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Developmental Effects after Inhalation Exposure of Gravid Rabbits and Rats to Ethylene Glycol Monoethyl Ether

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The effects of ethylene glycol monoethyl ether (EGEE) were determined on development *in utero*. Pregnant New Zealand White rabbits were exposed to air or 160 or 617 ppm EGEE for 7 hr/day from 1 to 18 days of gestation (dg). Virgin Wistar rats were exposed to 150 or 649 ppm EGEE or air 5 days/week for the 3 weeks immediately preceding their breeding. Sperm-positive rats were subsequently exposed to air or 202 or 767 ppm EGEE for 7 hr/day from 1 to 19 dg. Group sizes were 29 to 38 per concentration for both species.

Pregestational exposure of rats had no effect on mating success, and there was no effect of EGEE exposure on establishment of pregnancy in either species. Rabbits exposed to the both concentrations had decreased food intake and depressed weight gain. Exposure-related mortality occurred in the 617 ppm EGEE group of rabbits. The only toxic sign seen in rats was reduced weight gain after exposure to 767 ppm EGEE. Exposure induced high embryomortality at maternal toxic concentrations in rats and rabbits, while lower levels induced fetal growth retardation in rats but not in rabbits. Gestational exposure increased the incidence of anomalies and variations; these were primarily of soft tissues in rabbits and of skeleton in rats. Thus, significant evidence of terata, fetal growth retardation and embryomortality were induced in rabbits and rats at levels that were below or similar to those that induced maternal manifestation of toxicity. These data implicate EGEE as a teratogen.

Introduction

The alkyl ether derivatives of ethylene, diethylene and triethylene glycol are a class of solvents with numerous industrial and consumer applications. In general, they are soluble in most liquids, including water, ethers, alcohols, ketones and aliphatic hydrocarbons. Glycols, glycol ethers, and the glycol ether acetates are used in paints, lacquers, enamels, inks, dyes and in liquid soaps and dry cleaning fluids. They serve as surfactants, emulsion conditioners, degreasing agents and as the reaction medium in chemical synthesis. These chemicals are used in various extraction processes, as fixatives in perfumes, cosmetics, and soaps, and as desiccants. The National Institute for Occupational Safety and Health (NIOSH) estimates that 200,000 to 2,000,000 workers are exposed occupationally to each of at least eight separate glycols, glycol ethers, and ether acetates. The total number exposed probably is less than the sum of these estimates since some of the glycol ethers may be used interchangeably or in combination, or more than one may be used in a workplace.

Ethylene glycol monoethyl ether (EGEE, $HO\text{-}C_2H_4\text{-}O\text{-}C_2H_5$, Cellosolve, 2-ethoxyethanol; CAS: 110-80-5) was identified as an agent of concern for developmental toxicity because it is a widely used representative of this class of compounds and no reports were available on its teratogenic potential (*1*) before this study was initiated.

The limited female reproductive tract and/or developmental toxicity data available for EGEE were reviewed recently by Hardin (*2*). Embryotoxicity and teratogenicity have been demonstrated in mice, rats and rabbits following oral, inhalation or dermal exposure to EGEE. In those species examined for fetal changes, cardiovascular and/or skeletal defects were frequently observed. Overall, there were clear indications of reproductive and developmental toxicity including prenatal mortality, growth retardation and teratogenicity.

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Materials and Methods

Test Material

Production grade EGEE was supplied by Dow Chemical (Midland, MI) and was identified by Lot #5, TK #376. The purity of the material by gas chromatography (GC) was 99.993%. The infrared spectrum showed no peaks identifiable as other organic impurities.

Animals and Husbandry

Adult female Wistar rats weighing approximately 150 g (Hilltop Lab Animals, Inc., Scottdale, PA) and female New Zealand White rabbits weighing approximately 3.5 kg (White's Rabbitry, Kootenai, ID or Hatch's Rabbitry, Langley, WA) were used. Sexually mature males of the same strain were used for breeding. Mating units of one male and three or four females were established for rats. Animals were cohabitated for up to 10 consecutive days until approximately 30 females per group had positive evidence of mating. The day on which sperm were detected in the vaginal lavage of rats was considered day 1 of gestation (1 dg). Semen samples were collected, pooled, and diluted from at least three bucks daily for artificial insemination of 29 rabbits per group (*3,4*). The day of insemination was designated 0 dg.

Animals were housed in individual stainless steel wire cages before, during and after inhalation exposure, except during the mating period. Animals were fed Wayne Lab-Blox or Wayne rabbit diet, respectively, and water *ad libitum*. Animals did not have access to food or water during exposure. Food consumption was measured at intervals of 3 days or less during the experimental periods. To enable a twice-weekly weighing of animals in the three rat pregestational exposure groups during a 5-day period, it was necessary to stagger the weighing dates. Weights for groups that received their first weekly weighing on Monday, Tuesday and Wednesday, respectively, were designated A (Fig. 3). The second weekly weights obtained on Wednesday, Thursday and Friday, respectively, were designated B. This alternation continued on subsequent weeks. Rabbits were weighed before their initial exposure, prior to the daily exposures on 1, 6, 10, 14, 18, 22, 26 dg, and prior to killing at 30 dg. Rats were weighed at least twice weekly prior to initiating exposures and during the pregestational period. During the gestational exposure period, rats were weighed prior to the daily exposures on 1, 5, 9, 13 or 17 dg and before sacrifice at 21 dg. The survival, appearance and behavior of the females during the experimental period was noted. Humidity was not controlled, but there were no variations in diet, light cycle, etc., or use of pesticides, medications or other extraneous chemicals during the experimental period.

Following 3 to 4 weeks of quarantine and acclimatization, females were randomly divided by body weight into exposure groups. They were permanently and uniquely identified by tattooing the ear of rabbits or by attaching a consecutively numbered ear tag to rats. The rats were also identified as to pregestational exposure groups by subcutaneously injecting India ink in the forepaw: air, no mark; low, left forepaw; high, right forepaw. A similar marking of the hind paw was done after randomized assignment to gestational exposure groups.

Exposure System

The rationale for selection of the EGEE exposure concentrations was as follows: The low level was chosen to approximate the current federal (OSHA) Permissable Exposure Limit of 200 ppm (*5*). The high level was chosen on the basis of published toxicity reports following acute exposure (*6–10*). The concentration of 750 ppm was expected to produce minimal signs of toxicity in adult rats and rabbits.

Animals were exposed in individual stainless steel Battelle-designed chambers (U.S. Pat. 4,216,741; fabricated by Hazelton Systems, Inc., Aberdeen, MD) of approximately 2 m^3 ($\geq$ 2300 L) volume under dynamic air flow conditions (570 L/min). Details of the design and operation of these exposure chambers have been published previously (*11–13*). The volume occupied by the animals was approximately 90 L for 30 rabbits or 43 L for 144 rats per chamber. At least three chambers were employed per species. Animals were transferred to sanitized chambers and cage units at least once a week.

Chamber atmospheres were generated by metering liquid EGEE at controlled rates into heated (81C) stainless steel vaporization chambers. The resulting vapor mixed with the HEPA-filtered air stream being drawn into the exposure chamber. The concentration of EGEE in the chambers was analyzed daily during exposure using a gas chromatograph (Hewlett-Packard, Model 5840A) with a multiplexed sample valve capable of sampling from eight separate areas: each exposure chamber, the control chamber, the exposure suite and a chemical standard. Cycle time for all eight ports was approximately 32 min. Time-weighted mean analytical concentrations of EGEE in the chambers were determined. Chamber concentration calibrations were performed regularly using external standardization ("grab samples") as well as an internal standard device. Additional details of the exposure conditions and analytical procedures have been described by Andrew et al. (*14*).

Experimental Design

Three groups of rabbits were inseminated and exposed 7 hr/day from 1 dg through 18 dg to air, 160 ppm or 617 ppm EGEE (Table 1). Virgin rats were exposed 7 hr/day, 5 days/week for 3 weeks (pregestational

Table 1. Experimental design.

Animal	
New Zealand White rabbits	
Inseminated (artificially) mid afternoon	0 dg
Exposed to air, "Low" or "High" level for 7 hr/day	1–18 dg
Weighed at	1, 6, 10, 14, 18, 22, 26, 30 dg
Food consumption measured at 2 or 3 day intervals	0–29 dg
Sacrificed:	30 dg
Maternal organs weighed and examined for gross and histopathological changes; reproductive status determined; uterine contents examined for fetal mortality, growth retardation, or terata	
Wistar rats	
Exposed to air, "Low" or "High" level 7 hr/day, 5 days/wk	3 wks
Weighed at 3 or 4 day intervals	3 wks
Food consumption measured at 2 or 3 day intervals	3 wks
Bred (day sperm detected = 1 dg) for 9 consecutive nights	
Reexposed: Air to air, "Low" or "High" (A-A, A-L, or A-H)	1–19 dg
"Low" to air or "Low" (L-A or L-L)	1–19 dg
"High" to air or "High" (H-A or H-H)	1–19 dg
Weighed at	1, 5, 9, 13, 17, 21 dg
Food consumption measured at 2 or 3 day intervals	0–20 dg
Sacrificed:	
Maternal organs weighed and examined for gross and histopathological changes; reproductive status determined; uterine contents examined for fetal mortality, growth retardation, or terata	

exposure). They were then mated and exposed 7 hr/day from 1 dg through 19 dg (gestational exposure) (Table 1). Group size ranged from 29 to 38 at the initiation of exposure. For convenience, the exposure groups were denoted as "low level" and "high level" and the group exposed to filtered air as "air" (Table 2). Based upon the combination of pregestational and gestational exposures, one control and six experimental rat groups were formed. The rat groups are identified by the pregestational and gestational exposure in the presentation of results as follows.

Air-Air (A-A) (Control). 3-week pregestational exposure to filtered air followed by exposure to filtered air during days 1–19 of gestation.

Air-Low (A-L). 3-week pregestational exposure to filtered air followed by 202 ppm EGEE exposure during days 1–19 of gestation.

Air-High (A-H). 3-week pregestational exposure to filtered air followed by 767 ppm EGEE exposure during days 1–19 of gestation.

Low-Air (L-A). 3-week pregestational 150 ppm EGEE exposure followed by exposure to filtered air during days 1–19 of gestation.

Low-Low (L-L). 3-week pregestational 150 ppm exposure followed by 202 ppm EGEE exposure during days 1–19 of gestation.

High-Air (H-A). 3-week pregestational 649 ppm EGEE exposure followed by exposure to filtered air during days 1–19 of gestation.

High-High (H-H). 3-week pregestational 649 ppm exposure followed by 767 ppm EGEE exposure during days 1–19 of gestation.

Table 2. Exposure chamber concentrations of ethylene glycol monoethyl ether (EGEE).

Exposure group	Average daily concentrations, ppm ± SD[a]	
	Low EGEE	High EGEE
Rabbits (gestational)	160 ± 31	617 ± 49
Rats (pregestational)	150 ± 18	649 ± 50
Rats (gestational)	202 ± 11	767 ± 22

[a]The daily measurements contributing to each entry ranged from 9 to 19.

Animal Sacrifice and Examination Procedures

On 30 dg (rabbits) or 21 dg (rats), the animals were killed with carbon dioxide in a random order. The gravid uterus was removed and the maternal viscera were examined for evidence of gross pathology. The uterus was opened and contents examined for number and position of live fetuses, dead fetuses, and resorption sites. Each live fetus was examined grossly for any abnormality and weighed. Heads of half the fetuses were removed and placed in Bouin's fixative for subsequent examination by the freehand sectioning technique of Wilson (*15*). All rabbit fetuses and one-half of all rat fetuses (selected randomly) were examined under a dissecting microscope for internal anomalies using the fresh tissue dissection method of Staples (*16*). Fetal sex was determined by internal examination of the genitalia.

All fetal carcasses were eviscerated and placed in ethyl alcohol and subsequently processed and stained with Alizarin Red S for skeletal observations (*17*). All unusual observations were recorded. Once all fetal examinations were completed, the results were tabulated according to a predetermined classification of defects, namely: major malformations, minor anomalies, or common variants and/or deformations. These categories provided a standardized classification for relative severity of dysmorphology and/or dysgenesis. Results were compared with the background incidence published for these species and with the appropriate historical controls for this laboratory (*19–23*).

Histopathology

Necropsies were performed on all adult animals; liver, lung, spleen and kidneys were weighed. Internal abnormalities of the pregnant and nonpregnant animals were also recorded, e.g., adhesions, tumors or evidence of infection. Samples of the ovaries, uterus, liver, kidneys and lungs with trachea were preserved in 10% neutral buffered formalin. Standard paraffin sections were prepared and stained with hematoxylin and eosin prior to examination. Histopathological examinations were performed on tissues from 25% (approximately seven per group) of the pregnant animals, selected at random. The residual tissues and the tissues from the remaining 75% of the animals and from the nonpregnant animals were preserved for possible future examination.

Statistical Evaluation

The litter was considered as the basic experimental unit for statistical analyses (*24*). Comparisons of binary response variables among groups were done by chi-square test for independence or Fisher's exact probability test (*25*). Means represent the average of litter means. Analysis of variance (ANOVA) was the test of choice for continuous variables when several means were to be compared. Response proportions were analyzed by the analysis of variance technique with an arcsin transformation (θ) of the response proportion, p ($\theta = 2 \arcsin \sqrt{p}$) (*26*). If results of analysis of variance showed a significant treatment effect, then Duncan's multiple range test was used to make multiple comparisons among group means to investigate the possibility of a dose-response relationship. One way to express the results of Duncan's multiple range test is to divide the exposure groups into homogeneous subsets (indicated by superscripts in the tables and figures) which do not differ from each other with means ordered according to increasing size. Statistical computer package "Statistical Package for the Social Sciences (SPSS)" was used for most of the analyses. Hypotheses were tested at the 0.05 level of significance.

Results and Discussion

Exposure Atmosphere

Average daily exposure chamber concentrations of EGEE, obtained over the course of the various exposure periods, are indicated in Table 1. Once animal exposures were initiated, difficulties were experienced in obtaining the anticipated chamber concentrations of EGEE (200 and 750 ppm). The measured values with animals in the chambers were about 15 to 25% lower than the prescribed chamber concentrations obtained from empty chambers (Table 2). The causes were unclear and various corrective measures were taken. The differences in chamber concentrations likely represent loss of EGEE into or onto the animals, into moisture within the chamber, and, to some extent, onto all surfaces within the chamber as reported by Werner et al. (*27*) regarding their exposure of dogs to EGEE. Their experience and ours suggests that under the exposure and analytical conditions used, the concentration data obtained from GC monitoring probably represent the best measure of the concentrations to which the animals were exposed.

Maternal Observations

Rabbits. Food consumption (Fig. 1) differed dramatically among the three rabbit groups during the

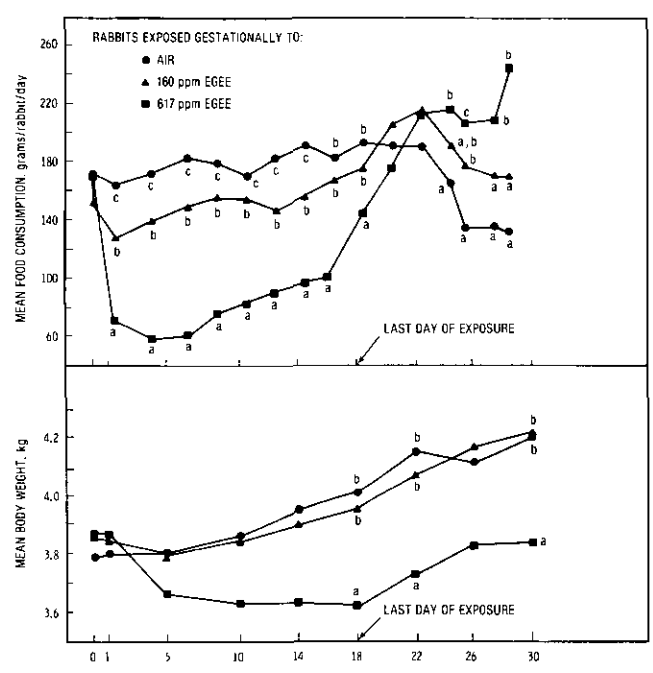

FIGURE 1. Summary of food consumption data and body weights for rabbits exposed to ethylene glycol monoethyl ether (EGEE) or air during gestation. Average daily exposure concentrations of EGEE are indicated. Groups with different superscripts differ significantly ($p < 0.05$) from each other by Duncan's.

exposure (1–18 dg) and post-exposure periods (19–30 dg). Both the 160 and 617 ppm EGEE-exposed groups ate significantly less than the air group throughout most of the exposure period. The 617 ppm EGEE group ate significantly more food than the air control group during the post-exposure period. Weight gain of pregnant does in the 617 ppm EGEE-exposed group differed dramatically (Fig. 1), presumably reflecting altered food intake and interruption of pregnancy. Subchronic inhalation exposure of nonpregnant rabbits at 400 ppm (6 hr/day, 5 days/week for 13 weeks) has been reported to cause decreased body weight in rabbits, but not in rats (*28*).

There was mortality in the 617 ppm EGEE-exposed rabbits. Four does in that group died during exposure between 10 and 13 dg, and one was found dead at 20 dg, 2 days after termination of exposure. Two air-exposed rabbits and the one in the 160 ppm EGEE-exposed group died apparently due to pneumonia. Based on the clinical history and gross necropsy findings, the deaths in the 617 ppm EGEE-exposed group were presumed to be caused primarily by the effects of the chemical on appetite and gastrointestinal function. It was not determined if this effect was direct or indirect. Ingestion of EGEE is reported to produce mild irritation of the gastrointestinal tract of several species (*6*), while in an *in vitro* preparation of rabbit intestine the muscle was "depressed" by EGEE (*29*). None of the five rabbits in the 617 ppm EGEE exposure group had grossly apparent pneumonia.

Mean relative doe liver weights (percent of body weight) increased in the order of air, 160 ppm and 617 ppm EGEE and each group differed significantly from the others. The relative kidney weight of only the 617 ppm EGEE exposure group was significantly increased compared with control. Mean weights of maternal lungs and spleens were unremarkable. Since these animals were pregnant, both the resulting endocrine milieu and/or the depressed food consumption and resulting wasting may have contributed to these organ weight changes.

Microscopic examination of rabbit liver and kidney sections showed no evidence of treatment-related changes. Uterine sections had slight to moderate infiltration of lymphocytes and/or heterophyls in the endometrium. This tended to be more prominent in the 617 ppm EGEE-exposed animals. In addition, most changes were present in all the 617 ppm EGEE-exposed rabbits and none of the control or 160 ppm EGEE-exposed rabbits examined (*14*). When compared to the controls, corpora lutea of 617 ppm EGEE-exposed does subjectively appeared smaller when viewed under a dissecting microscope.

Based upon the percent of does pregnant at sacrifice (Table 3), there was no evidence that daily exposure to EGEE from 1 dg to 18 dg altered rabbit fertility. If the data for the animals that died during the study were included, then the conception rates were 97, 86 and 92% for the air, 160 and 617 ppm EGEE-exposed groups, respectively.

Rats. Statistically significant differences in food consumption were seen only in the first two days of the pregestational exposure to 649 ppm EGEE (Fig. 2). In marked contrast to the food intake patterns of rabbits exposed to EGEE (Fig. 1), there was no effect on food consumption even in the rats exposed to 767 ppm EGEE during gestation (Fig. 2).

The body weights of the EGEE-exposed rats increased normally during the pregestational period (Fig. 3). Body weights of the two EGEE-exposed groups were significantly greater than that of the filtered air

Table 3. Reproductive status of rabbits exposed to EGEE or air.

Observations	Exposure concentrations of EGEE, ppm: Air (0)	Low (160 ppm)	High (617 ppm)
Percent pregnant	83	79	76
Number of litters examined	24	23	22
Mean corpora lutea per doe ± SD	11 ± 2	11 ± 2	10 ± 2
Mean implants per doe ± SD	9 ± 2	9 ± 2	8 ± 4
Total fetuses (live)	214	167*	0*
Mean live fetuses per litter ± SD†	9 ± 3[a]	7 ± 2[b]	0[c]
Mean resorptions per litter ± SD†	0.3 ± 0.6[a]	2 ± 1[b]	8 ± 4[c]
Number of litters w/resorptions	6	17*	22*

*$p \leq 0.05$ by chi-square compared with air group.
†Significant differences ($p < 0.05$) between groups by ANOVA. Groups with different superscripts (a,b,c) differ significantly ($p < 0.05$) from each other by Duncan's.

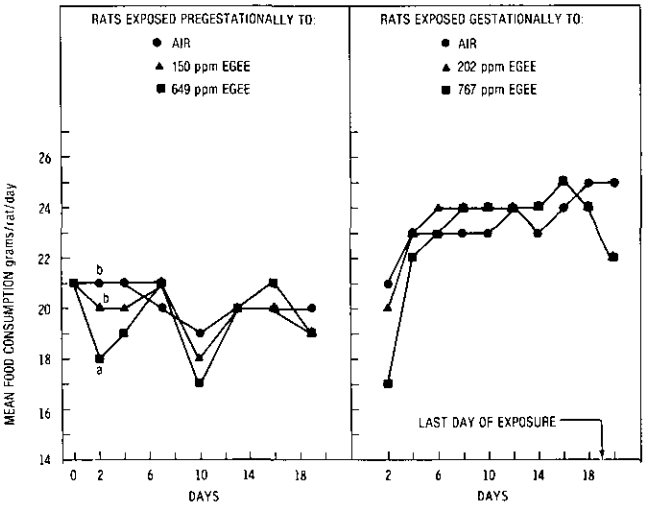

FIGURE 2. Summary of food consumption data for rats exposed to ethylene glycol monoethyl ether (EGEE) or air during pregestation and gestation. Average daily pregestational–gestational exposure concentrations of EGEE are indicated. Groups with different superscripts differ significantly ($p < 0.05$) from each other by Duncan's.

group at some weighing intervals (weeks 1B and 2B). However, the starting and final weights of the three groups did not differ significantly in the pregestational period. Thus, 3 weeks of exposure of nonpregnant rats to EGEE at up to 649 ppm did not appear to appreciably alter food consumption or growth. However, maternal body weights of rats exposed to 767 ppm EGEE during gestation were significantly reduced at 17 and 21 dg (Fig. 3). Body weight changes in rats generally reflected their reproductive status.

Mean relative dam liver weights (percent of body weight) of one group of rats exposed to 767 ppm EGEE gestationally (A-H) was significantly lower than those of the groups exposed to air, whereas one exposed to 202 ppm EGEE (A-L) was significantly greater than control. Mean relative weights of lungs and kidneys for the rats

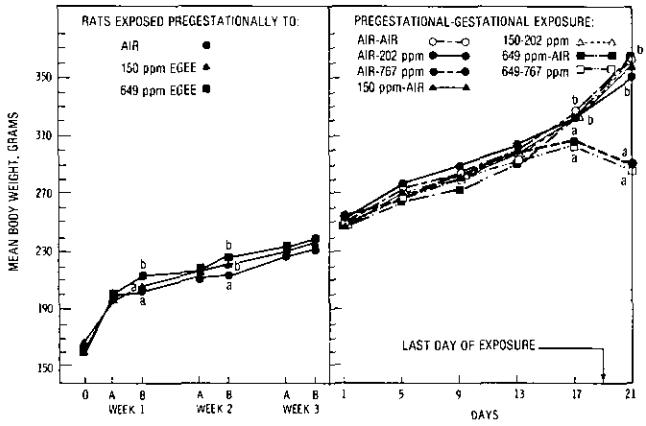

FIGURE 3. Summary of body weights for rats exposed to EGEE or air as noted for Fig. 2. Body weights on first three days of week designated as A and those on second 3 days of week designated as B. Groups with different superscripts differ significantly ($p < 0.05$) from each other by Duncan's.

exposed to 767 ppm EGEE during gestation (AH,HH) were significantly greater than the other groups. The relative weights of the spleens in the A-L, A-H and H-H groups were significantly greater than the A-A group. Since these animals were pregnant and since gestation may alter organ weights (*30*), these observations may not be biologically meaningful.

Microscopic examination of rat lung, liver, and kidney sections did not reveal any changes attributable to the exposure regimen. Uterine involution occurred in 15 of 16 rats examined following exposure to 767 ppm EGEE during gestation. These changes were not evident in the rest of the groups. The ovaries of the rats that received exposure to 767 ppm EGEE during gestation had significant changes indicative of corpora lutea regression. The changes were relatively smaller corpora lutea size, small luteal cells, and decreased uniformity of cells in the corpora lutea. The changes did not seem as pronounced as in the rabbits, as only 9 of 16 rats examined after gestational exposure to 767 ppm EGEE seemed to have evidence of corpora lutea regression.

Rats were mated for ten consecutive nights to obtain an adequate number of sperm-positive animals (Table 4). The EGEE-exposed females mated well; the percent sperm-positive for the 10 days of breeding was 68, 69, and 65% for the air, 150 and 649 ppm EGEE-exposed groups, respectively. The percent of sperm-positive rats that were pregnant was exceptionally high for the EGEE-exposed groups (about 95%), while only 82% of the controls were pregnant. In addition, when the percent of sperm-positive females pregnant at 21 dg was evaluated for each of the gestational exposure groups, only one group (A-L) had less than 84% pregnant (Table 4). Thus, these results are similar to those obtained in rabbits; exposure to EGEE did not appear to alter mating behavior, breeding performance, or fertility.

Table 4. Reproductive status of rats exposed to EGEE or air.

Observations	Pregestational-gestational exposure concentrations of EGEE, ppm						
	Air-Air (0–0)	Air-Low (0–202)	Air-High (0–767)	Low-Air (150–0)	Low-Low (150–202)	High-Air (649–0)	High-High (699–767)
Percent pregnant	87	76	84	97	92	97	94
Number litters examined	32	28	31	37	34	34	33
Mean corpora lutea per dam ± SD	17 ± 4	18 ± 5	16 ± 3	16 ± 3	16 ± 4	17 ± 4	16 ± 3
Mean implants per dam ± SD	13 ± 3	13 ± 3	12 ± 4	12 ± 3	13 ± 3	13 ± 3	13 ± 3
Total fetuses (live)	387	324	0*	444	402	418	0*
Mean live fetuses per litter ± SD†	12 ± 3[a,b]	12 ± 3[c]	0 ± 0[d]	12 ± 3[a]	12 ± 3[a,b,c]	12 ± 4[b,c]	0 ± 0[d]
Mean resorptions per litter ± SD†	0.5 ± 0.7[a]	1.0 ± 1.1[a]	12.0 ± 2.9[b]	0.4 ± 0.8[a]	0.9 ± 1.1[a]	0.8 ± 1.4[a]	12.5 ± 3.0[b]
Number litters w/resorptions	14	18	31*	10	18	17	33*

*$p \leq 0.05$ by chi-square compared with air-air group.

†Significant differences ($p < 0.05$) between groups by ANOVA. Groups with different superscripts (a,b,c) differ significantly ($p < 0.05$) from each other by Duncan's.

Fetal Observations

Rabbits. The most striking evidence of embryotoxicity is that the uteri of all pregnant does in the 617 ppm EGEE exposed group contained only early resorptions (Table 3). This 100% incidence of embryomortality was significantly different from the 160 ppm EGEE-exposed group. Therefore, the measures of reproductive status that relate to this parameter are also significant including: the 4-fold increase in number of litters with resorptions, a 4-fold increase in percent of litters with resorptions, and an 8-fold increase in resorptions per litters with resorptions. The number of implants per litter was not reduced, even though exposures were initiated at 1 dg.

Statistically significant effects were also evident among the same measures of reproductive status for the 160 ppm EGEE-exposed group (Table 3). Mean number of resorptions per litter was about six times that of the controls. The number of litters with resorptions and the percent of litters with resorptions was about three times that of the controls. The mean number of resorptions per litter with resorptions was about twice that of the air group.

Table 5. Fetal measures from rabbit litters exposed *in utero* to EGEE or air.

Observations	Exposure concentrations of EGEE, ppm		
	Air (0)	Low (160 ppm)	High (617 ppm)[a]
Number of litters	24	23	—
Mean body weight, g	47 ± 7	44 ± 11	—
Mean crown-rump length ± SD, mm	103 ± 5	101 ± 4	—
Mean placenta weight ± SD, g	5 ± 2	6 ± 1	—
Mean sex ratio (% males) ± SD	51 ± 14	59 ± 23	—

[a]No surviving fetuses.

Table 6. Summary of terata in fetal rabbits exposed *in utero* to EGEE or air.

Tissues and observations	Exposure concentration of EGEE, ppm		
	Air (0)	Low (160 ppm)	High (617 ppm)[b]
Total no. of litters	24	23	—
Total no. of fetuses	214	167	—
Major malformations[a]			
Craniofacial defect	0	1/1	
(acrania)	0	(4.4)	—
Ventral wall defects	0	4/4*	—
		(17.4)	—
Fused aorta and	0	5/5*	—
pulmonary artery		(21.7)	—
Spina bifida	0	1/1	—
		(4.4)	—
Diaphragmatic hernia	0	1/1	—
		(4.4)	—
Minor anomalies[a]			
Rib dysmorphology	4/4	5/5	—
	(16.7)	(21.7)	—
Renal changes	0	5/5*	—
		(21.7)	—
Forelimb flexure	0	3/2	—
		(8.7)	—
Cardiac dysmorphology	0	1/1	—
		(4.4)	—
Scoliosis	0	1/1	—
		(4.4)	—
Brachyury	0	1/1	—
		(4.4)	—
Common variations[a]			
Supernumerary ribs	155/23	164/23	—
(extra and rudimentary)	(95.8)	(100)	—
Extra ribs (only)	0	21/11*	—
		(47.8)	—
Vertebral variations	76/13	160/23*	—
		(100)	—
Altered fontanelle	0	2/2	—
		(8.7)	—
Sternebral variations	2/2	16/9*	—
	(8.3)	(39.1)	—
Ossification defects	4/3	3/3	—
	(12.5)	(13.0)	—

[a]Results are expressed as number of fetuses affected/number of litters affected; % litters affected in parentheses.
[b]No surviving fetuses.
*$p \leq 0.05$ by Fisher's exact compared with air group.

No statistically significant effects were observed on fetal size (weight or length), placenta weights, or sex ratios when the 160 ppm EGEE-exposed group was compared to filtered air controls (Table 5). Thus, there is no evidence of severe intrauterine growth retardation in these surviving fetuses.

Significant increases in the incidence of major malformations (ventral wall defects and fusion of aorta with pulmonary artery), minor anomalies (renal changes), and common skeletal variants (supernumerary ribs with associated vertebral variations and external defects) were seen in the 160 ppm EGEE-exposed group compared to the air controls (Table 6). Teratogenic effects have also been reported following similar exposure of Dutch Belted rabbits to 175 ppm (*31*).

Several of the terata observed (Table 6) were unusual and are briefly described herewith. There was one case of a "split" peritoneum in which the peritoneum had failed to close over two-thirds of the abdomen. The skin was intact, and there was no protrusion of viscera (hernia). The cardiac dismorphology seen was a rounded, or bulbous, heart without any other unusual characteristics. One case of "scrambled" sternebrae was seen in which most of the right and left portions of the sternum were displaced asymmetrically along the midline.

The problems associated with interpreting the biological significance of these and similar observations (Table 6 and 8) have been reviewed (*32,33*). In general, a dose-related increase in the incidence of any aberration was important, regardless of its classification.

Rats. A significant embryolethal effect (the induction of 100% resorptions) was observed to be associated with the 767 ppm gestationally exposed EGEE exposure (Table 4). This result resembles that noted above for rabbits and Nelson et al (*34*) have similarly reported concentration-related increases in embryomortality in rats exposed to 200 to 900 ppm EGEE on 7 to 13 and 14 to 20 dg. The mean numbers of resorptions per litter in the group gestationally exposed to 767 ppm EGEE was 25 times that of the air controls. Resorptions per litter in the group gestationally exposed to 202 ppm EGEE was about twice the control value. The effects of 765 ppm EGEE exposure on percent of litters with resorptions were obvious. In contrast to the rabbits exposed to EGEE, the categories related to resorptions in the 202 ppm EGEE-exposed groups, although higher, were not significantly different from the controls. The marked embryomortality induced in rats by exposure to high EGEE was surprising in the absence of any adverse effects on either food consumption or pregestational weight gain—a striking contrast to the rabbits.

Fetal body size was significantly reduced at sacrifice for the two groups gestationally exposed to 202 ppm EGEE (Table 7). There was a 16 to 21% reduction in body weight, and crown-rump length was reduced by 6 to 8%. Thus, exposure to EGEE at 200 ppm throughout gestation induced intrauterine growth retardation. There were no differences in placental weight or sex ratios among the various groups.

A significantly increased incidence of cardiovascular defects (transposed and retrotracheal pulmonary artery) was observed in the A-L, but not in the L-L group fetal rats. Although the specific defects were different, EGEE-exposed rabbit fetuses also had a significant incidence of cardiovascular malformations (fused aorta and pulmonary artery). Minor skeletal defects were the predominant effects observed in the 202 ppm EGEE-exposed rats, with a significant increase of both minor skeletal anomalies and common skeletal variants in rats.

The extensive observations of reduced skeletal ossification are consistent with fetal growth retardation (*35–37*). The minor skeletal anomalies consisted of various categories of rib dysmorphology, especially "knobby ribs" (Table 8). The increased incidence of common skeletal variants consisted of either supernumerary ribs and vertebrae, or reduced skeletal ossification. The incidence of extra and rudimentary ribs, as well as associated thoracic vertebrae, was significantly increased in the two 202 ppm EGEE-exposed groups compared to the three air groups, based on gestational exposures. Most of the individual skeletal variants listed in Table 8 were also significantly increased in the EGEE-exposed but not in the air-

Table 7. Fetal measures from rat litters exposed *in utero* to EGEE or air.

Observations	Pregestational-gestational exposure concentrations of EGEE, ppm						
	Air-Air (0–0)	Air-Low (0–202)	Air-High* (0–767)	Low-Air (150–0)	Low-Low (150–202)	High-Air (649–0)	High-High* (649–767)
Mean body weight ± SD, g†	3.8 ± 0.3[a]	3.0 ± 0.4[c]	—	3.9 ± 0.3[a]	3.2 ± 0.3[b]	3.9 ± 0.3[a]	—
Mean crown-rump length ± SD, mm†	36 ± 2[a]	33 ± 4[b]	—	37 ± 2[a]	34 ± 2[b]	37 ± 2[a]	—
Mean placenta weight ± SD, g	0.6 ± 0.1	0.7 ± 0.2	—	0.6 ± 0.1	0.7 ± 0.2	0.7 ± 0.1	—
Mean sex ratio (% males) ± SD	47 ± 16	44 ± 14	—	54 ± 15	47 ± 17	48 ± 12	—

*No surviving fetuses.

†Significant differences ($p < 0.05$) between groups by ANOVA. Groups with different superscripts (a,b,c) differ significantly ($p < 0.05$) from each other by Duncan's.

Table 8. Summary of terata in fetal rats exposed *in utero* to EGEE or air.

Observations	Pregestational-gestational exposure concentrations of EGEE, ppm						
	Air-Air (0–0)	Air-Low (0–202)	Air-high[b] (0–767)	Low-Air (150–0)	Low-Low (150–202)	High-Air (649–0)	High-High[b] (649–767)
Total no. of litters examined	32	28	—	37	34	33	—
Total no. of fetuses examined	386	324	—	441	397	420	—
Major malformations[a]							
Craniofacial defects	2/2 (6.3)	0	—	0	0	0	—
Minor anomalies[a]							
Rib dysmorphology	0	13/6* (21.4)	— —	1/1 (2.7)	6/6* (17.6)	0	—
Cardiovascular defects	0	6/4* (14.3)	— —	0	2/2 (5.9)	0	—
Polydactyly	0	0	—	0	1/1 (2.9)	0	—
Common variations[a]							
Supernumerary ribs	49/15 (46.9)	84/21* (75.0)	— —	30/16 (43.2)	110/24* (70.6)	41/15 (45.5)	— —
Reduced ossification	32/17 (53.1)	225/26* (92.9)	— —	17/11 (29.7)	275/33* (97.1)	22/13 (39.4)	— —
Sternebral variations	0	0	—	1/1 (2.7)	1/1 (2.9)	0	—
General edema	1/1 (3.1)	1/1 (3.6)	— —	0	0	0	—

[a]Results are expressed as number of fetuses affected/number of litters affected; % litters affected are in parentheses.
[b]No surviving fetuses.
*$p < 0.05$ by Fisher's exact compared with air-air group.

Table 9. Summary of significant effects of EGEE exposure.

Species	Observations	Exposure group	Direction of effect
Rabbit	Maternal food consumption	Low and High	↓
Rabbit	Maternal body weights	High	↓
Rabbit	Maternal mortality	High	↑
Rabbit	Maternal liver weight[a]	Low and High	↑
Rabbit	Maternal kidney weight[a]	High	↑
Rabbit	Histopathology of ovaries and uterus	High	↑
Rabbit	Incidence of resorptions/litter	Low and High	↑
Rabbit	Incidence of live fetuses/litter	Low and High	↓
Rabbit	Terata	Low	↑
Rat	Gestational body weight	A-H and H-H	↓
Rat	Maternal lung weight[a]	A-H and H-H	↑
Rat	Maternal liver weight[a]	A-L/A-H	↑/↓
Rat	Maternal kidney weight[a]	A-L, A-H and H-H	↑
Rat	Maternal spleen weight[a]	A-L, A-H and H-H	↑
Rat	Histopathology of ovaries and uterus	A-H and H-H	↑
Rat	Incidence of resorptions/litter	A-H and H-H	↑
Rat	Incidence of live fetuses/litter	A-H and H-H	↓
Rat	Fetal weight	A-L and L-L	↓
Rat	Fetal crown-rump length	A-L and L-L	↓
Rat	Terata	A-L and L-L	↑

[a]Relative weight, percent of body weight.

exposed groups. Subsequently, both cardiovascular malformations and skeletal variations were significantly increased in rat litters whose dams were exposed topically to EGEE (*38*). In contrast, inhalation exposure of rats at 50 and 250 ppm produced fetotoxicity without teratogenicity (*31*).

In summary, exposure of pregnant rabbits to EGEE resulted in conclusive evidence of embryotoxicity and teratogenicity in addition to maternal toxicity; the biologically and statistically significant findings are summarized in Table 9. Adverse maternal effects included a concentration-related depression in food consumption and consistent loss of body weight during exposure (Fig. 1). These effects may have contributed

to an increased incidence of maternal mortality at the 617 ppm EGEE exposure. Liver and kidney weights were elevated in the survivors. The most striking embryotoxic effect was the resorption incidence of 100% in all pregnant does surviving the 617 ppm EGEE exposure. The histopathological changes in the ovaries and uteri of 617 ppm EGEE-exposed does were consistent with the early interruption of pregnancy. A significantly increased incidence of embryomortality accompanied by a corresponding decrease in live fetuses per litter was seen even in those exposed at 160 ppm EGEE. Surprisingly, however, growth retardation was not seen in the surviving rabbit fetuses. Morphological changes detected included major malformations, minor anomalies, and variations of the soft tissue and skeleton of the rabbit fetuses.

A similar spectrum of statistically significant effects was seen in pregnant rats exposed to EGEE (Table 9). Alterations in maternal organ weights were not considered to be presumptive indications of toxicity because of the gestational influence and inconsistent changes. Food consumption was not affected, but body weight was reduced in those rats exposed gestationally to 767 ppm EGEE (Figs. 2 and 3). However, this effect probably reflected the total incidence of interrupted pregnancy at this level and was probably not a true measure of maternal toxicity. Increased embryomortality occurred only at 767 ppm EGEE in the rats, in contrast to the concentration-related incidence in rabbits. Again, histological examination of ovaries and uteri revealed evidence of early interruption of pregnancy in those groups exposed at 767 ppm EGEE during gestation with high incidences of resorptions. There was growth retardation of the live rat pups from the 202 ppm EGEE exposure group since weight, length and skeletal ossification were reduced. Growth retardation was not seen in the surviving rabbit pups. It is particularly noteworthy that an increased incidence of terata was seen in rats at the 202 ppm EGEE gestational exposures which did not induce maternal toxicity. The incidence of major malformations was not significantly elevated, but the incidence of minor anomalies and variations was significantly elevated. These involved both soft tissue and skeletons.

Conclusion

Thus, taken together these data implicate EGEE as being a teratogen in both rabbits and rats. Significant incidences of terata, intrauterine growth retardation, and embryomortality were induced at levels that were below or were similar to those that induce maternal manifestations of toxicity. Based upon these bioassay data and those from other laboratories, EGEE has exhibited sufficient evidence of teratogenicity and embryotoxicity that it should be regarded as presenting similar risks to humans.

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