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Carcinogenesis Studies of Dichlorvos in Fischer Rats and B6C3F1 Mice

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Dichlorvos (dichlorovinyl dimethyl phosphoric acid ester) is a cholinesterase inhibitor used widely as a contact and stomach insecticide for control of internal and external parasites. Carcinogenesis studies were conducted by administering dichlorvos in corn oil by gavage 5 times a week for 103 weeks to groups of 50 male and 50 female Fischer rats at 0, 4, or 8 mg/kg body weight, to groups of 50 male B6C3F1 mice at 0, 10, or 20 mg/kg, and to groups of 50 female B6C3F1 mice at 0, 20, or 40 mg/kg. During the course of the studies, body weights and survival rates of the male and female rats and mice were not different from those of their respective controls; females of both species appeared to gain more weight than controls. Neoplasms induced by dichlorvos included adenomas of the exocrine pancreas (male rats), mononuclear cell leukemia (male rats), and squamous cell papilloma of the forestomach (male and female mice; two other female mice had squamous cell carcinomas). Lesions observed in female rats that may have been due to dichlorvos administration included adenomas of the exocrine pancreas and fibroadenomas of the mammary gland. The results demonstrated that dichlorvos is carcinogenic for Fischer rats and B6C3F1 mice.

Key words: Dichlorvos — Carcinogenesis — Rats —Mice

Dichlorvos (dichlorovinyl dimethyl phosphoric acid ester, DDVP), a volatile liquid vinyl ester of phosphoric acid with anticholinesterase activity, is available in emulsifiable and oil-soluble concentrates, aerosols, granules, baits, and impregnated resin strips. As a contact and stomach insecticide dichlorvos is used for control of internal and external parasites in livestock, in flea collars for household pets, in indoor and outdoor areas to kill insects, and in domestic animals and humans as an anthelmintic. Due to its high vapor pressure it is especially effective in closed areas.[1)]

Dichlorvos is readily absorbed by inhalation, oral, or percutaneous exposure. This organophosphate insecticide is metabolized mainly in the liver to dimethyl phosphate and dichloroacetaldehyde, which are further degraded to dichloroacetic acid, dichloroethanol, dimethylphosphoric acid, and other water-soluble metabolites.[1–4)] The biologic half-life is rapid, and toxic effects are likely to be influenced by the metabolic products in addition to dichlorvos.

Dichlorvos has been shown to be an alkylating agent by the NBP[4] test.[5)] The insecticide induced gene mutations in *Salmonella typhimurium* strains TA100 and TA-1535, *Serratia marcescens*, *Escherichia coli* WP2, and *Saccharomyces cerevisiae* in the absence of rat liver S9.[6)] The mutagenic responses to dichlorvos were reduced in both bacterial and fungal systems in the presence of liver S9. However, using 30% male Syrian hamster or male Sprague-Dawley rat S9 mixes a more comparable mutagenic response was observed in TA100[7)]; dichlorvos was not mutagenic for TA98 with or without S9.[7,8)] In mammalian cell systems, dichlorvos induced increases in sister chromatid exchanges and chromosome aberrations in Chinese hamster ovary cells[8,9)] and lung fibroblasts,[10)] polyploidy in Chinese hamster V79 cells,[11)] and mutations in mouse lymphoma cells.[8)] Metabolites of dichlorvos such as desmethyldichlorvos, dimethyl phosphate, dichloroethanol, dichloroacetaldehyde, and dichloroacetic acid were negative in NBP tests,[5)] whereas dichloroacetaldehyde was positive in base-pair substitution *Salmonella* tests[12)]; not all have been adequately evaluated as yet in *Salmonella*.

In *in vivo* mutagenicity studies, dichlorvos, was negative in sex-linked lethal mutations in *Drosophila* Muller-5 and crossing over tests,[13)] in host-mediated tests with *Saccharomyces cerevisiae* in mice, and in tests for micronuclei, gene mutation, dominant lethals[14)] and chromosome aberrations in bone marrow cells and spermatocytes in mice.[15–18)] Other investigators reported that dichlorvos induced sperm abnormalities,[19)] depletion of testicular germinal epithelium,[20)] and dominant lethal mutations[21)] in mice and chromosomal aberrations in bone marrow cells of Syrian hamsters.[22)] After acute intoxication by dichlorvos, increased chromosome damage was found in human blood cells.[23)] Thus, dichlorvos has been shown to be mutagenic in a wide variety of *in vitro* systems,[8)] with more mixed results *in vivo*.

Previous carcinogenesis studies in rats of dichlorvos administered by inhalation at 4.7 mg/m^3 [24)] and in the

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[4] The abbreviations used are: NBP, nitrobenzylpyridine; NCI, National Cancer Institute; IARC, International Agency for Research on Cancer; NTP, National Toxicology Program.

diet at 234 mg/kg[25] did not report any carcinogenic effect. In studies using male and female Osborne-Mendel rats and B6C3F1 mice, diets containing 150–635 ppm dichlorvos also did not induce any statistically significant increases in tumor incidences.[26] However, in the dichlorvos-exposed mice biologically important lesions were observed in the esophagus: 2 squamous-cell carcinomas, 1 squamous cell papilloma, and 3 cases of focal hyperplasia of the esophageal epithelium.[26] These histopathologic findings were considered unusual.[6] According to an IARC Working Group, "the available data do not allow an evaluation of the carcinogenicity of dichlorvos to be made"[6]; this same conclusion of "inadequate evidence" was reached by another IARC Working Group in 1987.[27] Bremmer *et al.*[28] recorded and interpreted the available mutagenesis and carcinogenesis data (including our preliminary findings[8]; note: the incidence data given in their paper do not correspond with our histopathology findings) and concluded that "dichlorvos does not present a carcinogenic or mutagenic risk for man." Different views on the carcinogenicity data have been published[6, 27, 29, 30]; most indicate that the earlier studies were inadequate,[6, 27] flawed,[30] inadequately reported or unpublished, or showed unequivocal carcinogenicity.[29, 30] According to the EPA (using all the available data) "dichlorvos has been classified as a carcinogen based on oncogenic effects in mice and rats."[30]

Thus, the present studies were designed, undertaken, and are reported here in an attempt to better define the carcinogenic potential of dichlorvos.

MATERIALS AND METHODS

Dichlorvos, obtained from Shell Development Company (Houston, TX), was analyzed by infrared, ultraviolet/visible, and nuclear magnetic resonance spectroscopy to establish its chemical identity. Examinations by elemental analysis, water analysis, thin-layer chromatography, and gas chromatography showed that the chemical was 99% pure (further details are available in Ref. 8).

Male and female F344/N rats and B6C3F1 mice, 4–5 weeks old, were obtained from Charles River Laboratories (Portage, MI). They were housed in groups of five in polycarbonate cages with heat-treated hardwood chips bedding (Beta Chips, Northeastern Products Corp., Warrensburg, NY) in a room with controlled temperature (22°C ± 1°C), humidity (50% ± 10%), and light (12 h). Food (NIH 07 open formula natural ingredient rat and mouse ration) and water were given *ad libitum*. After a 3-week observation period the animals were divided randomly into groups of 50/species/sex. Using data, results, and evaluative findings from shorter-term (13-week) experiments,[8] the long-term (2-year) studies were designed. Male and female rats were given dichlorvos in corn oil by gastric intubation at 0, 4, or 8 mg/kg body weight, male mice at 0, 10, or 20 mg/kg, and female mice at 0, 20, or 40 mg/kg, 5 times a week for 103 weeks. The lower doses selected for male mice compared to female mice were chosen because dichlorvos at 80 mg/kg killed 50% of males and only 10% of females in the 13-week studies. The gavage route of administration was chosen because this permits the most accurate knowledge of the amount of chemical given, because dichlorvos has been shown to be unstable when mixed in feed or in water[8] or in air, and because metabolic pathways of dichlorvos given to rats orally or by inhalation are similar.[31] The animals were observed two times per day. Body weights were recorded once per week for the first 14 weeks of the studies and once every 4 weeks thereafter. Animals were killed and necropsied at 104 weeks, or when found dead or moribund. At necropsy, all organs and tissues were examined for grossly visible lesions. Tissues were preserved in 10% neutral buffered formalin, trimmed, embedded in paraffin, sectioned, stained with hematoxylin and eosin, and examined microscopically for histopathological changes. Only incidences of lesions that are significantly different between control and dosed groups are reported.

Survival analyses were performed according to the method described by Cox[32] and Tarone.[33] Tumor incidences were analyzed by survival-adjusted statistical methods described by Haseman,[34] Dinse and Haseman,[35] and Gart *et al.*[36] These included logistic regression analysis (appropriate for incidental tumors) and life table tests (appropriate for neoplasms considered to be associated with cause of death).

RESULTS

Rat studies Mean body weights and survival of dichlorvos-treated male and female rats were comparable to those of their respective controls throughout the study. At the end of the 2-year studies the mean body weights in the control, low dose, and high dose groups of male rats were 462 g, 457 g, and 446 g, and those of female rats were 327 g, 349 g, and 335 g; from the groups of 50, the numbers of surviving males were 31, 25, 24, and females were 31, 26, and 26, respectively.

Dichlorvos-associated neoplasms in rats were considered to be in the pancreas, hematopoietic system, and mammary gland. In the dichlorvos-exposed male and female rats, adenomas of the exocrine pancreas were increased compared with those in the respective controls (Table I). Most exposed male rats with adenoma of the pancreas had multiple adenomas (36% in controls versus 60–73% in treated groups) whereas all but one female had single adenomas. The increase in incidence of acinar

Table I. Lesions of the Acinar (Exocrine) Pancreas, Mononuclear Cell Leukemia, Mammary Gland in Rats Administered Dichlorvos by Gavage for Two Years

	Vehicle control	4 mg/kg	8 mg/kg
Male rats			
Number examined histopathologically[a]	50	49	50
Pancreas acinar atrophy	17	14	18
Acinar hyperplasia	37	45[b]	39
Acinar adenoma (single)	16	8	13
(multiple)	9	22[b]	20[b]
Acinar adenoma (total)	25	30[b]	33[b]
Mononuclear cell leukemia	11	20[c]	21[c]
Female rats			
Number examined histopathologically[a]	50	48	50
Pancreas acinar atrophy	5	6	15[b]
Acinar hyperplasia	21	23	30
Acinar adenoma (single)	2	3	5
(multiple)	0	0	1
Acinar adenoma (total)	2	3	6
Mononuclear cell leukemia	17	21	23
Mammary gland fibroadenoma	9	19[b]	17[b, d]
Multiple fibroadenoma	0	6[b]	3
Carcinoma	2	2	0
Mammary neoplasms (total)	11	20[c]	17

a) Numbers less than 50 were due to autolysis or cannibalism.
b) $P<0.05$.
c) $P<0.02$.
d) Including 1 rat having mammary gland adenoma.

adenoma in female rats was not statistically significant; yet the increase was three times that in concurrent controls. Hyperplasia was also increased in the low dose group of males. Hyperplasia and adenomas of the exocrine pancreas are considered part of a morphologic continuum, and some [37] therefore propose the option of combining preneoplastic and neoplastic lesions for evaluation. If these lesions had been combined, greater differences would have been seen between control and exposed rats, and females would have a much lesser occurrence than do male F344 rats.[37] Adenomas are distinguished from hyperplasia by greater heterogeneity in growth pattern, loss of normal acinar structure, and larger size of affected area. Pancreatic atrophy was increased in the high dose female rats; the lesions were focal and generally minimal in severity.

Increased incidences of mononuclear cell leukemia was observed in dosed male and female rats (Table I). In the male rats the increase in incidence was dose-related and statistically significant. For female rats the marginal numerical increase was not statistically significant (trend = 0.08 and pairwise comparison = 0.1).

The incidence of mammary gland fibroadenomas in the dichlorvos-treated female rats was significantly greater than that in the controls (Table I). Incidences of multiple fibroadenomas were seen in 9 exposed female rats whereas none were observed in the controls. No increase was observed in malignant mammary gland neoplasms. When all mammary gland neoplasms were considered appropriately together,[27, 38] i.e., fibroadenoma, adenoma, and carcinoma combined, the incidence in the 4 mg/kg dose group was significantly increased compared with that in vehicle controls, the increase in the 8 mg/kg group was not statistically increased compared to controls, and the trend was marginal ($P=0.07$).

Noteworthy nonneoplastic effects included moderately increased incidences of cytoplasmic vacuolization of the liver (male rats: controls, 7/50; low dose, 13/50; high dose, 19/50, $P<0.01$), multifocal atrophy of hepatocytes (female rats: 5/50; 12/50, $P<0.05$; 11/50), and cytoplasmic vacuolization of the adrenal cortex (male: 3/50; 8/50; 13/50, $P<0.01$; female: 9/50; 17/50, $P<0.05$; 12/50).

Mouse studies Mean body weights of dosed and vehicle control male and female mice were generally comparable throughout the studies. There were no significant differences in survival between any groups of either sex. At the end of the 2-year studies the average body weights of males in the control, low dose, and high dose groups were 44.2 g, 44.3 g, and 44.4 g and of females 39.4 g, 40.7 g,

Table II. Incidence of Forestomach Lesions in Mice in the Two-year Gavage Studies of Dichlorvos

	Vehicle control	Low dose	High dose
Male		10 mg/kg	20 mg/kg
Number examined histopathologically	50	50	50
Hyperplasia	11	5	9
Squamous cell papilloma	1	1	5
Female		20 mg/kg	40 mg/kg
Number examined histopathologically[a)]	49	49	50
Hyperplasia	6	7	5
Squamous cell papilloma	5	6	18[b)]
Carcinoma	0	0	2
Papilloma or carcinoma	5	6	19[b)]

a) Numbers less than 50 were due to autolysis or cannibalism.
$P<0.01$.

and 43.4 g; the numbers of surviving males were 35, 27, 29 and females 25, 29, 34, respectively.

Neoplasms associated with dichlorvos exposure in mice were restricted to the forestomach. Squamous cell papillomas of the forestomach were observed in 5 high dose male and in 18 high dose female mice compared with 1 in male and 5 in female controls (Table II). The relatively high incidence of papillomas seen in the control female mice is atypical, and the biologic explanation is not known. Forestomach carcinomas were also observed in 2 high dose female mice. The dichlorvos exposure concentrations in males were one-half that given to female mice, and may explain the difference observed between the sexes. Incidences of forestomach hyperplasia were not increased in the dichlorvos-exposed mice (Table II). The squamous cell papillomas were characterized by pedunculated branching structure consisting of a central core of connective tissue covered by thick stratified squamous epithelium. Some papillomas were sessile with elongated rete pegs rather than the typical branching pattern. These lesions are not typically seen in vehicle control male (23/1703; $1\% \pm 2\%$) or female mice (16/1709; $0.9\% \pm 2\%$) in NTP studies. No squamous cell carcinomas have been observed in corn oil vehicle control female B6C3F1 mice in NTP studies.

In female mice two commonly occurring site-specific tumors showed marginal decreases ($P<0.05$) in incidence. These were in the pars distalis of the pituitary galnd (12/45, one had a carcinoma; 6/45; 6/44) and lymphoma (16/50; 11/50; 9/50).

DISCUSSION

In the present studies, body weights and survival rates of male and female Fischer rats and of male and female B6C3F1 mice were not different from those of their respective controls. From the data it appeared that the male and female rats and mice could have been given higher doses of dichlorvos than the ones used in these studies. Nonetheless, given that both nonneoplastic and neoplastic lesions were induced by dichlorvos these long-term studies are considered appropriate for indicating that dichlorvos is carcinogenic to experimental animals.

In this study the incidence of pancreatic adenomas in the male vehicle control group was 50%. This incidence was greater than the historical incidence of 9% at the laboratory,[8)] the overall NTP historical incidence of 6%,[39)] and a retrospective evaluation incidence of 37%.[40)] The reason for the high vehicle control incidence was clearly related to the sampling method used. In the present study one cross section and one horizontal section of the pancreas were examined whereas commonly in other studies only one cross section was examined. Thus, a significantly larger area of the pancreas was examined in the present study than in the other NTP studies. Nonetheless, in the male rats given dichlorvos in corn oil both single and multiple adenomas occurred at a significant higher rate than in the vehicle controls. In the dichlorvos-treated female rats, although the increase over the controls was marginal (Table I), the increase is considered of biological importance and supportive of the findings in male rats.

Male F344 rats are more sensitive to exocrine pancreas adenoma development than female F344 rats and corn oil appears to enhance the incidence of exocrine pancreas adenomas in both sexes. In a retrospective histopathologic evaluation of nearly 800 pancreata the numbers of rats with adenoma of the exocrine pancreas was greater in the corn oil controls (male, 73/195, 37.4%; female, 9/197, 4.6%) compared to the untreated controls (male 28/193, 14.5%; female, 2/199, 1.0%).[37)] The mechanism of action of corn oil in pancreatic acinar cell

carcinogenesis in F344 rats remains unknown, yet Haseman *et al.*[39] and Eustis and Boorman[40] reported that body weight, laboratory, animal source, and the brand, lot, or peroxide level of corn oil had no bearing on the enhancement of pancreatic adenoma incidence by corn oil in F344 rats. In the induction of exocrine pancreatic adenomas by dichlorvos in corn oil, it remains unknown whether a synergistic effect was exerted.

Exocrine pancreatic cell atrophy also was observed in both vehicle control and dosed male and female rats, and the incidence was statistically greater in high dose female rats than in vehicle controls. The atrophy in dosed female rats was typical of that occurring naturally in untreated rats, and it is uncertain how the increased incidence is related to dichlorvos.

Mononuclear cell leukemia occurs in F344 rats.[41,42] As Haseman *et al.*[39] reported, the major association with corn oil administration was a lower ($P<0.001$) incidence of mononuclear cell leukemia in male F344 rats. In the present studies, the control and dichlorvos-exposed male rats received equal volumes of corn oil. Thus, despite the negative association,[39] increases were observed in both dose groups of male rats compared to controls. Dieter *et al.*,[43] using a transplantable mononuclear cell leukemia model, demonstrated that oral administration of dichlorvos to host male Fischer rats stimulated the growth of transplanted leukemic cells, supporting the finding of the present study. Dichlorvos had no effect on the incidence of mononuclear cell leukemia in female rats.

The incidences of mammary gland fibroadenomas in the dichlorvos-exposed female rats were increased compared to concurrent controls. Importantly, multiple fibroadenomas of the mammary gland were observed only in those given dichlorvos (Table I). When mammary gland neoplasms were evaluated together, only the incidence in the low dose group remained statistically greater than that in the vehicle controls. This lack of a dose-response relationship in the absence of any survival difference weakens the biologic importance of this finding. Further, the female controls had a slightly lower incidence (22%) than usually observed at this laboratory (31%) or across laboratories (28%).

In mice, squamous cell neoplasms of the forestomach occurred more frequently in both dichlorvos-exposed male and female mice than in controls; the incidence in high dose (20 mg/kg) male mice was somewhat greater than that in vehicle controls, whereas the incidence in the high dose (40 mg/kg) female mice was greatly increased compared with vehicle controls. Perhaps the observed difference for forestomach lesions between male and female mice can be explained logically by the fact that males were given only one-half the amount of dichlorvos administered to the females. Historically, this is an uncommon lesion in mice, occurring on average in only one corn oil control mouse for every two studies. The control male mice in these studies imitated the historic controls whereas the group of female mice had an incidence of 10%, outside the range at this laboratory (0–4%) or across studies (0–8%). Significantly two 40 mg/kg female mice had squamous cell carcinomas of the forestomach, a malignant lesion yet to be observed in a control female mouse.

Body weight and survival data of the study showed that the male mice might have been able to accept a dose level of 40 mg/kg without any deleterious effects on body weight or survival. There is the likelihood that 40 mg/kg dichlorvos would have induced a higher incidence of squamous cell neoplasms of the forestomach in male mice, as was seen in the female mice. In an earlier carcinogenesis study[26] dichlorvos administered in the diet at 318 or 635 ppm (41 or 81 mg/kg per day) for 78 weeks did not induce significantly greater incidences of neoplasms in male and female B6C3F1 mice than in controls. In those feed experiments dichlorvos exposure was discontinued for the last 30 weeks of the study,[26] and if one considers that mouse skin papillomas[44] and neoplastic lesions of rat forestomach[45] have been shown to regress after cessation of treatment, then one could speculate that squamous cell papillomas of mouse forestomach could do likewise. This is not entirely consistent, however, with the 3 cases of focal hyperplasia of the esophageal epithelium, as well as 3 uncommonly occurring squamous cell neoplasms (1 papilloma and 2 carcinomas) observed in dichlorvos-feed exposed mice.[26] A further complicating factor is that dichlorvos is not stable in the rodent diet.

The lesions of the esophagus and of the forestomach in these two experiments are biologically comparable given that the squamous cell epithelial lining these two organs is continuous,[46] the tumor types are the same, and for the former the feed exposure route would allow more consistent contact with the esophagus, whereas for the latter the gastric intubation route permits greater concentrated action.

Altogether, these studies demonstrate that dichlorvos may be a direct-acting carcinogen effective at the site of application in B6C3F1 mice. The direct-acting carcinogenic effect of dichlorvos is supported by the mutagenic effects on bacterial and mammalian cells *in vitro*, since the addition of liver S9 to the cultures diminished to some extent the mutagenic effect.[8] However, in rats the effects were distant from the dichlorvos application site, involving pancreas acinar adenomas, mononuclear cell leukemia, and mammary gland fibroadenomas. Since these tumors occur spontaneously in Fischer rats dichlorvos might have promoted the expression of these tumors. In the understanding of the mechanisms of multi-stage processes of carcinogenesis,

the term "promotion" has no meaning. It describes an experimental phenomenon, not mechanisms of carcinogenesis.[47] Promoters such as 12-O-tetradecanoylphorbol-13-acetate and benzoyl peroxide, like many carcinogens, have been shown to involve in one or more stages of the multistage carcinogenic processes which include DNA damage, chromosomal abnormalities, gene rearrangements, oncogen activation, disorders of differentiation, disruption of DNA repair systems, etc. That is why promoters are considered essentially weak carcinogens.

Dichlorvos has been shown to exhibit activities typical of a carcinogen, i.e., alkylating, mutagenic, and clastogenic. As its half-life *in vivo* is short, the effective dose of dichlorvos reaching the target tissues could be small. This may be hypothesized to explain why its carcinogenic effect is expressed in tissues with high spontaneous tumor incidence in rats. The mutagenic, clastogenic, and carcinogenic actions of dichlorvos probably involve alkylation of DNA or protein either by the methyl group or by the phosphoryl center with the dichlorovinyl moiety, as methylation of biologic macromolecules has been demonstrated in both *in vitro* and *in vivo* studies with dichlorvos.[12, 48-54] Involvement of the nucleus of the oxo acid of phosphorus in mononuclear cell leukemogenesis was postulated by Dieter *et al.*[43] Mutagenicity studies of desmethyldichlorvos, dichloroacetaldehyde, monomethyl phosphate, dimethyl phosphate, dichloroethanol, and dichloroacetic acid are being conducted to shed further light on the possible mechanism(s) involved. In summary, dichlorvos caused or was associated with neoplastic responses in rats (pancreas, hematopoietic system, and possibly the mammary gland) and in mice (forestomach).

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