 No 4, 451 - 456.
5. Registrant rebuttals.

C. Background

1. Initial Peer Review Meeting Findings

The chemical was originally evaluated by the Peer Review Committee on July 1, 1987. At this time, the Committee concluded that DDVP met the criteria of the B2 classification (probable human carcinogen), i.e., induction of oncogenic response in two rodent species including forestomach squamous cell carcinoma, an uncommon tumor, in mice.

The Committee's decision was primarily based on the results of the NTP bioassay in B6C3F1 mice and Fischer 344 rats. The following is an excerpt from the Committee's report dated 9/25/87:

"In B6C3F1 mice, dichlorovos induced a statistically significant increase in forestomach squamous cell papillomas and combined forestomach squamous cell carcinomas and papillomas in high-dose females. This tumor type (squamous cell papilloma) was also increased in high-dose males but was significant only for a positive dose-related trend.

In Fischer 344 male rats, administration of dichlorovos 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 fibro-adenomas 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. Dichlorovos has been shown to be mutagenic in the Ames Salmonella assay and mouse lymphoma assay and has been reported to alkylate DNA. In addition, a major metabolite of DDVP, dichloro-acetaldehyde has been reported to be a more potent mutagen in the Ames Salmonella assay than dichlorovos, itself.

Positive SAR information is also available. Of particular relevance, is that dichloropropene (Telone II), structurally similar to DDVP, 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 DDVP on this organ. Since DDVP did not demonstrate either excessive dermal or eye irritation (Toxicity 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 pair-wise comparison and exceeded the historical control range."

2. FIFRA Scientific Advisory Panel Evaluation

The Peer Review Committee's decision was presented to the FIFRA Scientific Advisory Panel (SAP) on September 23, 1987. The Panel did not agree with the Committee's overall assessment of the weight-of-the-evidence on DDVP. "The SAP concluded that DDVP should be classified as a Category C oncogen since: 1) only benign tumors were induced by DDVP, 2) they were not dose-related, and 3) DDVP was not mutagenic in in vivo assays (although it was mutagenic in several in vitro test systems both with and without metabolic activation)."

3. Second Peer Review Committee Meeting - Evaluation of SAP Findings

The Peer Review Committee met on September 29, 1987 to examine the issues raised by the SAP with respect to the classification of the carcinogenicity of DDVP. The following is an excerpt from the Committee's report dated 3/16/1988:

"Upon reconsideration, the Committee concluded that the results of the NTP bioassays indicate that DDVP demonstrates sufficient evidence of carcinogenicity in the male rat and in the female mouse since: 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 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) the presence of some forestomach carcinomas (which are rare) was seen in the female mouse, 4) significant positive trend was seen for forestomach papillomas in male mice at a dose that did not achieve an MTD, 5) supporting evidence 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 was available indicating that DDVP is positive for mutagenicity in vitro in bacterial and mammalian cells both with and without metabolic activation. The Committee, thereby, confirmed their initial classification of DDVP as a B2 oncogen."

4. Third Peer Review Committee Meeting

The Peer Review Committee met again on June 2, 1988 to discuss the impact of the April 18, 1988 meeting of the NTP Panel of Experts on the classification of the carcinogenicity of DDVP.

In this meeting the first and second peer review documents on DDVP, the comments of the SAP, and the transcript of the NTP Panel of Experts meeting of July 14, 1987 were made available to the Committee for review. The following is an excerpt from the Committee's report dated 8/17/1988:

"The relevant points discussed at the April 18, 1988 NTP Panel of Experts meeting were: a) the incidences of pancreatic acinar cell adenomas after additional sectioning of pancreata from F344 rats from the 2-year studies; and b) effects of DDVP administration on growth of transplantable rat mononuclear cell leukemias in male F344 rats.

a. Results of the Recut of the Pancreas

Longitudinal sections were cut from the pancreas of male and female rats of all test groups. There was a positive dose-related trend for total adenomas for male rats. There was no historical control data to compare these results with, since NTP does not routinely do longitudinal sectioning.

6

b. Effects of DDVP on the Growth of Transplantable Rat Mononuclear Cell Leukemias in Male F344 Rats

DDVP was positive in this model. There was a strong structure activity relationship for this effect with other dialkyl esters of phosphoric acid.

The NTP scientists concluded from the results discussed under points a and b above that the increased incidence of mononuclear cell leukemias in DDVP treated rats was treatment-related and from the results of the longitudinal sectioning of the pancreas that the evidence for oncogenicity in male F344 rats should be downgraded from clear evidence to some evidence of oncogenicity. The downgrading of the level of evidence was based upon both the incidence of mononuclear cell leukemia and of pancreatic acinar cell adenomas. The level of evidence in female rats remained at equivocal. The results of the mouse study were not discussed and therefore, the level of evidence remained at clear for female mice and at some for male mice based upon an increased incidence of stomach papillomas.

The HED Peer Review Committee considered the above information at their meeting and concluded that the classification of DDVP should remain a B2. They based this conclusion upon the following points:

- a. Results of the transplantable rat mononuclear cell leukemia model indicate that the incidence of mononuclear cell leukemia found in DDVP treated F344 rats was treatment-related.
- b. Although the results of longitudinal sectioning of the pancreas diminished the significance of the pancreatic acinar adenomas in male rats, the incidence of animals with multiple adenomas was still increased with DDVP treatment.
- c. DDVP is a direct acting mutagen and alkylates DNA."

The Committee considered this to be an interim classification until the evaluation of the results of oncogenic studies performed in Japan in which the test chemical DDVP was administered to rats and mice in drinking water, and the evaluation of several in vivo mutagenicity studies on DDVP which have been reported in the open literature as positive.

5. Fourth (Present) Peer Review Committee Meeting

The purpose of this meeting was the reconsideration of the NTP rat study in light of the recent NTP Panel of Experts report, evaluation of new oncogenicity studies with DDVP administered by inhalation (CFE rats) or in drinking water (Fischer 344 rats and B6C3F1 mice), and other ancillary information.

a. Reconsideration of the NTP Studies

The NTP studies were considered by the Peer Review Committee on several occasions and constituted the cornerstone in the evaluation and classification of the oncogenic potential of this chemical.

In their meeting of July 14, 1987, the NTP Panel of Experts recommended the reexamination of pancreata of male and female rats using longitudinal sections.

The longitudinal sectioning of pancreata resulted in an increased number of pancreatic acinar tumors in the control animals, thus diminishing the statistical significance of this lesion. The NTP analysis of the combined data indicated a statistically significant difference between the treated and control groups with a positive dose-related trend using the logistic regression analysis. However, according to the HED statisticians the increase in pancreatic acinar tumors was neither significant in the Fischer Exact test for pair-wise comparison, nor positive in the Cochran-Armitage test for dose-related trend, which are typically used for testing dose groups having no survival disparities. The incidence of animals with multiple pancreatic adenomas was still increased with DDVP treatment. Historical control data were

provided from four studies previously done by the NTP (Boorman G. A., et al., 1987).

b. Two-Year Inhalation Oncogenicity Study in the CFE Rats

In this study, groups of 50 CFE rats per sex per dose were exposed to concentrations of 0.05, 0.5, or 5.0 mg/m³ of technical DDVP 23 hours a day for 2 years. The treatment did not alter the spontaneous tumor profile in this strain of rats. The MTD seems to have been reached based upon significant body weight decrease in mid- and high-dose males ($p < 0.01$) up to week 76, and significant weight depression in the high-dose females ($p < 0.01$) throughout the study.

c. Two-Year Drinking Water Oncogenicity Studies in the Fischer 344 Rats and B6C3F1 Mice (MRID No. 410418 - 01 and - 02)

These two studies were considered deficient both in conduct and reporting and they were not amenable to statistical analysis. Major deficiencies observed in these two studies included incomplete histopathologic evaluation, absence of water consumption data required to determine the actual test chemical intake, and failure to include individual animal data in the final report.

In the rat study, groups of 51 animals per sex per dose were administered DDVP in drinking water at concentrations of 0, 140, or 280 ppm for 2 years followed by 4 weeks recovery period. In this study there appeared to be an increased incidence of mononuclear cell and lymphocytic leukemia in treated males, and of mammary gland fibroadenomas in females.

In the mouse study, groups of 50 animals per sex per dose were administered DDVP in drinking water for two years. In this study there appeared to be an increased incidence of malignant fibrous histiocyctomas and thymomas in males.

D. Weight-of-the-Evidence

The Committee considered the following points to be of importance in a weight-of-the-evidence determination of the oncogenic potential of DDVP.

1. "In B6C3F1 mice, DDVP induced a statistically significant increase in forestomach squamous cell papillomas and combined forestomach squamous cell carcinomas and papillomas in high-dose females. This tumor type (squamous cell papilloma) was also increased in male mice of the high dose but was significant only for a positive dose-related trend." Historical control data indicated that squamous cell forestomach carcinomas are relatively uncommon ($\leq 0.5\%$). However, there were no historical control data for corn oil gavage. The Committee questioned the relevance of this type of tumor to man.
2. DDVP did not demonstrate either excessive dermal or eye irritation (Category IV and III respectively). Therefore, the forestomach tumors seen in the mouse study were probably not due to irritancy of the material.
3. In Fischer 344 rats, administration of DDVP by oral gavage was associated with a numerical increase* in the incidence of pancreatic acinar adenomas, and a statistically significant increase in leukemia (of all types and sites) in males at both dosage levels. The increase in leukemia exhibited a significant positive dose-related trend. Although the results of longitudinal sectioning of pancreas diminished the statistical significance of pancreatic acinar adenomas, the incidence of animals with multiple pancreatic adenomas was still increased with DDVP treatment. Historical control data (Haseman et al., 1985; Eustis and Boorman, 1985) indicated that the incidence of pancreatic acinar adenomas and leukemia was outside of historical control range.
4. In Fischer 344 rats, administration of DDVP by oral gavage was also associated with "an increase in lung alveolar/bronchioalveolar adenomas in male which showed a positive

* The increase in pancreatic tumors was statistically significant with a positive dose-related trend before the recut.

dose-related trend but was not significant by pairwise comparison; an increase in fibroadenomas of the mammary gland in females which was significant by pairwise comparison at the low dose; and an increase in combined mammary gland tumors (fibroma, fibroadenoma, carcinoma, adenocarcinoma or adenomas) in females which was significant at the low dose." However, the incidence of lung and mammary gland tumors was within the historical control range as reported by Haseman et al., 1985 and Eutis and Boorman, 1985.

5. Data generated by the administration of DDVP to rats and mice in drinking water, though deficient and not amenable for statistical analysis, was useful in identifying a qualitative trend, in that the treatment induced some tumors similar to those induced in the oral gavage studies indicating that these tumors, observed in the earlier studies, are treatment-related. In the drinking water studies, the treatment appeared to increase the incidence of mononuclear cell leukemia and lymphocytic leukemia combined in male rats, and mammary gland fibroadenomas in female rats. In male mice, the treatment was associated with a numerical increase in malignant fibrous histiocyctomas and thymomas.
6. Results of the transplantable rat mononuclear cell leukemia model suggest that the incidence of mononuclear cell leukemia found in DDVP-treated Fischer 344 rats was treatment-related. However, the committee questioned whether this model has ever been validated and whether it is currently considered an accepted assay.
7. Administration of DDVP to CFE rats by inhalation did not alter the spontaneous tumor profile in this strain of rats.
8. DDVP has been reported to be a direct acting mutagen. In addition to its mutagenic activity in several in vitro testing systems, there is suggestive evidence that DDVP may have the potential to be active in vivo. There have been reports in the open literature suggestive of alkylating activity in testes, some dominant lethal effects in mice, induction of abnormal sperm, and depletion of germinal epithelium in male mice.

9. Dichloroacetaldehyde, a product of hydrolytic or oxidative cleavage of DDVP, has been reported in the open literature to be mutagenic in the reverse mutation assay in the Salmonella typhimurium and in the dominant lethal assay in the mouse.
10. DDVP is structurally related to tetrachlorovinphos (Gardona), phosphamidon and in particular 1,3-dichloropropene (Telone II), in that they all contain a vinyl or vinylidene chloride moiety. Vinyl chloride is a known human oncogen, and vinylidene chloride was positive in some animal studies. However, aspects of the reported carcinogenicity of vinylidene chloride appear conflicting and indicate sex, species and strain specificity. The mutagenic and oncogenic potential of vinyl and vinylidene chloride is well documented in the open literature and may be attributable to the initial formation of unstable oxiranes (Greim et al., Biochem. Pharmacol., 24 (1975) 2013; Bonse and Henschler, CRC Crit. Revs Toxicol., 4 (1976) 395-409). Data available on pesticidal chemical analogues provide evidence of mutagenic and oncogenic activity. Tetrachlorovinphos was classified as C oncogen (10/22/87), phosphamidon was classified as C oncogen (1/9/89), and dichloropropene was classified as B-2 oncogen (8/23/89).

E. Conclusions

The Committee agreed, based upon the available information, to classify DDVP as a group C oncogen, possible human carcinogen, in accordance with the Agency's Guideline for the classification of chemical carcinogens. This downgrading from the previous classification as group B-2 was due to: 1) erosion of the evidence on the pancreatic acinar adenomas in male rats, 2) upgrading and consideration of the negative inhalation study in the CFE rats, and 3) questions regarding the biological significance of the primary tumors in the NTP studies, i.e. leukemia in rats (variable tumors in historical controls) and forestomach tumors in mice and its relevance to man.

The group C classification is supported by the following:

1. In B6C3F1 mice, dichlorovos induced a statistically significant increase in forestomach squamous cell papillomas and combined forestomach squamous cell carcinomas and papillomas in high-dose females. This tumor type (squamous cell papilloma) was also increased in high-dose males but was significant only for a positive dose-related trend.
2. In Fischer 344 rats, DDVP was associated with a statistically significant increase, with a positive dose-related trend, in leukemia (of all sites and types) in males at both dosage levels. This evidence is supported by the results of the transplantable rat mononuclear cell leukemia model. The treatment was also associated with a numerical (not statistically significant) increase in pancreatic acinar adenomas in males. The incidence of animals with multiple pancreatic acinar adenomas was also increased.
3. The group C classification is further supported by the evident in vitro and suggestive in vivo mutagenic activity of DDVP and its hydrolytic- or oxidative-cleavage product dichloroacetaldehyde, and the structural similarity of DDVP to other chemical oncogens particularly 1,3-dichloropropene.

Quantification of human risk was also recommended. This decision was based on the fact that DDVP induced different types of tumors in either or both sexes of two rodent species including an uncommon tumor in mice, induced mutagenic response in several of in vitro and in vivo systems and was considered a direct-acting mutagen, and is structurally similar to known chemical mutagens/oncogens.

Quantification of human risk will be limited to oral and dermal routes of exposure. The potency estimate Q_1^* , will be based upon the geometric mean of the female mouse stomach tumors (forestomach, papilloma squamous, and squamous cell carcinoma) and the leukemia in male rats (memo by B. Fisher dated 9/12/1989).