

¹ Biotechnics, Inc., Hillsborough, North Carolina, USA

² Laboratory of Reproductive Toxicology, Institute of Environmental Toxicology, Mitsukaido, Ibaraki, Japan

³ National Center for Environmental Assessment, Office of Research and Development, Environmental Protection Agency, Washington, DC, USA

⁴ University of California, Berkeley and Lawrence Berkeley National Laboratory, Berkeley, California, USA

⁵ Laboratory of Experimental Pathology, National Institute of Environmental Health Sciences, NIH, DHHS, Research Triangle Park, North Carolina, USA

Bromoethane, chloroethane and ethylene oxide induced uterine neoplasms in B6C3F1 mice from 2-year NTP inhalation bioassays: pathology and incidence data revisited

CATHERINE A. PICUT¹, HIROAKI AOYAMA², JAMES W. HOLDER³, LOIS SWIRSKY GOLD⁴, ROBERT R. MARONPOT⁵, and DARLENE DIXON⁵

With 10 figures and 4 tables

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Address for correspondence: Dr. DARLENE DIXON, National Institute of Environmental Health Sciences, P.O. Box 12233, MDC2-09, Research Triangle Park, North Carolina 27709 USA; Fax: 919-541-7666, e-mail: dixon@niehs.nih.gov

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Summary

Chloroethane, bromoethane and ethylene oxide represent a unique set of chemicals that induce endometrial neoplasms in the uterus of B6C3F1 mice following an inhalation route of exposure. The results of the NTP's chronic bioassays with these three compounds resulted in an unusually high incidence of uterine epithelial neoplasms in B6C3F1 mice (chloroethane 86%, bromoethane 56%) and a lower incidence for ethylene oxide (10%). The uterine neoplasms were classified as adenomas, adenocarcinomas, and squamous cell carcinomas for bromoethane, and as adenocarcinomas for both chloroethane and ethylene oxide. The adenocarcinomas and squamous cell carcinomas were invasive into the myometrium and the serosa, and metastasized to a wide variety of organs. Metastatic sites included most commonly the lung, lymph nodes, and ovary at unusually high rates of metastases (79% for chloroethane and 38% for bromoethane). Because of the dramatically high rates of uterine neoplasms (induced by chemicals given by the inhalation route) and metastases, a re-evaluation of the pathology and incidence data was undertaken. The earlier results were confirmed. The mechanism of uterine carcinogenesis by chloroethane, bromoethane and ethylene oxide is unclear.

Introduction

Out of 750 chemicals that induce tumors in the Carcinogenic Potency Database, only twelve have been shown to induce tumors of endometrial origin in mice (GOLD et al. 1997; GOLD et al. 1999; GOLD et al. 2001; <http://potency.berkeley.edu>). In only three of the twelve (chloroethane, bromoethane and ethylene oxide) was the chemical administered by inhalation, and all three were conducted by the National Toxicology Program (NTP) in B6C3F1 mice at Battelle Northwest Laboratories in the early 1980's. With chloroethane and bromoethane, the results of the chronic bioassays were dramatic and demonstrated a high incidence of malignant uterine neoplasms of endometrial origin in the high dose female mice [chloroethane 86% (43/50), bromoethane 56% (27/48)] and a high metastatic rate for the malignant uterine neoplasms [chloroethane 79% (34/43), bromoethane 38% (9/24)]. Results for ethylene oxide were less dramatic for the uterus, as the incidence of uterine neoplasms was 10% (5/49) in the high dose; there was only one metastatic uterine tumor; and the uterus was only one of several target sites. None of these chemicals induced uterine tumors in rats, even though the uterus is a more common

target site in rats than mice (GOLD et al. 2001). The reason why this carcinogenic activity is directed toward the uterine endometrial cells of mice is unclear. All three chemicals are mutagenic and direct alkylating agents.

Uterine neoplasms are exceedingly uncommon in control mice. Uterine endometrial neoplasms rarely occur spontaneously, with the historical control incidence in B6C3F1 mice reported as 0–2% (HASEMAN 1999; NIEHS 2000), and as 1.7% in chamber controls at the study laboratory in 1980s (NTP 1987). Chemically-induced uterine neoplasms are also rare. A total of 12 chemicals among the 750 positive chemicals in the Carcinogenic Potency Database induced uterine neoplasms in mice (table 1). These are bromoethane, chloroethane, ethylene oxide, 1–2 dichloroethane, dacarbazine, trichloropropane, trimethylphosphate, diethylstilbesterol, glycidol, (N-6)-(Methylnitroso) adenosine, procabazine-HCl and vinyl acetate.

Due to the unusual pathologic findings of endometrial neoplasia associated with inhalation exposures to chloroethane, bromoethane and ethylene oxide in 3 separate NTP 2-year bioassays, a re-evaluation of the pathol-

ogy and incidence data from these studies was undertaken. Possible confounding factors that might have affected the results were considered, and did not appear relevant: purity of the 3 chemicals was high; none of the control mice developed uterine tumors, thus ruling out possible contamination (NTP 1987; NTP 1989a,b). This report describes the morphologic appearance of the endometrial tumors resulting from inhalation exposure to chloroethane, bromoethane and ethylene oxide. The results of the chronic bioassays for these three chemicals provide an opportunity to examine a large set of endometrial neoplasms and to evaluate and report the morphologic appearance of adenomas, adenocarcinomas, and squamous cell carcinomas arising in chronic bioassays following chemical exposure by the inhalation route.

Material and methods

Animal care and maintenance: The 2 year chronic inhalation studies for chloroethane, bromoethane and ethylene oxide were performed at Batelle Pacific Northwest Labora-

Table 1. Chemicals that induce uterine tumors in mice in the carcinogenic potency database.

Chemical	Route	Strain	Uterine tumor type	Experiment time (wks)	Incidences
Chloroethane	inhalation	B6C3F1	carcinoma	100	0/49, 43/50
Bromoethane	inhalation	B6C3F1	adenocarcinoma, adenoma, and squamous cell carcinoma	105	0/50, 4/50, 5/47, 27/48
Ethylene oxide	inhalation	B6C3F1	adenocarcinoma and adenoma	104	0/48, 2/47, 5/49
1,2-Dichloroethane	gavage	B6C3F1	endometrial stromal polyp and stromal sarcoma	90	0/50, 5/50, 5/50
Glycidol	gavage	B6C3F1	adenocarcinoma and carcinoma	104	0/50, 3/50, 3/50
1,2,3-Trichloropropane	gavage	B6C3F1	adenoma and adenocarcinoma	104	0/50, 5/50, 3/51, 9/55
trimethylphosphate	gavage	B6C3F1	adenocarcinoma	103	0/20, 7/50, 13/49
Procabazine, HCl	intraperitoneal	B6C3F1	adenocarcinoma	85	0/15, 14/35, 8/35
Diethylstilbesterol	diet	B62 ^a	mesothelioma	153	0/67, 0/71, 0/72, 0/70, 0/70, 0/69, 2/66, 7/71
Dacarbazine	intraperitoneal	Swiss	adenocarcinoma	61	0/154, 4/14
Vinyl acetate	water	Swiss	malignant tumor	151	1/37, 2/37, 8/37
(N-6)-(Methylnitroso) adenosine	water	Swiss	not specified	104	0/16, 5/20

^a This is a monohybrid cross offspring ($F_1 \times F_1$) ($B6CF_1 \times B6CF_1$); $B6CF_1$ is a cross between C57BL/6 \times BALB/c.

tories (Richland, WA). Research was conducted in compliance with the Animal Welfare Act, and other Federal statutes and regulations relating to animals and experiments involving animals, and adhered to principles stated in the 1996 "Guide for the Care and Use of Laboratory Animals" (National Academy Press, Washington, DC), in facilities that are fully accredited by the Association for the Assessment and Accreditation of Laboratory Animal Care, International.

The mice used in these studies were obtained from the Frederick Cancer Research Facility (Frederick, MD). Animals were shipped to the study laboratory at 5–6 weeks of age and were quarantined for three weeks. Thereafter, a complete necropsy was performed on five animals of each sex to assess their health status. The rodents were placed on study at 8–9 weeks of age. The mice were housed individually and fed *ad libitum* during non-exposure periods. Water was available at all times.

Study design for bromoethane: Groups of 49 or 50 B6C3F1 mice of each sex were exposed in chambers to bromoethane vapor at concentrations of 0 (chamber controls), 100, 200 or 400 ppm for 6 hours per day, 5 days per week for 103 or 104 weeks.

Study design for chloroethane: Groups of 50 B6C3F1 mice of each sex were exposed to chloroethane vapor at concentrations of 0 (chamber controls) or 15,000 ppm for 6 hours per day, 5 days per week for 100 weeks.

Study design for ethylene oxide: Groups of 50 B6C3F1 mice of each sex were exposed to air containing 0 (chamber controls), 50, or 100 ppm ethylene oxide, 6 hours per day, 5 days per week for 102 weeks.

Chemical procurement, analyses and delivery system: Chloroethane was obtained from Matheson Gas Products (East Rutherford, NJ) or Air Products, Inc. (Tamaqua, PA). Purity and identity analyses were conducted at Midwest Research Institute (Kansas City, MO) and Batelle Pacific Northwest Laboratories (Richland, WA). The purity of the chloroethane was determined by gas chromatography to be at least 99.5% pure. Samples of chloroethane were obtained from the generation reservoir after generation of study atmosphere, and it was determined that there was no evidence of decomposition or degradation of chloroethane in the study atmospheres.

Bromoethane was obtained from Dow Chemical Company (Midland, MI). The purity and identity analyses for bromoethane were conducted at Midwest Research Institute (Kansas City, MO). The purity of bromoethane was evaluated by elemental analysis, water analysis, and titration of the acidic components with 0.01 N sodium hydroxide in ethanol solution to the phenolphthalein endpoint, and gas chromatography, and determined to be greater than 98%. The purity of the study material was also determined by sampling the material from the generation reservoir after generation of study atmospheres. It was concluded that the study chemical remained stable in the generation reservoir during the generation of bromoethane study atmospheres.

Ethylene oxide was obtained from Union Carbide, Linde Division, Torrance, California and Somerset, N.J. The ethylene oxide was analyzed periodically by infrared spec-

troscopy and gas chromatography, and the purity was greater than 99%. No degradation of the chemical was observed over the course of studies.

Pathology evaluation: For the three Technical Reports (NTP 1987, 1989a,b), complete histological examination was carried out on all animals dying prematurely and on those animals surviving to term. All organs and tissues were examined for grossly visible lesions. Tissues were preserved in 10% neutral buffered formalin, embedded in paraffin, sectioned, and stained with hematoxylin and eosin.

Quality assurance and Pathology Working Group (PWG): When the pathology evaluation was completed by the laboratory pathologist for the Technical Reports, the slides, paraffin blocks and residual wet tissues were audited for accuracy of labeling and animal identification and for thoroughness of tissue trimming. All tissues with a tumor diagnosis, all potential target tissues and all tissues from a randomly selected 10% of the animals were re-evaluated microscopically by a quality assessment pathologist. The quality assessment report and slides were submitted to a Pathology Working Group according to the procedure described, in part, by MARONPOT AND BOORMAN 1982 and BOORMAN et al. 1985. The final pathology data reported in the Technical Reports represents a consensus of contractor pathologist, the quality assurance pathologist and the NTP Pathology Working Group. Because of the unusual results in the mouse uterus, a re-evaluation of the final pathology data and slides was conducted in 2002 by a panel of one M.D. and four veterinary pathologists. For bromoethane and chloroethane, this panel examined all original slides of the uterus and any non-uterine tissues in which the data reported a metastatic neoplasm. For ethylene oxide, all of the original slides of the uterus were examined. No recuts of any wet tissues were examined for any of the three chemicals. This panel confirmed by unanimous decision the diagnoses in the final pathology data.

Results

Re-evaluation of the pathology and incidence data for chloroethane, bromoethane and ethylene oxide in both mice and rat chronic bioassays confirmed the following: chloroethane induced only uterine tumors and only in mice; bromoethane induced only uterine tumors in mice, but in male rats induced tumors at other sites; and ethylene oxide induced tumors at multiple sites in both sexes of rats and mice. The carcinogenicity results for mouse uterus were thus most extreme for chloroethane, since the incidence was highest (86%), the metastases most frequent, and the uterus was the only site in any sex-species group. Bromoethane also had high tumor yields and metastases for mouse uterus.

Body weight and survival: For bromoethane, mean body weight in the high dose (400 ppm) female mice was generally 6–16% lower than controls. There was a reduced survival in the female mice in the high dose group only (36/50, 37/50, 37/49 and 23/49 at study termination

in the 0, 100, 200 and 400 ppm respectively). Bromoethane did not induce tumors in male mice.

For chloroethane, mean body weight of exposed female mice was 5–13% lower in the single dose group than that of controls throughout the study. The survival of the exposed groups of female mice was significantly lower than control (32/50 controls; 2/50 exposed). The majority of exposed female mice died as a result of uterine tumors. The study in male mice was considered inadequate because the mice, particularly exposed mice, died early from an ascending urinary tract infection. Female mice did not have such infection.

For ethylene oxide, there was no treatment related effect on body weight. Final mean body weights in exposed mice were 95–102% of those of the controls. The survival of female mice in the control group, 50 ppm and 100 ppm group was 25/50, 24/50 and 31/50, respectively. Ethylene oxide also induced malignant neoplasms in female mice in the mammary gland and hematopoietic system and induced neoplasms of the lung and benign neoplasms of the Harderian gland in female and male mice.

Histopathology of uterine neoplasms

Bromoethane: In the bromoethane carcinogenicity study, there was an increase in the incidence of neoplasms of the uterine endometrium, namely adenomas, adenocarcinomas and squamous cell carcinomas (table 2). The increased incidence of uterine tumors was statistically significant in the high dose group. One animal in the high dose group (400 ppm) had both a uterine adenoma and a uterine squamous cell carcinoma. All

other animals in the treatment groups had only one type of uterine epithelial cell neoplasm.

Adenomas were exophytic polypoid masses arising from the endometrium, and consisted of glands lined by a single layer of well differentiated cuboidal to columnar epithelial cells supported by a moderate amount of fibrous stroma (fig. 1). The cells were confined by a basal lamina and demonstrated no loss of polarity, and no invasion into the deep endometrial stroma or myometrium. The mitotic rate was low (0–1 mitotic figures/high powered field) (fig. 2). The lack of tissue invasion and the lack of cellular anaplasia was the basis for classifying these endometrial masses as benign adenomas rather than adenocarcinomas.

The adenocarcinomas induced by bromoethane were generally larger than the adenomas and invaded the myometrium (fig. 3). Commonly, the tumor involved the parietal and visceral peritoneum. Lymphatic and/or vascular invasion in the uterine sections was not a reliable indicator for malignancy, as this feature was rarely noted in routine uterine sections. The neoplastic cells varied from well-differentiated small cuboidal epithelial cells forming glandular patterns to large pale anaplastic cells forming sheets and nests (figs. 4 and 5). The mitotic rate varied from moderate to high (moderate – 2–3 mitotic figures/high powered field; high – 4 or more mitotic figures/high powered field). The stroma of these invasive adenocarcinomas was abundant and typical of a scirrhous reaction to proliferating neoplastic epithelial cells in other organs (fig. 4). Nine out of 24 (38%) adenocarcinomas metastasized to either the lung (4), regional lymph nodes (5), urinary bladder (2), pancreas (1), and/or adrenal gland (1). Intravascular tumor emboli were commonly seen in the lungs.

Table 2. Incidences of uterine tumors of epithelial origin in mice in the 2 year inhalation study of bromoethane.

Neoplasm	0 ppm	100 ppm	200 ppm	400 ppm
Adenoma	0/50	1/50	1/47	6/48 ^c
Adenocarcinoma	0/50	2/50	3/47	19/48 ^a
Squamous cell carcinoma (SCC)	0/50	1/50	1/47	3/48
Adenoma, Adenocarcinoma or SCC ^b	0/50	4/50	5/47	27/48 ^a

^a P < 0.001

^b combined incidence

^c One animal in this group also had a squamous cell carcinoma of the uterus.

Fig. 1. Adenoma of the uterus of female mouse exposed to 400 ppm bromoethane. The adenoma is a well-delineated nodular mass occluding the lumen with no invasion of the deep endometrium or myometrium. H&E. ×3.3.

Fig. 2. Higher magnification of the adenoma shown in figure 1. The cells of this mass are cuboidal, well differentiated, and form glands. The mitotic rate is low. H&E. ×66.

Fig. 3. Adenocarcinoma of the uterus of female mouse exposed to 400 ppm bromoethane. The mass obscures the lumen and invades into the myometrium. H&E. ×2.5.

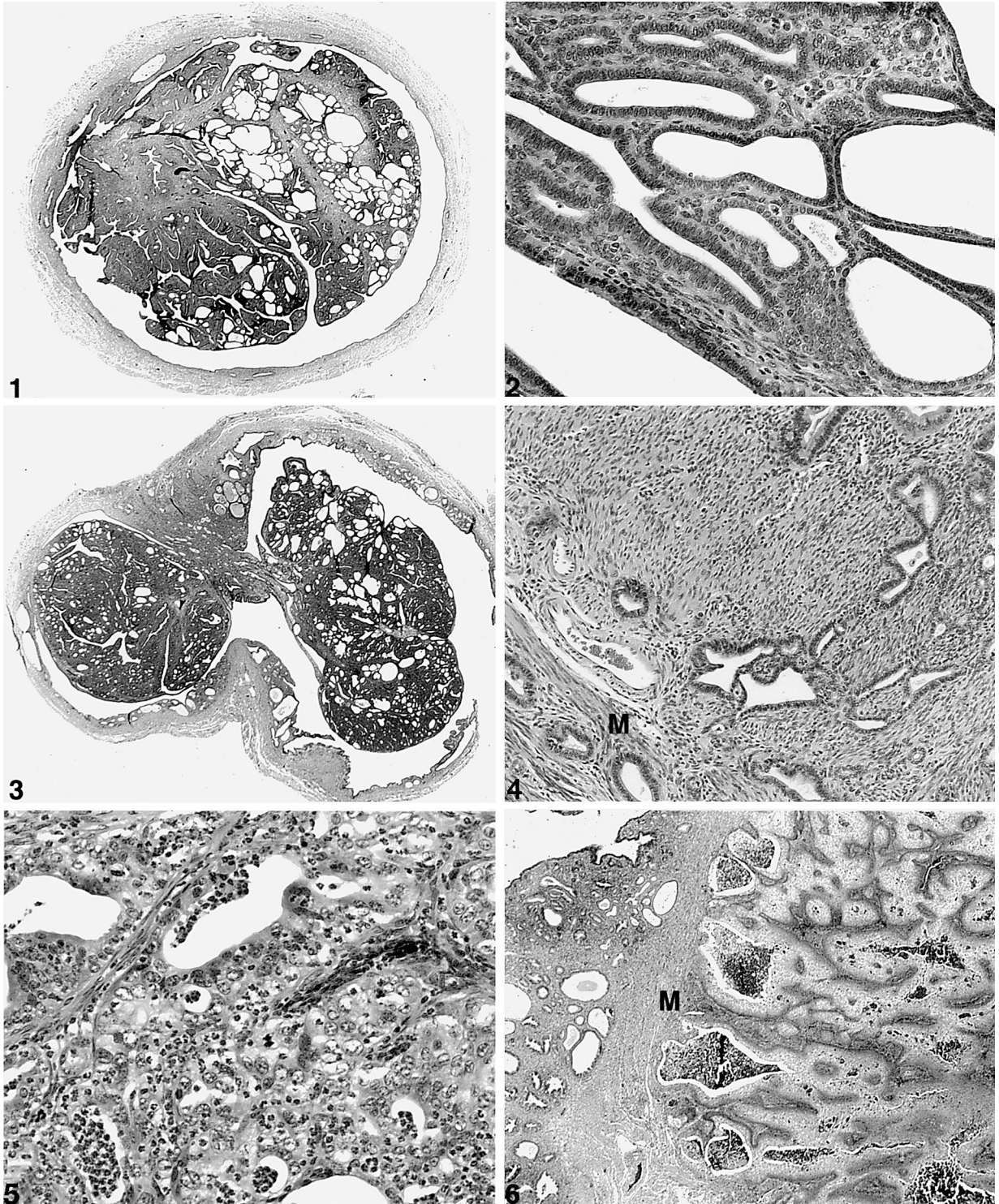


Fig. 4. Higher magnification of the adenocarcinoma shown in figure 3. The cells are invading the myometrium forming glandular patterns and are associated with a marked scirrhous reaction. M – Myometrium. H&E. $\times 33$.

Fig. 5. High magnification of an anaplastic adenocarcinoma of the uterus in a female mouse exposed to 400 ppm bromoethane. The neoplastic cells are ovoid with vesicular nuclear chromatin, have little polarity, and a moderate mitotic rate. H&E. $\times 80$.

Fig. 6. Squamous cell carcinoma from the uterus of a female mouse exposed to 400 ppm bromoethane. The neoplasm is highly invasive involving the myometrium (M). H&E. $\times 6.6$.

Five neoplasms of the uterine endometrium induced by bromoethane exposure contained a predominant (greater than 90%) cellular component of squamous cells, and these neoplasms were classified as squamous cell carcinomas (fig. 6). One of these five squamous cell carcinomas metastasized to the lung. In these tumors, glandular pattern formation was always evident in some parts of the mass, suggesting that these squamous cell carcinomas were secondary and arising within adenocarcinomas. As with the adenocarcinomas, lymphatic and/or vascular invasion in the uterine sections was rarely noted and was not a reliable indicator for malignancy.

Chloroethane: A highly significant increased incidence (86% or 43/50) of uterine adenocarcinomas of endometrial origin was clearly associated with chloroethane exposure in female mice (table 3). The adenocarcinomas were similar to those seen with bromoethane exposure. The mitotic rate varied from moderate to high. There was squamous differentiation in many of the adenocarcinomas induced by chloroethane, but the squamous differentiation was less prominent than the glandular patterns and comprised less than 20% of the epithelial component of the neoplasms (figs. 7 and 8). Thirty-four (34) of these 43 chloroethane-induced adenocarcinomas metastasized to a wide variety of organs, primarily lung (23/43), ovary

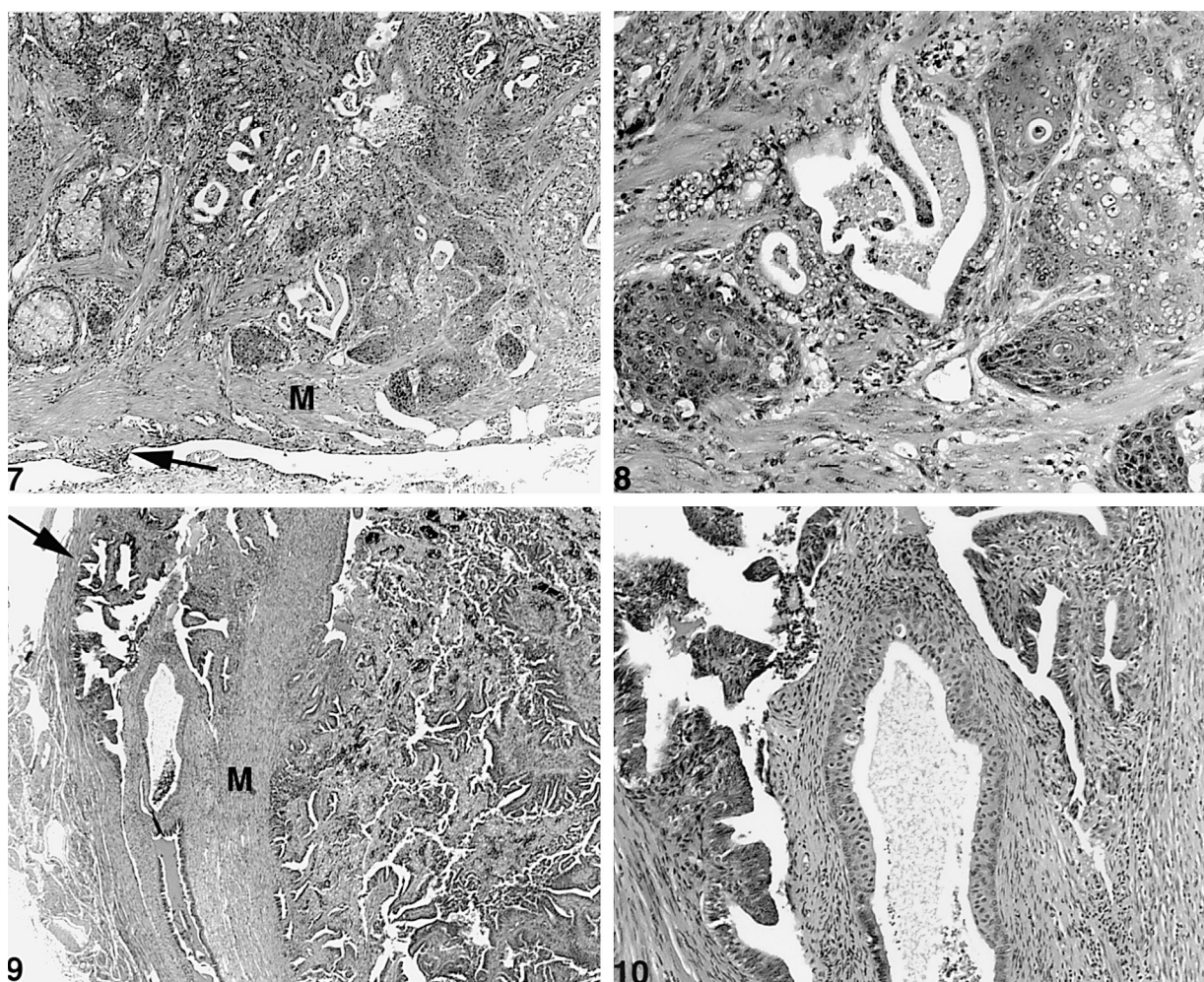


Fig. 7. Adenocarcinoma with squamous differentiation of the uterus in a female mouse exposed to 15,000 ppm chloroethane. The adenocarcinoma extends into the myometrium (M) and to the serosa (arrow). H&E. $\times 16$.

Fig. 8. Higher magnification of the adenocarcinoma of figure 7 demonstrating squamous cells forming islands and nests, and cuboidal cells forming glandular patterns. H&E. $\times 50$.

Fig. 9. Adenocarcinoma with squamous differentiation of the uterus of a female mouse exposed to 100 ppm ethylene oxide. The adenocarcinoma invades the myometrium (M) and extends to the visceral peritoneum (arrow). Note the papillary projections of neoplastic cells into glandular lumina, typical of the pattern seen in the adenocarcinomas induced by ethylene oxide. H&E. $\times 10$.

Fig. 10. Higher magnification of the neoplasm in figure 9. There is squamous differentiation of the neoplastic cells lining one gland. The majority of the cells however are cuboidal to columnar. H&E. $\times 33$.

Table 3. Incidences of uterine tumors of epithelial origin in mice in the 2 year inhalation study of chloroethane.

Neoplasm	0 ppm	15,000 ppm
Adenocarcinoma	0/49	43/50 ^a

^a P < 0.001

Table 4. Incidences of uterine tumors of epithelial origin in mice in the 2 year inhalation study of ethylene oxide.

Neoplasm	0 ppm	50 ppm	100 ppm
Adenocarcinoma	0/49	2/47	5/49 ^a

^a P < 0.001

(22/43), and lymph nodes (18/43), and to a lesser extent to the kidney, adrenal gland, pancreas, mesentery, urinary bladder, spleen, heart, colon, stomach, gallbladder, small intestine, ureter and liver. The degree of squamous differentiation by the uterine adenocarcinomas did not correlate with the occurrence or pattern of metastasis.

Ethylene oxide: Uterine glandular adenocarcinomas were increased in the high dose females exposed to ethylene oxide (table 4). The incidence of 5/49 (10%) adenocarcinomas exceeded the overall mean historical incidence in untreated control female B6C3F1 mice (8/2,055; 0.4%) and the historical incidence for chamber controls in inhalation experiments at the study laboratory (4/236; 1.7%) (NTP 1987). The incidence of uterine neoplasms for ethylene oxide was thus lower than for bromoethane and much lower than for chloroethane. The adenocarcinomas were lobular masses occluding the lumen of the uterus and generally infiltrating slightly into the myometrium, and in one case to the serosa. The masses were comprised of large cuboidal to columnar cells densely packed into papillary folds projecting into glandular lumina. The cells had large vesicular nuclei with prominent eosinophilic nucleoli with a low to moderate mitotic rate. Compared to the adenocarcinomas induced by bromoethane or chloroethane, these ethylene oxide induced adenocarcinomas had less supporting stroma, were less invasive into myometrium, and the cells were generally larger with more vesicular nuclei. Squamous differentiation was seen in one of the adenocarcinomas induced by ethylene oxide (figs. 9 and 10). One of the adenocarcinomas induced by ethylene oxide metastasized to the peritoneum, lung and lymph nodes.

Discussion

The high incidences of uterine neoplasms and metastases were confirmed in a re-evaluation by a

panel of pathologists in 2002. Bromoethane, chloroethane, and ethylene oxide were also administered by inhalation to F344 rats, but none of these chemicals induced uterine tumors in rats despite the fact that the uterus is a more frequent target site in rats than mice (GOLD et al. 2001).

Bromoethane, chloroethane and ethylene oxide are mutagenic alkylating agents. Bromoethane is a chemical in the class of halogenated alkanes, which encompasses a wide range of industrial and pharmaceutical agents. Haloalkanes are highly lipophilic and readily cross the blood-brain barrier (KLAASSEN 1996). Bromoethane has limited commercial use as an ethylating agent in organic syntheses, for ethylation of gasoline, and has been formerly used as an anesthetic. To a much lesser extent it was formerly used as a fruit and grain fumigant, refrigerant, and a solvent. Bromoethane occurs naturally and is formed by marine algae.

In the NTP 2-year bioassay bromoethane induced adenomas, adenocarcinomas, and squamous cell carcinomas of uterine endometrial epithelium (NTP 1989b). The squamous cell carcinomas induced by bromoethane invariably had small zones of acinar formation by cuboidal cells, suggesting that these squamous cell carcinomas were arising from or within a primary adenocarcinoma. Since greater than 90% of the epithelial component of the mass was squamous cells, these tumors were classified as squamous cell carcinomas. Nine of 24 adenocarcinomas and squamous cell carcinomas metastasized to the urinary bladder, lymph nodes, lung, pancreas, and/or adrenal glands. In addition to the neoplastic effect on the uterus, bromoethane caused an increased incidence of alveolar/bronchiolar neoplasms of the lung in male mice compared to controls (adenomas or carcinomas, combined: 7/50; 6/50; 12/50; 15/50) in the two-year chronic bioassay (NTP 1989b).

Chloroethane, also known as ethyl chloride, is also in the class of halogenated alkanes. It is a strong alkylating agent that was primarily used in the manufacture of tetraethyl lead anti-knock gasoline additives, ethylcellulose plastics, dyes and pharmaceuticals. It is also used as a topical anesthetic, and as an industrial refrigerant (FISHBEIN 1979; NIOSH 1983). It is not known to occur naturally.

In the NTP 2-year bioassay, chloroethane induced uterine adenocarcinomas of endometrial origin. Several of the adenocarcinomas induced by chloroethane exhibited small zones of squamous differentiation. Since the predominant cell type in these tumors was acinar cell, these tumors were appropriately classified as adenocarcinomas rather than squamous cell carcinomas. In addition to the uterine tumors in this two-year bioassay, chloroethane resulted in a marginally increased incidence of hepatocellular neoplasms in the exposed female mice (3/49 in control; 8/48 in high dose) (NTP 1989a).

Ethylene oxide, also an alkylating agent, is mutagenic and clastogenic. It is a major industrial chemical used primarily as an intermediate in the manufacture of other chemicals; e.g., ethylene glycol, a major component of

automotive and other antifreeze products, polyester resins, nonionic surfactants, and specialty solvents (NIOSH 1981). Ethylene oxide is a metabolite of ethylene and occurs naturally. The most highly exposed individuals are hospital workers and workers involved in sterilization and manufacturing.

Ethylene oxide exposure resulted in an increased incidence of uterine adenocarcinomas in female mice. One of seven adenocarcinomas exhibited squamous differentiation and one of the adenocarcinomas metastasized. Aside from causing uterine adenocarcinomas in mice, ethylene oxide also induced alveolar/bronchiolar carcinomas in female and male mice, cystadenomas of the Harderian gland in male and female mice, and malignant lymphoma in female mice, compared to controls (NTP 1987).

The papillary features in the uterine adenocarcinomas induced by ethylene oxide are similar to the human endometrial adenocarcinomas typically associated with obesity, diabetes, and hypertension, and are estrogen-related (COTRAN et al. 1989). The finding that one of seven of the ethylene oxide induced tumors exhibited squamous differentiation is similar to the human situation, where squamous differentiation is seen in 10–20% of human endometrial adenocarcinomas (COTRAN et al. 1989). In contrast to the neoplasms induced by ethylene oxide, the adenocarcinomas induced by chloroethane or bromoethane are histologically and behaviorally more similar to the human serous carcinomas. Serous carcinomas are highly aggressive endometrial neoplasms that occur primarily in elderly women, are nonestrogen related, and carry a poor prognosis (SMITH AND MCCARTNEY 1985).

The mechanism by which bromoethane, chloroethane or ethylene oxide cause uterine tumors following inhalation exposure is not clear. Most of the mechanistic studies assessing the induction of endometrial tumors with these chemicals have been done with chloroethane. It has been suggested that the specificity for chloroethane to cause uterine tumors in mice may depend on the species-specific metabolism of chloroethane in mice. The conjugation of chloroethane with glutathione is considered the major route of metabolism of this chemical in the mouse at high concentrations (above 6000 ppm), and studies show a significant depletion of glutathione in the liver, uterus, kidney, and brain of chloroethane-exposed mice but not rats (POTTENGER et al. 1992). The glutathione conjugates formed during the metabolism of chloroethane are suspected as being the excitotoxins responsible for hyperexcitability, seizures and nervous disorders in mice exposed to chloroethane. FEDTKE et al. (1994a) postulate that the hyperexcitability and CNS disorder caused by these GSH-conjugates may lead to central hormonal effects that eventually result in uterine tumor development.

Limited studies in mice have been done to help determine if the uterine tumors caused by chloroethane or bromoethane are hormonally related as FEDTKE et al.

(1994a) proposes. BUCHER et al. (1995) studied changes in blood concentration of sex hormones of mice prior to and during a 21 day exposure to concentrations of bromoethane or chloroethane. No consistent pattern of change was found in blood concentration of estradiol or progesterone. Thus the findings reported by BUCHER et al. (1995) suggest that early changes in circulating estradiol or progesterone are not important contributing factors in the uterine neoplasia caused by these chemicals (BUCHER et al. 1995). However, this does not rule out the possibility that hormonal imbalance is still responsible for tumor development in these mice.

Other possible mechanisms of carcinogenesis for chloroethane, bromoethane or ethylene oxide may be by a direct genotoxic effect. Chloroethane is metabolized in part, not only by a glutathione dependent pathway, but also by a cytochrome P450 dependent metabolic pathway. There is a potential role of this oxidative metabolic pathway for the expression of mouse tumors, since this oxidative route of metabolism results in the formation of acetaldehyde which is a genotoxic carcinogen (FEDTKE et al. 1994b).

We conclude that chronic inhalation exposure of mice to bromoethane, chloroethane or ethylene oxide results in increased incidences of endometrial tumors as evidenced by histologic evaluation of uterine tissue. Adenomas were induced by bromoethane, and were exophytic polypoid masses arising from the endometrium, and consisted of glands lined by a single layer of well differentiated cuboidal to columnar epithelial cells. Adenocarcinomas were induced by bromoethane, chloroethane and ethylene oxide. Those adenocarcinomas induced by bromoethane or chloroethane were invasive with neoplastic cells exhibiting varying degrees of pleomorphism and anaplasia supported by a substantial fibrous stroma. On the other hand, the adenocarcinomas induced by ethylene oxide were less invasive and formed large lobular masses occluding the lumen of the uterus. The ethylene oxide induced adenocarcinomas were generally comprised of large vesicular cells with prominent eosinophilic nucleoli. Squamous cell carcinomas were induced by exposure to bromoethane. These squamous cell carcinomas were comprised predominantly (greater than 90%) of squamous cells but generally had a small glandular component. Also there was a high rate of metastasis of malignant endometrial tumors in mice from chloroethane (79%) and bromoethane (33%) studies. As far as the mechanism(s) by which these tumors occur, further studies are necessary to better understand the biochemical/molecular events important in the development of chloroethane, bromoethane, and ethylene oxide induced uterine neoplasia in B6C3F1 mice. Studies may include the effects of these chemicals on hormone receptor expression in mouse uterine tissue, on circulating hormone levels, and/or on chemical modulation of hormone receptor expression within the tumor tissue itself.

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