Special Report: Reproductive and Developmental Toxicity of Ethylene Glycol and Its Ethers[1]

Polymers of Ethylene Glycol are linked via ether linkages with various alcohols or via ester linkages to various fatty acids in many cosmetic ingredients. Ethylene Glycol, when reacted with an alkyl alcohol, forms an ethylene glycol monoalkyl ether. These compounds are metabolized in the human body by alcohol dehydrogenase and aldehyde dehydrogenase to form corresponding acetaldehyde and acetic acid derivatives. Data are presented that show reproductive and developmental toxicity is associated with metabolites of ethylene glycol monoalkyl ethers, but not with the monoalkyl ethers themselves. Further, it is suggested that the toxicity of these metabolites is inversely proportional to the length of the alkyl chain in the original alkyl ether. In the case of the compounds used in cosmetics, most have alcohols or fatty acids linked to polyethylene glycol chains, not a single Ethylene Glycol moiety. Where Ethylene Glycol is linked to a fatty acid by an ester linkage, the resulting compound is chemically different from the monoalkyl ethers. Where Ethylene Glycol is linked to an alcohol via an ether linkage, the alkyl chain is large and complex, suggesting little or no potential toxicity. Overall, it was found that metabolites of ethylene glycol monoalkyl ethers are reproductive and developmental toxins. In general, however, the metabolites of concern are not expected to be formed in cosmetic formulations that contain polymers of ethylene glycol.

In their review of the safety assessments of various polyethylene glycol (PEG) derivatives, the Cosmetic Ingredient Review (CIR) Expert Panel expressed concern about the teratogenicity and testicular toxicity of the monomer, Ethylene Glycol, and its monoalkyl ethers. Safety assessments of PEG-derived cosmetic ingredients for which this concern is an issue include:

Ceteth-1, -2, -3, -4, -5, -6, -10, -12, -14, -15, -16, -20, -24, -25, -30, and -45 (CIR 1996a).

Oleth-2, -3, -4, -5, -6, -7, -8, -9, -10, -12, -15, -16, -20, -23, -25, -30, -40, -44, and -50 (CIR 1996b).

PEG-2, -3, -5, -10, -15, and -20 Cocamine (CIR 1996c).

PEG-2, -3, -4, -8, -9, -12, -20, -32, -75, -120, -150, and -175 Distearate (CIR 1996d).

PEG-7, -30, -40, -78, and -80 Glyceryl Cocoate (CIR 1996e).

PEG-5, -10, -20, -24, -25, -27, -30, -35, -40, -50, -55, -60, -75, -85, and -100 Lanolin; PEG-5, -10, -20, -24, -30, and -70 Hydrogenated Lanolin; PEG-75 Lanolin Oil; and PEG-75 Lanolin Wax (CIR 1996f).

PEG-5, -10, -16, -25, and -40 Soya Sterol (CIR 1996g).

To provide a perspective on the chemical structures of the compounds involved, and to form a basis for comparison with the structures of the PEG-derivatives listed above, this special report begins with a presentation of the structure of ethylene glycol and its monoalkyl ethers.

Ethylene Glycol, when reacted with an alkyl alcohol, a corresponding ethylene glycol monoalkyl ether is formed, as shown in Figure 1. Ethylene Glycol Monomethyl Ether and Ethylene Glycol Monoethyl Ether are, respectively, 2-Methoxyethanol and 2-Ethoxyethanol. The ethylene glycol monoalkyl ethers are metabolized by alcohol dehydrogenase and aldehyde dehydrogenase, resulting in aldehyde and acetic acid derivatives as shown in Figure 2. If the initial compound is 2-methoxyethanol, then the metabolites are methoxyacetaldehyde and methoxyacetic acid.

This special report includes data on Ethylene Glycol, 2-Methoxyethanol, and 2-Ethoxyethanol (the latter two are under the section Ethylene Glycol Ethers). 2-Butoxyethanol, a less harmful reproductive and developmental toxin, has been previously reviewed by the CIR Expert Panel which found that, in oral and inhalation reproduction toxicity studies in rodents, decreases in the number of viable litters, increases in resorptions, and ventricular septal defects and arterial defects have been reported. Dermal application failed to elicit any reproductive toxicity (Andersen 1996).

EXPOSURE LIMITS

The Occupational Safety and Health Administration (OSHA) and the American Conference of Governmental Industrial Hygienists (ACGIH) have established exposure limits for Ethylene Glycol, 2-Methoxyethanol, and 2-Ethoxyethanol (National Institute for Occupational Safety and Health [NIOSH] 1983; ACGIH 1986). The OSHA standards were based primarily on reports of blood, hepatic, renal, and central nervous system toxicity caused by 2-Methoxyethanol and 2-Ethoxyethanol; no reproductive studies were considered when the standards were adopted. The ACGIH threshold limit values for 2-Methoxyethanol and 2-Ethoxyethanol were changed to the present 5 ppm limits from 25 and 50 ppm, respectively, based on evidence of testicular toxicity. The ACGIH threshold limit value for 2-Butoxyethanol is included for the sake of comparison. These limits are given in Table 1 (NIOSH 1983).

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[1]Reviewed by the Cosmetic Ingredient Review Expert Panel. Rebecca S. Lanigan, former Scientific Analyst and Writer, prepared this report. Address correspondence to Dr. F. Alan Andersen, Director, CIR, 1101 17th Street, NW, Suite 310, Washington, DC 20036, USA.

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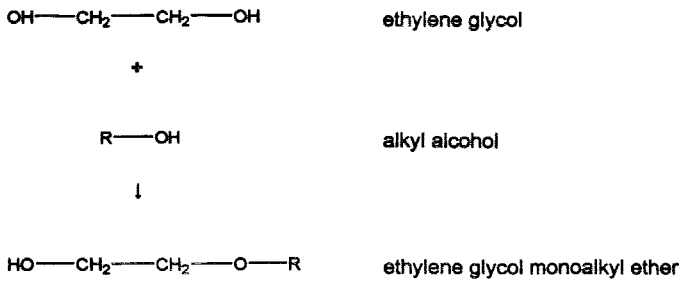

FIGURE 1
Reaction of Ethylene Glycol with an alkyl alcohol to form an ethylene glycol monoalkyl ether.

NIOSH proposed that 2-Methoxyethanol and 2-Ethoxyethanol have the potential to cause adverse reproductive effects in male and female workers. This recommendation was based on published studies in which dose-related embryotoxicity and other reproductive effects were found in a number of animal species exposed to the chemicals via different routes of administration; of particular concern were studies in which adverse effects were observed in pregnant animals exposed to concentrations below the government standards. As a result, NIOSH suggested that worker exposure be minimized whenever possible (NIOSH 1983).

ETHYLENE GLYCOL

Khera (1991) investigated the possible relationships between changes in maternal homeostasis and the incidence of fetal anomalies in pregnant Sprague-Dawley rats induced by maternotoxic doses of Ethylene Glycol. The first of three studies was an acid-base electrolyte study on gestational day (GD) 11 using five to eight cannulated rats per treatment group. Doses of 1250, 2500, or 5000 mg/kg Ethylene Glycol were administered orally

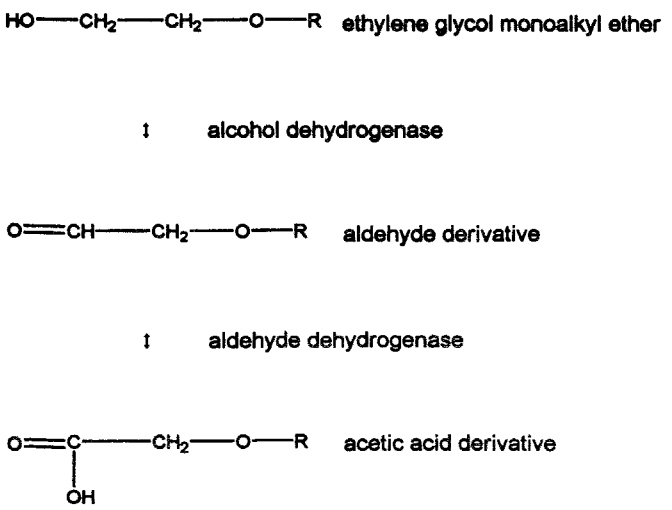

FIGURE 2
Metabolism of ethylene glycol monoethers.

or 3333 mg/kg was adminstered subcutaneously, either alone or simulaneously with an oral dose of 530 mg/kg aqueous $NaHCO_3$ (an endogenous agent known to correct metabolic acidosis). In all studies, drinking water was supplemented with 2.65 mg/ml $NaHCO_3$. Blood samples had hyperosmolality, metabolic acidosis, and dose-related increases in the anion gaps (comparable to controls) and osmolar gap (returned to normal at 9 hours). Urinalysis results included dose-related diuresis and a significant decrease in osmolality without significant changes in pH, Na^+, and/or K^+ values. Urine sediment from all groups (including controls) contained calcium oxalate crystals, which were more prevalent in the high dose groups.

In the second study, a teratology study, 2800 or 3333 mg/kg Ethylene Glycol doses were injected subcutaneously with or without $NaHCO_3$ on GD 11 using 10–15 dams per treatment group. Developmental effects were evaluated in term fetuses (GD 22); two thirds were examined for skeletal defects and the remainder for visceral anomalies. Three of 13 dams treated with 3333 mg/kg Ethylene Glycol alone died, but 2800 mg/kg was not maternolethal. In the 2800 mg/kg dose group, 55/136 fetuses had skeletal anomalies after Ethylene Glycol treatment alone. With $NaHCO_3$ administration, this number was 20/128. At the higher dose, the values for skeletal anomalies were 70/82 and 46/83, respectively. Control values were 11/106 ($NaHCO_3$) and 4/110 (water). Simultaneous treatment with $NaHCO_3$ significantly reduced the maternal toxicity, loss in fetal body weight, and the incidence of fetal skeletal defects. Malformations included retarded ossifications in sternebrae, vertebrae, metacarpals, and metatarsals, and a reduced number of ribs (eight to 12) or fused ribs.

In the third study, conceptuses in situ, still enclosed in the uterine capsule, were examined. A single dose of 3333 mg/kg Ethylene Glycol alone or with $NaHCO_3$ was given on GD 11 or multiple doses of 5000 mg/kg/day Ethylene Glycol were administered orally from GD 7–13. Dams (6 to 12 per group) were killed 24 or 48 hours after the single or last dose of Ethylene Glycol. Karyorrhexis and pyknosis of mesodermal cells in the allantoic bulb, an absence of chorioallantoic fusion, placental thrombosis, and maternal hemorrhage in the yolk sac cavity were detected (3333 mg/kg Ethylene Glycol alone). No lesions were found in the conceptuses and dams of control rats (water or $NaHCO_s$ alone). The total cross-sectional area of maternal vascular spaces increased following treatment with Ethylene Glycol, whereas that of the fetuses decreased. Simultaneous treatment with $NaHCO_3$ increased the surface area of gaseous exchange between the apposing maternal and fetal vascular channels. The higher dose of 5000 mg/kg Ethylene Glycol caused lesions in all but one conceptus, and reductions in the width of the labyrinth (consisting of larger maternal spaces and proportionately smaller and fewer allantoic villi) and the basal zone of the placenta were observed. Additionally, two of four conceptuses had intraplacental hematomas from a maternal hemorrhage. One such hematoma contained crystals morphologically similar to those of calcium oxalate.

TABLE 1
Exposure limit values (NIOSH 1983)

Compound	ACGIH threshold limit value[a]	OSHA permissible exposure limit[a]
Ethylene Glycol (vapor)	50 ppm (125 mg/m^3)	—
2-Methoxyethanol	—	25 ppm (80 mg/m^3)[b]
2-Ethoxyethanol	5 ppm (19 mg/m^3)[b]	200 ppm (740 mg/m^3)[b]
2-Butoxyethanol	25 ppm (120 mg/m^3)[b]	—

[a] Values as time-weighted averages for 8-hour work shifts during a standard 40-hour week.
[b] "Skin" notation, indicating the potential for skin absorption of toxic amounts.

The maternal metabolic acidosis and hyperosmolality detected in the first study were suspected to contribute towards the reductions in villigenesis, chorion size, and erythroblastic population in the allantois, as well as the increase in the incidence of fetal abnormalities and reduced fetal body weight observed in the second and third studies. Materno-embryonic gaseous exchange and fetal nutrition were reduced, impairing fetal development, by maternal factors due to treatment with Ethylene Glycol, including metabolic acidosis, hyperosmolality, hemorrhages in the ectoplacental cone, extraembryonic cavities, and around Reichert's membrane, and necrosis of the decidua basalis (Khera 1991).

Lamb et al. (1985) administered Ethylene Glycol in drinking water to male and female CD-1 albino mice in a continuous breeding program to determine reproductive function and offspring development. F_0 mice were treated with 0.25, 0.5, or 1.0% (*w/v*) Ethylene Glycol. Forty and 20 mice per sex made up the vehicle control group and each treatment group, respectively. Mice were treated during a 1-week premating period after which the male and female mice were randomly paired within each dose group and cohabited for 14 weeks while treatment continued. Newborn litters were evaluated and immediately killed by decapitation. At the end of the 14 weeks, males and females were separated and the litters saved during the following 3-week period. Treatment was also continued during this time and until the end of the study. Final litters (F_1) from control and high dose groups were weaned and evaluated for reproductive performance. At 70 days of age, 20 F_1 offspring per sex from the two groups were randomly paired and mated with nonsiblings, and the resulting litters were necropsied.

No clinical signs of Ethylene Glycol toxicity were observed in the F_0 generation; however, two females given 0.5% Ethylene Glycol and one male and two females in the control group died prematurely. At least one death in the 0.5% Ethylene Glycol group might have been due to the treatment, as the renal tubules contained a moderate number of oxalate crystals. Exposure to 1% Ethylene Glycol was associated with significant decreases in the number of litters (F_1) per fertile pair, the mean number of live births per litter, and the mean live pup weight as compared to controls (Table 2).

In the assessment of reproductive performance for the F_1 generation, fertility was 80% and 61% for control and 1% Ethylene Glycol treated mice, respectively; the difference was not statistically different, however. The lower live birth index and live pup weight were also not significant. No clinical signs of Ethylene Glycol toxicity were observed, but some offspring (F_2) had shorter snouts and wide-set eyes compared to controls, as well as shortened frontal, nasal, and parietal bones, one or more pairs of fused ribs, abnormally shaped or missing sternebrae and/or vertebrae, and twisting of the spine. Bones were smaller and had altered shapes compared to controls under low-magnification examination; however, no lesions were found at microscopic examination. Calcium oxalate crystals were not observed in the renal tubules of either group. The researchers concluded that

TABLE 2
Reproductive performance of mice given Ethylene Glycol (Lamb et al. 1985)

Treatment group	Pairs with litters	Surviving pairs	Litters per pair	Live pups per litter	Proportion of pups born alive	Live pup weight (g)
Control	40/40	38/40	4.9 ± 0.08	10.8 ± 0.5	0.96 ± 0.03	1.63 ± 0.02
0.25%	20/20	20/20	4.7 ± 0.02	10.4 ± 0.5	0.96 ± 0.02	1.64 ± 0.02
0.5%	20/20	18/20	4.9 ± 0.08	10.5 ± 0.6	0.97 ± 0.01	1.58 ± 0.02
1.0%	20/20	20/20	4.5 ± 0.2[b]	10.2 ± 0.3[a]	0.96 ± 0.02	1.53 ± 0.02[b]

[a] Significantly different from control ($p < .05$).
[b] Significantly different from control ($p < .01$).

TABLE 3
Teratologic evaluation of Ethylene Glycol—CD-1 mice (NCTR 1984a)

Ethylene Glycol dose (mg/kg/day)	% Adversely affected implants/litter	% Litters with ≥1 affected implant	% Malformed live fetuses/live litter	% live litters with ≥1 malformed live fetus
Control	11.17	72.0	0.25	4.0
750	19.65	83.3	10.0	66.67
1500	50.67	95.7	37.77	81.82
3000	64.41	100	56.54	95.65

Ethylene Glycol was a "weak reproductive toxicant, but a potential teratogen" (Lamb et al. 1985).

In another study using CD-1 mice, researchers administered 750, 1500, or 3000 mg/kg/day Ethylene Glycol in drinking water (10 ml/kg/day dose volume) to 23–25 pregnant mice per group on GD 6–15. The mice were killed on GD 17. No unscheduled maternal deaths or toxicity signs were observed except for piloerection and dose-related decreased body weight gain, body weight loss on GD 11–17, and decreased liver weight. The body weight loss was significant in the mid- and high-dose groups. At a dose of 1500 mg/kg/day Ethylene Glycol, the dams had a lower average number of corpora lutea and a decrease in the number of implantation sites/litter. The high dose caused 19.86% resorptions/litter (compared to 8.74% in the control group). A dose-related increase occurred in the number of resorptions per litter, and a significant decrease in the incidence of dead fetuses (dose-dependent) at the top two doses (Table 3). In general, significant increases were found at the mid- and high-doses in the percentage of adverse effects in conceptuses/litter during the post-implantation phase of pregnancy. Fetal body weight dropped in a dose-dependent manner. The most common fetal abnormalities observed were hydronephrosis, craniofacial anomalies (clefts of the face, lip, and palate) and skeletal dysmorphogenesis that included fused, short, branched or missing ribs, fused or misaligned sternebrae, incomplete ossification, and fused thoracic or lumbar arches (National Center for Toxicological Research [NCTR] 1984a).

In a second study by NCTR (1984b), the results were similar. Doses of 1250, 2500, or 5000 mg/kg/day Ethylene Glycol (10 ml/kg) were given in water on GD 6–15 to 27–29 CD rats per group. No unscheduled maternal deaths or distinctive clinical signs were observed other than piloerection. Dose-dependent decreases in maternal body weight, body weight gain, liver weight, and relative kidney weights were observed. These changes were significant in the mid- and high-dose groups. No significant differences were found between treatment groups and vehicle control groups in the number of corpora lutea/dam, number of implantation sites/dam, percentage of preimplantation loss, or the percentage of dead fetuses. However, the percent of nonlive implants/litter was increased in the high-dose group, and 21.0% resorptions per litter occurred (compared to 4.7% for controls; see Table 4). Common malformations included clefts of the lip, face, and palate, and varying degrees of skeletal dysplasia (defects of the cranium, ribs, and vertebral column) (NCTR 1984b).

A study conducted by the National Toxicology Program (NTP) reported that the no observable adverse effect levels (NOAEL) for maternal and developmental toxicity were 1000 and 2000 mg/kg/day Ethylene Glycol, respectively, when administered to artificially inseminated New Zealand white rabbits (NTP 1991). Doses of 100, 500, 1000, or 2000 mg/kg/day Ethylene Glycol (in water) were given by gavage on GD 6–19. The dose volume was 5 ml/kg body weight. Live fetuses were dissected from the uterus on GD 30 after examination of the maternal liver, kidneys, intact uterus, and counting of the corpora lutea. At the 2000 mg/kg dose, 42.1% (8/17) of does were killed on GD 9–25 due to acute renal failure, the pregnancy rate was 81.8%, three does delivered early, and one doe aborted on GD 20. The only significant effect of treatment observed at necropsy was

TABLE 4
Teratologic evaluation of Ethylene Glycol—CD rats (NCTR 1984b)

Ethylene Glycol dose (mg/kg/day)	% Adversely affected implants/litter	% Litters with ≥1 affected implant	% Malformed live fetuses/live litter	% live litters with ≥ 1 malformed live fetus
Control	6.02	42.9	1.37	7.14
1250	12.02	67.9	6.65	39.29
2500	29.52	89.7	25.11	68.97
5000	76.59	96.3	73.53	96.15

a slight increase in maternal absolute kidney weight. Upon further examination, renal lesions of the cortical tubules included intraluminal crystals, and epithelial necrosis. Pregnancy rates were 95.5, 95.7, 91.3, and 95.2% for control and three treatment groups (up to 1000 mg/kg/day), respectively. One doe delivered early in each dose group. Except for a significant increase in the number of implantation sites/litter and a slight increase in the live litter size at 500 mg/kg/day, no treatment-related effects on any gestational parameters were observed. However, the values at that dose were within historical control values. Treatment with Ethylene Glycol did not result in significant fetotoxicity or increase the incidence of fetal malformations at any dose tested using New Zealand white rabbits.

The same researchers compared various toxicity endpoints to determine the relative sensitivities of New Zealand white rabbits, Sprague-Dawley rats, and Swiss mice to oral doses of Ethylene Glycol during organogenesis (Table 5). For maternal toxicity, sensitivity was rabbits > mice > rats, but for developmental toxicity the order was mice ≫ rats ≫ rabbits (NTP 1991; Tyl et al. 1993).

Pregnant Fischer 344 rats were treated with 0.2, 0.4, and 1.0 g/kg/day Ethylene Glycol in the diet on GD 6–15 and killed on GD 21. A positive-control group received 500 mg/kg hydroxyurea in saline intraperitoneally on GD 11. The negative-control group received stock diet. Fetuses were delivered by caesarian section on GD 21 and examined for teratogenic effects. Clinical signs of maternal toxicity were not observed, and no significant differences were detected between control and treated maternal weight gain on GD 6–21. Fetal length, weight, the number of total implantations, and litter size were not different between controls and treated animals. Preimplantation loss was greater at 1.0 g/kg/day Ethylene Glycol, but the difference was not significant. No increased incidences of major fetal anomalies, skeletal or visceral, were observed after treatment with Ethylene Glycol;

TABLE 5
NOAELS for Ethylene Glycol

Species	Route and time of administration	NOAEL (mg/kg/day)	Reference
	Maternal Toxicity		
New Zealand white rabbit	Gavage on GD 6–19	1000	Tyl et al. 1993
Sprague-Dawley rat	Gavage on GD 6–15	<1250 (not determined)	Price et al. 1985
Fischer 344 rat	Dosed feed on GD 6–15	1000	Maronpot et al. 1983
CD rat	Inhalation on GD 6–15 (whole-body)	1000	Tyl et al. 1995b
CD rat	Gavage on GD 6–15	1000	Neeper-Bradley et al. 1995
CD-1 mouse	Gavage on GD 6–15	750	Price et al. 1985
CD-1 mouse	Drinking water (multigenerational)	400	Lamb et al. 1985
CD-1 mouse	Dermal application on GD 6–15	>3549	Tyl et al. 1995a
CD-1 mouse	Inhalation on GD 6–15	150 mg/m^3 (whole-body);	Tyl et al. 1995b
Swiss mouse	Gavage on GD 6–15	1500	Tyl et al. 1989
	Developmental Toxicity		
New Zealand white rabbit	Gavage on GD 6–19	2000	Tyl et al. 1993
Sprague-Dawley rat	Gavage on GD 6–15	<1250 (not determined)	Price et al. 1985
CD rat	Gavage on GD 6–15	500	Neeper-Bradley et al. 1995
CD rat	Inhalation on GD 6–15	150 mg/m^3 (whole-body)	Tyl et al. 1995b
Fischer 344 rat	Dosed feed on GD 6–15	200	Maronpot et al. 1983
CD-1 mouse	Gavage on GD 6–15	150	Neeper-Bradley et al. 1995
CD-1 mouse	Gavage on GD 6–15	<750 (not determined)	Price et al. 1985
CD-1 mouse	Drinking water (multigenerational)	800	Lamb et al. 1985
CD-1 mouse	Dermal application on GD 6–15	3549	Tyl et al. 1995a
CD-1 mouse	Inhalation on GD 6–15	<150 mg/m^3 (whole-body)	Tyl et al. 1995b
Swiss mouse	Gavage	150	Tyl et al. 1989

Note. GD, gestational day.

however, incidences of poorly ossified and unossified vertebral centra significantly increased in fetuses of the high dose group (Maronpot et al. 1983).

Ethylene Glycol (in water) was applied to the skin of pregnant CD-1 mice (30 per group) on GD 6–15 at dosages of 404, 1677, and 3549 mg/kg/day. The positive control was 3000 mg/kg/day Ethylene Glycol. Ethylene Glycol was applied to the clipped and shaved skin of the dorsum, below the scapulae caudal to the neck. Each test solution was applied as a 100-μl volume. The application site was then covered with sterile gauze and masking tape. Mice of the positive control group received 3000 mg/kg/day (300 mg/ml; 10 ml/kg) Ethylene Glycol by gavage. Dams were killed for necropsy on GD 18. Fetuses were removed, weighed, sexed, and examined for signs of developmental toxicity. Dams given dermal applications of Ethylene Glycol had no treatment-related maternal toxicity, differences in pre- or postimplantation loss, fetal body weights/litter, or increased incidences of fetal malformations. Dams of the positive control group had increased water consumption, reduced fetal body weights/litter, and increased fetal malformations and variations. Eight dams given Ethylene Glycol by gavage died before scheduled necropsy. The kidneys of mice of the positive control group had tubular cell degeneration, but no oxalate crystals. Minimal renal tubular lesions were observed in three mice. The maternal and developmental NOAELS were both 3549 mg/kg/day (Tyl et al. 1995a).

Tyl et al. (1995b) administered a respirable aerosol of 150–2500 mg/m^3 Ethylene Glycol (whole-body exposure) to pregnant CD rats and CD-1 mice on GD 6–15 (6 hours per day). Rats were killed on GD 21 and mice on GD 18. Dams were evaluated for body weight, the weights of the gravid uterus, kidneys, and liver, the number of ovarian corpora lutea, and status of implantation sites (i.e., number of resorptions, dead and live fetuses). Fetuses were dissected from the uterus, counted, weighed, sexed, and examined for external, visceral, and skeletal malformations. All rat dams survived to scheduled necropsy. The high dose caused a significant increase in absolute and relative liver weight, but other parameters were normal. No other signs of maternal toxicity were observed. Gestational parameters (i.e., pre- and post-implantation loss, live fetuses/litter, sex ratio, and fetal body weight/litter) did not differ from controls. Treatment-related increases did not occur in the incidence of individual malformations, pooled external, visceral, or skeletal malformations, or total malformations by fetus or litter. No increases occurred in the incidence of external or visceral variations. Reduced ossification of the humerus, zygomatic arch, metatarsals, and proximal phalanges of the hindlimb were observed in fetuses of rat dams given 1000 mg/m^3 and 2500 mg/m^3 Ethylene Glycol. The maternal NOAEL was 1000 mg/m^3, and the developmental NOAEL was 150 mg/m^3 (Tyl et al. 1995a).

All mouse dams survived to scheduled necropsy. One dam given the high dose had a totally resorbed litter. Dams given the 1000 mg/m^3 and 2500 mg/m^3 Ethylene Glycol had reduced body weight and weight gain during and after the exposure period, as well as reduced gravid uterine weight. Embryo/fetal toxicity occurred after treatment with 1000 mg/m^3 and 2500 mg/m^3. Signs of embryo/fetal toxicity included an increase in nonviable implantations/litter, a reduction in viable implantations/litter, reduced fetal body weights/litter, increases of individual and pooled malformations (external, visceral, and skeletal), and an increase of total malformations. Observed malformations included exencephaly, cleft palates, foreshortened and abnormal faces or facial bones, vertebral fusions, and forked, fused, or missing ribs. The maternal NOAEL was 150 mg/m^3 and the developmental NOAEL was 150 mg/m^3 (Tyl et al. 1995b).

The mechanism of Ethylene Glycol toxicity is not fully understood. In all cases, however, toxicity of Ethylene Glycol appeared to be due to its metabolism to more toxic compounds such as glycoaldehyde and glycolic acid by hepatic alcohol dehydrogenase (primarily) and other enzymes (Jacobsen and McMartin 1986; NTP 1993a). When enzyme activity was blocked, toxicity did not occur (Jacobsen and McMartin 1986; Khera 1991).

Carney et al. (1996) identified glycolic acid as the proximate toxicant for Ethylene Glycol developmental toxicity using CD rat whole embryo cultures. In this study, the 10.5-day conceptuses (10 per group) were cultured and exposed to 0.5–50.0 mM/L of Ethylene Glycol or glycolic acid for 46 hours. Other embryos were cultured in media containing 12.5–50.0 mM glycolic acid or sodium glycolate. The positive control was 1.0 mM sodium valproate. The lowest effect level of Ethylene Glycol was 50.0 mM/L, which caused a very slight decrease in morphology score. Glycolic acid caused a significant increase in the percentage of dysmorphic embryos. Disorganized patterns of visceral yolk sac vessels were also observed. When glycolic acid and sodium glycolate were added to the medium, the percentage of embryos with active yolk sac circulation was slightly, but not significantly decreased. Embryo viability was not affected. A significant increase in the percentage of dysmorphic embryos was observed after treatment with 12.5 mM glycolic acid and sodium glycolate.

The two major routes of potential human exposure to Ethylene Glycol are dermal and inhalation routes. However, investigators determined that Ethylene Glycol was poorly absorbed through the skin and its low vapor pressure made significant inhalation exposures less likely. Investigators concluded that normal human uses of Ethylene Glycol would result in negligible plasma concentrations of Ethylene Glycol that were well below the threshold limits for reproductive and developmental toxicity (Carney 1994; Sun, Frantz, and Beskitt 1995).

ETHYLENE GLYCOL ETHERS

The monoalkyl ethers of Ethylene Glycol cause reproductive and developmental toxicity (NTP 1993b). Studies have suggested that the metabolites of 2-Methoxyethanol and 2-Ethoxyethanol (such as methoxyacetaldehyde and methoxyacetic acid) are the toxic agents and the proximate teratogens (Foster et al. 1984; Miller et al. 1984; Gray et al. 1985; Ritter et al. 1985; Beattie and Brabec 1986; Miller 1987; Scott et al. 1989; Clarke, Duignan, and Welsch 1990; Chiewchanwit and Au 1994). The

metabolites (but not the parent monoalkyl ethers) attack rapidly dividing cell systems and other tissues with high rates of respiration and energy metabolism, often inhibiting mitochondrial respiration (Beattie and Brabec 1986). Methoxyacetic acid is more toxic than ethoxyacetic acid. These metabolites have the structure $RO(CH_2)_nCOOH$. Generally, an increase in n tends to decrease the fetotoxicity of the compound more than an increase in the -R chain length. For example, minor metabolites of 2-Methoxyethanol such as 4-methoxybutyric acid or 3-methoxypropionic acid are much less active than methoxyacetic acid in terms of embryotoxicity (Miller 1987).

Monoalkyl ethers of Ethylene Glycol are biotransformed to their acetaldehyde and acetic acid forms by liver alcohol and aldehyde dehydrogenases (Foster et al. 1984; Gray et al. 1985; Chiewchanwit and Au 1994). In treated rats during labelling studies, the 2-Ethoxyethanol ether linkage was cleaved to produce $^{14}CO_2$. Of the dose administered, 11.7% (ethoxy-labelled) or 4.6% (ethanol-labelled) was eliminated as expired $^{14}CO_2$ (Cheever et al. 1984). Small amounts of Ethylene Glycol have also been detected in the urine of F344/N rats after inhalation of the glycol ethers, indicating that the monomer is a minor metabolite (Kennedy, Chang, and Henderson 1993).

Dividing spermatocytes commonly are affected by treatment with 2-Methoxyethanol and 2-Ethoxyethanol (and their metabolites), resulting in severe testicular toxicity (Foster et al. 1983; Chapin and Lamb 1984; Chapin et al. 1984; Gray et al. 1985). Creasy et al. (1985) reported that 250 mg/kg 2-Methoxyethanol (single oral dose) did not affect Sprague-Dawley rat zygotene spermatocytes in stage XIV of meiosis, but after one day of exposure, 70% of pachytene spermatocytes in stage I were killed or depleted. Dividing spermatocytes were similarly affected whereas step I spermatids were not. In tubules containing large numbers of necrotic cells, fine vacuolation of the Sertoli cell cytoplasm was observed. Otherwise, Sertoli cells were not affected. A gradual reduction in susceptibility occurred toward midpachytene, and cells in stages VII–XI were unaffected by treatment (Creasy et al. 1985).

The glycol ethers inhibit intercellular communication. Doses of 17.5, 20, and 25 μl/ml 2-Methoxyethanol (0.13–0.3 M) apparently blocked the function of gap junctions in human palatal mesenchyme cells (HEPM) (Welsch and Stedman 1984a). Cell-cell communication was also inhibited in Chinese Hamster V79 cells by 2-Methoxyethanol and 2-Ethoxyethanol (Welsch and Stedman 1984b). Pulse-labeling and autoradiographic analysis results indicated that 2-Methoxyethanol had adverse effects on the treated HEPM cells. The cells were rounded, stained more intensely, and had a lower cell density than control culture cells. Inhibition of gap junction–mediated chemical messenger transfer did not occur as deduced from study of electron micrographs; rather, morphological effects suggested that gap junctions were not established at sites of cell-cell contact. Because intercellular communication is a fundamental event for the differentiation of embryonal tissues, the researchers hypothesized that this blocking action disrupts fetal organogenesis (Welsch and Stedman 1984a).

Oral Toxicity

Oudiz, Walsh, and Wiley (1993) used a mouse chimera assay to detect a decrease in cell proliferation of preimplantation embryos as a result of treatment with 2-Methoxyethanol. Male Swiss ICR mice were administered 750 mg/kg/day 2-Methoxyethanol in water by gavage for 5 days, 12 received 1,500 mg/kg/day 2-Methoxyethanol, and 22 control mice received only distilled water. The males were mated to unexposed females during weeks 1–7 posttreatment and embryos were retrieved 26 hours after mating. High-dose males had transient weight loss that returned to control values by the first week of the mating period. During week 4, mice treated with 750 mg/kg/day 2-Methoxyethanol had infertility. The same effects were noted in the high-dose group during weeks 3–6. The mean proliferation ratios of surviving four-cell embryos determined during the chimera assay were significantly less than the control ratio (0.51 ± 0.04) in the 750 mg/kg/day group for week 4 (0.41 ± 0.07) and the 1,500 mg/kg/day group for week 5 (0.46 ± 0.04). No dose-related differences were seen in the total chimera number seen (Oudiz, Walsh, and Wiley 1993).

In a second experiment within the same study, 16 mice each made up the control and treatment groups (50 and 200 mg/kg/day for 5 days). The males were mated as above. Mean body weights were not significantly altered compared to the control group (distilled water). Infertility also did not occur in the dosed mice. The mean proliferation ratios were significantly less for both the 50 and 200 mg/kg/day groups during week 4 (0.49 ± 0.01 and 0.48 ± 0.02, respectively) compared to that of the control group (0.50 ± 0.01). No dose-related differences in total cell number of chimera were observed. The week 4 proliferation ratio decreases in this and the previous experiment corresponded to the pachytene spermatocyte stage. Overall, changes in sperm were transmitted and expressed within the preimplantation embryo as a cell proliferation disadvantage in the chimera assay, even at exposure concentrations below those that adversely affect fertility (Oudiz, Walsh, and Wiley 1993).

Scott et al. (1989) treated pregnant *Macaca fascicularis* primates daily with 2-Methoxyethanol during organogenesis to assess the embryotoxic effects of the ether. Doses of 12, 24, or 36 mg/kg/day 2-Methoxyethanol were administered to four primates once daily from GD 20–45 by gavage in 15 ml water through a stomach tube. Two control groups were used (ethanol and untreated). The formation and elimination of methoxyacetic acid were followed in maternal blood samples. Hysterotomies were performed on GD 100. Fetuses were examined for gross abnormalities, fixed, x-rayed, dissected, and assayed for methoxyacetic acid in pharmacokinetic studies. At 12 and 24 mg/kg, 3 of 10 and 3 of 13 embryos died as a result of treatment, respectively. The dead embryos were retained in the uterus with minimal embryonic autolysis. One spontaneous abortion occurred in each of these two treatment groups. Dose-related maternal anorexia (slight to severe) and weight loss were recorded as well. Untreated control monkeys had a mean weight of 3.45 kg at the start of treatment that increased to 3.47 kg by the end of treatment. The alcohol control group mean weight increased from 3.53 to

3.63 kg. The 3.79, 3.48, and 4.25 kg mean weights of the 12, 24, and 36 mg/kg/day treatment groups decreased to 3.72, 3.28, and 3.88 kg, respectively. The high dose, 36 mg/kg/day, caused 100% embryo mortality (8/8) between GD 27–63 and one fetus was missing a digit from each forelimb. Fetuses that survived to GD 100 had no malformations or growth retardation. Methoxyacetic acid accumulated in maternal serum after repeated daily dosing. Results of transplacental studies indicated that the concentration of the metabolite was uniformly distributed in the embryo and extraembryonic fluids at a concentration similar to that in maternal serum and accumulated in the yolk sac at high concentration (Scott et al. 1989).

In a study by Foster et al. (1983), oral doses (in water) of 100–500 mg/kg/day 2-Methoxyethanol and 250–1000 mg/kg/day 2-Ethoxyethanol were administered to male Sprague-Dawley rats over 11 days. Sixteen hours after a single 500 mg/kg dose of 2-Methoxyethanol was given, mitochondrial damage was observed in spermatocytes. The degeneration of pachytene spermatocytes was seen within 24 hours after a single 100 mg/kg 2-Methoxyethanol dose. Similar results occurred at day 11 when 500–1000 mg/kg/day 2-Ethoxyethanol doses were given. Chromatin margination of spermatid nuclei occurred by day 4 after a 500 mg/kg/day dose of 2-Methoxyethanol and day 7 after 250 mg/kg/day. Spermatid and late spermatocyte populations were absent by day 11 for those doses. Progressive and dose-related depletion of the spermatocytes and early spermatid population occurred with continued dosing. Affected spermatocytes had general cellular shrinkage, increased cytoplasmic eosinophilia, and nuclear pyknosis. Mitochondrial swelling and disruption, cytoplasmic vacuolation, and early condensation of nuclear chromatin were detected. The NOAELs for this study were 50 and 250 mg/kg/day for 2-Methoxyethanol and 2-Ethoxyethanol, respectively. Rats treated with 250 or 500 mg/kg/day for 2-Methoxyethanol had dose-related decreased testicular and prostate weights. Testicular damage was generally reversible within one full maturation cycle, but totally atrophic tubules were observed in the testes of several rats, indicating a loss of spermatogonia. Doses of 500 or 1000 mg/kg/day 2-Ethoxyethanol produced degeneration of a severity similar to that caused by 100 mg/kg/day 2-Methoxyethanol. The degree of degeneration and depletion of the spermatocyte population was similar for 500 and 1000 mg/kg/day 2-Ethoxyethanol, but onset was more rapid at the lower dose (Foster et al. 1983). Similar effects were seen when primary mixed cultures of Sertoli and germ cells from the testes of Sprague-Dawley rats were exposed to 2 and 10 mM concentrations of methoxy- and ethoxyacetic acid, respectively; up to 50 mM 2-Methoxyethanol or 2-Ethoxyethanol did not induce degenerative changes (Gray et al. 1985).

An NTP study (1993b) of intraperitoneal (i.p.) and oral administration of undiluted 0.165 and 0.330 ml/kg 2-Methoxyethanol to pregnant Wistar rats resulted in 65% (i.p.) and 53.8% (oral) total embryotoxicity at 0.165 ml/kg and 100% embryotoxicity for both dose routes at 0.330 ml/kg. The control group had 4.4% total embryotoxicity, 190 total implants, 4.4% were dead and resorbed, and none were malformed. At the low dose, 105 (i.p.) and 101 (oral) implants were counted, respectively; 4.7 and 19.3% were dead and resorbed; and 62.8 and 45.2% of survivors were malformed. The high dose had 106 and 105 implants; 100% of survivors were malformed; and 20.1 and 15.1% were dead and resorbed. Eighty to 100% of the malformations were hydronephrosis (decreased with increasing dose) and heart, tail, and limb defects. Miscellaneous anomalies included microphthalmia, micrognathia, folded retina, cleft lip, and diaphragmatic hernia. The most common cardiac malformation was interventricular septal defect, with the lesion in the muscular portion of the septum. Other heart defects were levocardia, defects between the ascending aorta and right ventricle, truncus communus, dilated ductus arteriosus, and dilated aortic arch. Short or kinked tails occurred frequently. Limb defects, including short limb, bowed radius, and ventral polydactyly were observed and their incidence increased with the dose (Ritter et al. 1985).

In a 2-week study in which male F344/N rats were treated with 200, 400, 600, 1000, or 2000 mg/kg/day 2-Methoxyethanol in drinking water, the absolute right testis weight was 1.235 for control and 1.182, 0.667, 0.429, 0.372, or 0.316 g for dosed groups, respectively. The respective relative testes weights were 6.07 for control and 5.59, 3.29, 2.38, 2.51, and 2.35 g for dosed groups (NTP 1993b).

Nelson et al. (1989) reported that 2-Methoxyethanol administered in liquid diet increased embryonic intracellular pH in rats during critical stages of organogenesis. Concentrations above 73 mg/kg/day produced total embryomortality. At lower doses, cardiovascular malformations were observed. The teratogenic NOAEL was 16 mg/kg/day 2-Methoxyethanol. Approximately 36 mg/kg/day caused 50% embryomortality and decreased the survivor's performance in subsequent maze testing (Nelson et al. 1989).

Inhalation Toxicity

The toxicity of glycol ethers for dividing spermatocytes ultimately decreased testicular weights and size, caused degeneration of germinal epithelium of the testes, and increased flaccidity of the testes in rats and rabbits that had inhaled 30, 100, or 300 ppm (0.09, 0.31, or 0.93 mg/m^3) 2-Methoxyethanol 6 hours daily 5 days/week for 13 weeks (Miller et al. 1983; Rao et al. 1983).

In two of five male New Zealand white rabbits, inhalation of 30 ppm 2-Methoxyethanol 6 hours per day, 5 days per week, for 13 weeks caused a small to moderate reduction in testes size. One of five rabbits had degeneration of the testicular germinal epithelium and few spermatozoa. In the same study, a dose of 100 ppm 2-Methoxyethanol caused similar, but more severe effects in four of five (testes size reduction) and three of five rabbits (degeneration). Some tubules were normal in appearance, whereas others had no germinal elements. Small, flaccid testes and diffuse, severe degenerative changes were observed in the three surviving rabbits in the high-dose group. In male Sprague-Dawley rats used in the same study, reduced testes size

TABLE 6
Dose-finding teratology study—Sprague-Dawley rats (Nelson et al. 1981)

Concentration 2-Ethoxyethanol (ppm)	Dams killed (GD 7–13)	Dams delivered (GD 7–13)	Dams killed (GD 14–20)	Dams delivered (GD 14–20)	Day delivered
1200	46 resorptions (4),[a] 0 live fetuses	Not evaluated	Not evaluated	8 dead pups[b] (2), 20 resorption sites	24
900	40 resorptions (3), 0 live fetuses	Not evaluated	1 dead fetus (1), 11 live fetuses	31 dead pups (3), 36 sites	24
600	54 resorptions (6), 19 live fetuses	12 dead pups (11), 0 live pups	3 dead fetuses (2), 18 live fetuses	29 dead pups (6),[c] 62 sites	24
300	16 live fetuses (2), 0 dead or resorbed	5 dead pups (3),[d] 32 live pups	1 dead fetus (1), 3 live fetuses	11 dead pups (4),[d] 27 live pups	22.5
200	Not evaluated	Not evaluated	Not evaluated	21/32 pups survived (4)	22.5

[a]Number of dams in parentheses.
[b]Dead within 24 hours of delivery.
[c]One dam kept five pups alive for 10 days.
[d]These litters culled to eight pups/litter, and 20/24 pups survived.
GD, gestational day.

and treatment-related microscopic lesions only occurred at the highest dose (300 ppm). Moderate to severe degeneration of the germinal epithelium in the seminiferous tubules was observed. Reduced numbers of spermatozoa and degenerating spermatozoa were seen in the epididymides of all affected rats. A slight reduction in the amount of prostatic secretory material was also detected (Miller et al. 1983).

Rao et al. (1983) also observed reduced testes size and atrophic seminiferous tubules in Sprague-Dawley rats exposed to 300 ppm 2-Methoxyethanol vapor 6 h/day, 5 days/week for 13 weeks. Male and female rats (20–30 per group) inhaled 30–300 ppm 2-Methoxyethanol vapors as above. Males were bred to unexposed females to determine reproductive capability and dominant lethality. Females were bred to unexposed males. Exposure to 300 ppm 2-Methoxyethanol was enough to completely suppress fertility in males. Fertility was partially restored by 13 weeks after treatment. Body weights decreased at this concentration. No dominant lethal effect or impaired fertility was seen in males exposed to 30 or 100 ppm. No adverse reproductive effects were observed in females at any dose. The NOAEL for fertility and reproduction was 100 ppm 2-Methoxyethanol (Rao et al. 1983).

McGregor et al. (1983) found that sperm abnormalities in CD rats and B6C3F$_1$ mice caused by inhalation of 500 ppm (7 h/day for 1 or 5 days) before mating increased the number of pre- and post-implantation losses and decreased the pregnancy rate. Exposure to 25 ppm did not affect reproduction. No signs of clinical toxicity were observed in exposed rats and mice other than the failure to increase body weight during the testing period (McGregor et al. 1983).

Pregnant Sprague-Dawley rats were treated with 200–1200 ppm 2-Ethoxyethanol by inhalation 7 h/day on GD 7–13 or 14–20 in a preliminary dose-finding study. The dams were then either killed on GD 20 or allowed to deliver and rear surviving offspring. The only effect of treatment was slightly prolonged gestation (approximately 48 hours) in the dams exposed on GD 14–20. Dams whose pups died were killed and implantation sites were counted. (Results are found in Table 6.) In the second part of this experiment, rats were again exposed on GD 7–13 and 14–20. The treatment dose was 100 ppm. The number of surviving pups was not given, but gestation was increased by approximately 12 hours in treated dams. Offspring were subjected to behavioral testing, which included rotorod testing for neuromuscular ability, avoidance and operant conditioning, activity wheel testing, and other tasks. Groups of offspring were also used for neurochemical analysis. In general, offspring of treated rats were less active and had impaired performance in behavioral testing. Acetylcholine, norepinephrine, dopamine, and protein levels were altered in the brains of the offspring. Greater differences between the treatment groups and controls occurred in offspring of rats exposed to 2-Ethoxyethanol earlier in gestation (Nelson et al. 1981).

In later studies, it was observed that either paternally or maternally exposed rats did poorly in behavioral testing compared to controls and had neurochemical deviations when 25 ppm 2-Methoxyethanol was inhaled 7 h/day, 7 days/week for 6 weeks (Nelson et al. 1984).

Dermal Toxicity

Reproductive and developmental toxicity also occur after dermal application of glycol ethers. In a Chernoff-Kavlock assay, pregnant Alpk/AP rats were given 10 ml/kg/day of 3, 10, 30, or 100% 2-Methoxyethanol in physiological saline on GD 6–17.

TABLE 7
Chernoff-Kavlock assay—dermal application, rats (Wickramaratne 1986)

Reproduction parameters	% 2-Methoxyethanol				
	Saline control	3%	10%	30%	100%
No. dams with litter	10/10	10/10	6/10	0/10	All dams died
Group mean weight change (g)					
GD[a] 1–21	115.3 ± 12.9	113.3 ± 18.4	102 ± 13.07	No litters produced	All dams died
GD 7–17	80.6 ± 13.4	81.0 ± 16.8	68.3 ± 9.2	No litters produced	All dams died
No viable litters					
Day 1[b]	10/10	10/10	5/6	No litters produced	All dams died
Day 5	10/10	10/10	3/6	No litters produced	All dams died
Total litter size (live + dead)	116	112	30	No litters produced	All dams died
Mean no. live pups/litter					
Day 1	11.5 ± 2.84	11.2 ± 2.49	6.0 ± 4.2	No litters produced	All dams died
Day 5	11.3 ± 2.71	11.2 ± 2.49	4.0 ± 4.43	No litters produced	All dams died
% Survival (days 1–5)	98.5	100	43.75	No litters produced	All dams died
Mean live pup weight (g)					
Day 1	6.05 ± 0.9	6.33 ± 0.55	5.83 ± 0.55	No litters produced	All dams died
Day 5	9.61 ± 1.01	9.82 ± 1.55	10 ± 0.99	No litters produced	All dams died

[a]Gestational day.
[b]Postpartum day.

The test substance was applied to a 5 cm^2 area of shaved infrascapular skin for 6 hours per application. The site was covered by an occlusive patch. Control rats received saline. Rats were allowed to litter normally and rear their litters until day five postpartum. No adverse effects were observed after treatment with 3% 2-Methoxyethanol. Materno- and embryotoxicity (Table 7) were observed at the higher doses (Wickramaratne 1986).

Upon administration of a single bolus dose of 250 mg/kg 2-Methoxyethanol to CD-1 mice on GD 11, the ether was rapidly oxidized to methoxyacetic acid. Seventy percent of the fetuses had nonspecified digit anomalies. In order to mimic human dermal exposure to 2-Methoxyethanol, osmotic minipumps were implanted subcutaneously in pregnant mice on GD 11. Each pump delivered 27 mg/kg/h of the test chemical for 8 or 12 h. Maternal plasma, embryos, and exocoelomic/amniotic fluid were collected at 4, 8, 12, and 24 hours. When one pump was implanted for 8 or 12 hours, 31% or 35% of fetuses examined at GD 18 had digit malformations (micro-, syn-, ectro-, and polydactyly, respectively). With two pumps, the percentage increased to 87% or 85%. Peak concentrations of the metabolite, methoxyacetic acid, were detected 12 hours after exposure. Concentrations of 2.1 (one pump) or 5.2 (two pumps) mmol/kg methoxyacetic acid were found in collected embryos, but approximately 25% less was found in maternal plasma and >50% more in the exocoelomic/amniotic fluid (Clarke, Duignan, and Welsch 1990).

Hardin, Goad, and Burg (1984) applied 2-Ethoxyethanol and other ethylene glycol ethers in water to the skin of time-mated Sprague-Dawley rats on GD 7–16 and observed no toxic signs in the dams after treatment with 2-Ethoxyethanol. A dose volume of 0.25 ml 2-Ethoxyethanol was applied four times daily to 18 rats. This dose was chosen as a teratogenic dose based on the results of a previous study and served as the positive control for the remaining ethers tested. Dams were killed on GD 21. Dams treated with 2-Ethoxyethanol did have significant body weight loss from GD 7–21 and the gravid uterus weights were reduced also. Vehicle control results are in parentheses. Seven of 18 rats had totally resorbed litters (none). The number of implants per litter did not differ from the control. The number of dead implants per litter was 7.7 ± 5.1 (1.2 ± 1.2). In each litter with live fetuses, the number of live fetuses was 6.5 ± 4.1 for 11 litters (10.4 ± 2.6 for 17 litters). The mean fetal body weight was 3.0 ± 0.6 g (3.9 ± 0.6 g). Three fetuses in three litters had gross malformations (acaudia and imperforate anus), whereas none were found in the control group. Visceral abnormalities included cardiovascular malformations (aorta, ductus, ventricular septal defects, and abnormal subclavian), renal anomalies (hydronephrosis, hydroureter, ectopic kidney, and fused kidney), hydrocephalus and hemorrhage of the brain, ocular defects (microophthalmia, abnormal optic nerve, and folded retina), and testicular abnormalites (slight ectopic or undescended testes, and agenesis). The control group had no cardiovascular, neural, or

ocular malformations, but had some instances of hydronephrosis, and ectopic kidneys. The incidences were 10 of 10 litters (21 fetuses of 34) with malformations in the 2-Ethoxyethanol–treated group, and 3 of 17 litters (3 fetuses of 87) in the vehicle control group (Hardin, Goad, and Burg 1984).

PROPYLENE GLYCOL

In order to understand the structure activity relationship between Ethylene Glycol and Propylene Glycol, the CIR Expert Panel reviewed reproductive and developmental toxicity data on the latter chemical. Propylene Glycol and Polypropylene Glycol have previously been reviewed by the CIR Expert Panel, and information from that safety assessment is presented in *italicized text* below (Andersen 1994).

A continuous breeding reproduction study was conducted using COBS Crl:CD-1 (ICR)BR outbred Swiss albino mice (6 weeks old). The continuous breeding phase of the study (task II) was begun after the dose-setting study (task I) and involved three experimental groups (40 mice per group) and a control group of 80 mice. Experimental and control groups contained an equal number of male and female mice. The three experimental groups were given the following doses (in feed or water), respectively, during a 7-day premating period: 1.0% Propylene Glycol (daily dose 1.82 g/kg), 2.5% Propylene Glycol (daily dose 4.80 g/kg), and 5.0% Propylene Glycol (daily dose 10.10 g/kg). The animals were then randomly grouped as mating pairs, cohabited, and treated continuously for 98 days; data were collected on all newborns. If significant adverse effects on fertility were observed, a crossover mating trial (task III) was usually performed to determine whether F_0 males or females were more sensitive to the effects. Task III was not conducted and would have consisted of mating high-dose mice of each sex to control mice of the opposite sex and then analyzing the offspring. To perform an offspring assessment of reproductive function (task IV) following exposure to Propylene Glycol, the dam (from phase II) was dosed through weaning and F_1 mice were dosed until mating occurred at 74 ± 10 days of age. Mating pairs consisted of male and female offspring from the same treatment group (20/group/sex); F_2 litters were examined. In the continuous breeding phase (task II), there were no significant changes ($p < .05$) in mean live pup weight per litter between the control group and any of the treatment groups. In task IV, which was an offspring assessment of reproductive function, only the high-dose group (5% Propylene Glycol) was involved. There were no significant differences ($p < .05$) between control and experimental groups with respect to the following observations in task IV: mating index, fertility index, mean number of live pups per litter, proportion of pups born alive, and sex of pups born alive (Morrissey et al. 1989).

The effect of Propylene Glycol on the development of $B_6D_2F_1$ mouse zygotes in the pronuclear stage was evaluated; oocytes were fertilized in vitro. Samples of zygotes were incubated for 20 minutes (at 22°C) with 1.5, 3.0, and 6.0 M Propylene Glycol in phosphate-buffered saline. There were three zygote cultures per test concentration of Propylene Glycol, and each group of three was incubated with 0, 0.1, and 0.25 M sucrose, respectively. The three control cultures without Propylene Glycol were also incubated with 0, 0.1, and 0.25 M sucrose, respectively. Subsequently, the zygotes were incubated for 24 hours (at 37°C) under 5% CO_2, and the percentage of zygotes that cleaved to form two-cell embryos was determined. The percentage of zygotes that developed to two-cell embryos was not altered in control cultures or cultures exposed to 1.5 M Propylene Glycol (78% in both cultures), but was reduced in cultures exposed to 3.0 M Propylene Glycol (7%; $p < .05$). Embryonic development was inhibited completely in zygotes exposed to 6.0 M Propylene Glycol. The presence of sucrose in the incubation medium did not influence embryonic development (Damien, Luciano, and Peluso 1989). In a later publication by the same investigators, the data indicated that the exposure of $B_6D_2F_1$ mouse zygotes to ≥2.5 M Propylene Glycol for 2–7 minutes altered both intracellular pH and developmental potential. In that these effects were independent of volume changes noted in zygotes and therefore intracellular Propylene Glycol concentrations, the authors postulated that the toxicity of Propylene Glycol is mediated by direct alteration of the cell membrane (Damien, Luciano, and Peluso 1990).

Kavlock, Short, and Chernoff (1987) investigated the teratogenic potential of several substances, including Propylene Glycol. A group of 30 pregnant female CD-1 mice was given single oral doses of 10,000 ppm Propylene Glycol on days 8–12 of gestation. Fertility rates, numbers of maternal deaths, numbers of resorptions, average litter sizes, birth weights, and pup postnatal weight gain were monitored in an assessment of the maternal and perinatal effects of Propylene Glycol. The fertility rates of mice given Propylene Glycol were not significantly different from those of control mice. There were no maternal deaths or resorptions observed in any of the animals dosed with Propylene Glycol. All other parameters measured were not significantly different from control values. Propylene Glycol was not a teratogen in this test.

Propylene Glycol (dose 0.01 ml/g body weight) was also injected subcutaneously into each of 21 pregnant ICR/Jcl female mice (9–12 weeks old) on day 9, 10, or 11 of gestation. Days 9–11 correspond to the sensitive stage for the induction of fetal deaths and malformations in this strain of mice. The following malformations were noted in 5 of the 226 living fetuses: open eyelid (3 fetuses), polydactyly (1 fetus), and cleft palate (1 fetus). In a control group of 28 mice (same strain and weight range) injected subcutaneously with water (0.01 ml/g body weight) during pregnancy, the only malformation noted was exencephalus in 1 of 320 living fetuses. The incidence of malformations in 1,026 living fetuses from an untreated control group of 90 pregnant

mice (same strain and weight range) was 3 fetuses with polydactyly, exencephalus, and open eyelid, respectively (Nomura 1977).

Ascitic mouse ovarian tumor cells were used in an in vitro teratogenicity assay by Braun et al. (1982). Tumor cells were labeled with [^{3}H]thymidine in situ via an intraperitoneal injection of 0.2 mCi of radioactivity. Cells were harvested and suspended in phosphate-buffered saline (10^7 cells/ml). Various concentrations of Propylene Glycol were added to aliquots of the cells (all concentrations not stated) and incubated at 37°C for 30 minutes. The teratogenicity of the substances tested was assayed by the ability of the test substances to inhibit attachment of the tumor cells to Concanavalin A–coated plastic. The extent of attachment of the cells was measured by counting the radioactivity on the Concanavalin A–coated plastic. Propylene Glycol did not inhibit attachment of the tumor cells; the largest dose tested was 27,000 mg/L Propylene Glycol. Propylene Glycol was a nonteratogen in this test.

DISCUSSION

It is generally recognized that the ethylene glycol monoalkyl ethers are not themselves the toxic agent for reproductive or developmental toxicity, but rather it is one or more metabolites (Foster et al. 1984; Gray et al. 1985; Chiewchanwit and Au 1994). The ethylene glycol monoalkyl ethers are metabolized by alcohol dehydrogenase and aldehyde dehydrogenase, resulting in 2-methoxyethanol, for example, then the metabolites are methoxyacetaldehye and methoxyacetic acid. It is also apparent from the available data that the toxicity of the metabolites of ethylene glycol monoalkyl ethers is inversely proportional to the length of the alkyl chain, with methoxyacetic acid > ethoxyacetic acid > propoxyacetic acid > butoxyacetic acid (Gray et al. 1985). The CIR Expert Panel noted adverse reproductive and developmental effects of oral administration of 2-butoxyethanol and the absence of any reproductive toxicity when applied dermally in its earlier review, concluding that 2-butoxyethanol can be used safely (at concentrations up to 10%).

Implications for Ceteths

Given the methods of manufacture of the Ceteths (CIR 1996a), there is no likelihood of methoxyethanol, ethoxyethanol, etc., being present as an impurity. Because there likely would be ethylene glycol monomer linked to cetyl alcohol in preparations of the shorter chain length Ceteths and Ceteth-1 itself is listed as a cosmetic ingredient, it is appropriate to evaluate the potential toxicity of Ceteth-1. As shown in Figure 3, compared to the structure of the least active ethylene glycol monoalkyl ether, 2-Butoxyethanol, the structure of Ceteth-1 has a very long alkyl chain.

Because reproductive and developmental toxicity is inversely related to the chain length of the alkyl group, the Ceteths would appear to present even less risk than 2-Butoxyethanol. In addition, many of the Ceteths will contain only a polyethylene glycol base, further reducing the potential for any adverse effects of the kind seen for ethylene glycol monoalkyl ethers.

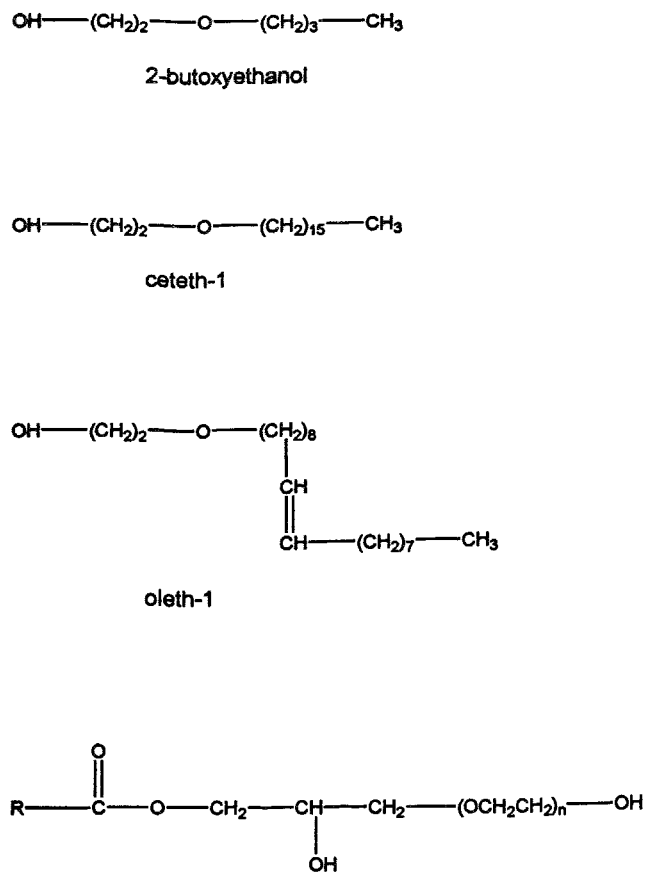

FIGURE 3
Structures of ceteth-1, oleth-1, and PEG-n glyceryl cocoate compared to 2-butoxyethanol.

Implications for Oleths

Given the methods of manufacture of the Oleths (CIR 1996b), there is no likelihood of methoxyethanol, ethoxyethanol, etc., being present as an impurity. Because there likely would be ethylene glycol monomer linked to oleyl alcohol in preparations of the shorter chain length Oleths, it is appropriate to evaluate the potential toxicity of Oleth-1. As shown in Figure 3, compared to the structure of the least active ethylene glycol monoalkyl ether, 2-Butoxyethanol, the structure of Oleth-1 has a very long alkyl chain.

Because reproductive and developmental toxicity is inversely related to the chain length of the alkyl group, the Oleths would appear to present even less risk than 2-Butoxyethanol. In addition, many of the Oleths will contain only a polyethylene glycol base, further reducing the potential for any adverse effects of the kind seen for ethylene glycol monoalkyl ethers.

Implications for PEGs Cocamine

Given the methods of manufacture of the PEGs Cocamine (CIR 1996c), there is no likelihood of methoxyethanol, ethoxyethanol, etc., being present as an impurity. In particular, the data

presented above relates exposures of ethylene glycol monoalkyl ethers to subsequent reproductive and developmental toxicity. PEG Cocamines are polyethylene glycol ethers of the primary aliphatic amine derived from coconut oil, and are chemically different from alkyl ethers. This suggests that the toxicity concerns would not exist for the PEGs Cocamine.

Implications for PEGs Distearate

Given the methods of manufacture of the PEGs Distearate (CIR 1996d), there is no likelihood of methoxyethanol, ethoxyethanol, etc., being present as an impurity. In particular, the data presented above relate exposures of ethylene glycol monoalkyl ethers to subsequent reproductive and developmental toxicity. PEG Distearates are diesters of polyethylene glycol, and are chemically different from alkyl ethers. This suggests that the toxicity concerns would not exist for the PEGs Distearate.

Implications for PEGs Glyceryl Cocoate

Given the methods of manufacture of the PEGs Glyceryl Cocoate (CIR 1996e), there is no likelihood of methoxyethanol, ethoxyethanol, etc., being present as an impurity. In addition, the PEGs Glyceryl Cocoate, the partial esters of polyoxyethylated glycerol with stearic acid, generally conform to the formula in Figure 3.

where RCO- represents the coconut oil–derived fatty acids (mainly C_{18}) and n has an average value equal to the number in the name. Prior to the reaction with the RCO- group, polyethylene glycol is combined via an ether linkage with glycerol. Although the PEGs Glyceryl Cocoate each have a polyethylene glycol base, not ethylene glycol, it should be recognized that ethylene glycol monomer could be present in any polymerization process that seeks to create chain lengths of less than 10. For that reason it is appropriate to evaluate the potential toxicity of PEG-1 Glyceryl Cocoate. As shown in Figure 3, compared to the structure of the least active ethylene glycol monoalkyl ether, 2-Butoxyethanol, the structure of PEG-1 Glyceryl Cocoate has a very long complex chain that includes an ester linkage and an hydroxyl group. Because reproductive and developmental toxicity is inversely related to the chain length of a simple alkyl group, the PEGs Glyceryl Cocoate would appear to present no such risk. In addition, the PEGs Glyceryl Cocoate would contain mostly a polyethylene glycol base, further reducing the potential for any adverse effects of the kind seen for ethylene glycol monoalkyl ethers.

Implications for PEGs Lanolin

Given the methods of manufacture of the PEGs Lanolin (CIR 1996f), there is no likelihood of methoxyethanol, ethoxyethanol, etc., being present as an impurity. Although the exact structures of lanolin, hydrogenated lanolin, lanolin oil, or lanolin wax are not known, such extracts are usually long-chain compounds. When combined via an ether linkage with polyethylene glycol, it is not likely that any of them would present the simple R-group appearance of methyl, ethyl, propyl, or even butyl. It is also unlikely that the Lanolin moieties would be metabolized (e.g., via β-oxidation) to simple methyl, ethyl, etc., alkyl groups. In addition, most of the polyethylene glycol chain lengths used in making the various PEGs Lanolin are 10 or longer, suggesting that there would be very little monomer linked by an ether group to the lanolin moiety.

Implications for PEGs Soya Sterol

Given the methods of manufacture of the PEGs Soya Sterol (CIR 1996g), there is no likelihood of methoxyethanol, ethoxyethanol, etc., being present as an impurity. In particular, PEG-5, -10, -16, -25, and -40 Soya Sterol would not be expected to be reproductive toxins as they are ethers of soybean oil sterols such as stigmasterol, γ-sitosterol, and campesterol, and are chemically different from alkyl ethers. They have a polyethylene base, not ethylene glycol. In addition, most of the polyethylene glycol chain lengths used in making the various PEGs Soya Sterol are 10 or longer, suggesting that there would be very little monomer linked by an ether group to the soybean oil sterols, further reducing the potential for any adverse effects of the kind seen for ethylene glycol monoalkyl ethers.

CONCLUSION

Metabolites of ethylene glycol monoalkyl ethers are reproductive and developmental toxins. In general, these metabolites of concern are not expected to be formed in cosmetic formulations that contain polymers of ethylene glycol.

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