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

007543

OCT 13 1989

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Dichlorvos (DDVP) - Submission of Drinking Water Oncogenicity Studies in the Rat and Mouse

Tox Chem No.: 328
Project No: 9-1211
Record Nos: 242820
242821

FROM: William B. Greear, M.P.H. William B. Greear 9/27/89
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TO: Mark Boodee, RM Team #81
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THRU: Marion P. Copley, D.V.M., Section Head Marion Copley 9/27/89
Review Section II
Toxicology Branch I - Insecticide, Rodenticide Support
Health Effects Division
10/6/89

Conclusions

The following two studies are Core-Supplementary. Due to the number and nature of the deficiencies and discrepancies in the studies (see DERs attached) it is improbable, possibly impossible that the studies could be upgraded. Even if the studies were upgraded, there would be no impact on the classification of dichlorvos.

Requested Action

The Amvac Chemical Corporation has requested that EPA take into consideration a rat and a mouse drinking water oncogenicity study during the special review of dichlorvos.

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Background

Under a cover letter dated March 21, 1989, Steven D. Jellinek representing the Amvac Chemical Corporation, has submitted the following two studies for evaluation:

1. An Assessment of the Carcinogenic Potential of Dichlorvos Administered in Drinking water to Male and Female F344 Rats for Two Years.

2. An Assessment of the Carcinogenic Potential of Dichlorvos Administered in Drinking Water to Male and Female B6C3F1 Mice for Two Years.

The studies have been evaluated and the DERS are attached.

Note: Additional information concerning the rat study was sent by FAX transmission from J. Mennear to M. Copley on June 6, 1989. A copy of this transmission is attached to the DER of the rat study.

Attachment

Reviewed By: William B. Greear, M.P.H. William B Greear 6/1/89 007543
Review Section II, Toxicology Branch I - IRS (H7509C)
Secondary Reviewer: Marion P. Copley, D.V.M. [illegible] 6/1/89
Review Section II, Toxicology Branch I - IRS (H7509C)

DATA EVALUATION REPORT

Study Type: Guidelines Series 83-2 - Oncogenicity - Mouse

TOX Chem No.: 388
MRID No.: 410418-01

Accession No.: N/A

Test Material: Dichlorvos

Synonyms: DDVP; Vapona; Oko; Dedevap; Herkol; Dimethyl 2,2-dichlorovinyl phosphate; 2,2-Dichlorovinyl dimethyl phosphate

Sponsor: Amvac Chemical Corporation

Testing Facility: Biosafety Research Center
Food, Drugs and Pesticides
Shizuoka, Japan

Title of Report: An Assessment of the Carcinogenic Potential of Dichlorvos Administered in Drinking Water to Male and Female B6C3F1 Mice for Two Years.

Author: Yoichi Konishi, John H. Mennear, and Bruce K. Bernard

Study No.: N/A

Report Issued: March 12, 1989

Conclusions:

The study is incomplete; however, certain general trends were observed. Body weight gain and water consumption were decreased in both treated groups. There was a dose-related decrease in the absolute and relative weight of the liver and gonads of males. The absolute and/or relative weight of the pancreas was decreased in treated females. Other weight changes were apparent. Testicular atrophy was increased in males in the high-dose group. 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 histopathology data are not amenable to statistical analysis because it is unknown how many animals received histopathological examination. Additional histopathology should be conducted.

Classification: Core-Supplementary

A. Materials:

1. Test Compound - Dichlorvos; Description: Not provided; Lot No.: Not provided; Purity: 97.26%; Contaminants: [redacted]

2. Test Animals - Species: Mouse; Strain: B6C3F1; Age: 7 weeks; Weight: Average males 23.2 g, average females 18.5 g; Source: Charles River, Japan, Inc.

B. Study Design:

1. Animal Assignment - Animals were assigned to the following test groups:

Test Group		Dose in Water (ppm)	Main Study 102 Weeks Male	Main Study 102 Weeks Female	10-Week Range-Finding Study Male	10-Week Range-Finding Study Female
1.	Control	0	50	50		
2.	Low (LDT)	400[1]	50	50		
3.	High (HDT)	800[2]	50	50		
		First Range-Finding Study				
1.	Control	0			10	10
2.	Low (LDT)	25			11	11
3.		50			12	11
4.		100			12	12
5.		200			12	11
6.	High (HDT)	400			12	12
		Second Range-Finding Study				
1.	Low (LDT)	1600			10	10
2.		3200			11	9
3.		5800			11	9
4.	High (HDT)	10,000			10	9
		Third Range-Finding Study				
1.	Low (LDT)	400			11	10
2.	High (HDT)	800			11	9

2. Drinking Water Preparation - During the first range-finding study, the dosing solutions were prepared weekly. In the second and third range-finding studies and the 102-week study, dosing solutions were prepared 6 days a week. A standard basal diet (CE-2, CLEA) was available ad libitum.

1 Calculated to be 23.0 and 56.8 mg/kg/day for males and females, respectively.
2 Calculated to be 47.8 and 102.3 mg/kg/day for males and females, respectively.

Results - At the time the study was conducted, the stability of DDVP in tap water was not ascertained. However, the stability of DDVP in tap water has been determined by the Environmental Toxicology Laboratory, Nippon Soda Company, Odawara, Japan on November 14, 1988. It was reported that solutions containing 140 to 800 ppm of dichlorvos were allowed to stand for up to 6 hours at 23 ± 1 °C and the amount of DDVP recovered ranged from 90.6 to 100.9 percent. Therefore, the dosing solutions used by the investigator were probably stable in the range of 140 to 800 ppm. The stability of dosing solutions outside of this range has not been addressed.

3. Animal Maintenance (2-Year Study) - The animals were acclimated to laboratory conditions for 1 week prior to study initiation. The animals were housed in groups of 9 to 12 (range-finding studies) and 10 (2-year study) in aluminum cages in a room(s) maintained at, approximately, a temperature of 24 °C and relative humidity of 65 percent. A 12-hour lighting cycle was maintained. The number, if any, of air changes per hour was not reported.

4. Statistics:

Survival Analysis - The probability of survival was estimated by the product-limit procedure of Kaplan and Meier (1958). Analysis for possible dose-related effects on survival used the method of Cox (1972) for testing two groups for equality and Tarone's (1975) life table test for a dose-related trend.

Calculation of Incidences of Lesions - The incidence of lesions was presented as a ratio of the number of animals bearing lesions at a specific anatomic site to the number of animals necropsied and the site examined grossly and/or microscopically.

[This reviewer believes that the incidence of lesions as defined above is scientifically unsound. The amount of information obtained from a gross examination of tissues is quite different from the amount of information that is obtained from microscopic examination of the tissues. Statistical analysis of such data would be improper and misleading.]

Analyses of Tumor Incidence - Three statistical methods were used to analyze the tumor incidence data: life table tests, incidental tumor analysis, and Fisher Exact/Corchran-Armitage trend analyses. Tests of significance included pairwise comparisons of each treated group with controls and tests for overall dose-response trends.

5. Quality Assurance - Not provided.

C. Methods and Results:

1. Observations - The animals were observed for clinical signs of toxicity and mortality on a daily basis.

Results:

Range-Finding Studies - First Study: One female died in each of the 25, 50, and 200 ppm groups. No signs of toxicity were observed. Second Study: All animals treated with 5000 and 10,000 ppm DDVP died. Seven of 11 males and 1 of 9 females dosed with 3200 ppm DDVP died. One male in the 1600 ppm group died. Clinical signs of toxicity were not reported. Third Study: No deaths occurred. Clinical signs of toxicity were not reported.

Two-Year Study - No record of clinical signs could be found by the authors. The number of animal surviving to 102 weeks is provided in the table below.

No. of Animals Alive/No. of Animals (Percent)

Sex	Control	LDT	HDT
Male	32/50(64%)	33/50(66%)	42/50(84%)
Female	33/50(66%)	25/50(50%)	40/50(80%)

2. Body Weight - Body weights were determined weekly in the range-finding studies. For the 2-year study, mean body weights were determined weekly for the first year and every 2 weeks thereafter.

Results:

Range-Finding Studies - First Study: Unremarkable. Second Study: Body weight gain of treated animals was less than controls in the First Study. Third Study: Unremarkable.

Two-Year Study - Mean body weights of treated mice were lower than controls and males were more severely affected than females. The response was dose-related. Only graphical (mean) data were provided. Individual animal data were not provided as requested.

3. Food Consumption, Water and Compound Intake - Water consumption was determined weekly during the first year and every 2 weeks thereafter in the 2-year study.

Results - Water consumption was reduced in the male 400 and 800 ppm groups by 45 and 60 percent, respectively, and in the femal 400 and 800 ppm groups by 25 and 35 percent, respectively. The mean data were provided only in graphical form. Individual animal water consumption data were apparently not collected.

4. Ophthalmological Examinations were not conducted.

5. Hematology/Clinical Chemistry examinations were not conducted.

6. Urinalysis was not conducted.

7. Sacrifice and Pathology:

Range-Finding Studies - All animals were necropsied. Organ weights were determined for the brain, heart, lung, liver, spleen, kidneys, pancreas, and male gonads. All major organs were stated to be examined microscopically.

Two-Year Studies - All animals were necropsied unless their bodies were extensively autolyzed. Organ weights were determined for the liver, kidneys, brain, spleen, heart, lung, pancreas, and male gonads. Tissues were preserved in 10% formalin, embedded in paraffin, sectioned and stained with H&E. All major organs were stated to be examined microscopically.

Results:

a. Organ Weight:

Range-Finding Studies - First Study: Unremarkable. Second Study: The relative weights of the lungs were increased in males and females in the 3200 ppm group when compared to the low-dose group. Third Study: Unremarkable.

Two-Year Study - The absolute weight of the liver, spleen, brain, heart, lung, and gonads of males in the 800 ppm group were significantly (statistically) lower than the controls. The absolute weight of the liver of males in the 400 ppm group was lower than controls. A dose-related decrease in absolute and relative liver weight and gonad weight occurred in males. The relative weights of the spleen and lung were also decreased in the male 800 ppm groups. There was a statistically significant decrease in the absolute weight of the kidneys and pancreas of females in the 800 ppm group. The absolute weight of the liver of females in the 800 ppm group was lower

than the controls. The relative weight of the pancreas of females in the 800 ppm group was lower than the controls.

b. Gross Pathology:

Range-Finding Studies - First Study: Unremarkable. Second Study: Dark red discoloration of the lungs and intestinal hemorrhage was reported to be observed in animals that died prior to scheduled termination. Third Study: Not reported.

Two-Year Study - Reported in conjunction with microscopic pathology (see section below).

c. Microscopic Pathology:

1) Non-Neoplastic:

Range-Finding Studies - First Study: Unremarkable. Second Study: Pulmonary congestion and hemorrhage, and hemorrhage and necrosis of the duodenum and small intestine were observed in animals dying prior to scheduled termination. Surviving animals only exhibited pulmonary congestion. Third Study: Unremarkable.

Two-Year Study - Atrophy of the testes was increased in treated males. It occurred in 0, 5 and 4 males in the control, low-, and high-dose groups, respectively. Squamous-cell hyperplasia of the stomach was increased in treated males. It occurred in 0, 4, and 3 males in the control, low-, and high-dose groups, respectively. Pneumonia was increased in treated females. It was present in 10, 16, and 20 females in the control, low-, and high-dose groups, respectively. Squamous-cell hyperplasia of the stomach occurred in one female in the high-dose group. This lesion was not observed in the female control and low-dose groups. Uterine cysts were observed in 2, 2, and 5 females in the control, low-, and high-dose groups, respectively.

[It is unknown how many of the observations were based on macroscopic or microscopic observations.]

2) Neoplastic:

Two-Year Study - In Tables 10 and 11 Incidences of Neoplastic Lesions in Male/Female Mice Dosed with Dichlorvos for Two Years (see copies attached),

it was indicated that necropsies were conducted on 49, 47 and 47 males in the control low-, and high-dose groups, respectively, and on 41, 38 and 43 females in the control, low-, and high-dose groups, respectively.

Data on the occurrence of squamous-cell papilloma and carcinoma are listed in the table below:

Occurrence of Squamous-Cell Papilloma and Carcinoma of the Stomach in B6C3F1 Mice

	Control		LDT		HDT	
	Male	Female	Male	Female	Male	Female
Squamous-Cell Papilloma	0	2	1	1	1	0
Squamous-Cell Carcinoma	0	0	1	0	0	0

Data on the occurrence of malignant fibrous histiocytomas of the subcutis is provided in the table below:

Occurrence of Malignant Fibrous Histiocytoma of the Subcutis in Male and Female B6C3F1 Mice

	Control		LDT		HDT	
	Male	Female	Male	Female	Male	Female
Malignant Fibrous Histiocytoma	1	1	4	0	3	0

Data on the occurrence of thyomas in male mice are listed in the table below:

Occurrence of Thyoma in Male B6C3F1 Mice

	Control	LDT	HDT
Thyoma	0	4	1

[There is absolutely no indication of how many animals received histopathological examinations. It is noted that in the female groups many animals were so severely autolyzed that necropsy data could not be obtained. The question arises: Why were so many animals lost to autolysis if the

animals were observed daily? It is also noted that individual animal survival data, i.e., date of death or sacrifice, were not submitted as requested. Are the data available, missing or not recorded? These observations suggest that a rigorous schedule of daily observation of individual animals was not maintained. In Tables F1 and 2 Individual Animal Tumor Pathology of Male/Female Mice in the Two-Year Drinking Water Study of Dichlorvos: Control, Low and High Dose, no tissue information was submitted for several animals. No reason was provided. The only indication that certain animals, e.g., control male #035, low-dose male #100, control females #157, 160 and 180, low-dose females #219, 230, and 1249 and high-dose females #279 and 292, were actually on test was the animal number with the week of sacrifice. Were the tissues lost? Were the animals lost? These issues need to be addressed by the authors.]

D. Discussion:

In the 2-year study, survival was not affected by treatment. Body weight gains of treated mice were reduced when compared to controls. The response showed a dose-response relationship and males were more severely affected than females. Water intake was reduced from 25 to 60 percent in treated mice. There were several differences between the treated and control groups with respect to organ weights. Dose-related decreases in the absolute and relative weights of the liver and gonads occurred in treated males. Males in the high-dose group also exhibited decreases in the absolute and relative weight of the spleen and lung, and decrease in the absolute weight of heart and brain. The absolute and relative weight of the pancreas was decreased in females in the high-dose group. The relative weight of the pancreas was also decreased in females in the low-dose group. The absolute weight of the liver and kidneys was decreased in females in the high-dose group. There were greater numbers of treated males with atrophy of the testes and squamous-cell hyperplasia of the stomach than controls. The number of females with pneumonia was greater in the treated groups. Squamous-cell hyperplasia was present in one female in the high-dose group. There was an increase in the number of treated males with malignant fibrous histiocytoma of the subcutis. There was an increase in the number of males in the low-dose group with thymomas.

There were several problems encountered in the evaluation of this study. Following are several of the problems:

- Individual animal data were not provided.
- There were no records available on the observations of the animals throughout the study.
- There is no information indicating the number of animals that received a hirtopathological examination, thus preventing the calculation of the frequency in which lesions occurred.
- Many of the animals were severely autolyzed, thus cause of death due to the presence of lesions could not be established.
- For several animals, it was reported that no tissue information was submitted. This was not satisfactorily explained.
- It was indicated that tumor incidence was statistically analyzed. However, it was based on the number of animals necropsied and not on the number of animals that received a histopathological examination. Thus, the analysis is misleading.

If possible, additional histopathology should be conducted. At the very least, the authors should submit information on the number of animals/tissues that received a histopathological examination.

Core Classification: Supplementary

Justification of Classification:

The study is incomplete to such an extent that the information may be at best only suggestive of gross trends.

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Pages 12 through 14 are not included in this copy.

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Reviewed By: William B. Greear William B. Greear 6/22/89
Review Section II, Toxicology Branch I - IRS (H7509C)
Secondary Reviewer: Marion P. Copley, D.V.M. Marion Copley 9/27/89
Review Section II, Toxicology Branch I - IRS (H7509C)

007543

DATA EVALUATION REPORT

Study Type: Guideline Series 83-2 - Oncogenicity - Rat

TOX Chem No.: 338
MRID No.: 410418-02

Accession No.: N/A

Test Material: Dichlorvos

Synonyms: DDVP; Vapona; Oko; Dedevap; Herkol; Dimethyl 2,2-dichlorovinyl phosphate; 2,2-Dichlorovinyl dimethyl phosphate

Sponsor: Amvac Chemical Corporation

Testing Facility: Biosafety Research Center
Food, Drugs, and Pesticides
Shizuoka, Japan

Title of Report: An Assessment of the Carcinogenic Potential of Dichlorvos Administered in Drinking Water to Male and Female F344 Rats for Two Years.

Author: Makoto Enomoto

Study No.: N/A

Report Issued: March 12, 1989

Conclusions:

This is a 104-week feeding study with a 4-week recovery period.

The study report is incomplete to such an extent that the data should only be used to identify suggestive trends. The data provided on the occurrence of lesions has been incorrectly compiled, resulting in the improper tabulation of incidence data which is misleading. However, there appeared to be a marginal increase in the incidence of mononuclear cell and lymphocytic leukemia in treated males and a slight increase in the incidence of fibroadenoma of the mammary gland in females; nevertheless, the pathology data are not amenable to statistical analysis. Additional histopathology should be conducted and the actual incidence of histological lesions should be tabulated and statistically analyzed if possible.

Classification: Core-Supplementary

A. Materials:

1. Test Compound - Dichlorvos; Description: not provided; Lot No.: not provided; Purity: 97.26%; Contaminants: [redacted]

2. Test Animals - Species: Rat; Strain: F344; Age: 6 weeks;* Weight: males - 86 to 92 g, females - 74 to 85 g; Source: Charles River, Japan, Inc.

B. Study Design:

1. Animal Assignment - Animals were assigned to the following test groups:

	Test Group	Dose in Drinking Water (ppm)	Main Study 108 Weeks** Male	Main Study 108 Weeks** Female	6-Week Range-Finding Study Male	6-Week Range-Finding Study Female
1.	Control	0	51	51		
2.	Low (LDT)	140[1]	51	51		
3.	High (HDT)	280[2]	51	51		
1.	Control	0			10	10
2.	Low (LDT)	5***			10	10
3.		10***			10	10
4.		20***			10	10
5.		40***			10	10
6.	High (HDT)	80***			10	10

*Range-Finding Study Age: 7 weeks, Weight: males (106-135 g), females (80-95 g).
**104-Week exposure followed by a 4-week recovery period.
***Doses expressed as mg/kg/day.
[1]Calculated to be 8.3 and 10.4 mg/kg/day for males and females, respectively.
[2]Calculated to be 17.5 and 21.8 mg/kg/day for males and females, respectively.

2. Drinking Water Preparation - Daily doses were prepared by dissolving the appropriate amount of DDVP in 10 mL of tap water. In the main study, body weight and water intake were utilized to determine the amounts of DDVP to use to get a suitable volume of 10 mL/100 g body weight. In the range-finding study, it was determined that DDVP decomposed within 450 minutes, therefore, the rats were forced to consume 10 mL of the test solution within 4 to 5 hours. After that tap water was available ad libitum. A standard basal commercial diet (CE-2, CLEA) was available ad libitum.

Results - At the time the study was conducted, the stability of DDVP in tap water was not ascertained. However, the stability of DDVP in tap water has been determined by the Environmental Toxicology Laboratory, Nippon Soda Company, Odawara, Japan on November 14, 1988. It was reported that solutions containing 140 to 800 ppm of DDVP were allowed to stand for up to 6 hours at 23 ± 1 °C and the amount of DDVP recovered ranged from 90.6 to 100.9 percent. Therefore, the dosing solutions used by Dr. Enomoto were sufficiently stable with rats receiving approximately 140 and 280 ppm DDVP in dosing solutions.

3. Animal Maintenance - The animals were quarantined and acclimated to laboratory conditions for 1 week prior to testing. The animals were housed in groups of 2 or 3 in plastic cages in a room maintained at a temperature of 23 ± 3 °C and relative humidity of 55 ± 10 percent. Lighting was provided for a 12-hour on/12-hour off cycle. It was stated that the animals received "more than 10 times air refresh." Sterilized bedding was "refreshed" at 3-day intervals.

4. Statistics were not provided in the original report. However, in the rewritten report the following analyses were conducted.

   Survival Analyses - The probability of survival was estimated by the product-limit procedure of Kaplan and Meier (1958). Analysis for possible dose-related effects on survival used the method of Cox (1972) for testing two groups for equality and Tarone's (1978) life table test for a dose-related trend.

   [Note: The incidence of lesions was presented as the ratio of the number of animals bearing the lesion at a specific anatomic site to the number of animals necropsied and the site examined grossly and/or microscopically. This reviewer believes that the incidence of lesions as defined above is generally scientifically unsound. The amount/type of information obtained from a gross examination of tissues is quite different from the amount/type of information that is obtained from microscopic examination of tissues for most tissues - see Discussion section of the DER.]

   Statistical analysis of the incidence of lesions as defined above would appear to be improper.

5. Quality Assurance - not provided.

C. Methods and Results:

1. Observations - The animals were observed for clinical signs of toxicity and mortality. The frequency at which these observations were made was not stated in the main study. However, observations of animals for clinical signs of toxicity were made daily for the range-finding study.

Results:

Range-Finding Study - Two males and two females in the 80 mg/kg/day, one female in the 40 mg/kg/day, and one male in the 5 mg/kg/day groups died. Low activity and lacrimation were observed in rats in the 80 mg/kg/day group.

Main Study - The number of animals surviving up to week 104 is provided in the table below (abstracted from Table 4 of the report):

No. of Deaths/No. of Animals (Percent)

	Dose Level		
Sex	Control	Low	High
Male	43/51 (86%)	36/48 (75%)	37/48 (77%)
Female	42/49 (86%)	36/48 (75%)	41/51 (80%)

Survival was comparable among control and treated groups.

During the first 2 weeks of the study, the treated animals exhibited chromodacryorrhea, and females in the high-dose group had tremors and were hypersensitive to touch. During the last 3 months, the incidence and severity of lacrimation was increased in animals in the treated groups.

2. Body Weight - Weekly body weights were determined.

Results:

Range-Finding Study - Group mean body weights were reported for 10 rats/sex/group. Males and females in the 80 mg/kg/day group exhibited decreased body weight gain.

Main Study - During the last few months of the study, mean body weights of males in the high-dose group and females in the low- and high-dose groups were decreased when compared to controls (see Figure 1, attached). The data provided in Table 4 (mean body weight) appears to have been censored in that the number of animals in each

group reported for week 0 is not 51, the starting number of animals per group. The investigator should explain why the data were censored and indicate from which animals the data were deleted.

[It is noted that individual animal body weights, although requested, were not submitted. It is unknown whether this was an oversight or whether the data do not exist. This should be addressed.]

3. Food Consumption, Water, and Compound Intake - Food consumption and water intake were stated to be determined on a weekly basis.

   Results:

   Range-Finding Study - It was stated that animals in the 80 mg/kg/day group had decreased food and water intake; however, no data were provided.

   Main Study - No data were provided on food consumption and water intake as requested. Average compound intake data were provided for several weeks (28 intervals). The average daily dose was stated to be calculated as 8.3 and 17.5 mg/kg/day for males in the low- and high-dose groups and 10.4 and 21.8 mg/kg/day for females in the low- and high-dose groups based on water consumption and body weight.

4. Ophthalmological examinations were not conducted.

5. Hematology/Clinical Chemistry examinations were not conducted.

6. Urinalysis was not conducted.

7. Sacrifice and Pathology

   Range-Finding Study - At the end of 6 weeks, the animals were sacrificed and microscopically examined. Organ weights were determined for the brain, thymus, lung, heart, liver, spleen, adrenals, kidneys, testes, and ovaries. In addition to these organs, the thyroids, trachea, esophagus, salivary glands, pancreas, lymph nodes, stomach, and small and large intestines were histologically examined.

   Main Study - All animals that died and that were sacrificed on schedule were subject to gross pathological examination and the CHECKED (X) tissues were collected for histological examination. The (XX) organs in addition were weighed.

X	Digestive System
	Tongue
	Salivary glands*
X	Esophagus*
X	Stomach*
X	Duodenum
X	Jejunum*
X	Ileum*
X	Cecum*
X	Colon*
	Rectum*
XX	Liver*
	Gallbladder*
X	Pancreas
	Respiratory
X	Trachea*
XX	Lung*

X	Cardiovasc./Hemat.
	Aorta*
XX	Heart*
X	Bone marrow*
X	Lymph nodes*
XX	Spleen*
XX	Thymus*
	Urogenital
XX	Kidneys*
X	Urinary bladder*
XX	Testes*
	Epididymides
X	Prostate
	Seminal vesicle
XX	Ovaries
X	Uterus*

X	Neurologic
XX	Brain*
X	Periph. nerve
X	Spinal cord (3 level)
XX	Pituitary*
X	Eyes (optic n.)*
	Glandular
XX	Adrenals*
	Lacrimal gland
X	Mammary gland
	Parathyroids*
X	Thyroids*
	Other
X	Bone*
X	Skeletal muscle*
X	Skin
X	All gross lesions and masses
X	Harderian glands
X	Subcutis

The above tissues were histologically examined in all animals found dead and sacrificed in a moribund condition. The above tissues were also examined in 20 animals of the control and high-dose groups and in 10 animals of the low-dose group. It was stated that in addition, the liver, lung, kidney, and endocrine organs were examined in 10 males and 10 females in the low-dose group.

Results:

a. Organ Weight

Range-Finding Study - In the original submission, absolute organ weights were only provided for females. Data on male organ weights were provided in this submission. Females in the 80 mg/kg/day group displayed lower absolute organ weights than the control and remaining treatment groups. This was probably due to the large decrease in body weight at termination. Relative organ weights were calculated by this reviewer. There appeared to be increases in the relative weights of the brain, adrenals, and kidneys in the 80 mg/kg/day group. However, a dose-response was not apparent and it is doubtful that these differences are biologically significant. (In the initial submission both the relative and absolute weight of the lungs in the 80 mg/kg/day group were approximately one order of magnitude lower than the control and other test groups.

This error has been corrected.) Males in the 80 mg/kg day group exhibited decreases in the absolute weight of the lung, heart, brain, liver, kidney, thymus, and gonads. The relative weight of the brain was increased in males in the 80 mg/kg/day group. The organ weight changes did not appear to be dose-related and were only marginal changes.

Main Study - The absolute and relative weight of the liver was decreased in males in the high-dose group. [Individual animal organ weight data were not provided as requested.]

b. Gross Pathology

Range-Finding Study - Data were not provided; however, it was stated that there was no evidence of treatment-related changes.

Main Study - Data on macroscopic and microscopic lesions were combined; therefore, making an analysis of strictly macroscopic effects impossible.

c. Microscopic Pathology

1) Non-neoplastic

Range-Finding Study - No data were provided. However, it was stated that minimal interstitial cell pneumonia of the lung and calcification of the corticomedullary area of the kidney were observed in both control and treated animals and were not attributed to treatment.

Main Study - A major difficulty was encountered in the evaluation of the data. It was stated by J.H. Mennear in the report that "Both Drs. Enomoto and Konishi indicated that pathology focused primarily on tumors, therefore, nontumor pathology reporting is probably not complete." This statement certainly places a limitation on the usefulness of the nontumor pathology data presented in this report. Two examples of this problem follow:

- In Table E2 Summary of Non-neoplastic Lesions in Female Rats in the Two-Year Drinking Water Study of Dichlorvos, it was indicated that hyperplasia of pituitary chromophobe cells was slightly elevated in high dose females (control 4/49, 8%; low dose 2/42, 5%; high dose 7/51, 14%). In Table F2 Individual Animal Tumor Pathology of Female Rats in the Two-Year Drinking Water

Study of Dichlorvos: Control, Low Dose, High Dose it is shown that only 18, 14, and 16 females in the control, low-, and high-dose group had their pituitary gland examined microscopically, respectively. Hyperplasia of the chromophobe cells was only detected in those animals that received microscopic examination. The incidence of this lesion as presented by the author is incorrect as well as misleading. The incidence statistic for this specific lesion should be based solely on the number of animals that have undergone microscopic examination and should not include those animals that have only received gross necropsy. It is doubtful that hyperplasia of chromophobe cells of the pituitary could be diagnosed by merely conducting a gross necropsy. The incidence of this lesion would more properly have been displayed as 4/18 (22%), 2/14 (14%), and 7/16 (44%) in the female control, low-, and high-dose group, respectively. Tabulation of the incidence data in this manner indicates a possible effect in the high-dose group, which was masked by the way the data was presented by the authors in the report. This section of the report should be corrected by the author to provide proper incidence data on the occurrence of lesions in animals that have had their tissues examined microscopically.

- A second example is that for several tissues, e.g., stomach, liver, etc., the number of animals that have had a specific tissue site examined microscopically has not been reported in any table in the report. One example of this problem can be seen in the reporting of the incidence of hyperplasia of the Harderian gland. It was indicated that the incidence of this lesion was elevated in treated females (control 0; low dose 3/42, 7%; high dose 4/51, 8%). It is not indicated any place in the report how many rats had their Harderian gland examined microscopically. Because the Harderian gland is not listed in the protocol as one of the tissues that would be microscopically examined, one can only assume that the lesion was visible on macroscopic examination. Only then was the tissue (possibly) examined microscopically for confirmation of the gross diagnosis. Again, it is quite apparent that the data should be corrected to provide the incidence of the occurrence of each specific lesion in those animals that have undergone microscopic examination.

It was also noted that the author has deviated from the protocol. The protocol implies that the endocrine glands from 20 animals/sex in the low-dose group would be examined microscopically. According to Table F1, the number of males in the low-dose group that had their thyroid examined microscopically was 16, even though sufficient animals survived to termination to examine 20 microscopically. A second deviation from protocol exists. It was stated that all animals in the control and high-dose groups would receive a complete histological examination of certain tissues. In the case of the thyroid, the number of males in the control and high-dose group that had their thyroid examined was 22/49 (45%) and 13/49 (27%). This type of deviation from protocol exists for many other tissues, e.g., adrenal, pituitary, pancreas, mammary gland, kidney, etc.

2) Neoplastic

Main Study - [Refer to the problems discussed above, especially the deficient histopathology examination, because they also pertain to this area of the study.]

The total incidence of mononuclear cell leukemia and lymphocytic leukemia discovered in male and female rats are provided in the table below:

Incidence of Mononuclear Cell Leukemia and Lymphocytic Leukemia in Dichlorvos-Treated F344 Rats

	Control		Low		High	
	M	F	M	F	M	F
Mononuclear Cell Leukemia	3/51 (6%)	5/28 (18%)	7/48 (15%)	2/20 (10%)	6/48 (13%)	5/26 (19%)
Lymphocytic Leukemia	1/51 (2%)	0/28 (0%)	2/48 (4%)	2/20 (10%)	2/48 (4%)	0/26 (0%)
Mononuclear Cell and Lymphocytic Leukemia (Combined)	3/51 (6%)	5/28 (18%)	9/48 (19%)	2/20 (10%)	8/48 (17%)	0/26 (0%)

Note: There is an error in the data and probably in the statistical analysis that was conducted. In Table F1, the number of male rats in the control group with mononuclear cell and lymphocytic leukemia is 4 (animals 011, 026, 042, and 049).

The Fischer Exact test was p = 0.125 and p = 0.214 for mononuclear cell leukemia in males in the low and high dose, respectively, and p = 0.048 and p = 0.082 for mononuclear cell and lymphocytic leukemia (combined) in males in the low and high-dose group, respectively. (The analysis was based on the data presented in the table.) The reliability of the statistical analysis is questionable. The table indicates that 6 cases of mononuclear cell leukemia occurred in 6 of 48 males examined in the high-dose group. This is misleading. In the male high-dose group, males 026, 027, 028, 031, 036, 038, 039, 040, 041, 045, 046, 047, 049, and 050 did not receive any microscopic examination according to Table F1. The number of males in the high-dose group that had one or more tissues examined microscopically was 34. On perusal of Table F1 it is quite apparent that the microscopic examination for many of the 34 males was incomplete. It is questionable whether mononuclear cell leukemia could be reliably diagnosed by a mere gross necropsy examination. In summary, information on the incidence of mononuclear cell leukemia is incomplete and the statistical analysis of the data is incorrect as well as misleading.

The author has conducted a "special" analysis of the incidence of mononuclear cell leukemia in the spleens of males that were microscopically examined. The information is presented in the table below:

Mononuclear Cell Leukemia in Male F344 Rats in Which the Spleen was Examined Microscopically

	Control	Low	High
Mononuclear Cell Leukemia	3/27 (11%)	7/20 (35%)	6/26 (23%)
Fischer Exact test		p = 0.053	p = 0.214

The incidence of mammary gland tumors in animals that were examined microscopically was calculated from Tables F1 and F2 to be as follows:

Mammary Gland Tumors in Male and Female F344 Rats

	Control		Low (140 ppm)		High (280 ppm)	
	M	F	M	F	M	F
Fibroadenoma	1/4 (25%)	0/13 (0%)	*	0/1 (0%)	1/2 (50%)	3/13 (23%)
Adenoma	1/4 (25%)	0/13 (0%)		0/1 (0%)	0/2 (0%)	0/13 (0%)
Cystadenoma	0/4 (0%)	3/13 (23%)		0/1 (0%)	0/2 (0%)	1/13 (8%)
Adenocarcinoma	0/4 (0%)	1/13 (8%)		0/1 (0%)	0/2 (0%)	0/13 (0%)

*Mammary gland not examined microscopically.

The incidence data provided is poor due to incomplete histopathological examination. However, there was a slight increase in the occurrence of mammary gland fibroadenoma in females in the high-dose group.

Neoplastic lesions of the pancreas were not observed in 17 and 12 males in the control and high-dose group and 10 females in each of the control and high-dose groups that were microscopically examined.

Data were not provided on neoplastic lesions of the forestomach. However, hyperkeratosis of the esophagus (forestomach) occurred in 2, 0, and 1 males in the control, low-, and high-dose groups, respectively. No lesions of the esophagus/forestomach were detected in females. As usual, it is unknown how many animals underwent a histopathological examination of this tissue site.

It was reported that the incidence of chromophobe adenoma of the pituitary gland was increased in low-dose females. The incidence was reported as: control 3/49, 6%; low dose 6/42, 14%; and high dose 2/51; 4%. Based on the number of females that had their pituitary gland examined microscopically, the incidence is: control 3/18, 17%; low dose 6/14, 43%; and high dose 2/6, 13%. This is one more instance in which the data were improperly presented and statistically analyzed by the author.

It was stated that there was an increased incidence of bronchioalveolar adenoma of the lung in females in the high-dose group. This lesion occurred in 3 of 24 females (13%), microscopically examined in the high-dose group. There were no entries for the lung in the individual animal tumor data for the control and low-dose group.

It was noted that a female in the high-dose group (#051) was diagnosed to have a C-cell adenoma of the thyroid. This specific diagnosis is questionable since the tissue was not examined microscopically.

In Table F1 it was indicated that there were 47 males in the high-dose group with interstitial cell tumor of the testes. This is incorrect; there were 48 when tabulated correctly. In 18 of these males, the diagnosis was not confirmed microscopically.

It was also noted that there are duplicate sets of pages for pages 161-176.

The tables (Tables 7 and 10) on the incidence of neoplastic lesions are attached.

D. Discussion:

Survival was comparable among the control and treated groups. Animals in both treated groups exhibited chromodacryorrhea during the first 2 weeks of study. Females in the high-dose group had tremors and were hypersensitive to touch. During the last 3 months of the study, treated animals exhibited an increase in the incidence and severity of lacrimation. Mean body weight of males in the high-dose group and females in the low- and high-dose groups were lower than controls during the last few months of the study.

There was a decrease in the absolute and relative weight of the liver in males in the high-dose group. Data on the incidence of microscopic liver lesions could not be determined because the number of animals examined microscopically was not provided. It could not be determined if gross pathological changes occurred due to the manner in which data were presented. It was indicated that nontumor pathology reporting is probably not complete. It was indicated that the investigators primarily focused on tumor pathology. Therefore, this information is of limited reliability. There was an increased number of males and females in the high-dose group with ductal hyperplasia of the pancreas; however, the incidence and therefore its significance could not be definitively determined. There was a marginal increase in the number of males in the treated

groups with mononuclear cell leukemia and mononuclear cell leukemia and lymphocytic leukemia combined. Incidence data submitted on this tumor type are not complete because the histopathological examination was incomplete. Data provided on the incidence of mammary gland tumors in females are incomplete. Only mammary tissues taken from 13, 1, and 13 females in the control, low-, and high-dose groups were examined, respectively. The reported incidence of fibroadenoma in females was 0, 0, and 23 percent in the control, low, and high dose, respectively. There was no occurrence of pancreatic and forestomach neoplasms reported by the investigators.

In summary, the study is incomplete and the report is misleading. Tumor incidence data were obtained by grouping the animals which received a histopathological examination with those that received only a gross necropsy examination. Several errors in reporting the data were discovered. There were also incidences in which a tissue was reported to have a specific type of tumor although the tumor was not confirmed by microscopic examination. This study is of limited use. However, there appears to be a marginal increase in mononuclear cell and lymphocytic leukemia in male rats. In addition, there appeared to be a slight increased incidence of fibroadenoma of the mammary gland in female rats. These gross trends must be considered even though confidence in the depth of the histopathological examination is low.

It was stated that wet tissues, microscopic slides, and their corresponding tissue blocks are stored at the Agricultural Chemical Institute, Nitokuro, Hiro, Japan. It is recommended that a complete histopathological examination be conducted, the data be retabulated, and the actual incidence of histological lesions be provided.

E. Discrepancies:

1. It should be noted that for several groups, the number of animals per group used to calculate percent survival is not 51, the number of animals per group at initiation of the study. This difference was not addressed by the authors in the "Summary, Results, or Conclusion" section of the report. On page 158, it was stated that data on animal #019, 036, and 051 of the male low-dose group were not used in tumor or survival analyses because they were misdosed during the experiment. Yet it is stated that "Thereafter they were maintained at the proper dose levels and killed at scheduled termination." This requires explanation. During what time period were they misdosed? Were they misdosed for such a lengthy period that the data actually needed to be censored? In the male high-dose group, animal #011 and 012 died prior to termination and the animals were severely autolyzed.

This can probably be attributed to the absence of animal observations on the days surrounding the day of death. Animal #031 of the male high-dose group was misdosed and dropped from the analysis. Again, the reasons for censoring the data need to be addressed. In the female control group, animals #030 and 050 died prior to termination and the animals were severely autolyzed. The date of deaths was not recorded. Again, this can probably be attributed to the absence of animal observations on the days of death, days preceding death, or days following death. In the female low-dose group, animals #019, 020, and 021 were missing. The treatment of these data was not addressed.

2. An error exists. In Table F1 Individual Animal Tumor Pathology of Male Rats in the Two Year Drinking Water Study of Dichlorvos Low Dose, it indicated that 11 (not 12) males in the low-dose group died prior to week 104 out of 48 animals used in the analysis. Whereas in Table 4 Mean Body Weights and Survival of Rats in the Two-Year Drinking Water Studies of Dichlorvos, 36/48 males in the low-dose group survived to Week 104, i.e., 12 deaths occurred prior to Week 104.

3. Discrepancies exist among Table 7 Incidences of Neoplastic Lesions in Male Rats Dosed with Dichlorvos for Two Years and Table E1 Summary of Nonneoplastic Lesions in Male Rats in the Two Year Drinking Water Study of Dichlorvos and Table F1 Individual Tumor Pathology of Male Rats in the Two Year Drinking Water Study of Dichlorvos: Low Dose. In Table 7 it was indicated that 48 males in the low-dose group were necropsied, in Table E1 it was indicated that 50 low-dose males were necropsied, and in Table F1 it was indicated that the lungs and bronchi of 51 low-dose males were examined. <u>This discrepancy requires explanation</u>.

4. It was noted that in Table F2 pages 171 and 172 are duplicates with the exception that in one table the total number of thyroid tissues examined was 45 on page 171 but was 47 on page 172. Page 172 appears to be correct.

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F. Deficiencies

1. The animals were not given a complete histopathology examination.

2. The method of getting the animals to drink the dosing solutions prior to decomposition of dichlorvos was not explained.

3. Individual animal data on food and water consumption were not reported.

4. The method for calculating the average daily intake of dichlorvos was not adequately described.

5. Individual animal organ and body weight data were not reported.

6. The method in which the incidence of tumors was statistically analysed was improper.

7. There was incomplete reporting of non-tumor pathology.

8. There were numerous deviations from the histopathology protocol.

9. No quality assurance statement was included in the report.

Core Classification: Supplementary

Justification of Classification:

The study is incomplete to such an extent that the information may be at best only suggestive of gross trends.

R:53709:Greear:LCBI-7:KENCO:05/31/89:06/09/89:DD:vo:rw
R:53715:Greear:LCBI-7:KENCO:06/21/89:10/27/89:DD:GJ:VO:DD

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