Foregastomach Lesions in Rats and Mice Administered 3-Chloro-2-methylpropene by Gavage for Two Years

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ABSTRACT

The carcinogenicity of 3-chloro-2-methylpropene (CMP), a chemical intermediate and insecticide, was studied because of possible human exposure and because of its structural relationship to vinyl chloride and allyl chloride. CMP in corn oil was administered by gavage to groups of 100 male and 50 female Fischer 344/N rats at 0, 75, or 150 mg/kg body weight and to groups of 50 male and 50 female B6C3F, mice at 0, 100, or 200 mg/kg body weight, 5 times a week for 103 weeks. The body weights of the two CMP treated groups of rats were 3-15% lower than the controls; the survival rates were similar. The body weights and survival rates of the CMP-exposed male and female mice were not different from the respective controls throughout the study. CMP administration resulted in dose-related increases in the incidence and severity of forestomach basal cell hyperplasia and the incidence of forestomach squamous cell papillomas in both sexes of rats and mice. In the two groups of CMP-exposed male mice the incidences of squamous cell carcinoma of the forestomach were also increased. Invasion or metastasis of the squamous cell carcinomas to other organs was observed in 2 male mice treated at 100 mg/kg and in 3 male mice and one female mouse treated at 200 mg/kg. The data show that CMP is a carcinogen for the forestomach in rats and mice and acts at the tissue site of contact and support genetic toxicity findings that CMP is a direct-acting alkylating agent.

INTRODUCTION

CMP, a monomethylated allyl compound, is a colorless, volatile liquid, used mainly as an intermediate for the production of plastics, pharmaceuticals, and other organic chemicals. CMP is also used as an insecticide and fumigant for grains, tobacco, and soil. It is toxic by inhalation, skin application, and ingestion and is irritating to the eyes and respiratory tract (1-3).

In structure-activity relationship studies of allyl and allylic compounds Eder et al. (4) and Neudecker et al. (5) demonstrated that AC was mutagenic in tests with Salmonella typhimurium strain TA100 and was positive in 4-(p-nitrobenzyl)pyridine tests for alkylating activities. The mutagenicity and alkylating activity of AC was enhanced by monomethylation. The monomethylated compounds studied included CMP, 3-chloro-1-butenone, and 1-chloro-2-butenone. These authors also demonstrated that the mutagenic potencies of these compounds correlated well with their alkylating activities.

Carcinogenesis studies have demonstrated that followed by 12-O-tetradecanoylphorbol-13-acetate promotion 3 times a week, a single dose of AC initiated skin papilloma development in 7 of 30 female Swiss mice whereas of the 90 controls receiving 12-O-tetradecanoylphorbol-13-acetate alone, 6 had papilloma (6). AC failed to induce papillomas when it was applied repeatedly to mouse skin (6). Administration of AC in corn oil by gavage for 78 weeks caused a marginally increased incidence of forestomach squamous cell papillomas and squamous cell carcinomas in B6C3F, mice; the incidences were 4% (2/46) and 0% (0/50) in male mice treated at 172 and 199 mg/kg, respectively, and 6% (3/47) and 6% (3/45) in female mice treated at 129 and 258 mg/kg, respectively. Forestomach lesions were not observed in groups of 50 male and 50 female Osborne-Mendel rats gavaged at 55-77 mg/kg (7). A borderline tumorigenicity of AC was also demonstrated in a pulmonary adenoma bioassay when the compound was injected i.p. 3 times a week for 8 weeks at 5.9 g/kg to A/ST mice (8). Carcinogenicity of monomethylated allylic compounds has not been reported in the literature. The purposes of the present studies were to investigate the carcinogenicity of CMP, a monomethylated compound, because of potential human exposure and to provide data for analysis of structure-activity relationships of allyl and allylic compounds.

MATERIALS AND METHODS

CMP was obtained in two lots: lot 110967 from Aldrich Chemical Co. (Milwaukee, WI) and lot P091781 from Pfaltz and Bauer (Stamford, CT). Analyses of infrared, ultraviolet/visible, and nuclear magnetic resonance spectra confirmed that the compounds in the two lots were identical to CMP. During the process of synthesizing CMP by substitutive chlorination of isobutylene, DVC [CHCl=C(CH₃)—CH₃]—CH₃ is also formed. It is difficult to remove the DVC from CMP by distillation (9). The two lots of CMP used in the present studies contained 5 and 3.6% DVC, respectively. Details of chemical analyses have been described elsewhere (10). The first lot was used for the first half and the second lot the latter half of the 2-year experimental period.

Male and female 5-week old F344/N rats and 6-week old B6C3F, mice were obtained from Charles River Breeding Laboratories (Kingston, NY) and divided into groups of 50 animals each. The animals were housed 5 per cage in polycarbonate cages with hardwood chips as bedding in a controlled room with 12-h light, 23 ± 1°C temperature, and 50 ± 20% humidity. The rats were quarantined for 3 weeks and the mice 2 weeks before the start of the experiments. All animal husbandry operations were conducted under NIH guidelines (11).

Each group of male and female rats were administered CMP in corn oil by gavage at 0, 75, or 150 mg/kg, 5 times a week for 103 weeks. Groups of male and female mice were given the compound at 0, 100, or 200 mg/kg by the same route on the same schedule. Dose volumes administered were 5 ml/kg for rats and 10 ml/kg for mice. Body weights were recorded weekly for the first 13 weeks and then monthly throughout the 2-year study period. A complete necropsy was performed on each animal found dead or in a moribund condition during the course of the study or at the end of the treatment period. All gross lesions as well as at least one tissue section of each organ in the animals were examined microscopically.

Differences in survival were analyzed by life table methods (12). Tumor incidence data were analyzed by survival-adjusted methods (13) and by Fisher’s exact tests and Cochran-Armitage trend tests based on the overall proportion of tumor-bearing animals (14). All reported P-values for tumor data are one-sided and those for survival are two-sided.
RESULTS

Body weights of the 150-mg/kg group of male rats were 10–15% lower than those of the vehicle controls after the tenth week of the study period, whereas those of the 75-mg/kg group of male rats and the 150-mg/kg group of female rats were only slightly (3–7%) reduced relative to the vehicle controls. There were no significant differences in survival between the CMP-treated male and female rats and the controls.

The incidences of forestomach lesions in the male and female rats are shown in Table 1. Forestomach basal cell hyperplasia and papillomas were observed at increased incidences in the CMP-treated male and female rats. In the control rats the basal cell hyperplasia was generally focal and minimal (Fig. 1) to mild (Fig. 2) while in the treated rats the basal cell hyperplasia was more diffuse and severe (Fig. 3). The incidence of forestomach papilloma was significantly higher in the 150-mg/kg groups of male and female rats. Microscopically, the papillomas consisted of arborized finger-like projections from the surface. The projections had a core of fibrovascular tissue contiguous with the submucosa and were covered by hyperkeratotic squamous epithelium. In most instances, the papillomas were pedunculated and the arborized projections arose from a single stalk (Fig. 4). Forestomach squamous cell carcinomas were observed in 2 of 48 male rats treated at 150 mg/kg but not in any other groups. Metastasis was not observed. Squamous cell carcinomas were characterized by downward projecting sheets, nests, and anastomosing cords of squamous tumor cells that invaded the underlying structures. The invading masses of cells originated at the base of papillomas.

Table 1 Incidences of forestomach lesions in rats gavaged with CMP

<table>
<thead>
<tr>
<th>Incidence of lesions</th>
<th>Vehicle control</th>
<th>75 mg/kg</th>
<th>150 mg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Basal cell or epithelial hyperplasia</td>
<td>19/50 (38)*</td>
<td>41/50 (82)*</td>
<td>44/48 (90)*</td>
</tr>
<tr>
<td>Squamous cell papilloma</td>
<td>1/50 (2)</td>
<td>5/50 (10)</td>
<td>30/48 (63)*</td>
</tr>
<tr>
<td>Squamous cell carcinoma</td>
<td>0/50 (0)</td>
<td>0/50 (0)</td>
<td>2/48 (4)</td>
</tr>
<tr>
<td>Female</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Basal cell hyperplasia</td>
<td>24/50 (48)</td>
<td>42/50 (84)*</td>
<td>45/50 (90)*</td>
</tr>
<tr>
<td>Squamous cell papilloma</td>
<td>1/50 (2)</td>
<td>1/50 (2)</td>
<td>10/50 (20)*</td>
</tr>
<tr>
<td>Squamous cell carcinoma</td>
<td>0/50 (0)</td>
<td>0/50 (0)</td>
<td>0/50 (0)</td>
</tr>
</tbody>
</table>

* Numbers in parentheses, percentage.

P < 0.01 compared with vehicle controls.
The mean body weights of the 200-mg/kg group of male and the 2 CMP-treated groups of female mice were slightly (5–9%) lower than those of the controls. Survival of the control male mice was significantly lower ($P < 0.05$) than that of the 100-mg/kg group of male mice. Survival rates of the 3 groups of female mice were not significantly different from each other.

The incidences of forestomach lesions in mice are shown in Table 2. The incidences of forestomach epithelial hyperplasia and squamous cell papilloma showed significant dose-related increases in the CMP-treated male and female mice. The incidences of squamous cell carcinoma of the forestomach were also increased in the CMP-treated male mice. Evidence of metastasis or invasion of other organs was observed in 2 of the 100-mg/kg group and 3 of the 200-mg/kg group of male mice and one of the 200-mg/kg group of female mice. The microscopic characteristics of squamous cell neoplasms of mice were similar to those described in rats.

No other neoplasms in the dosed male or female rats or mice were regarded as being caused by the administration of CMP.

**DISCUSSION**

The discovery that vinyl chloride was carcinogenic in animals in 1971 (15) and in humans in 1974 (16) has focused attention on the potential mutagenicity and carcinogenicity of the structurally analogous aliphatic and olefinic halogenated hydrocarbons (17). Many halogenated aliphatic and olefinic hydrocarbons have since been shown to be carcinogenic in animals (18). The present studies assessed the carcinogenicity of another member of the olefinic halogenated hydrocarbon series: CMP.

The results of the 2-year studies demonstrated that CMP administered to rats and mice by gavage induced proliferative lesions in the forestomach. Male and female rats administered CMP at 150 mg/kg and male and female mice given CMP at 100 or 200 mg/kg had increases in forestomach squamous cell papillomas. The CMP-dosed male mice also had increases in the incidence of forestomach squamous cell carcinoma. The compound also caused forestomach basal cell or epithelial hyperplasia in rats and mice.

Halogenated alkenes are thought to undergo epoxidation reactions that are catalyzed by the cytochrome P-450-dependent polysubstrate monooxygenase system. The resultant epoxides may react with tissue macromolecules, leading to toxicity, mutagenicity, and/or carcinogenicity (19–21). Halogenated hydrocarbons with more than two carbon atoms, such as AC, have also been postulated to be activated via the epoxidation pathway (22).

Neudecker *et al.* (5) and Eder *et al.* (23) reported that CMP was a direct-acting mutagen as its mutagenic activity in Salmonella strain TA100 assays was reduced in the presence of rat liver S9. These results suggested that CMP is not metabolically activated to an epoxide. The present studies demonstrated that CMP is a carcinogen and that administration of CMP by gavage to rats and mice induced neoplasms only at the site of application, i.e., forestomach. The results are consistent with the notion that CMP is direct-acting.

The relationship between basal cell hyperplasia and development of neoplasm in the forestomach is not clear. In a study in which male F344/N rats were administered 10 doses of CMP by gavage in a 2-week period, generalized forestomach mucosal hyperplasia and hyperkeratosis were observed. Other known animal forestomach carcinogens such as ethyl acrylate, diglycidyl resorcinol ether, 1,2-dibromo-3-chloropropane, and 1,2-dibromoethane given by the same route and schedule also caused forestomach proliferative lesions whereas such lesions were not observed in rats administered nonforestomach carcinogens such as dichloroethane and corn oil (24). Two-year chronic administration of CMP by gavage to rats and mice, as demonstrated in the present studies, also induced proliferative lesions in the forestomach. The development of forestomach papillomas and carcinomas was probably related to the chronic interaction between CMP, a mutagen and alkylating agent, and the proliferating forestomach basal and epithelial cells. Such interaction may increase the risk for transcription errors leading to neoplastic transformation. An association of cell proliferation and forestomach carcinogenesis has been proposed in studies of butylated hydroxyanisole (25, 26) and ethyl acrylate (27).

The structure-activity relationships of allyl and allylic compounds are summarized in Table 3. Data reported thus far showed that propene (propylene) was not mutagenic in *Escherichia coli* tests (28) and was not carcinogenic in rats and mice in inhalation studies (29, 30). Chlorine substitution enhanced the mutagenic and carcinogenic potential of propene. The chlorine-substituted compounds 1-chloropropene and AC were mutagenic in Salmonella assays (4, 5, 31) and induced a marginal increase in forestomach tumors in mice when administered p.o. (6, 7). The mutagenicity of AC was enhanced by monomethylation, i.e., the mutagenic potentials of CMP, 3-chloro-1-butene, and 1-chloro-2-butene in Salmonella TA100 assays were greater than those of AC. Dimethylated AC such as 3-

<table>
<thead>
<tr>
<th><strong>Table 2</strong> Incidences of forestomach lesions in mice gavaged with CMP</th>
<th>Incidence of lesions</th>
<th>Male</th>
<th>Female</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Vehicle control</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>100 mg/kg</td>
<td>200 mg/kg</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Epithelial hyperplasia</td>
<td>0/49 (0)*</td>
<td>14/49 (29)*</td>
<td>15/49 (31)*</td>
</tr>
<tr>
<td>Squamous cell papilloma</td>
<td>3/49 (6)</td>
<td>19/49 (39)*</td>
<td>30/49 (61)*</td>
</tr>
<tr>
<td>Squamous cell carcinoma</td>
<td>0/49 (0)</td>
<td>5/49 (10)*</td>
<td>7/49 (14)*</td>
</tr>
<tr>
<td><strong>100 mg/kg</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Epithelial hyperplasia</td>
<td></td>
<td>6/48 (12)</td>
<td>13/44 (30)*</td>
</tr>
<tr>
<td>Squamous cell papilloma</td>
<td>4/50 (8)</td>
<td>15/48 (31)*</td>
<td>29/44 (66)*</td>
</tr>
<tr>
<td>Squamous cell carcinoma</td>
<td>0/50 (0)</td>
<td>1/48 (2)</td>
<td>2/44 (5)</td>
</tr>
</tbody>
</table>

* Numbers in parentheses, percentage.
* $P < 0.01$ compared with vehicle controls.
* $P < 0.05$ compared with vehicle controls.
* DU, data unavailable.

**Table 3 Structure-activity of allyl and allylic compounds**

<table>
<thead>
<tr>
<th>Compound</th>
<th>Structure</th>
<th>Mutagenicity</th>
<th>Carcinogenicity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Propene</td>
<td>CH$_3$–CH–CH$_3$</td>
<td>*</td>
<td>–</td>
</tr>
<tr>
<td>1-Chloropropene</td>
<td>CHCl–CH–CH$_3$</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Allyl chloride</td>
<td>CH$_2$–CH–CHCl</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>3-Chloro-2-methylpropene</td>
<td>CH$_2$–C–CH$_2$Cl</td>
<td>+++</td>
<td>++</td>
</tr>
<tr>
<td>3-Chloro-1-butene</td>
<td>CH$_2$–CH–CHCl</td>
<td>+++</td>
<td>DU*</td>
</tr>
<tr>
<td>1-Chloro-2-butene</td>
<td>CH$_2$–CH–CHCl</td>
<td>+++</td>
<td>DU</td>
</tr>
<tr>
<td>3-Chloro-2-methyl-1-butene</td>
<td>CH$_2$–C–CHCl</td>
<td>++</td>
<td>DU</td>
</tr>
<tr>
<td>1-Chloro-2-methyl-2-butene</td>
<td>CH$_2$–C–CH$_2$Cl</td>
<td>++</td>
<td>DU</td>
</tr>
</tbody>
</table>

* Negative.
* $P < 0.01$ compared with vehicle controls.
* $P < 0.05$ compared with vehicle controls.
* DU, data unavailable.

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chloro-2-methyl-1-butene and 1-chloro-2-methyl-2-butene were slightly less mutagenic than monomethylated allylic compounds (4, 5). By demonstrating the carcinogenicity of CMP the present report as summarized from that of the National Toxicology Program (10) show that along with increased mutagenicity and alkylating potential (4, 5) monomethylation of an allylic compound such as CMP also enhances its carcinogenicity.

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