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Propylene Glycol Monomethyl Ether (PGME): Inhalation Toxicity and Carcinogenicity in Fischer 344 Rats and B6C3F1 Mice

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ABSTRACT

A series of inhalation studies with propylene glycol monomethyl ether (PGME) vapor were undertaken to characterize its subchronic toxicity in mice and chronic toxicity/oncogenicity in rats and mice. Groups of male and female Fischer 344 rats and B6C3F1 mice were exposed to 0,300, 1,000, or 3,000 ppm vapor from 1 week to 2 years. Primary treatment-related effects included: initial sedation of animals exposed to 3,000 ppm and its subsequent resolution correlating with induction of hepatic mixed function oxidase activity and S-phase DNA synthesis; elevated mortality in high-exposure male rats and mice (chronic study); elevated deposition of alpha$_{2U}$-globulin (α_{2U}-G) and associated nephropathy and S-phase DNA synthesis in male rat kidneys; accelerated atrophy of the adrenal gland X-zone in female mice (subchronic study only); and increased occurrence and/or severity of eosinophilic foci of altered hepatocytes in male rats. No toxicologically relevant statistically significant increases in neoplasia occurred in either species. A numerical increase in the incidence of kidney adenomas occurred in intermediate-exposure male rats; however, the association with α_{2U}-G nephropathy, a male rat specific effect, indicated a lack of relevance for human risk assessment.

Keywords. Propylene glycol monomethyl ether; PGME; glycol ether; carcinogenicity; inhalation; toxicity; rats; mice; alpha$_{2U}$-globulin nephropathy; altered heptocellular foci; cell proliferation.

INTRODUCTION

Propylene glycol monomethyl ether (PGME) is one of a family of glycol ether solvents with a wide variety of consumer product and industrial applications. It is the most volatile of its chemical family; therefore, humans are most likely to be exposed by inhalation of vapor. Commercial PGME is a clear liquid typically consisting of a mixture of 2 isomers: at least 97% as 1-methoxy-2-propanol and the remainder as 2-methoxy-1-propanol. To minimize odor and potential eye irritation, the American Conference of Governmental Industrial Hygienists (ACGIH) threshold limit value and short-term exposure limit for PGME have been set at 100 parts per million (ppm) and 150 ppm, respectively (1).

In acute inhalation studies of PGME vapor, concentrations greater than 6,000 ppm caused marked sedation in rats and mice and death in mice (7, 8). In previous inhalation subacute studies in rats and mice (25) and subchronic studies in rats and rabbits (21), 3,000 ppm PGME also caused sedation or anesthesia during the first week of the exposure period. Increases in liver weights were also noted in rats and mice exposed to 3,000 ppm (21, 25). Additionally, re-examination of histologic slides from the previous 2-week study revealed an increase in hyaline droplets and mitotic figures in proximal tubular epithelium in kidneys of male rats exposed to 3,000 ppm (The Dow Chemical Company, unpublished data).

The metabolism and pharmacokinetics of PGME in rats have been previously characterized (25, 28). The absorption of the water-soluble PGME vapor is primarily limited by respiration and the major routes of metabolite elimination are expired air for the major isomer and urine for the minor isomer. The major isomer is metabolized via O-demethylation to propylene glycol which may subsequently be metabolized to CO_2 via the tricarboxylic acid cycle, or contribute to glycogen formation via the glycolytic pathway. The dealkylation reaction was saturated at exposures above 1,500 ppm. The minor isomer may be metabolized by alcohol dehydrogenase to 2-methoxypropionic acid that, as discussed by Miller et al (26), may have a different toxicological profile than PGME. However, at less than 3% of the test material, the minor isomer was found to have no measurable impact on the toxicity of PGME (26). Blood levels of PGME in exposed rats were found to decrease dramatically following exposure to 3,000 ppm vapor for 10 days suggesting the induction of the enzymes in metabolism of PGME during this period (28).

The present series of studies were primarily undertaken to characterize the chronic toxicity/oncogenicity of PGME vapor in rats and mice and subchronic toxicity of PGME in mice. Further, the potential mechanisms of toxicity of PGME and/or its resolution were also examined in rats and mice exposed to vapor from 1 week to 18 months.

MATERIALS AND METHODS

Test Material and Exposure Conditions

PGME used in all studies was obtained from The Dow Chemical Company (Plaquemine, LA). Samples were periodically characterized by gas chromatography with flame ionization detection and infrared spectroscopy. In addition, test material was assayed for water content by Karl Fisher titration, and for peroxide by polarography. Test material purity was $\geq$99.96%, with isomer concentrations of 97.39–97.49%

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for 1-methoxy-2-propanol, and 2.46–2.59% for 2-methoxy-1-propanol. No impurities were present at more than 0.1%. Water was found in the test material at 210–473 ppm, and hydrogen peroxide was below detection limits (0.5–3 ppm).

In all studies, animals were whole-body exposed to PGME vapor under dynamic airflow conditions, which provided approximately 12 changes/hour of chamber air, and maintained normal oxygen concentration. Vapor was generated by metering liquid PGME into a glass J-tube assembly, through which was passed a preheated stream of compressed air. The compressed air was heated to the minimum extent necessary to vaporize all of the test material (approximately 65, 105, and 120–160°C for 300, 1,000, and 3,000 ppm chambers, respectively). Compressed air and PGME vapor were diluted with room air to achieve a flow rate of 2,900 Lpm and the desired PGME vapor concentration (27). The analytical concentration of PGME in the breathing zone of the animals was measured, twice per hour, with a MIRAN 1A infrared spectrophotometer (Foxboro Analytical, Norwalk, CT). In addition, the amount of PGME used daily for each chamber was recorded, and the nominal concentrations of PGME vapor were calculated. The mean time-weighted average chamber concentrations were within 0.5% of the target concentrations for all of the studies. The distribution of PGME at the extremes of the animal breathing zones was measured periodically and generally found to be within 1–2% of the reference sample line.

Animals

Male and female rats and mice were purchased from Charles River (Portage, MI). Following acclimation for 14 days animals were randomized by body weight and identified by either an ear tag or an implanted microchip (BioMedic Data Systems, Inc; Maywood, New Jersey). Rats were housed 2 per stainless steel wire cage, and mice, singly. Animals were maintained under environmentally controlled conditions (12-hour photoperiod, 22 ± 2°C, 40–60% relative humidity). Water and Purina Certified Rodent Chow #5002 (Purina Mills, Inc, St. Louis, MO) were freely available, except that feed was withheld during exposure periods. Rodents were 10–12 weeks of age at study start of the 1- and 2-week studies and 6–8 weeks of age at the start of all studies 13-weeks or longer. In all studies, animals were observed at least daily, and in the 13-week and chronic studies, they were weighed weekly for the first 13 weeks, and at 4-week intervals, thereafter; except that no in-life body weights were recorded for Group B animals after osmotic minipumps were implanted.

These studies were conducted in accordance with the Environmental Protection Agency's TSCA Good Laboratory Practice Procedures (10) and were reviewed and given full approval by the Institutional Animal Care and Use Committee.

Study Design(s)

The series of PGME inhalation exposures of rats and mice encompassing the overall assessment are listed in Table 1. Routine, nonclinical toxicity studies were conducted to characterize subchronic toxicity in mice (13-week duration), chronic toxicity, and oncogenicity in rats, and oncogenicity in mice. Additional groups of male and female rats and mice used to examine S-phase DNA synthesis and MFO activity were exposed to vapor at 1 or more of the exposure levels used in the toxicity studies/bioassays for 1, 2, 13, 26, 52, or 78 weeks. Following the appropriate exposure period, data on a standard set of parameters in the toxicity study/bioassays were evaluated (Group A) and data on hepatic and/or renal S-phase DNA synthesis (Group B), hepatic MFO activity (Group C), and, in male and/or female rats, renal α_{2U}-G (Group D) was measured. Subgroupings of animals (A–D) are also summarized in Table 1. In the 1- and 2-week studies, animals received five separate 6-hour exposures over 7 days and 9 exposures over 11 days, respectively. In all remaining studies exposures were for 6 hours/day, 5 days/week.

Routine Toxicity Studies: In the mouse subchronic toxicity study, additional data was obtained on a number of hematological and clinical chemistry parameters, terminal body weights, and the weights of brain, heart, lungs, liver, kidney, and testes. Organ weights relative to body weights were calculated. An extensive set of tissues from control and high-exposure groups of mice were preserved in

TABLE 1.—Summary of routine, and mechanistic studies.

		Number of rats (R) and mice (M) per exposure level (males/females)											
		1 and 2 wk		13 wk		6 mo		12 mo		18 mo		24 mo	
PPM	Group	R	M	R	M	R	M	R	M	R	M	R	M
0	A*	—	—	—	10/10	—	—	—	—	—	—	50/50	50/50
	B	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	10/10	10/10	—	—
	C	5/5[φ]	5/5[φ]	5/5	5/5	5/5	5/5	5/5	5/5	10/10	10/10	—	—
	D	5/2	5/2	5/5	—	5/0	—	5/0	—	—	—	—	—
300	A	—	—	—	10/10	—	—	—	—	—	—	50/50	50/50
	B	—	—	5/5	5/5	5/5	5/5	5/5	5/5	10/10	10/10	—	—
	C	—	—	5/5	5/5	—	—	—	—	—	—	—	—
	D	—	—	5/5	—	5/0	—	5/0	—	—	—	—	—
1,000	A	—	—	—	10/10	—	—	—	—	—	—	50/50	50/50
	B	—	—	—	—	5/5	5/5	5/5	5/5	10/10	10/10	—	—
	C	—	—	—	—	—	—	—	—	—	—	—	—
	D	—	—	—	—	5/0	—	5/0	—	—	—	—	—
3,000	A	—	—	—	10/10	—	—	—	—	—	—	50/50	50/50
	B	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	10/10	10/10	—	—
	C	5/5[φ]	5/5[φ]	5/5	5/5	5/5	5/5	5/5	5/5	10/10	10/10	—	—
	D	5/2	5/2	5/5	—	5/0	—	5/0	—	—	—	—	—

*Group A: routine study, Group B: cell proliferation in liver and kidneys, Group C: hepatic MFO induction, Group D: α_{2U}-g nephropathy evaluation.
[φ]Hepatic enzyme induction determined after 2-week exposure only.

phosphate-buffered 10% formalin, processed, stained with hematoxylin and eosin, and examined using light microscopy.

In the rat chronic toxicity and carcinogenicity study, blood and urine samples were obtained from 10 animals/sex/dose at 6, 12, and 18 months, and from 20 animals/sex/dose at 24 months for hematology, urinalysis, and clinical chemistry evaluations. Similar parameters were evaluated in the mouse oncogenicity study except that urinalysis and clinical chemistries were not included. Selected organs were weighed at the scheduled necropsies and a complete set of tissues from control and high-exposure animals were collected, processed, stained, and examined histopathologically. In addition, the lungs, liver, kidneys, gross lesions, and nasal tissues (chronic rat study only) of intermediate- and low-exposure group rats and mice were also examined histopathologically.

In liver cross-sections from the left lateral, right lateral and right medial lobes, foci of hepatocellular alteration in rats were graded considering their number, size, and cellular character. Foci were separated into basophilic and eosinophilic types based on tinctorial properties consistent with those employed by others (14, 17, 29, 32). Basophilic foci were graded based on the following criteria: *Very slight*: 1 to 3 tigroid foci; *Slight*: more than 3 tigroid foci; *Moderate*: at least 1 large atypical basophilic focus. Eosinophilic foci were graded based on the following criteria: *Very slight*: 1 to 4 foci, none larger than a liver lobule. Hepatocytes generally larger and paler than surrounding hepatocytes and little cellular atypia; *Slight*: 5–14 small foci or at least 1 focus between 1 and 3 lobule(s) in size and minimal cellular atypia; *Moderate*: 15–24 foci or at least 1 focus between 3 to 6 lobules in diameter and little cellular atypia. Prominent cystic degeneration and/or angiectasis and possible small areas of defined tissue compression. *Severe*: $\geq$25 foci or at least one focus larger than 6 lobules in diameter with little cellular atypia. Prominent cystic degeneration and/or angiectasis and possible areas of poorly defined tissue compression. *Very Severe*: Many foci and/or foci over 6 lobules in diameter with little atypia. Prominent cystic degeneration and/or angiectasis and areas of tissue compression along the margin of the focus. When cystic degeneration was observed within a focus of altered hepatocytes, only the surrounding altered focus was recorded.

In kidneys, the severity of chronic progressive glomerulonephropathy (CPG) in rats was graded based on the following criteria: *Very slight*: rare basophilic or protein filled tubules; lower limit of detectability, affected less than 5% of parenchyma. *Slight*: affected 5–50% of parenchyma; probably not clinically significant. *Moderate*: affected 50–80% of the kidney parenchyma, may have affected animal's health. *Severe*: affected 80–100% of the kidney parenchyma; may have been fatal.

Alpha$_{2U}$-Globulin (α_{2U}-G) Nephropathy: Alpha$_{2U}$-globulin (α_{2U}-G) nephropathy was evaluated after 6 and 12 months of exposure. Briefly, rats were anesthetized, and perfused with a 1.5% glutaraldehyde/4.0% formaldehyde solution. Kidneys were immersed in cold (4°C) fixative, and all subsequent processing was done at that temperature. Kidneys were embedded in methacrylate, sectioned at 2 μm and stained with Lee's methylene blue-basic. An additional 2 μm-thick section from each kidney was cut and processed for immunohistochemical staining of α_{2U}-G according to methods outlined by Burnett et al (6). Both sections were evaluated by light microscopy.

S-Phase DNA Synthesis: S-Phase DNA synthesis was determined in rats and mice using the 5-bromo-2-deoxyuridine (BrdU) incorporation immunohistochemical quantitation method outlined by Goldsworthy et al (13). Briefly, ALZET osmotic minipumps (Alza Corp., Palo Alto, CA) containing BrdU were implanted subcutaneously at the start of the last week of exposure. The liver, kidney, and duodenum (positive control) were excised and preserved in formalin. Tissues were embedded in paraffin, and histologic slides were stained immunohistochemically for identification of nuclear BrdU incorporation. The percent of cells with labeled nuclei or labeling index (LI) was calculated after counting 1,000 randomly selected hepatocytes or renal cortical epithelial cells.

Enzymes: The concentration of cytochromes P-450 and the activities of several mixed function oxidase enzymes were evaluated using the methods outlined by Burke et al (5), Omura and Sato (30), and Simmons and McKeem (31). Briefly, after humane euthanasia, livers were excised, weighed, frozen in liquid nitrogen, and stored at −80°C. Microsomes were subsequently prepared by differential centrifugation as described by Guengerich (16). CYP1A1 activity was determined by measuring the O-dealkylation of ethoxyresorufin (EROD), CYP2B1/CYP2B2 activity was determined by measuring the O-dealkylation of pentoxyresorufin (PROD), and total cytochromes P450 were measured by CO-binding difference spectroscopy. Protein levels were quantitated using the method outlined by Lowry et al (23).

Statistical Analysis

Descriptive statistics (means and standard deviations only) of chamber concentrations, temperatures, relative humidities and air flows as well as white blood cell differential counts were calculated (where appropriate) for the various studies. Body and organ weights (absolute and relative), urinalysis, clinical chemistry, hematology, enzyme, and/or S-phase DNA synthesis data were evaluated by Bartlett's test for equality of variances ($\alpha = 0.01$; 37). Then, based on the outcome, exploratory data analyses were performed by either a parametric or nonparametric analysis of variance (ANOVA)($\alpha = 0.10$; 33, 19) followed respectively by Dunnett's test or the Wilcoxon Rank-Sum test with a Bonferroni correction for multiple comparisons ($\alpha = 0.05$, 2-sided; 37, 19, 24). Statistical outliers were identified by a sequential test ($\alpha = 0.02$, 2-sided; 15), but only excluded from analysis for documented, scientifically sound reasons, unrelated to treatment. In the chronic studies, the incidence of histopathologic observations from all animals scheduled for the terminal sacrifice was statistically analyzed. For tissues where every rat or mouse in all exposure groups was examined, the incidences of specific observations were tested first for deviation from linearity using ordinal spacings of the doses ($\alpha = 0.01$; 2). If linearity was not rejected, the data were tested for a linear trend using the Cochran-Armitage Trend test ($\alpha = 0.02$, 2-sided; 2). If the trend was statistically significant, or if significant deviations from linearity were found,

incidences for each exposure group were compared to the control group using a pairwise chi-square test with Yates' continuity correction ($\alpha = 0.05$, 1-sided; 12). For tissues that were not examined from all animals in the intermediate groups, statistical analyses were limited to the pairwise comparisons of the control and high exposure groups using the pairwise chi-square test with Yates' continuity correction.

Differences in mortality patterns were tested by the Gehan-Wilcoxon procedure (4) for all animals scheduled for terminal sacrifice. If a significant effect was identified for all dose groups ($\alpha = 0.10$) then individual analyses were run comparing each exposure concentration to control ($\alpha = 0.05$).

Results

Clinical Findings

In all studies, rats and mice exposed to 3,000 ppm PGME displayed decreased activity, incoordination, and transient sedation during the first week of exposures. Recovery was evident approximately 1 to 2 hours postexposure. During the second week, (where exposures continued), these effects abated and no further sedation was noted. Significantly, in the chronic toxicity/oncogenicity study rats, exposure-induced sedation returned following approximately 12–18 months of the exposure period. A similar recurrence of sedation was not evident in chronically exposed mice.

Mortality

There was no treatment-related effect on mortality in studies of less than 24 months duration. In the chronic studies, cumulative mortality was increased, albeit not statistically significant, in male rats and male mice in the last few months of exposure to 3,000 ppm PGME. In male rats, cumulative mortality at study termination was 40, 32, 40, and 58% (Figure 1a) and in male mice cumulative mortality was 18, 20, 18, and 34% (Figure 1b) for the 0, 300, 1,000, and 3,000 ppm groups, respectively. The mortality of female rats and mice was unaffected by treatment, ranging from 15–32% (data not presented). There was no exposure-related increase in any specific cause of death that were generally typical for the strain and age rats and mice used in these studies (3, 4, 35, 36).

Body Weights

In the chronic toxicity/oncogenicity studies, PGME exposure caused a statistically significant decrease in body weights of exposed rats and mice (data not shown) relative to controls during the exposure period (in-life phase of the study). Although no consistent body weight effects were observed in male rats, female rats exposed to 3,000 ppm for more than 118 days had statistically significant body weight decrements of up to 7%. Mice of both sexes exposed to 3,000 ppm PGME had statistically identified decrements in mean in-life body weights (2–7%) over much of the 2-year study. Statistically identified decrements in in-life body weights were also noted, though less frequently, in mice of both sexes exposed to 1,000 ppm vapor. Despite these changes during the dosing period, no statistically identified body weight differences of treated rats or mice of either sex were observed at study termination.

Clinical Pathology

In the subchronic mouse study there were no significant hematologic or clinical chemistry effects attributable to treatment. Likewise, in the chronic studies, no hematological effects in rats or mice, and no urinalysis findings (rats only) were attributable to treatment. Several parameters measured in exposed rats (only), however, were altered relative to controls. Serum creatinine was increased 78% and urea nitrogen was increased 100% in males exposed to 3,000 ppm for 24 months. Serum alkaline phosphatase (AP) activities were increased 16–79% in male rats exposed to 3,000 ppm for 6 through 24 months, and to 1,000 ppm for 24 months. Serum enzymes associated with hepatocellular degeneration (alanine aminotransferase and aspartate aminotransferase) were mildly but inconsistently elevated in high-exposure group males during the first year. However, in the second year of the exposure period, treatment-related increases in these liver enzymes were not apparent. There were no other treatment-related changes in clinical chemistry parameters.

Morphologic Pathology, Alpha$_{2U}$-Globulin, DNA Synthesis, and Enzymes

Thorough gross and histopathologic examinations were completed on all animals in the 13-week study in mice and

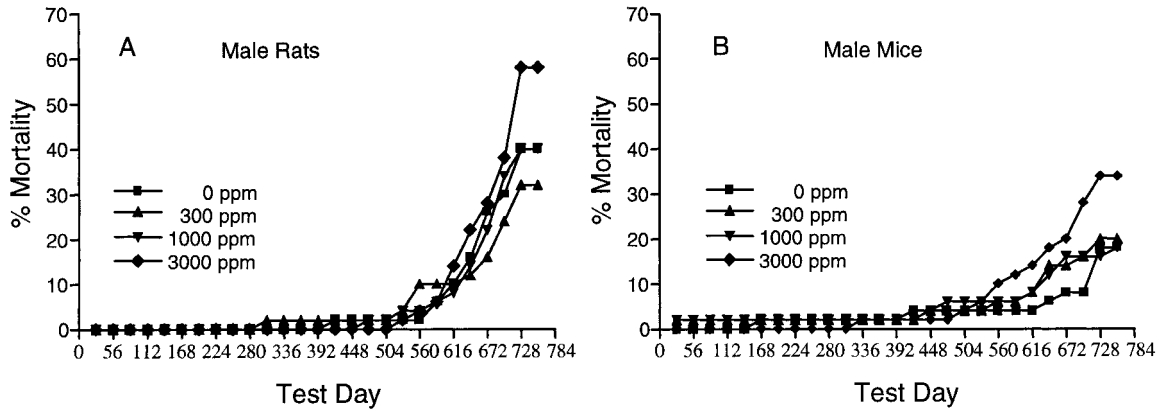

FIGURE 1.—Cumulative mortality, (A) male rats, (B) male mice. Data plotted as percent mortality of total number of animals in each dose group. No statistically identified differences in mortality patterns by Gehan-Wilcoxon procedure, alpha = 0.05.

the 24-month studies in rats and mice. In rats, direct effects of exposure were observed in liver of males at 1000- and 3000-ppm exposure levels and in kidneys of male and females in the 3,000-ppm exposure level in the chronic study. In mice, the only morphologic change attributable to exposure to PGME vapor was an exacerbation of spontaneous adrenal cortical change in females following 13 weeks' exposure (see later).

Liver: Liver weights were generally not significantly increased in rats and mice after 1 week of exposures (dosing), but were increased in high-exposure group animals following 2 weeks up to 2 years of dosing. In general, male rats, male mice, and females of both species exposed to 3,000 ppm had increases in absolute liver weight of approximately 10%, 8%, and 6% relative to controls, respectively (data not shown).

Male rats exposed to ≥1,000 ppm PGME for 24 months had a slight increase in the incidence of grossly observed dark foci in the liver. This correlated with the histologic finding of increased incidence and/or severity of eosinophilic hepatocellular foci (Table 2). Cystic degeneration of the liver (spongiosis hepatis), independent of its common occurrence within eosinophilic foci, was also slightly increased in 3,000-ppm males. Eosinophilic foci often had prominent cystic degeneration and/or angiectasis that, in many cases, expanded the size of the focus and caused mild tissue compression on the edge of the foci. Large foci of this type were characteristic of those found in the intermediate- and high-exposure group males (Figure 2). Basophilic foci were generally of the common, spontaneous, "tigroid" variety and were graded as very slight to slight in severity, reflecting their small size and relative normalcy of their appearance. Hepatocellular morphology in these foci was only slightly atypical. Often, the larger eosinophilic foci with cystic degeneration were large enough to be recognized upon gross examination as "dark foci." No treatment-related lesion were observed in mice following 24 months of exposures to PGME vapor.

Increases in hepatic S-phase DNA synthesis were also noted in high-exposure male rats (Figure 3a) and, to a lesser degree, in high-exposure male mice (Figure 3b) relative to controls. Following 1 week of exposure to 3,000 ppm vapor, the LI was increased 49-fold and 7-fold in male rats and mice, respectively. The lack of statistical significance in male mouse data reflected the relatively large variability in these data. The initial response of 3,000 ppm exposure group female rats (1.3-fold increase in LI) and female mice (2.0-fold increase) was less robust, but generally sustained through the subchronic and chronic exposure periods. The response in the liver of high-exposure males of both species rapidly declined by the second week. In subsequent studies with longer exposure periods, hepatic S-phase synthesis generally remained only slightly higher in male rats than in female rats with the exception of the response observed following 52 weeks of exposure where the response in males was considerably higher than in females. Hepatic S-phase synthesis in high-exposure group male mice remained similar to that found in females following longer-term exposure periods.

TABLE 2.—Incidence of selected nonneoplastic and neoplastic microscopic liver lesions in rats inhaling PGME vapors for 2 years.

	Chamber control	300 ppm	1,000 ppm	3,000 ppm
Male				
Number examined	50	50	50	50
Basophilic foci	34 (1.7)[a]	34 (1.8)	31 (1.6)	29 (2.1)
Eosinophilic foci	26 (2.5)	32 (2.5)	37 (2.6)*	39 (3.7)*
Cystic degeneration	9 (1.3)	5 (2.1)	9 (1.6)	16 (2.1)
Adenoma, hepatocellular	0	1	1	2
Carcinoma, hepatocellular	0	1	1	0
Female				
Number examined	50	50	50	50
Basophilic foci	47 (1.9)	45 (2.0)	43 (2.2)	46 (2.2)
Eosinophilic foci	29 (2.1)	22 (2.4)	29 (2.0)	34 (2.3)
Cystic degeneration	1 (1.0)	0	0	0
Adenoma, heptocellular	2	0	1	1

[a] Average severity grade of lesion in affected animals: 1, very slight; 2, slight; 3, moderate; 4, severe; 5, very severe.
*Significantly different from chamber controls at $p < 0.05$, *t*-test.

Hepatic MFO activity in rats and mice exposed to 3,000 ppm PGME was increased to the greatest extent relative to controls during the first 13 weeks of the exposure period. CYP2B1/2 dependent PROD activity was highest (2–4-fold control activity) following 2 weeks' exposure in male (Figure 4a) and female (Figure 4b) rats, and subsequently regressed to near control levels at approximately 52 weeks of exposure. The latter coincided with the gradual return of visible sedative effects. In male mice exposed to PGME for 2 weeks, PROD activity was increased over 5-fold that of controls (Figure 4c) remaining approximately 1.8- to 3-fold higher than control levels for up to 18 months. In female mice, PROD activity was approximately 2-fold higher than control levels for the whole 18-month period examined (Figure 4d). In both species, CYP1A1-dependent ethoxyresorufin O-demethylase (EROD) activity was induced only slightly. The total hepatic Cytochrome P-450 content of rats and mice generally reflected the level of PROD activity over the 18 months of the exposure period examined.

Kidneys: Kidney weights of high-exposure group male rats were similar to controls for the first 6 months of the exposure period, then increased with increasing time of exposure: 5.4% at 12 months, 7.4% at 18 months, and 22.5% at 24 months. In females, from the second week of exposure onward, kidney weight increases varied in a narrow range from 4.0% (not statistically identified) at 13 weeks, to 9.4% at 12 months, and 4.8% following 24 months. No treatment-related effects on kidney weight were observed in exposed male or female mice.

A mild α_{2U}-G nephropathy was identified histologically in male rats. During the first 2 weeks of exposure to 3000 ppm PGME, immunohistochemical staining for α_{2U}-G was increased (Figure 5a, b). There was alteration of the normal fan shaped intracellular arrays of secondary lysosomes within epithelial cells of the P2 segment, characterized by rhomboidal crystalloid and large round globular shapes. Although α_{2U}-G staining was more prominent following 2 weeks of exposures than following 1 week of exposures, evidence of damage to tubular epithelium was more prominent following 1 week. Morphologic findings associated with 3,000 ppm exposure included multifocal tubular basophilia, slight attenuation of cell height, extrusion of necrotic cells or debris into tubular lumens, and occasional mitotic figures. Following 13 weeks exposure, morphologic findings in the 3,000-ppm group males compared to those at 2 weeks were similar, but less severe, and α_{2U}-G staining was about twice

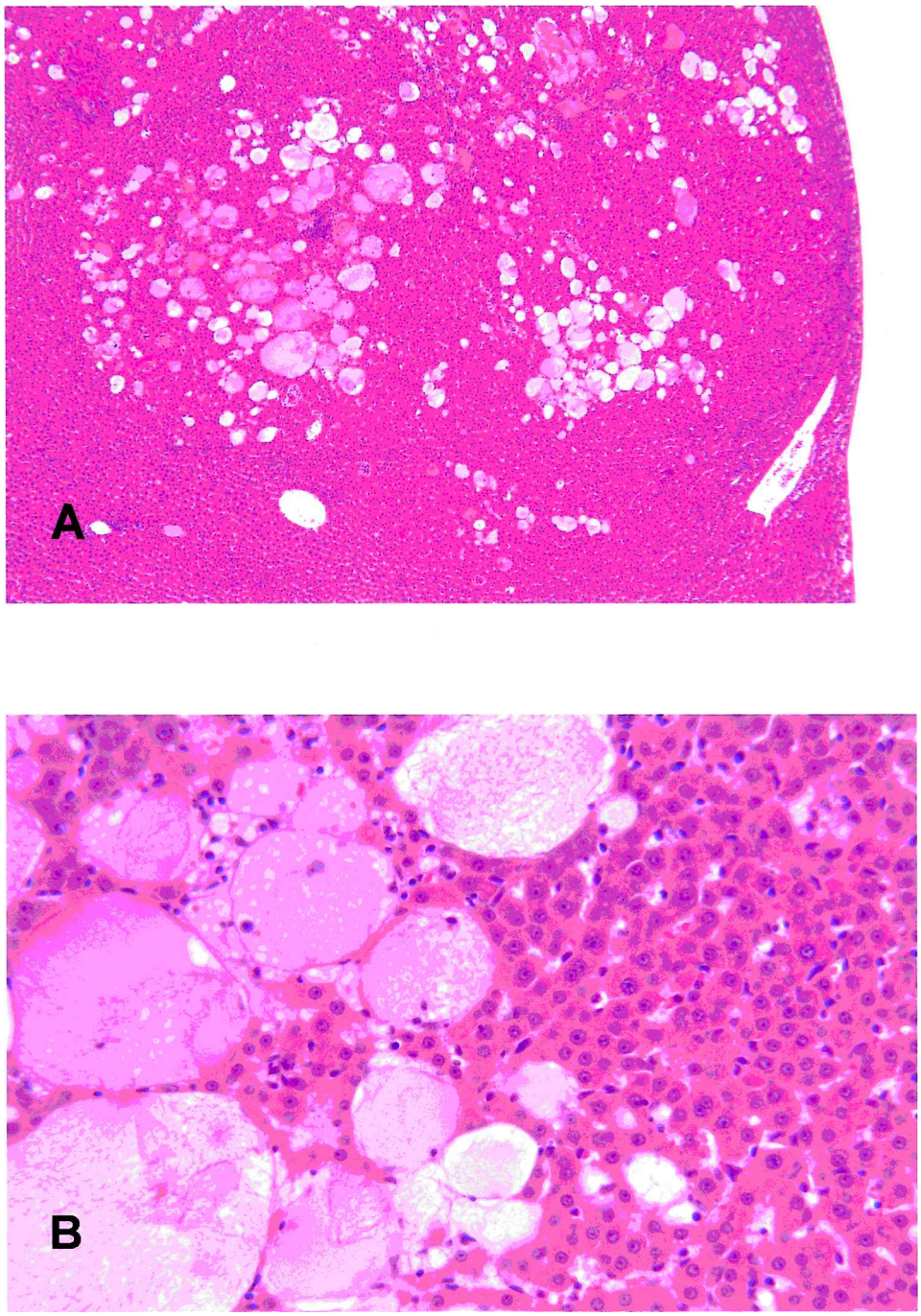

FIGURE 2.—Liver from male rat exposed to 3,000 ppm PGME for 2 years. A) Large eosinophilic focus of cellular alteration, H&E, ×19. B) Same lesion, detail of cystic degeneration and hemorrhage within the altered focus, H&E, ×300.

that of controls, involving about half of the cortical tubules. At 300 ppm, 2 of 5 males had a very slight increase in staining. Following 6 and 12 months of exposure, the morphologic alteration of retained α_{2U}-G remained in 1,000- and 3,000-ppm male rats. Female rat kidneys displayed no treatment-related histopathological changes following 1, 2, and 13 weeks of exposure; therefore, the kidneys of female rats were not evaluated for α_{2U}-G deposition in the chronic study, and were not evaluated for kidney cell proliferation (Group B) beyond the 6-month time point.

The histological appearance of the kidneys of treated male rats following longer exposure periods was not distinguishable from spontaneous CPG, with the exception of morphologic changes in secondary lysosomes. CPG was significantly exacerbated following exposure to 3,000 ppm PGME vapor for 24 months, an effect that was much more pronounced in male than in female rats (Table 3). No associated increase in incidence of renal epithelial tumors was observed at any exposure level in rats. No treatment-related effects were observed in the kidneys of mice.

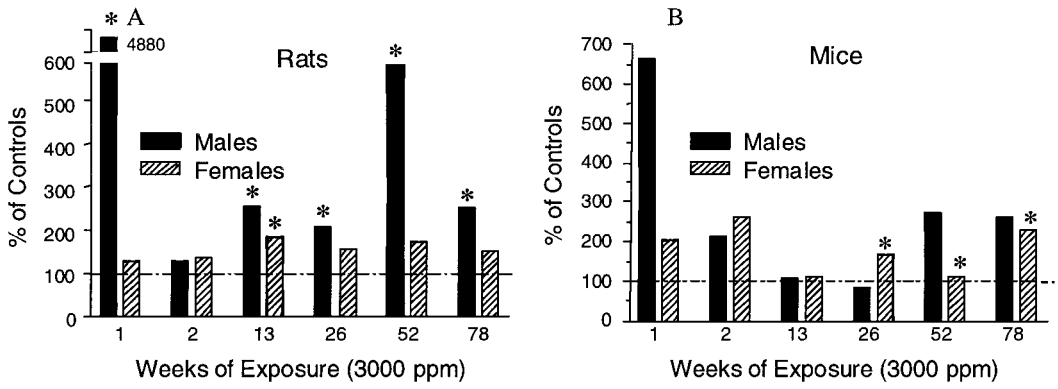

FIGURE 3.—BrdU hepatocyte labeling in rats (A) and mice (B) exposed to 3,000 ppm PGME for up to 78 weeks. *= Statistically identified as different from controls by Dunnett's Test, alpha = 0.05.

The level of S-phase DNA synthesis in the kidneys of male rats exposed to 3,000 ppm PGME was elevated 9-fold relative to controls following one week of exposure (Figure 6). The degree of this difference decreased with increasing duration of exposure until there were no statistical differences between the LI of exposed and control rats following 6 months of exposure. As the rats aged, the levels of S-phase synthesis in controls increased substantially and became more variable, consistent with the expected, age-related increase in background CPG. BrdU renal labeling indices were unaffected in female rats through 6 months of exposure (last time point examined), and in mice following 13 weeks of exposure (data not shown).

Other Tissue Changes: An accelrated atrophy of the adrenal gland X-zone occurred in all female mice exposed to 3,000 ppm and 2 of 10 females exposed to 1,000 ppm for 13 weeks. No treatment-related pathological changes were found in the adrenal gland of male mice after 13 weeks nor in either sex of rats exposed to these levels of vapor for 24 months.

DISCUSSION

Inhalation of relatively high concentrations of PGME vapor by rats and mice resulted in a number of treatment-related changes, some of which were modified by adaptive changes in the liver of exposed animals. A striking effect of exposure

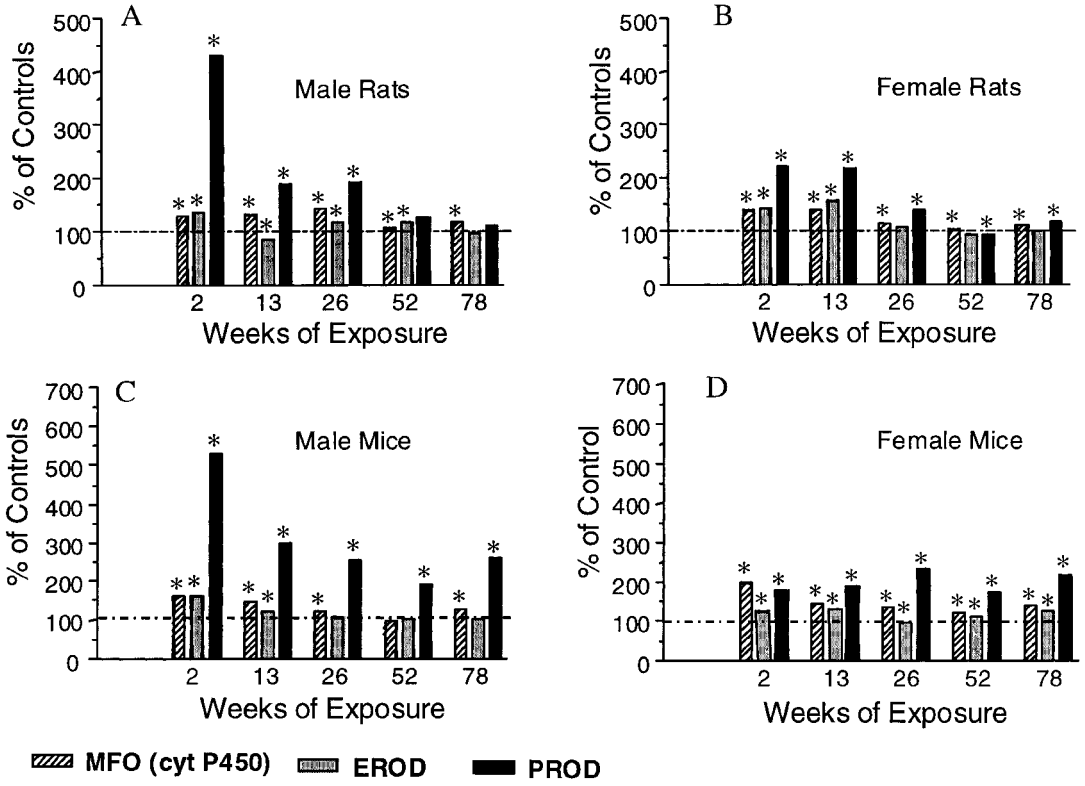

FIGURE 4.—Enzyme induction in (A) male and (B) female rats, and (C) male and (D) female mice exposed to 3,000 ppm PGME. *= Statistically identified as different from controls by Dunnett's Test, alpha = 0.05.

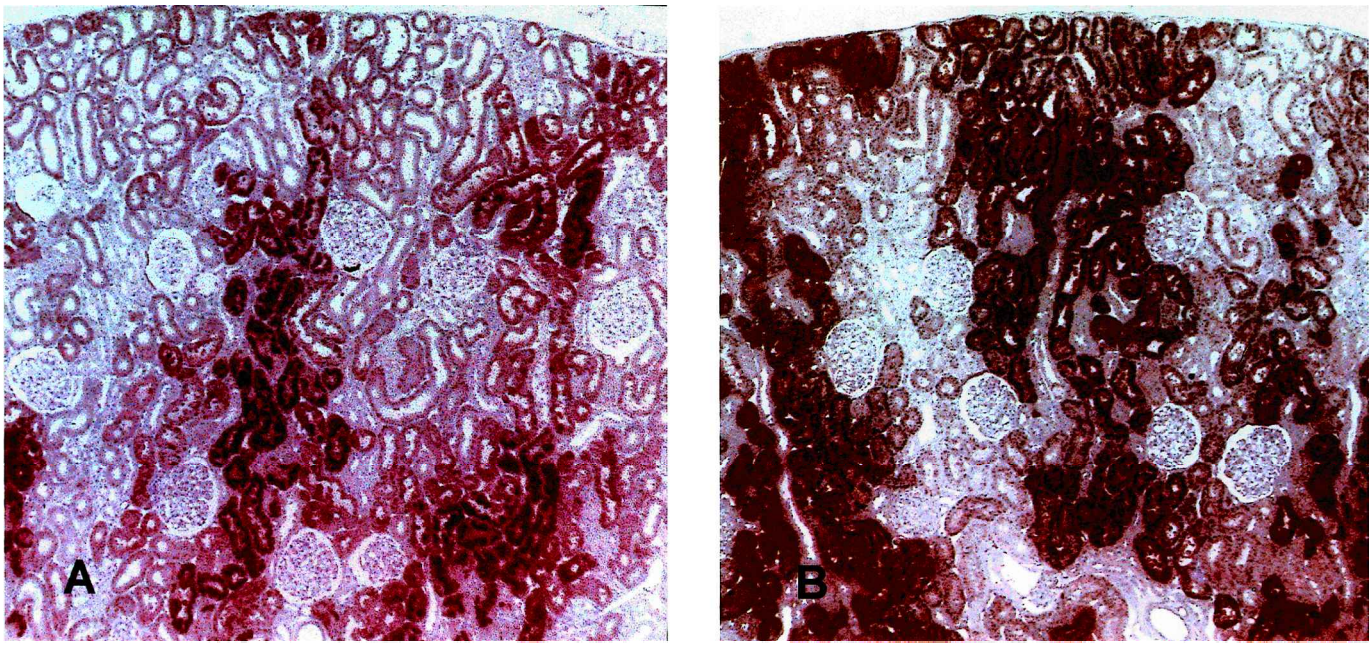

FIGURE 5.—A) Normal extent of immunohistochemical α_{2U}-g staining in young control male rat kidney, ×47. B) Male rats exposed to 3,000 ppm PGME for 2 weeks have a larger portion of α_{2U}-g immunohistochemically stained cortical tubule cells, ×47.

to 3,000 ppm vapor was a transient sedation of all exposed animals during the first week of exposure. Resolution of sedation during the second week of exposure coincident with increases in liver weight, increases in S-phase DNA synthesis, and induction of MFO, especially in males. The lack of reported necrosis in liver of rodents exposed to PGME for 2 to 13 weeks (21, 26) along with the known metabolism of PGME supports that hepatocyte proliferation is associated with enhanced metabolism of PGME (26). As diagrammed in Figure 7, MFO based O-dealkylation of PGME, likely via CYP2B1/2 enzyme(s) as reflected by PROD activity, and an adaptive increase in the number of hepatocytes in exposed rats and mice is hypothesized to decrease PGME blood levels associated sedation. This is consistent with the resolution of sedation observed in rats and mice exposed to 3,000 ppm vapor and the reported decreases in blood levels and MFO induction reported in rats repeatedly administered PGME via oral gavage by Morgott and Nolan (28). In rats, the apparent decrease in hepatic CYP-450 enzyme activity following more prolonged exposures (PROD activity in Figures 4a and b), provide an explanation for the observed resumption of sedation during exposures to PGME vapor. In mice, the lack of renewed sedation following long-term exposures is consistent with the maintenance of relatively consistent enzyme activity (PROD in Figures 4c and d). Upon chronic exposure to PGME, histopathologic changes were only observed in liver of exposed rats, consisting of increased eosinophilic foci of altered hepatocytes with or without cystic degeneration. Any relationship to chronic metabolic induction and/or the diminution of this in the last year of life was unclear.

An additional histopathologic change noted in rats was the occurrence of α_{2U}-G nephropathy in males and CPG in both

TABLE 3.—Incidence of chronic progressive glomerulonephritis (CPG) and renal cortical neoplasms in rats inhaling PGME vapors for 2 years.

	Chamber control	300 ppm	1,000 ppm	3,000 ppm
Male				
Number examined	50	50	50	50
CPG	50 (2.9)[a]	49 (2.7)	50 (2.7)	50 (3.3)*
Adenoma	0	0	1	0
Adenocarcinoma	0	0	2	1
Female				
Number examined	50	50	50	50
CPG	46 (1.5)	47 (1.4)	48 (1.5)	47 (1.7)*
Adenoma	0	0	0	1

[a] Average severity grade of lesion in affected animals: 1, very slight; 2, slight; 3, moderate; 4, severe; 5, very severe.
*Significantly different from chamber controls at $p < 0.05$, t-test.

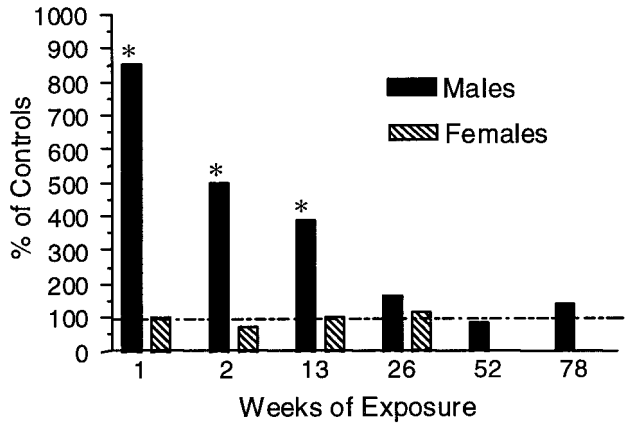

FIGURE 6.—BrdU kidney cortical cell labeling in rats exposed to 3,000 ppm PGME. *= Statistically identified as different from controls by Dunnett's Test, alpha = 0.05.

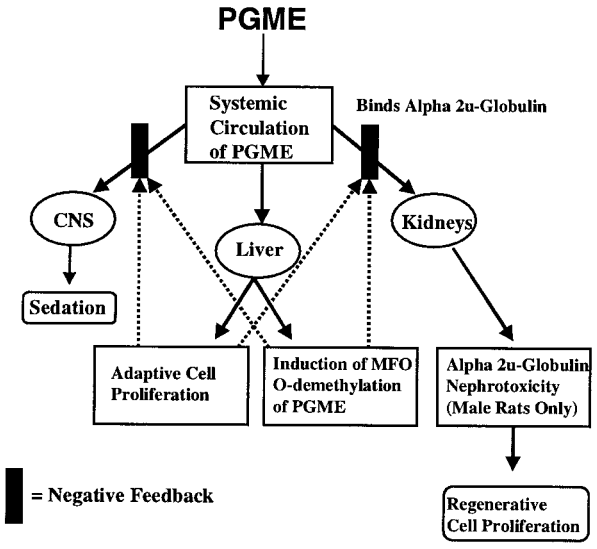

FIGURE 7.—Mechanistic pathways of PGME exposure in rats and mice.

sexes exposed to 3,000 ppm PGME. CPG is a very common spontaneous disease in Fischer 344 rats, especially in males, and has many morphologic features in common with the nephropathy caused by prolonged α_{2U}-G retention in the kidneys of male rats (11). The effect of the α_{2U}-G nephropathy superimposed on CPG in the chronic study appeared simply as an exacerbation of the spontaneous disease. The increases in renal α_{2U}-G deposition and S-phase DNA synthesis early in the life of the male rats suggests that such a pattern of nephrotoxicity may result in increased chronic renal disease. As reviewed by the EPA (11), α_{2U}-G associated nephropathy is considered to be unique to the male rat.

The increased potential for hepatic biotransformation and subsequent elimination of PGME during the second week of exposure causing resolution of sedative effects would also have decreased levels of PGME available to bind with α_{2U}-G (Figure 5). Further, the production of α_{2U}-G by the male rat naturally declines beginning at approximately 5 months of age (11). These factors likely contributed to the observed decline in S-phase synthesis reflective of regeneration in renal tubular epithelium, indicative of regenerative synthesis and increased α_{2U}-G deposition relative to controls over a period of 2 to 26 weeks of exposure. The lack of an exact temporal coordination of these events may relate to the dynamics of development of toxicity and tissue regeneration in the affected cells. A minimal, yet statistically significant increase in S-phase synthesis of renal tubular epithelium was observed in male rats following 13 weeks of exposures to 300 ppm (LI 300 ppm = 9.5 vs control = 5.7) was not associated with any other treatment effect on function or morphology indicating an apparent lack of biological significance. Finally, a small, yet statistically significant, increase in CPG severity in female rats exposed to 3,000 ppm for 2 years may reflect minimal nephrotoxicity or simply random variability.

The occurrence of 3 renal epithelial tumors in the 1,000-ppm exposure male rats (6%) exceeded the incidence in historical controls for similar studies conducted in, or sponsored by, this laboratory: 3/530 (0.56%; range, 0–1) and 0/531 (0%) for control males and females, respectively; 5/1972 (0.25%; range, 0–1) and 6/1975 (0.3%; range, 0–1) in treated males and females, respectively. The incidence of Fischer 344 rats with benign or malignant renal cortical epithelial neoplasms in other laboratories was approximately 1% in males and 0.1% in females (22). Although rare, spontaneous renal cortical epithelial neoplasms have also occurred sporadically in Fischer-344 rats of both sexes used in shorter subchronic studies in this and other laboratories (18). The association with α_{2U}-G-mediated nephropathy indicate that these tumors are of little to no relevance to human risk assessment (34). The very low incidence of renal cortical epithelial tumors in high-exposure male and female rats was within the historical control range, and, therefore regarded as spontaneous.

In the subchronic mouse study, the only treatment-related effect was an accelerated atrophy of the adrenal gland X-zone in all female mice exposed to 3,000 ppm and 2 of 10 females exposed to 1,000 ppm. The X-zone normally atrophies at first pregnancy or, in nulliparous mice, between 39 and 200 days, depending on the strain (9). Because the X-zone normally atrophies in male mice at puberty (6 weeks of age), this tissue was not available for examination in males. Although the mouse adrenal X-zone may be involved in steroidogenesis (20), its accelerated atrophy has no known effects, and is of unclear toxicological significance.

In conclusion, PGME induces an adaptive hepatic response in rats and mice characterized by increased S-phase DNA synthesis, likely related to cell proliferation, and induction of MFO enzyme activity. The resultant increased metabolism of PGME leads to an amelioration of PGME-induced sedation observed during the first week of repeated exposure to high concentrations of this chemical. Partial regression of these adaptive changes over time in rats resulted in a return of clinical evidence of sedation. In male rats, exposure to high concentrations of PGME also causes a minimal α_{2U}-G nephropathy and associated increases in renal tubular epithelial cell proliferation, possibly modulated by the induction and regression of PGME metabolism. PGME exposure for up to 24 months did not result in any statistically identified increase in the incidence of neoplasia in either species; however, an increased incidence of kidney cortical-epithelial tumors above historical control levels was noted in intermediate-exposure group male rats. The association with α_{2U}-G nephropathy indicated a lack of relevance for human risk assessment. A NOEL of 300 ppm was established in rats based on altered hepatocellular foci in males. NOAEL of 1,000 ppm was established in mice, based on accelerated adrenal X-zone atrophy in females after 13 weeks of exposures and slight body weight decreases in both sexes.

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