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Prenatal Ethylene Glycol Monomethyl Ether (EGME) Exposure Produces Electrocardiographic Changes in the Rat[1]

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Prenatal Ethylene Glycol Monomethyl Ether (EGME) Exposure Produces Electrocardiographic Changes in the Rat. TORAASON, M., AND BREITENSTEIN, M. (1988). *Toxicol. Appl. Pharmacol.* **95**, 321–327. The purpose of the present study was to determine if electrocardiographic (EKG) changes observed in fetuses exposed *in utero* to ethylene glycol monomethyl ether (EGME) persisted beyond the fetal/neonatal period. Groups of pregnant Sprague–Dawley rats were gavaged on gestation days 7–13 (sperm = day 0) with 0, 50, or 75 mg/kg EGME. Body weight prior to delivery was reduced and gestation was prolonged in EGME-treated dams. EGME treatment reduced the percentage of pregnant dams that delivered, litter size, and pup weight. There were no survivors beyond 3 days of age in the 75 mg/kg EGME group. The number of litters surviving through weaning and weight gain of male and female offspring through 8 weeks of age were reduced in the 50 mg/kg EGME group. In this same group, heart weight was unaffected, but heart/body weight ratios were increased when rats were 8 weeks old. EKGs were obtained from unanesthetized and unrestrained rats at 3 and 6 weeks of age. Prenatal EGME exposure increased the QRS interval in 3- and 6-week-old rats, and increased the T wave in 6-week-old rats. Thirty-six and 54% of 3- and 6-week old litters, respectively, had one or more individuals that were classified as having an intraventricular conduction delay (double R wave and QRS interval of 14 msec or longer). No microscopic heart abnormalities were associated with the observed intraventricular conduction delay.

Ethylene glycol monomethyl ether (EGME), also known as 2-methoxyethanol, is used as a solvent in lacquers, enamels, varnishes, inks, and dyes and as an anti-icing additive in fuels and other fluids (NIOSH, 1983). EGME is a teratogen in rats and mice, and the developing cardiovascular system is particularly sensitive (Hardin, 1983; Nagano *et al.*, 1981). At low EGME treatment levels, malformations of the cardiovascular system occur in rats in the virtual absence of other major visceral abnormalities (Toraason *et al.*, 1985, 1986c). In addition to physical malformations, cardiac functional alterations have also been detected following prenatal exposure to EGME (Toraason *et al.*, 1985, 1986a). We previously reported electrocardiographic (EKG) changes on gestation day 19 in rat fetuses exposed to EGME on gestation days 7–13 (Toraason *et al.*, 1985). The present investigation extends this work by evaluating EKGs of 3- and 6-week-old rats exposed *in utero* (days 7–13) to EGME. The purpose of the study was to determine if intraventricular conduction delays observed in fetuses persisted in survivors beyond the fetal/neonatal period.

MATERIALS AND METHODS

Maternal treatment. Female Sprague–Dawley rats [Crl:CDBR], time-mated by Charles River Breeding

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Labs (Wilmington, MA), were housed individually in wire-mesh cages and provided NIH-07 rat chow (Ziegler Bros., Garden, PA) and tap water *ad libitum*. Dams were maintained on a 12-hr light–12-hr dark photoperiod at 23 ± 2°C and 55 ± 15% relative humidity. On day 5 of gestation (sperm = day 0), dams were ear-tagged and divided randomly into control ($N = 25$), 50 ($N = 30$), or 75 ($N = 30$) mg/kg EGME treatment groups. Dams were weighed daily and treated by gavage with EGME in a constant dose volume of distilled water (10 ml/kg) on days 7–13 of gestation. EGME (CAS 109-86-4) was purchased (Cat. No. E182) from and certified (98% pure) by Fisher Scientific Co. (Fair Lawn, NJ).

EKG recording system. EKGs were obtained using a Grass model 79D EEG and Polygraph Data Recording System (Grass Instruments Co., Quincy, MA). The unit included a Wide Band ac EEG Preamplifier, Model 7P5 with Lead Selector, Model 7LSA. A Polygraph dc Driver Amplifier, Model 7DAF, was used to drive the pen oscillograph. Sensitivity of the preamplifier was 50 μV/cm. Low-frequency response limit was 10 Hz. The dc Driver Amplifier provided output of the EKG signal to two separate channels having different high-frequency response limits. These limits were set at 35 and 75 Hz. The analog output of the dc Driver Amplifier ranged from 0 to 2.8 V and was digitized using the Data Acquisition and Signal Analysis System (DASA) of Gould Electronics (Cleveland, OH) installed in an IBM-AT computer. The digitized signal that was obtained at a high-frequency response of 75 Hz was somewhat noisy and precise measurements were often difficult. The 35-Hz limit provided a signal that was free of noise, but lacked the definition provided by the 75-Hz limit. The two signals were used in tandem to measure P-R, R-R, and QRS intervals, and the T wave. Beats per minute (BPM) were calculated by dividing 60 sec by the R-R interval. QRS amplitude was obtained from the 75-Hz signal and recorded in volts. Pilot work indicated that a sampling rate of 2000 samples/sec would provide adequate resolution of the EKG signal. However, after taking EKGs on all the 3-week-old rats, it was determined that a sampling rate of 2500 samples/sec would provide maximum resolution. Subsequently, this sampling rate was used with 6-week-old rats. EKGs were recorded for 3 sec.

EKG evaluation. When all offspring were 3 and 6 weeks of age, EKGs were obtained by holding unanesthetized rats by the scruff of the neck, and inserting platinum needle electrodes (Grass Instruments Co., Quincy, MA) 1-2 mm at the base of the right forelimb, and left hindlimb, to obtain the standard lead II EKG. The right hindlimb served as a ground. Strip-chart recordings of EKGs were reviewed for rhythm variations and abnormal or missing peaks. Duration of the T wave, P-R, QRS and R-R intervals, and the amplitude of the QRS were determined from the digitized signals using DASA software.

Care and necropsy of offspring. Offspring were housed with their mothers until they were 25–27 days old. At this time litters were ear-tagged, split according to sex, and held in box cages with nonpyrogenic bedding for 2 more weeks. Offspring were then housed in pairs in wire-mesh cages for the remainder of the study. Body weights of offspring were recorded when they were 1, 3, 7–11, and 17–19 days old, and 6 and 8 weeks old. All offspring were killed by a lethal dose of CO_2 when they were 8 weeks old. Hearts were removed, trimmed of major vessels, washed, weighed, and fixed in Bouin's solution. Hearts were sectioned and stained with hematoxylin and eosin for histopathological evaluation. Microscopic evaluation was performed on hearts harvested from all EGME-exposed offspring and on an equal number of randomly selected control offspring by Dr. Jim E. Proctor (Experimental Pathology Laboratories, Inc., Ross, OH).

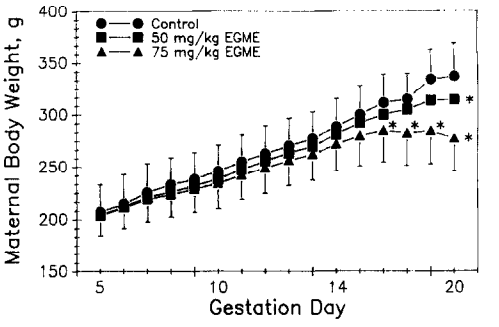

FIG. 1. The effect of EGME treatment on gestation days 7–13 on body weight of pregnant rats. Values are means ± SD. *Values are significantly different from control value, $p < 0.05$.

Statistical analysis. The litter was considered the statistical unit for variables obtained from offspring with the exception of postnatal survival where total numbers of offspring were compared. Maternal body weight, delivery day, and litter size were compared using ANOVA and Duncan's Multiple Range Test. EKG intervals and amplitudes, and heart and body weights were compared using the Mann–Whitney Rank Sum test. Number of pregnant dams that delivered, incidence of EKG aberrations, and the number of survivors from day 1 to day 3 and day 3 to 8 weeks were compared using Fisher's Exact test with Bonferroni correction for multiple comparisons.

RESULTS

Maternal Toxicity and Reproductive Outcome

Pregnant dams treated with EGME exhibited a small but significant reduction in body weight during the last few days of gestation (Fig. 1). The reduced body weight is likely

TABLE 1

REPRODUCTIVE OUTCOME IN DAMS TREATED WITH EGME

	EGME (mg/kg)		
	0	50	75
Pregnant/litters delivered	14/14	21/20	17/3*
Mean litter size Day 1	10 ± 2	6 ± 3*	2 ± 2*
Survival index			
Live offspring Day 3/Day 1	133/146	63/121*	0/7
Live offspring 8 weeks/Day 3	132/133	51/63	0/0

Note. Litter size is presented as mean ± SD. All other numbers are totals. Litters not delivered were resorbed.
* Significantly different than control, $p < 0.05$.

due to resorption of fetuses, since both EGME treatment groups produced fewer litters of smaller size compared to the control group (Table 1). Furthermore, there was no effect on body weight gain during or shortly after EGME treatment (days 7–13). Prolonged gestation in dams treated with EGME is shown in Fig. 2. The mean ± SD of the gestation period was 21.3 ± 0.6 days for the controls, 22.8 ± 0.6 days for the 50 mg/kg EGME group, and 22.3 ± 1 days for the 75 mg/kg EGME group. All dams that did not deliver by gestation day 26 were killed and examined for resorbed litters.

Growth and Survival of Offspring

EGME treatment had a dose-dependent effect on postnatal survival (Table 1). None of the offspring in the 75 mg/kg EGME group lived beyond 1 day of age, and nearly half the pups born alive in the 50 mg/kg EGME group did not live to 3 days of age. At birth and during the first 6 weeks of life there were no significant differences in body weight between the controls and offspring from dams treated with 50 mg/kg EGME (Fig. 3). At 8 weeks of age, however, both males and females treated with EGME weighed significantly less than controls.

Cardiac Effects

Heart weights were slightly, but not significantly, greater in EGME-treated rats than

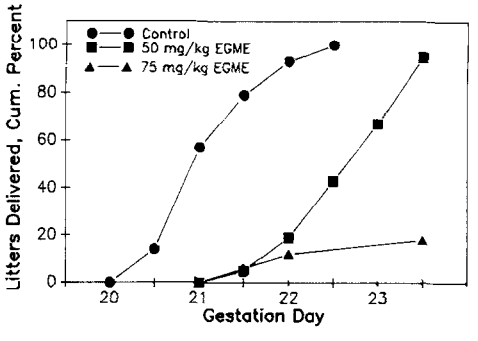

FIG. 2. The effect of EGME treatment on gestation days 7–13 on gestation length and cumulative percentage of dams that delivered. EGME treatment significantly ($p < 0.05$) prolonged gestation and reduced the percentage of pregnant dams that delivered.

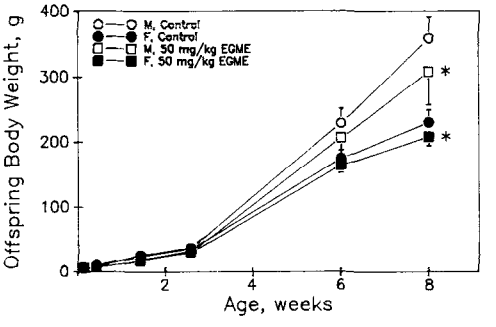

FIG. 3. The effect of EGME treatment on gestation days 7–13 on postnatal body weight in male and female rats. Values are means ± SD. *Values are significantly different from control value, $p < 0.05$.

TABLE 2

HEART AND BODY WEIGHTS OF 8-WEEK-OLD EGME-EXPOSED AND CONTROL RATS

	EGME (mg/kg)	
	0	50
Males/litters	67/13	23/11
Body wt. (g)	353 ± 31	308 ± 54*
Heart wt. (g)	1.3 ± 0.1	1.5 ± 0.4
Heart/body wt. ratio (g/kg)	3.8 ± 0.2	5.1 ± 2.0*
Females/litters	65/13	28/11
Body wt. (g)	231 ± 18	213 ± 16*
Heart wt. (g)	1.0 ± 0.1	1.1 ± 0.1
Heart/body wt. ratio (g/kg)	4.2 ± 0.3	5.0 ± 0.7*

Note. Weights are means ± SD.

* Significantly different than control, $p < 0.05$.

in controls (Table 2). However, heart/body weight ratios were significantly greater in EGME-exposed rats than in controls. No microscopic abnormalities that were associated exclusively with EGME-exposed rats were noted.

EKG Observations

Lead II EKGs from three 6-week-old rats are shown in Fig. 4. The analog EKG signal from each rat was digitized and drawn by a computer-driven printer-plotter. Commercially available software was used to measure duration and amplitude of waves and intervals. Figures 4A and 4B are from the same control rat. The 4A signal was recorded with maximum frequency response of 75 Hz, whereas, the maximum frequency response was 35 Hz for Fig. 4B. The waves were used in tandem to determine the initiation and termination of the intervals indicated. Examples of an EKG aberration that occurred exclusively in EGME-exposed rats are illustrated in Figs. 4C and 4D. Signal 4C is an example of a QRS that met the minimum requirements indicative of an intraventricular conduction delay—there is a small double R wave and the QRS duration is 14.0 msec or greater. Fourteen milliseconds was arbitrarily chosen because it is 3 standard deviations greater than the mean QRS interval obtained from the 6-week-old control rats. Signal 4D is an example of a much more dramatic delay with a QRS duration of 17 msec.

EKG observations from control and EGME-exposed rats are summarized in Table 3. Three-week-old rats were still with their mothers and not yet individually identified; therefore, the male and female EKG measurements recorded at this time were pooled. QRS intervals were significantly increased in both 3- and 6-week-old rats exposed to EGME. Duration of T waves were significantly increased in 6-week-old rats. The absence of intraventricular conduction delays in control rats prevented meaningful statistical comparison to EGME-exposed rats. How-

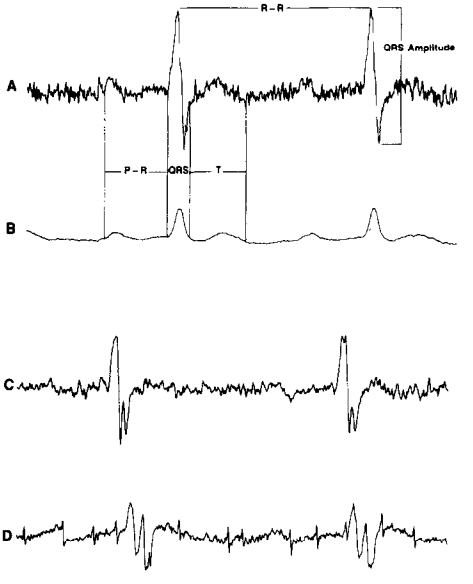

FIG. 4. Printer-plotter reconstruction of digitized EKG signals obtained from 6-week-old rats. (A) is a normal EKG obtained at maximum frequency response of 75 Hz and is somewhat noisy. (B) is the same signal recorded with the maximum frequency response limited to 35 Hz, and is clear but lacks detail. The two signals were used in tandem to identify the onset and completion of the waves and intervals noted. (C) and (D) are two examples of prolonged QRS intervals (14 and 17 msec, respectively) with a characteristic double R wave indicating an intraventricular conduction delay.

ever, 36 and 54% of 3- and 6-week-old litters, respectively, had one or more offspring that were classified as having an intraventricular conduction delay. QRS amplitude tended to be greater in EGME-exposed rats, but the increases were not significant. The only arrhythmia observed was an SA block (starting and stopping of atrial pacemaker), which occurred infrequently in both control and exposed rats.

TABLE 3

EKG OBSERVATIONS IN OFFSPRING EXPOSED TO EGME ON DAYS 7–13 OF GESTATION

	EGME (mg/kg)	
	0	50
3-Week-old males and females/litters	133/13	53/11
EKG intervals (msec)		
P-R	40 ± 4	39 ± 2
QRS	12 ± 1	14 ± 1*
T wave	28 ± 3	32 ± 6
Heart rate (beats/min)	434 ± 45	420 ± 44
IV conduction delay	0/0	5/5
SA block	1/1	1/1
6-Week-old males/litters	67/13	23/11
EKG intervals (msec)		
P-R	42 ± 2	45 ± 5
QRS	11 ± 1	13 ± 2*
T wave	32 ± 2	38 ± 8*
QRS amplitude (volts)	0.77 ± 0.2	0.98 ± 0.34
Heart rate (beats/min)	439 ± 45	419 ± 41
IV conduction delay	0/0	3/3
SA block	1/1	2/1
6-Week-old females/litters	66/13	28/11
EKG intervals (msec)		
P-R	40 ± 1	42 ± 3
QRS	11 ± 1	13 ± 2*
T wave	32 ± 3	36 ± 5*
QRS amplitude (volts)	0.77 ± 0.2	0.84 ± 0.3
Heart rate (beats/min)	456 ± 50	407 ± 70
IV conduction delay	0/0	4/4
SA block	2/2	0/0

Note. Ratios are number of number of offspring/litters. EKG intervals, QRS amplitude and heart rate are presented as mean ± SD.

* Significantly different than control, $p < 0.05$.

DISCUSSION

The EGME treatments used in the present study did not appear to be maternally toxic since the reduction in body weight of dams was apparently due to resorbed fetuses. However, the EGME treatments markedly affected reproductive outcome by prolonging gestation and producing fewer and smaller litters, all of which have been observed previously (Toraason *et al.*, 1986a,b). On the basis of the marked effects of EGME treatment on reproductive outcome, the reduced survival rate during the first 3 days of life is not a surprise. Although survivors beyond 3 days of age appeared healthy during the course of the study, the reduced body weight at the time of necropsy suggests that functional deficits may have taken a toll. In addition to the cardiovascular effects observed in the present study, prenatal EGME exposure has been shown to produce neurochemical deviations in 21-day offspring and behavioral alterations in 60-day-old offspring (Nelson *et al.*, 1984). Therefore, the reduced body weight could be a gross manifestation of other physiological or biochemical anomalies.

The present investigation of cardiovascular effects was aided by the discovery that neonatal rats, and to some extent juvenile or young adult rats, will remain relatively still when held gently by the scruff of the neck. This handling procedure greatly expedited the recording of EKGs and allowed it to be performed in unrestrained and unanesthetized rats. Three-week-old rats, almost without exception, were cooperative when held in this manner. About one-half of the 6-week-old animals, however, resisted by twisting or turning the lower portion of their bodies. Resistant rats were partially restrained by firmly grasping a large piece of back-skin between the first three fingers and the base of the thumb. This study had been originally attempted using anesthetized offspring to obtain EKGs. EGME-exposed rats, however, died from a dose of anesthetic (pentobarbital)

that was tolerated by control rats (unpublished results). Therefore, the present handling procedure avoided the complexities of varying the anesthetic dose while at the same time drastically reducing the stress associated with traditional forms of animal restraint.

Digitization and computer analysis of EKG signals were necessary to detect the QRS aberration shown in Fig. 4. Average heart rate was over 400 beats/min, and the duration of the QRS was only 11–12 msec with conduction delays increasing this interval by 2–5 msec. On standard strip-chart recorders normally used for EKG recording, voltage shifts lasting 2–3 msec are difficult to detect. Despite the short duration, the appearance of conduction delays in juvenile rats was identical to delays observed on gestation day 19 in rat fetuses exposed *in utero* to EGME (Toraason *et al.*, 1985).

Although recording the EKG with only a single lead (lead II) makes interpretation of the QRS difficult, the observed QRS aberration resembles a right-bundle-branch block in humans (Dubin, 1974; Goldman, 1982). The lead II EKG in Fig. 4D is characterized by a missing Q and a double R wave. The second R wave could arise from conduction of the excitation impulse around the blocked right bundle branch. Right-bundle-branch blocks are relatively common in humans and frequently involve congenital lesions of the septum (Goldman, 1982). This fact is significant since ventricular septal defects are one of the most frequently observed cardiac lesion produced by prenatal EGME exposure in rats (Toraason *et al.*, 1985, 1986c). Furthermore, bundle-branch blocks have been associated with prolonged QRS intervals and abnormal T waves, and both of these were significantly increased in EGME-exposed rats. In our previous study (Toraason *et al.*, 1985) when EGME-exposed fetuses were examined, there was a significant increase in the number of rats with prolonged QRS intervals in addition to the characteristic double R wave.

Right-bundle-branch block is sometimes exhibited in humans with cardiac hypertrophy or congenital septal defects, but it is often found in the absence of any clinical evidence of heart disease (Goldman, 1982). The intraventricular conduction delays present in survivors in the present study did not appear to be incompatible with life. Although conduction delays may not have been responsible for the mortality in the EGME-exposed group, it is likely that more severe cardiac malformations including ventricular septal defects were responsible for a significant number of deaths. In a previous investigation treatment of dams with 50 mg/kg EGME produced a variety of major cardiac malformations in 50% of their fetuses (Toraason *et al.*, 1986c).

In rats surviving to 8 weeks of age, there were no cardiac lesions detected microscopically that were associated with EGME exposure. Hearts from both control and EGME-exposed rats were first examined in blind, and then rats with conduction delays were reexamined without any specific lesion being noted. The lack of correlation between cardiac dysfunction and morphological changes following prenatal exposures to developmental toxicants has been previously reported. We observed no association between a variety of cardiac malformations and intraventricular conduction delays in day 19 fetuses exposed to EGME (Toraason *et al.*, 1985). Robinson and Cameron (1984) did not detect EKG changes in rats with ventricular septal defects due to prenatal nitrofen exposure. Lau *et al.* (1986) did detect EKG changes in rats treated prenatally with nitrofen, but these changes did not appear to be associated with physical malformations. Abnormal EKGs produced by prenatal trypan blue exposure were also unattributable to physical malformations (Grabowski and Tunstall, 1977; Watkinson *et al.*, 1983).

Hearts from rats exposed prenatally to EGME weighed more than hearts from control rats, but the increase was not statistically significant. When the reduced body weights of EGME-exposed rats were taken into con-

sideration, by calculating heart/body weight ratios, the ratios were greater in EGME-exposed rats than in controls. In addition, QRS duration was significantly prolonged in EGME-exposed rats, and QRS amplitude was increased, though not significantly. Increased heart weight, prolonged QRS duration, and exaggerated QRS amplitude are all suggestive of hypertrophy. However, in the absence of anatomical evidence the findings are only suggestive and do not substantiate cardiac hypertrophy as an effect of EGME exposure.

In summary, doses of EGME that were not maternally toxic had a severe effect on reproductive outcome. In addition to producing physical malformations, EGME produced functional changes in fetal rats, and these changes persisted through at least 6 weeks of age. Although not evident grossly or histologically, the specific cardiac abnormality was an intraventricular conduction delay that had characteristics similar to those of a right-bundle-branch block. The consequence of this EKG aberration on the long-term health of rats is unknown.

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