Inhalation Carcinogenesis of Various Alkylation Agents

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ABSTRACT—A series of earlier studies showed that inhalation exposures of rats to three water-reactive electrophilic compounds produced brisk yields of nasal cancer even when the animals were exposed for only 30 days (6 hr/day x 5 days/wk). In addition, carcinogenic potencies of the compounds appeared to relate to their chemical reactivities as measured by hydrolysis rates. For a further study of this phenomenon, inhalation exposures were conducted with five additional water-reactive compounds: $\beta$-propiolactone [(BPL) CAS: 57-57-8], methylmethane sulfonate [(MMS) CAS: 66-27-3], ethylchloroformate [(ECF) CAS: 541-41-3], dichloroacetyl chloride [(DCAC) CAS: 79-36-7], and propylene oxide [(PO) CAS: 75-56-9] on male Sprague-Dawley rats. The hydrolysis rates of these compounds span 6 orders of magnitude. The compounds were administered for 30 days (6 hr/day x 5 days/wk) with the use of exposure concentrations that were inversely proportional to the respective hydrolysis rates. With this protocol, all compounds except PO (the slowest reacting compound) produced nasal cancer in rats. The concentrations of MMS and BPL employed in the studies produced similar nasal cancer yields, indicating that the carcinogenic potencies of these compounds in rat nasal mucosa were proportional to their hydrolysis rates. The nasal cancer yields of DCAC and ECF were less than expected. DCAC hydrolyzes so rapidly at in vivo temperatures (half-life <0.01 min) that it may not reach target DNA in reactive form. Why the exposures to ECF produced yields of nasal cancer not predicted by its reactivity is not clear. The studies produced similar nasal cancer yields, indicating that the carcinogenic potencies of these compounds in rat nasal mucosa were proportional to their hydrolysis rates. The nasal cancer yields of DCAC and ECF were less than expected. DCAC hydrolyzes so rapidly at in vivo temperatures (half-life <0.01 min) that it may not reach target DNA in reactive form. Why the exposures to ECF produced yields of nasal cancer not predicted by its reactivity is currently under investigation. These results combined with our earlier results demonstrate that the carcinogenic potencies of some inhaled reactive electrophilic compounds are related to their hydrolysis rates.—JNCI 1987; 79:285-289.

Rats exposed via inhalation for 30 days (6 hr/day x 5 days/wk) to bis(chloromethyl) ether (1), epichlorohydrin (2), or dimethylcarbamyl chloride (3) developed carcinomas of the nasal mucosa. Similar tumor responses were observed at exposure levels that were roughly inversely proportional to the chemical reactivities of the respective compounds, as measured by their hydrolysis rates. To investigate further the relationship between carcinogenic potency to the rat nasal mucosa and the chemical reactivity of inhaled direct-acting gaseous electrophilic compounds, we studied the carcinogenic effects of the following additional direct-acting compounds: BPL (CAS: 57-57-8), PO (CAS: 75-56-9), MMS (CAS: 66-27-3), ECF (CAS: 541-41-3), and DCAC (CAS: 79-36-7).

BPL has been used in organic synthesis for numerous purposes and has been shown to be carcinogenic in guinea pigs and mice by both skin painting and sc injection (4-7). PO produces carcinomas of the stomach by ig gavage (8) and sarcomas by sc injection (9, 10). A 2-year inhalation exposure of F344 rats to PO showed a mild carcinogenic effect (11). MMS is frequently used to induce mutations in cell culture systems (12) and it produces tumors in rats when given ip (13) and sc (14). ECF is used in research laboratories in peptide synthesis (15) and to modify purine bases (16). Its carcinogenic potential is not known. DCAC has been produced in substantial quantities for industrial use (17). Its carcinogenic potential is unknown. The rates of hydrolysis of these compounds are given in Table 1.

MATERIALS AND METHODS

The test chemical BPL was obtained from Sigma Chemical Co. (St. Louis, MO), and the rest of the chemicals were obtained from Aldrich Chemical Co. (Milwaukee, WI). The purities of all compounds were greater than 95%, and all were used as supplied. All of the chemicals are liquids at room temperature. Test atmospheres of the chemicals were generated by passing an airstream over the liquids in a generating flask and then feeding the effluent vapor into the chamber air supply. By varying the flow of air through the flasks, stable chamber concentrations were achieved. Animal exposures were done in 1.0-m$^3$ or 1.3-m$^3$ dynamic exposure chambers (18) for 30 days (6 hr/day x 5 days/wk). Chamber concentrations of the chemicals were analyzed at half-hour intervals during the daily exposures by means of a Wilks Miran infrared gas analyzer (Wilks Scientific Corp., South Norwalk, CT), with the use of appropriate wavelengths for each compound.

The 30-day exposure concentrations were selected on the basis of published hydrolysis rates. Table 1 lists the hydrolysis rates and target concentrations for all of the compounds studied. Only one concentration (50 ppm) was used for MMS because of the cost of this compound. Target concentrations of 0.5, 1, and 2 ppm were selected for DCAC because of the extreme difficulties that would have been encountered in maintaining and analyzing the very low concentrations (<1 ppb) proportional to its very rapid hydrolysis rate.

ABBREVIATIONS USED: BPL = $\beta$-propiolactone; DCAC = dichloroacetyl chloride; ECF = ethylchloroformate; MMS = methylmethane sulfonate; PO = propylene oxide; $T_s$ = half-life (period required for the hydrolysis of one-half the mass of a given compound).

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Groups of 9- to 10-week-old male Sprague-Dawley rats (Charles River Breeding Laboratories, Wilmington, MA), each rat weighing 325 ± 16.8 g, were used throughout. They were first quarantined for 2 weeks. During this time, complete serologic and pathologic analyses were done on randomly selected animals. These analyses were found to be negative. Purina Lab Chow (Ralston Purina Co., St. Louis, MO) and water were provided ad libitum, except during exposure periods. All animals were observed daily, weighed monthly, and allowed to die spontaneously or sacrificed when moribund. A complete necropsy was performed on each animal. The nasal passages were flushed with 10% neutral buffered Formalin; then the entire head and other organs were fixed in the same fixative. The head was then decalcified; and stepwise cross-sections were taken in the dorsoventral plane perpendicular to the long axis of the skull, beginning just posterior to the nostrils and extending caudal as far as the orbit. Histologic sections were taken from each lobe of the lung and from the trachea, larynx, liver, kidneys, testes, and any other organs exhibiting gross pathology. Paraffin sections, 4-5 μm thick, were prepared in the usual fashion and stained with hematoxylin-eosin and with special stains, if necessary, for histologic examination.

RESULTS

The observed mean daily concentrations for each of the exposures plus or minus the standard deviation are given in table 1.

Mortality for animals exposed to 20 ppm BPL reached nearly 20% during the 1st week of exposure, and for this reason the exposures were discontinued. There was also significant mortality among the animals exposed to 1,720 ppm PO after eight exposures, and exposures were discontinued at that time. All other exposures were con-ducted for the anticipated 30 days. The mortality curves for all groups are given in text-figures 1 and 2.

The primary lesions induced by the alkylating agents were seen in the upper respiratory tract and limited to the anterior portion of the nasal cavity. These lesions involved the nasomaxillary turbinates, lateral walls, nasal septum, and the olfactory epithelium in the dorsal meatus. The respiratory epithelium was affected by these treatments to a greater extent than was the squamous epithelium or olfactory epithelium; the respiratory epithelium showed necrosis, extensive ulceration, and acute inflammation. In some cases, there were moderate-to-severe squamous metaplasia and dysplastic lesions, leading to tumorigenesis. Most of the animals dying at the higher exposure concentrations of a given chemical showed severe rhinitis with seropurulent exudate filling the nasal cavity. The olfactory epithelium in the ethmoidal regions showed inflammatory reaction, with and without seropurulent exudate. The trachea and larynx did not show neoplastic changes. Bronchiectasis and acute pneumonic lesions were noticed in the lungs of older animals.

High incidences of nasal tumors occurred among those groups of rats receiving 5 and 10 ppm BPL and 50 ppm MMS. Low incidences of nasal tumors occurred among those groups receiving 20 ppm BPL for 5 days and among those groups receiving the highest concen-
trations of DCAC and ECF (table 2; text-fig. 3). No nasal tumors were observed in any other treated groups or in the control group (table 3).

Animals exposed for 30 days to 10 or 5 ppm BPL exhibited a dose-dependent response for nasal tumors (text-fig. 3). Although the absolute incidence of nasal tumors was higher in the 5-ppm-exposed group (66 vs. 48%), the slopes of the mortality-corrected tumor incidence curves were similar for both exposure groups and the median time to tumor was much shorter for the 10-ppm-exposed group (447 vs. 680 days). The tumorigenic potency of BPL was demonstrated by the observation of an 8% nasal tumor incidence among animals exposed to 20 ppm BPL for only 5 days (table 2).

At 50 ppm, MMS exhibited a potent carcinogenic effect on the nasal epithelium. Of 80 exposed animals, 47 (59%) developed tumors of the nasal mucosa (table 2) with the first carcinoma seen in an animal dying at 256 days. In 2 animals dying with nasal squamous cell carcinomas, there were metastatic lesions in the lungs. Two other animals showed benign tumors in the larynx and trachea. The slope of the mortality-corrected nasal tumor incidence curve for animals exposed to MMS was very similar to the slopes of the curves for animals exposed to 5 and 10 ppm BPL (text-fig. 3).

ECF at the highest concentration (6 ppm) produced a single squamous cell carcinoma in an animal dying at 700 days. Similarly, 2 animals exposed to the highest concentration of DCAC (2 ppm) exhibited nasal carcinomas. These animals died 701 and 887 days after the initial DCAC exposure. No nasal tumors were observed in any groups receiving PO; however, 2 animals exposed to PO died with adenomas of the lung. Air-control animals developed no tumors in any part of the respiratory tract.

The data on tumors arising outside the respiratory tract, given in table 2, parallel those of historical colony controls.

**DISCUSSION**

The lesions and the tumors produced by the compounds studied are highly site specific, involving primarily the respiratory epithelium of the anterior portion of the nasal cavity and the olfactory epithelium in the dorsal meatus. The carcinomas were predominantly of squamous cell type, involving the nasomaxillary turbinates, septum, and the lateral walls. The mucosa of the posterior portion of the nasal cavity, which contains the ethmoidal region with olfactory epithelium, did not show neoplastic lesions but only mild-to-severe rhinitis, with or without seropurulent exudate.

The location of the major reactive and neoplastic lesions in the anterior region and the absence of such lesions in the posterior region suggest that the magnitude of local deposition of the tested chemicals played a significant role in the induction of nasal tumors.

**Table 2.—Median life-span and time of tumor appearance following exposure to alkylating agents**

<table>
<thead>
<tr>
<th>Treatment (exp=exposures)</th>
<th>No. of animals</th>
<th>Median lifespan, days</th>
<th>Animals with tumors, No. (%)</th>
<th>Time of tumor appearance, days</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>BPL</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5 ppm×30 exp</td>
<td>50</td>
<td>652</td>
<td>33 (66)</td>
<td>680</td>
</tr>
<tr>
<td>10 ppm×30 exp</td>
<td>50</td>
<td>352</td>
<td>24 (48)</td>
<td>447</td>
</tr>
<tr>
<td>20 ppm×5 exp</td>
<td>50</td>
<td>624</td>
<td>4 (8)</td>
<td>780</td>
</tr>
<tr>
<td><strong>PO</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>435 ppm×30 exp</td>
<td>50</td>
<td>655</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>870 ppm×30 exp</td>
<td>50</td>
<td>635</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>1,740 ppm×8 exp</td>
<td>50</td>
<td>519</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><strong>MMS</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>50 ppm×30 exp</td>
<td>80</td>
<td>496</td>
<td>47 (59)</td>
<td>513</td>
</tr>
<tr>
<td><strong>ECF</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.5 ppm×30 exp</td>
<td>50</td>
<td>576</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>3 ppm×30 exp</td>
<td>50</td>
<td>573</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>6 ppm×30 exp</td>
<td>50</td>
<td>617</td>
<td>1 (2)</td>
<td>700</td>
</tr>
<tr>
<td><strong>DCAC</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.5 ppm×30 exp</td>
<td>50</td>
<td>528</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>1 ppm×30 exp</td>
<td>50</td>
<td>524</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>2 ppm×30 exp</td>
<td>50</td>
<td>595</td>
<td>2 (4)</td>
<td>794</td>
</tr>
<tr>
<td><strong>Air×30 exp</strong></td>
<td>98</td>
<td>613</td>
<td>0</td>
<td>0</td>
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</tbody>
</table>

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TABLE 3.—Specific types of tumors and other lesions of the nasal mucosa in rats exposed to various alkylating agents

<table>
<thead>
<tr>
<th>Treatment</th>
<th>No. of animals examined</th>
<th>Squamous metaplasia</th>
<th>Polypl or papillomas</th>
<th>Squamous cell carcinoma</th>
<th>Adenocarcinoma</th>
<th>Mixed carcinoma</th>
<th>Osteogenic sarcoma</th>
<th>Tumors in other organs</th>
</tr>
</thead>
<tbody>
<tr>
<td>BPL</td>
<td>5 ppm×30 exp</td>
<td>50</td>
<td>24</td>
<td>24</td>
<td>11</td>
<td>21</td>
<td>—</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>10 ppm×30 exp</td>
<td>50</td>
<td>24 (48)</td>
<td>26</td>
<td>21</td>
<td>5</td>
<td>20</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>20 ppm×5 exp</td>
<td>50</td>
<td>4 (8)</td>
<td>37</td>
<td>14</td>
<td>4</td>
<td>1</td>
<td>—</td>
</tr>
<tr>
<td>FO</td>
<td>455 ppm×30 exp</td>
<td>50</td>
<td>0</td>
<td>40</td>
<td>5</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>870 ppm×30 exp</td>
<td>49</td>
<td>0</td>
<td>45</td>
<td>12</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>1,740 ppm×8 exp</td>
<td>49</td>
<td>0</td>
<td>38</td>
<td>10</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>MMS, 50 ppm×30</td>
<td>80</td>
<td>47 (59)</td>
<td>49</td>
<td>5</td>
<td>11</td>
<td>33</td>
<td>2</td>
<td>2</td>
</tr>
</tbody>
</table>

a One adenoma (lung), 1 lymphed leukemia, 1 hepatocellular carcinoma (liver), 1 hemangiomia (spleen), 1 islet cell adenoma (pancreas), 1 solid cell carcinoma (thyroid), 3 fibroma (subcutaneous), 1 fibrosarcoma (subcutaneous).
b One adenocarcinoma (thyroid), 1 cortical adenoma (adrenal), 1 papilloma (skin), 1 squamous cell carcinoma (skin), 2 fibromas (subcutaneous), 1 lipoma (subcutaneous).
c Four malignant lymphomas, 1 hemangiomia (spleen), 3 cortical adenomas (adrenal), 2 cortical adenocarcinomas (adrenal), 1 phaeochromocytoma (adrenal), 1 squamous cell carcinoma (zymbal gland), 1 fibroma (subcutaneous), 1 lipoma (subcutaneous), 1 papilloma (skin), 1 fibroadenoma (mammary gland).
d Two adenomas (lung), 5 malignant lymphomas, 1 myeloid leukemia, 1 hepatocellular carcinoma (liver), 1 adenocarcinoma (adrenal), 1 Leydig cell tumor (testes), 4 fibromas (subcutaneous), 3 fibrosarcoma (subcutaneous).
e Four malignant lymphomas, 1 granulocytic leukemia, 1 hepatocellular carcinoma (liver), 2 phaeochromocytomas (adrenal), 1 adenoma (thyroid), 1 squamous cell carcinoma (Zymbal gland), 1 Leydig cell tumor (testes), 5 fibromas (subcutaneous), 1 fibrosarcoma (subcutaneous).
f Two adenomatous polyps (larynx), 2 adenomatous polype (trachea), 1 malignant lymphoma, 1 adenoma (adrenal), 1 adenocarcinoma (adrenal), 1 papilloma (skin), 2 adenomas (thyroid), 1 islet cell adenoma (pancreas), 3 fibroadenomas (mammary gland), 1 adenocarcinoma (salivary gland), 1 adenoma (salivary gland), 2 lipomas (subcutaneous), 9 fibromas (subcutaneous), 1 adenoma (parathyroid).
g Two malignant lymphomas, 2 hepatocellular carcinomas (liver), 1 phaeochromocytoma (adrenal), 2 adenomas (thyroid), 1 fibrosarcoma (subcutaneous), 1 squamous cell carcinoma (skin).
h One malignant lymphoma, 1 hemangiomia (spleen), 2 cortical adenomas (adrenal), 1 islet cell adenoma (pancreas), 1 adenoma (thyroid), 1 Leydig cell tumor (testes), 1 fibroma (subcutaneous), 2 papillomas (skin), 1 lipoma (subcutaneous), 1 fibroadenoma (mammary gland), 3 lymphosarcomas.
i One adenoma (lung), 3 malignant lymphomas, 1 hepatocellular carcinoma (liver), 2 cortical adenomas (adrenal), 2 cortical adenocarcinomas (adrenal), 1 phaeochromocytoma (adrenal), 1 islet cell adenoma (pancreas), 2 adenomas (thyroid), 2 Leydig cell tumors (testes), 4 fibromas (subcutaneous), 1 fibrosarcoma (subcutaneous), 1 polyp (intestine).
j Two malignant lymphomas, 2 adenomas (thyroid), 2 fibroadenomas (mammary gland), 2 fibromas (subcutaneous), 4 lipomas (subcutaneous).
k Two malignant lymphomas, 1 adenocarcinoma (salivary gland), 2 squamous cell carcinomas (Zymbal gland), 2 papillomas (skin), 1 squamous cell carcinoma (skin), 4 fibromas (subcutaneous), 2 fibrosarcomas (subcutaneous).
l One hemangioendothelial sarcoma (liver), 1 phaeochromocytoma (adrenal), 1 adenoma (kidneys), 1 squamous cell carcinoma (Zymbal gland), 1 sebaceous acinar carcinoma (Zymbal gland), 2 fibroadenomas (mammary gland), 2 forestomach papillomas, 2 papillomas (skin), 1 squamous cell carcinoma (skin), 4 fibromas (subcutaneous), 3 lipomas (subcutaneous).
m Four malignant lymphomas, 3 cortical adenomas (adrenal), 2 phaeochromocytomas (adrenal), 2 adenomas (thyroid), 3 Leydig cell tumors (testes), 1 squamous cell carcinoma (Zymbal gland), 5 fibromas (subcutaneous), 2 fibrosarcomas (subcutaneous), 2 lymphosarcomas.

The nasal tumor responses of the MMS- and BPL-exposed groups support the hypothesis that the carcinogenic potencies of some water-reactive electrophilic compounds are proportional to their chemical reactivities as measured by hydrolysis rate. The hydrolysis rate of BPL is about 9.5 times faster than that of MMS (21, 22). Therefore, exposures to 5 ppm BPL should have elicited the same tumor response as exposures to 50 ppm MMS. In our studies, exposures to 50 ppm MMS produced nasal tumors at a faster rate than did exposures to

— Crucial role in tumorigenesis. Thus the absence of tumors in posterior portions of the mucosa could be attributed to the lack of effective dose in those parts of the nasal mucosa. It is generally thought that the pattern of deposition of an inhaled gas in the respiratory tract is a function of water solubility. It is noteworthy, therefore, that BCME, a water-insoluble, direct-acting compound studied in our previous work (1), exerted its carcinogenic effect on the olfactory epithelium of the posterior region of the nasal cavity and on the lungs.
5 ppm BPL (text-fig. 3). However, the slopes of the tumor responses for these groups were very similar, as were the overall tumor yields (66 vs. 59%). Since literally any set of concentrations could have been chosen for these exposures, the fact that the relative concentrations selected on the basis of hydrolysis rates gave similar tumor responses suggests that the relative chemical reactivities of these compounds play a role in their relative carcinogenic potencies.

The relative chemical reactivities of DCAC and PO may also play a role in the relative carcinogenic potencies of these compounds. Dichloroacetyl chloride hydrolyzes very rapidly \( T_\text{h}=0.004 \text{ min at } -20^\circ C \) (19). It is unlikely that sufficient amounts of this compound would survive transit across the aqueous media in the rat nasal mucosa to target DNA. It may be that electrophilic compounds with very rapid hydrolysis rates are less carcinogenically potent than expected because of competing reactions with these compounds by water and nucleophiles other than DNA. Propylene oxide, however, reacts very slowly \( T_\text{h} \approx 5,000 \text{ min at } 37^\circ C \) (23). When inhaled, this compound may not react with sufficient rapidity with target DNA to produce the initial lesions necessary for carcinogenesis. Propylene oxide has been shown to elicit a marked carcinogenic response when given by other routes of administration (8-10). It may be that PO must remain in contact with cells for longer periods than are possible under inhalation exposure regimes for a carcinogenic response to be elicited.

The lack of a brisk nasal tumor response in groups exposed to ECF is not currently understood. The hydrolysis rate of this compound \( 19 \text{ min at } 30^\circ C \) (20) would seem to be in the correct range for a tumorigenic response. Higher concentrations of this compound may be necessary to elicit tumorigenesis. The mortality-corrected tumor responses to BPL at 5 and 10 ppm are consistent with a concentration-squared relationship. If the same relationship is true for ECF, then small increases in exposure concentrations could yield large tumor responses.

Further studies are under way concerning the relationships between the chemical reactivities of electrophilic compounds and their tumorigenic potencies in rat nasal mucosa. The extent and types of adducts formed by these compounds, as well as their effects on nasal cell cycle kinetics, are receiving particular attention.

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