Lung Neoplasms in Rodents after Chronic Administration of Dimethyl Hydrogen Phosphite


ABSTRACT

Dimethyl hydrogen phosphite (DMHP), an intermediate in the production of insecticides or herbicides, was administered by p.o. gavage for 2 yr to male Fischer 344/N rats and male and female B6C3F1 mice at doses of 0, 100, or 200 mg/kg and to female Fischer 344/N rats at doses of 0, 50 or 100 mg/kg. Dose related toxicity was seen in the lungs of treated male and female rats. The lung lesions were most prevalent in the high dose male rat group which received a dose twice that given to the high dose female rats. Lung lesions included alveolar epithelial hyperplasia, chemically related pneumonia, alveolar-bronchiolar adenoma, alveolar-bronchiolar carcinoma, and squamous cell carcinoma. DMHP also caused neoplastic and nonneoplastic lesions of the forestomach in male rats; a similar but less pronounced effect was observed in female rats. Nonneoplastic lesions associated with administration of DMHP included mineralization of the cerebellum in male rat and focal calcification of the testis in male mice. Under the conditions of this study, there was clear evidence for carcinogenicity for male rats, equivocal evidence for carcinogenicity in female rats, and no evidence for carcinogenicity in either male or female mice. DMHP caused the highest incidence of lung tumors in the male rat of all chemicals studied to date in the National Cancer Institute-National Toxicology Program Carcinogenesis Testing Program.

INTRODUCTION

DMHP2 was one of several compounds which was nominated in 1976 to the National Toxicology Program by the US Armed Forces for toxicology and carcinogenesis studies. These compounds were being considered for use in experimental situations as simulants to mimic the physical properties but not the neurotoxic properties of anticholinesterase agents (nerve gas). Nerve gas simulants are used in the military to test equipment including facemasks and chemical detection systems; DMHP is no longer being considered as a candidate simulant (1). DMHP is used as an intermediate in the production of insecticides and herbicides, as an additive to lubricants, and as a stabilizer in oil and plaster (2, 3). There is little or no information available in the literature on the mutagenicity, toxicity, or carcinogenicity of DMHP. This study was performed to evaluate the toxicity and carcinogenicity of DMHP given p.o. to Fischer 344/N rats or B6C3F1 mice for 2 yr.

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1To whom requests for reprints should be addressed. The National Toxicology Program Technical Report on Dimethyl Hydrogen Phosphite may also be obtained by writing to: Public Information, National Toxicology Program, P. O. Box 12233, Research Triangle Park, NC 27709.
2The abbreviations used are: DMHP, dimethyl hydrogen phosphite; NCI, National Cancer Institute; NTP, National Toxicology Program.

MATERIALS AND METHODS

Animals. Male and female Fischer 344/N rats and B6C3F1 mice were obtained from Charles River Breeding Laboratories, Portage, MI. At the start of the chronic study, rats were 7 wk old and mice were 6–8 wk old. Within each species and sex, animals were allocated to cages and dose groups using a table of random numbers. The animals were housed by species and sex, 5/cage in solid bottom polycarbonate cages provided with hardwood chips (P. J. Murphy Forest Products Corp., Rochelle Park, NJ). The cages were covered with polyester filters (Snow Filtration, Cincinnati, OH). Tap water and NIH 07 Open Formula (Zeigier Brothers, Gardners, PA) were available ad libitum. The animals were maintained in a room that was kept at 22–24°C with a humidity of 30–70% and with 12–15 room air changes/h. A 12-h fluorescent light cycle was used throughout the experiment. All animals were checked twice daily for morbidity and mortality. Moribund animals were killed and necropsied. Clinical signs and body weights by animal were recorded every 4 wk.

Chemicals. Dimethyl hydrogen phosphite (lot KCO31247) was supplied by the US Army Chemical Systems Laboratory, Aberdeen, MD. The chemical was found to be 97–98% pure based on elemental analysis (4). The infrared or nuclear magnetic resonance spectra were consistent with literature spectra (4). Trimethyl phosphate was found to be a contaminant (1%).

Animals were randomized into treatment groups of 50 each for controls, low dose and high dose. DMHP was administered by gavage 5 days/wk for 103 wk at doses of 0, 100, or 200 mg/kg to male rats and male and female mice and 0, 50, or 100 mg/kg to female rats. Control animals received corn oil alone. The doses for this chronic study were based on a previous 13-wk study (4).

Pathology. Complete necropsies were done on all animals, unless precluded by autolysis or cannibalism. Thus the number of organs or tissues examined microscopically may have varied and may not necessarily have equaled the number of animals started on the study. Tissues were preserved in 10% neutral buffered formalin, embedded in paraffin, sectioned, and stained with hematoxylin and eosin. The following tissues were examined microscopically: tissue masses; skin; mesenteric and mandibular lymph nodes; mammary gland; salivary gland; thyroid; parathyroid; esophagus; stomach; duodenum; jejunum; ileum; colon; cecum; rectum; liver; gallbladder (mice); pancreas; spleen; kidneys; adrenals; urinary bladder; seminal vesicles-prostate-testes or ovaries-uterus; nasal cavity; brain; pituitary; eyes; and spinal cord.

Statistical Methods. Differences in survival were analyzed by life table methods (5). For the analysis of tumor incidence data, two different procedures were used to assess dose-response trends and to make pairwise comparisons between dose groups and controls: (a) life table analysis (appropriate for fatal tumors) and (b) Peto's (6) incidental tumor test (appropriate for tumors observed at necropsy in animals dying from an unrelated cause). Except where noted, results reported as "significant" are significant (P < 0.05) by both procedures. The multiple comparison procedures of Williams (7, 8) and Dunnett (9) were used to assess the statistical significance of differences in body weights.
LUNG NEOPLASMS IN RODENTS

Table 1
Survival and mean body weight of rats and mice in the 2-yr study of dimethyl hydrogen phosphate

<table>
<thead>
<tr>
<th>Survival and mean ± SE body wt (g)</th>
<th>Initial</th>
<th>6</th>
<th>12</th>
<th>18</th>
<th>24</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Dose (mg/kg)</strong></td>
<td><strong>No. surviving</strong></td>
<td>50</td>
<td>50</td>
<td>48</td>
<td>45</td>
</tr>
<tr>
<td><strong>Male rats</strong></td>
<td><strong>Vehicle control</strong></td>
<td>139.2 ± 2.4</td>
<td>391.4 ± 2.8</td>
<td>467.8 ± 3.4</td>
<td>494.2 ± 4.9</td>
</tr>
<tr>
<td>100</td>
<td>142.8 ± 2.8</td>
<td>391.0 ± 2.8</td>
<td>461.2 ± 3.2</td>
<td>477.4 ± 4.2</td>
<td>29</td>
</tr>
<tr>
<td>200</td>
<td>139.4 ± 2.9</td>
<td>376.9 ± 4.1</td>
<td>419.1 ± 4.6</td>
<td>432.2 ± 4.5</td>
<td>23</td>
</tr>
<tr>
<td><strong>Female rats</strong></td>
<td><strong>Vehicle control</strong></td>
<td>110.7 ± 1.5</td>
<td>215.6 ± 1.7</td>
<td>251.6 ± 2.8</td>
<td>292.6 ± 3.8</td>
</tr>
<tr>
<td>0</td>
<td>107.6 ± 1.8</td>
<td>214.5 ± 2.2</td>
<td>248.6 ± 3.4</td>
<td>290.1 ± 4.5</td>
<td>33</td>
</tr>
<tr>
<td>100</td>
<td>107.2 ± 2.1</td>
<td>213.5 ± 1.8</td>
<td>240.0 ± 2.5</td>
<td>274.8 ± 3.7</td>
<td>26</td>
</tr>
<tr>
<td><strong>Male mice</strong></td>
<td><strong>Vehicle control</strong></td>
<td>22.9 ± 0.2</td>
<td>39.2 ± 0.6</td>
<td>44.3 ± 0.6</td>
<td>46.4 ± 0.8</td>
</tr>
<tr>
<td>100</td>
<td>23.1 ± 0.2</td>
<td>38.9 ± 0.5</td>
<td>42.7 ± 0.6</td>
<td>39</td>
<td>45.4 ± 0.7</td>
</tr>
<tr>
<td>200</td>
<td>22.9 ± 0.2</td>
<td>38.0 ± 0.5</td>
<td>40.1 ± 0.6</td>
<td>44</td>
<td>43.9 ± 0.6</td>
</tr>
<tr>
<td>100</td>
<td>18.9 ± 0.2</td>
<td>29.2 ± 0.4</td>
<td>34.7 ± 0.7</td>
<td>40.5 ± 0.9</td>
<td>34</td>
</tr>
<tr>
<td>200</td>
<td>19.1 ± 0.2</td>
<td>28.7 ± 0.5</td>
<td>34.8 ± 0.7</td>
<td>40.6 ± 0.9</td>
<td>34</td>
</tr>
<tr>
<td>200</td>
<td>18.9 ± 0.2</td>
<td>29.4 ± 0.6</td>
<td>35.2 ± 0.8</td>
<td>40.2 ± 1.0</td>
<td>35</td>
</tr>
</tbody>
</table>

* P < 0.05 versus vehicle controls.
* P < 0.01 versus vehicle controls.

RESULTS

Body Weights and Survival. Mean body weights of high dose male rats were significantly lower (P < 0.01) than those of the vehicle control males after 6 mo of dosing. Low dose male and high dose female rats showed a marginal depression in weight gain. The survival of high dose male rats was significantly lower than that of the vehicle controls, and the decrease in survival was attributed to the toxicity of the chemical.

The survival of high dose male rats was significantly lower than that of the vehicle controls; survival in the control and low dose male mice were comparable, and no differences in survival were seen in control and dosed female mice. Mean body weights of high dose male rats were 5–10% less than those of the vehicle controls after wk 28; mean body weights of low dose male mice and low and high dose female mice were comparable to those of the corresponding control groups (Table 1). No dose-related clinical signs were seen in dosed male and female rats or mice.

Lung Lesions. Nonneoplastic and neoplastic lung lesions were increased in dosed male and female rats (Table 2). The nonneoplastic lesions were described as alveolar epithelial hyperplasia, adenomatous hyperplasia, or chronic interstitial pneumonia. These terms were used to diagnose a complex treatment related lesion characterized by hyperplasia of the alveolar epithelium and thickening of the septal walls around terminal bronchioles and adjacent alveoli. When the thickening of the interstitium was a prominent feature the diagnosis of interstitial pneumonia was used. Interstitial pneumonia was found in 0 of 10 vehicle controls, 4 of 19 low dose, and 18 of 24 high dose male rats that died early in the study.

Seven vehicle control males were diagnosed as having interstitial pneumonia. These changes were unlike those found in the treated animals and were characterized as focal collections of macrophages often with mild histiocytic-plasmacytic cuffing of vessels adjacent to the macrophages. In the treated rats the interstitial pneumonia was usually centriacinar and consisted of thickening of the alveolar septal walls, hyperplasia of the lining pneumocytes, and collections of alveolar macrophages within alveolar lumini (Fig. 1).

Adenomatous hyperplasia was a focal expansive lesion characterized by extensive proliferation of well-differentiated pneumocytes. This lesion was considered hyperplastic rather than neoplastic because the underlying supporting tissues of the lung remained intact and cytomorphological evidence of neoplasia was lacking. The expansive nature of the lesion plus proliferative infoldings into alveolar spaces distinguished this lesion from the commonly observed focal hyperplasia of the alveolar epithelium. The latter is usually seen as a minimal or mild lesion following type 1 pneumocytic injury.

Squamous cell carcinomas, alveolar-bronchiolar adenomas, alveolar-bronchiolar carcinomas, and alveolar-bronchiolar adenomas or carcinomas (combined) occurred with a significant (P < 0.05) positive trend in male rats and increased incidence of these tumors in high dose male rats was also significant (P <
Squamous cell papilloma or squamous cell carcinoma, the high-dose effect was not statistically significant (Table 2). Alveolar-bronchiolar adenomas were characterized by focal areas of increased cellularity which caused compression of the adjacent parenchyma (Fig. 2). The cells formed solid, glandular, or papillary patterns with obliteration of the underlying alveolar structure. There was little cellular atypia and mitotic figures were uncommon (Fig. 3).

Grossly alveolar-bronchiolar carcinomas were yellow or white firm masses involving one or more lobes of the lung. Microscopically these neoplasms were composed of polyhedric cells usually arranged in a papillary pattern, although tubular and solid trabecular patterns were also observed. Alveolar-bronchiolar carcinomas showed more cellular atypia, invasion of adjacent lung parenchyma, and a scirrhous response. The alveolar-bronchiolar carcinomas metastasized to the mediastinal tissues in three high dose and one low dose male rat. No metastases were seen in the female rats with carcinoma of the lung.

Five high dose male rats had lung tumors composed entirely of squamous cells (Fig. 4). For this reason, these tumors were diagnosed as squamous cell carcinomas. The criteria for distinguishing proliferative lesions of the rat lung have been previously described (10). Squamous cell carcinomas appeared grossly as white to green lung masses. Microscopically these lung masses were characterized by squamous differentiation, cellular atypia, abundant keratin, and invasion of surrounding tissues (Fig. 4). Several metastasized to the heart (Fig. 5). One of the animals with a squamous cell carcinoma also had an alveolar-bronchiolar carcinoma involving a separate lobe. Similar lung lesions were not seen in dosed male mice.

Forestomach. Nonneoplastic and neoplastic lesions of the forestomach were increased in male and female rats (Table 3). There was an increase in hyperkeratosis, epithelial hyperplasia, squamous cell papilloma, and squamous cell carcinoma in treated male rats. In high dose female rats, there was an increased incidence of epithelial hyperplasia, and one squamous cell papilloma and one squamous cell carcinoma were also seen in this group.

The epithelial hyperplasia was characterized as diffuse focal thickening of the basal layer, often with acanthosis. Papillary projections lined by squamous epithelium and with fibrovascular cores were diagnosed as squamous cell papillomas. In lesions where the squamous cells invaded the submucosa, the diagnosis of squamous cell carcinoma was made. Forestomach lesions were not seen in the treated mice.

Cerebellum. Focal mineralization in the granular layer of the cerebellum was present in 12 of 49 (24%) high dose male rats but not in any of the other groups of male or female rats or mice. The mineralization was characterized by multiple spherical basophilic concretions up to 1 mm diameter. The concretions tended to occur in clusters in the granular layer. No association between the presence of concretions and cell damage was found, nor did the concretions appear to be associated with vessels.

Testes. Focal calcification was observed at increased incidences in dosed male mice (vehicle control, 2 of 50, 4%; low dose, 9 of 47, 19%; high dose, 24 of 50, 48%). These lesions in the treated animals appeared as circular oblong deposits with obliteration of the underlying cellular features. The shape and location of the deposits suggested mineralization of seminiferous tubules.

**DISCUSSION**

Dimethyl hydrogen phosphate is a chemical that caused non-neoplastic and neoplastic lesions in the rat lung and stomach after p.o. administration but no dose-related neoplastic lesions in the mouse. The lung tumors seen in the rat included alveolar-bronchiolar adenomas, alveolar-bronchiolar carcinomas, and squamous cell carcinomas. Hyperplasia of the alveolar epithelium of the lung was often observed. The incidence of squamous cell papilloma and squamous cell carcinoma of the forestomach was increased in high dose male rats.

Lung tumors are relatively uncommon in Fischer 344/N rats both in untreated and vehicle controls (11). The NTP program-wide historical incidences for lung tumors in male Fischer 344/N rats receiving corn oil vehicle are alveolar-bronchiolar adenomas, 3.0% (34 of 1143); alveolar-bronchiolar carcinoma, 1.4% (16 of 1143); and squamous cell carcinoma, 0.2% (2 of 1143).

DMHP caused lung tumors in 24 of 50 high dose male rats and this incidence was well above the incidence of lung tumors in the concurrent control group (0%; 0 of 50) and the historical incidence listed above. Alveolar-bronchiolar carcinomas were seen in 3 of 50 high dose female rats; the high dose in the female rats was 100 mg/kg, one-half of the dose given to high dose male rats. It is interesting to note that the incidence of all lung lesions in female rats receiving 100 mg/kg was similar to that of the males receiving 100 mg/kg, suggesting that the sexes may be equally susceptible to the pulmonary changes.

Examination of the results of nearly 300 NCI-NTP carcinogenicity studies (12, 13) revealed only a single chemical, 5-nitroacenaphthene (14), that produced a carcinogenic response in the male rat lung. In this earlier feeding study the incidence of lung tumors observed in the two dosed groups (7 of 38 and 3 of 45) was considerably lower than that seen in the current study with DMHP (1 of 50 and 24 of 50). Thus DMHP has shown the highest incidence of lung tumors in the male rat of all chemicals studied to date in the NCI-NTP Carcinogenesis Testing Program. Lung tumors are a leading cause of both morbidity and mortality in humans (15, 16). Inhalation exposure to chemicals or cigarette smoke has been the most frequent route of exposure associated...
with lung disease (17–20). These DMHP rodent studies illustrate that mammalian lung tumors may also arise after p.o. administration of a chemical.

The mechanisms for DMHP induction of lung tumors in the rat are not known. Other organophosphorous compounds have been shown to be toxic to the rat lung (21) but the carcinogenic response seen with DMHP is an unusual finding. NTP has initiated studies to determine the distribution of the chemical and metabolites to the lung, and these studies might help to explain the mechanisms involved in lung toxicity and carcinogenicity and the reasons why the rat appears to be more susceptible than the mouse to lung tumor formation.

DMHP was not mutagenic in Salmonella typhimurium strains TA98, TA100, TA1535, or TA1537 in the presence or absence of liver S9. DMHP did not produce sex-linked recessive lethal mutations in Drosophila melanogaster (4). Further short-term testing is needed to identify the potential of DMHP to interact with or alter cellular genetic material.

Under the conditions of these gavage studies, there was clear evidence of carcinogenicity in male F344/N rats receiving dimethyl hydrogen phosphate, as shown by increased incidences of alveolar-bronchiolar adenomas, alveolar-bronchiolar carcinomas, squamous cell carcinomas of the lung, and squamous cell papillomas or carcinomas of the forestomach. There was equivocal evidence of carcinogenicity in female F344/N rats receiving dimethyl hydrogen phosphate, as shown by marginally increased incidences of alveolar-bronchiolar carcinomas of the lung and of neoplasms of the forestomach, but there was no evidence of carcinogenicity for male or female B6C3F1 mice receiving this chemical.

REFERENCES


Fig. 1. Interstitial pneumonia in a high dose male rat. The lesion is subplural and involves several acini. H & E; original magnification, × 100.

Fig. 2. Small bronchiolar-alveolar adenoma. There is minimal compression and loss of the underlying alveolar architecture. H & E; original magnification, × 66.
Fig. 3. Bronchiolar-alveolar adenoma. The lesion has a papillary pattern. The cells are well differentiated and there is little cellular atypia. H & E; original magnification, ×200.

Fig. 4. Squamous cell carcinoma arising in the lung. This lesion is characterized by well-differentiated keratinocytes and abundant keratin production. H & E; original magnification, ×22.
Fig. 5. Squamous cell carcinoma metastatic to the heart. Islands of well-differentiated keratinocytes are found in the wall of the left ventricle. H & E; original magnification, x 21.
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