TERATOLOGY 32:33–39 (1985)

Electrocardiographic Study of Rat Fetuses Exposed to Ethylene Glycol Monomethyl Ether (EGME)

MARK TORAASON, BARBARA STRINGER, PAUL STOBER, AND BRYAN D. HARDIN
Department of Health and Human Services, U.S. Public Health Service, Centers for Disease Control, National Institute for Occupational Safety and Health, Division of Biomedical and Behavioral Science, Experimental Toxicology Branch, Cincinnati, Ohio 45226

ABSTRACT The widely used industrial solvent ethylene glycol monomethyl ether (EGME) is teratogenic to rats and mice, inducing a variety of heart and major vessel abnormalities. In the present study, electrocardiography was used to evaluate heart function in day 20 rat (Sprague-Dawley) fetuses from mothers treated on gestation days 7–13 (sperm = day 1) with 0, 25, or 50 mg/kg EGME by gavage in 10 ml/kg water. The increased incidence of fetuses with cardiovascular malformations (primarily right ductus arteriosus and ventricular septal defect) and abnormal electrocardiograms (EKG) was dose dependent. The most prevalent EKG abnormality was a prolonged QRS wave. Mean QRS intervals were not significantly increased by EGME exposure, but there were significantly more litters in the 50-mg/kg EGME group that had one or more fetuses with QRS complexes of 40 msec or longer. The enhanced duration and the appearance of the aberrant QRS's suggested the presence of an intraventricular conduction delay in these fetuses. Heart rate and other EKG characteristics such as the P wave or P-R and Q-T intervals were not significantly affected by exposure to EGME. There did not appear to be an association between abnormal EKG's and fetal heart dysmorphology.

Ethylene glycol monomethyl ether (EGME), also known as 2-methoxyethanol, has a wide variety of industrial applications. EGME is used as a solvent in lacquers, enamels, varnishes, inks, and dyes and as an anti-icing additive in fuels and other fluids (NIOSH, '83). There is potential for exposure to EGME not only for workers during manufacturing processes, but also for consumers who use the many products that contain it. EGME is readily absorbed through the skin and acts primarily on the central nervous, renal, and hematopoeitic systems (Hardin, '83). High doses can cause respiratory arrest and renal failure. The reproductive effects in rats and mice treated with EGME include dose-dependent embryo lethality and teratogenesis (Hardin, '83; Nelson et al., '82; Nagano et al., '81). In addition to a variety of skeletal abnormalities, EGME causes heart and great vessel malformations. The apparent sensitivity of the developing cardiovascular system to EGME prompted us to look further at the effects on the heart of prenatal EGME exposure.

Several studies have used electrocardiography to evaluate heart function following prenatal insult. The most comprehensive work has been done by Grabowski and Payne ('80, '82, '83) with the insecticide Mirex. Maternal Mirex treatment induces a high incidence of perinatal deaths in rats but few visible malformations. Electrocardiograms (EKG) revealed a significant incidence of cardiovascular problems in fetuses (Grabowski and Payne, '80, '82) and neonates (Grabowski and Payne, '83). The cardiovascular problems were correlated with edema in fetuses and some deaths in neonates. First- and second-degree heart blocks were the most persistent EKG aberrations observed (Grabowski and Payne, '80, '82). In the chick, the same dose of epinephrine that caused physical malformations caused atrioventricular blocks and intraventricular conduction de-

Received November 21, 1984; accepted March 5, 1985.

Mention of products or company names does not constitute endorsement by the National Institute for Occupational Safety and Health.

lays (Rajala, '80; Rajala et al., '83, '84). The EKG changes were evident within a few hours of treatment, but the physical malformations were not apparent until several days later. Trypan blue (Grabowski and Tunstall, '77; Watkinson et al., '83) and Nitrofen (Robinson and Cameron, '84) have also been shown to induce abnormal EKG's in fetal rats, although an association between functional and morphological changes was not apparent. These studies demonstrate that the EKG can detect functional alterations in the heart following prenatal exposure to teratogens. In the present study, electrocardiography was used to evaluate heart function in fetuses exposed to EGME.

MATERIALS AND METHODS

Maternal treatment

Female Sprague-Dawley rats, time-mated by Charles River Breeding Labs (Wilmington, MA), were housed individually in wire mesh cages and provided with Purina rat chow and tap water ad libitum. Dams were maintained on a 12-hr light/12-hr dark photoperiod at 23°C ± 2°C and 55%, ± 15% relative humidity. Dams were weighed daily and treated by gavage with 0, 25, 50, or 100 mg/kg EGME in 10 ml/kg of distilled water on days 7–13 of gestation (sperm = day 1). This interval covers the period of cardiovascular vulnerability (Wilson, '73). Treatment levels were based on a pilot study that indicated that between 50 and 200 mg/kg EGME was embryolethal. Eleven rats were assigned to the control group and eight rats to each of the three exposed groups. EGME (Cat. No. E182) was purchased from and certified by Fisher Scientific Company (Fair Lawn, NJ).

EKG recording system

EKG's were obtained using a Grass model 79D EEG & Polygraph Data Recording System (Grass Instruments Co., Quincy, MA). The basic unit included a Wide Band A.C. EEG Preamplifier, model 7P5 with Lead Selector, model 7LSA. A Polygraph D.C. Driver Amplifier, model 7DAF, was used to drive the pen oscillograph. Sensitivity of the preamplifier was 50 μV/cm. Low-frequency response was 10 Hz, and high-frequency response was 75 Hz. EKG's were recorded at a strip chart speed of 50 and 100 mm/sec for 5–10 sec.

Fetal electrocardiograms

On day 20 of gestation, mothers were anesthetized with 40 mg/kg pentobarbital (i.p.), and a laparotomy was performed. Fetuses with placentas attached were removed one at a time through a small slit in the uterus. Each fetus was placed on its back on a bed of absorbent paper. Fetuses were kept warm with an infrared heat lamp. EKG's were taken by positioning a flexible arm holding four platinum needle electrodes and electrical cable directly above the fetus. The electrodes were inserted 1–2 mm into the fetus at the base of each limb. Electrodes in the right forelimb, left forelimb, and left hindlimb comprised the standard limb leads I, II, and III. The right hindlimb served as the location of the ground. Only the lead II recording was evaluated in the present study because it gave the most distinctive signal. Time to obtain one EKG was about 1 min.

EKG evaluation

Each EKG record was evaluated subjectively (in blind) for rhythm variations and abnormal or missing peaks. Objective analysis involved measuring the duration of P, P-R, QRS, QT and R-R (Fig. 1) using a Zeiss videoplan microcomputer (Carl Zeiss Inc., NY) connected to a stylus and digitizing board. The R-R interval was used to calculate heart rate. Generally all intervals were measured for five sequential beats, but this was not always the case. Nondiscernible waves could not be not measured, and in several fetuses less than five beats were measured. One control and one 25-mg/kg fetus were not included in EKG analysis because of the poor quality of the recordings.

Fetal examination

After completion of the EKG measurement, placentas were detached from fetuses, and fetuses were weighed and examined for external physical defects. Fetuses were then preserved intact in Bouin's solution and examined in blind for visceral abnormalities by Dr. Phillip T. Goad, Intox Laboratories, Inc. (Redfield, AR) using the Wilson technique (Wilson, '65).

Statistical analysis

Means of EKG intervals were calculated for each fetus and then for each litter. The Kruskal-Wallis test (Hollander and Wolfe, '73) was used to assess group differences for P, P-R, QRS, and Q-T intervals. This test was also used to compare the number of implants per litter, fetuses per litter (alive and dead), fetuses as a percent of implants, and the mean fetal weight. The incidence of EKG

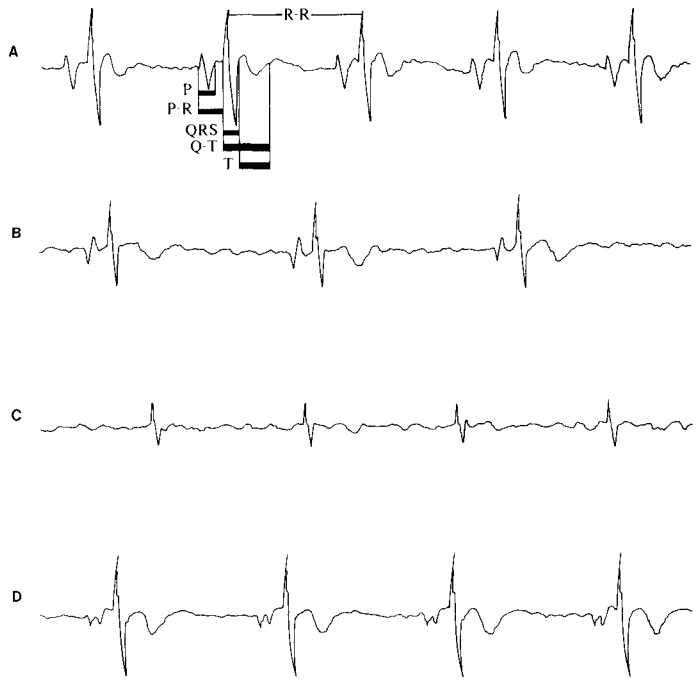

Fig. 1.A: Normal lead II EKG from a fetal rat. Chart speed was 100 mm/sec. P, P-R, QRS, QT, and T intervals are shown. The R-R interval was used to calculate heart rate. B, C, D: Fetal rat EKG's with an inverted P wave, missing P wave, and undefined P wave, respectively.

abnormalities in litters exposed to EGME was compared to the control group using one-sided Fisher's exact test (Siegel, '56) and a Bonferroni correction (Milliken and Johnson, '84) for multiple comparisons to a common control. Visceral defects in fetuses were too rare to make statistical analysis meaningful.

RESULTS

Reproductive outcome

Treatment of pregnant dams with 100 mg/kg EGME caused all fetuses to be resorbed early in gestation. Since this investigation was concerned with electrocardiographic study of live fetuses, no further consideration is given to this exposure group. The reduced number of litters in the 50 mg/kg group was due to the fact that two dams thought to be pregnant at the time of treatment were found not to be when sacrificed (Table 1).

Treatment of pregnant dams with 25 or 50 mg/kg EGME did not affect their physical appearance or general behavior. There was a small dose-dependent decrease in maternal body weight on day 20 in the 25- and 50-mg/kg EGME groups (Table 1). The reduced weight was due largely to the reduced number of fetuses per litter, although there was also a small but nonsignificant decrease in fetal body weight in the two exposed groups. The greatest number of resorptions was found in the 50 mg/kg EGME group, but the groups were not statistically significantly different (Table 1).

Physical malformations

Five of the eleven control litters were examined for visceral malformations. None of the control fetuses had cardiovascular malformations, but there was a dose-dependent increase in cardiovascular defects in fetuses treated with EGME (Table 2). The most common defect was ventricular septal defect followed by right ductus arteriosus, both of which were found only in the 50-mg/kg group. Cardiovascular malformations are rare in rats (Kameyama, L.C. et al., 1980; Palmer, 1972), but defects similar to those seen in these EGME-exposed fetuses are also associated with ethylene glycol monoethyl ether (EGEE) exposure (Andrew et al., 1984; Hardin et al., 1982, 1984). One dam in the 50 mg/kg group had only two live fetuses; both had gastroschisis. These were the only grossly

TABLE 1. Reproductive outcome in dams treated with EGME on days 7–13 of gestation[1]

	Dose EGME (mg/kg)		
	0	25	50
Dams treated	11	8	8
Litters	11	8	6
Maternal body wt (gm)	322 ± 24	306 ± 33	289 ± 39
Implants	135	80	56
Resorptions	9	6	18
Live fetuses	123	74	37
Dead fetuses[2]	3	0	1
Fetal body wt (gm)	2.8 ± .6	2.5 ± .4	2.3 ± .5

[1]Body weights are mean ± S.D. All other values are total number of observations.
[2]Dead fetuses appeared normal, but a heartbeat was not evident.

TABLE 2. Physical malformations in fetuses exposed to EGME on days 7–13 of gestation[1]

	Dose EGME (mg/kg)		
	0	25	50
Litters/fetuses examined	5/55	8/74	6/38
Cardiovascular (CV) malformations			
Double aortic arch	0	1/1	0
Right aortic arch	0	0	2/4
Right ductus arteriosus	0	0	3/4[2]
Right azygos vein	0	0	1/1
Abnormal aorta	0	0	1/1[2]
Abnormal subclavian	0	0	1/1[2]
Ventricular septal defect	0	0	2/5[2]
Total CV malformations	0	1/1	3/7[2]
Hydronephrosis	1/1	4/7	3/3[2]
Folded retina	0	0	1/1
Gross malformations[3]	0	0	2/1

[1]Data are number of litters/fetuses exhibiting the malformation.
[2]Includes one grossly malformed fetus.
[3]Two fetuses from one litter had gastroschisis.

malformed fetuses in the study. One of these fetuses had multiple malformations of the heart and major vessels, while the other had a normal heart (Table 2).

EKG observations

Figure 1A shows a normal lead II EKG for a 20-day rat fetus. A distinctive characteristic of the lead II fetal rat EKG is the symmetrical, diphasic P and T waves. The diphasic P wave is absent in the adult rat (Detweiler, '81). Diphasic P wave in humans is an indication of atrial hypertrophy (Dubin, '74), and the relative size of the positive or negative deflection is an indication that the hypertrophy resides in the right or left atrium. In the rat fetus, the diphasic P wave could be due in part to the closeness of the limb leads to the heart. There is little precedent for evaluating fetal rat EKG's based on variations in the P wave other than the appearance of multiple or misplaced P's in an arrhythmia. Nevertheless, P waves were scored abnormal if they were inverted (Fig. 1B), that is, having a negative deflection followed by a positive deflection rather than the normal positive-negative sequence; if they were not clearly detectable or missing (Fig. 1C); or if they had an undefined appearance (Fig. 1D). Using these criteria, all three groups had individuals with abnormal P waves, and there were no significant differences among them.

The normal lead II QRS complex in the fetal rat is diphasic, the Q wave is absent, R and S waves are of approximately equal but opposite amplitude, and there is usually a notch on the downsweep of the R wave (Fig. 1A). In the present study, there was a consistent type of variation in the QRS complex in fetuses exposed to EGME. The notch in the downsweep of the R wave occurred below the isoelectric point in the downsweep of the S wave, and the R peak was shortened or absent (Fig. 2A). In several fetuses, the S wave notch resembled a double peak (Fig. 2B), and was usually followed by an overshoot of the S wave above the isoelectric line. In some of these fetuses, the aberrant QRS complexes appeared irregularly; that is, three or four normal QRS's occurred between abnormal ones. There were significantly more litters in the 25-mg/kg and 50-mg/kg groups with fetuses having aberrant QRS's than in the control group (Table 3).

The ST interval is absent in the normal fetal rat (Fig. 1A). The T wave is also diphasic. Although the T wave varied in amplitude considerably from fetus to fetus, there was no apparent consistent variation that could be easily defined and therefore considered abnormal.

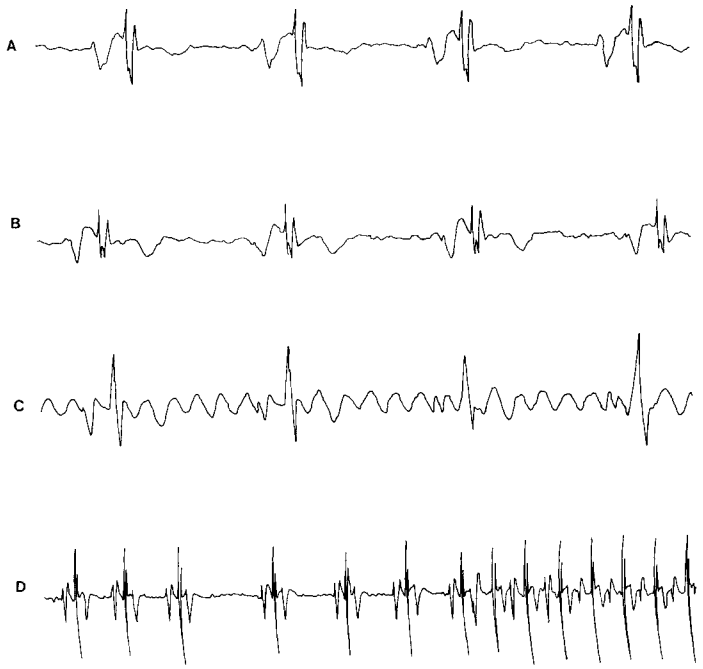

Fig. 2.A,B: Fetal rat EKG's with abnormal QRS complexes apparently owing to intraventricular conduction delay. C: Fetal rat with atrial flutter. D: Fetal rat with an SA block. Chart speed was set at 50 mm/sec to emphasize the arrhythmia.

Two fetuses (one control, one 25 mg/kg) had atrial flutter (several P waves occurring in rapid succession) (Fig. 2C). One fetus in the 50-mg/kg group had an arrhythmia that would be best described as an SA block (stopping or slowing of the atrial pacemaker) (Fig. 2D).

P,P-R, QRS, and Q-T intervals and heart rate are shown in Table 3. There were no significant differences among the groups. Though the mean QRS values were not significantly different, there were significantly more litters in the 50-mg/kg group that had fetuses with prolonged QRS intervals. Seven fetuses in this group had QRS durations of 40 msec or longer, and the QRS's of these fetuses were abnormal in appearance. In the 25-mg/kg group, three of the seven aberrant QRS's were longer than 39 msec. Only one control had a QRS longer than 39 msec. The prolonged control QRS was normal in appearance, but the fetus had severe bradycardia with a heart rate of 30 beats per minute.

DISCUSSION

Electrocardiography revealed a specific functional defect that occurred more frequently than any physical malformation. Nineteen fetuses exposed to EGME had aberrant QRS waves. The duration of the QRS complex in 11 of these fetuses was prolonged. In humans, a QRS duration greater than 120 msec indicates a conduction delay and is referred to as a bundle branch block (Dubin,

TABLE 3. EKG observations on fetuses exposed to EGME on days 7–13 of gestation

	Dose EGME (mg/kg)		
	0	25	50
EKG intervals (msec[1])			
P	37 ± 2	36 ± 1	37 ± 2
P-R	64 ± 5	62 ± 4	64 ± 4
QRS	34 ± 1	34 ± 1	36 ± 2
Q-T	143 ± 12	152 ± 8	149 ± 9
Heart rate (beats/min)	154 ± 11	152 ± 10	163 ± 17
EKG abnormalities[2]			
Number evaluated	11/122	8/73	6/37
Aberrant QRS	1/1	5/7*	4/12*
QRS ⩾ 40 msec	1/1	2/3	4/7*
Aberrant P	10/19	7/26	4/12
Atrial flutter	1/1	1/1	0
SA block	0	0	1/1

[1]EKG intervals and heart rate are presented as mean ± S.D. Means were computed for each fetus, then for each litter, and then across litters.
[2]EKG Abnormalities presented as number of litters/fetuses.
*Significantly different than control ($p < 0.05$).

'74). Fetal rats with a QRS duration greater than 39 msec were arbitrarily classified as having an intraventricular conduction delay. Thirty-nine milliseconds is considered a conservative cut-off point since it is five standard deviations longer than the mean QRS interval of the control group. Nonetheless, 19% of the fetuses in the 50-mg/kg EGME group had apparent conduction delays. This contrasts with the control group where only one fetus (<1%) had a prolonged QRS complex (41 msec), which was probably due to the severe bradycardia rather than a conduction delay.

There did not appear to be an association between the abnormal QRS's and any specific morphological defect. Only four of the fetuses with conduction delays had heart malformations, which were multiple and included major vessel abnormalities. Furthermore, four fetuses with heart defects had normal EKG's. Two of these fetuses had a single major vessel abnormality and two had only ventricular septal defect. Robinson and Cameron ('84) were also unable to detect EKG changes following Nitrofen exposure in rat fetuses that had ventricular septal defect. Perhaps ventricular septal defect in the fetal rat is not readily identified with the lead II EKG.

The ventricular conduction block was easily identified by the doubled-peaked S wave and the increased duration of the QRS complex in fetuses exposed to EGME. This effect is markedly different than the numerous arrhythmias produced by Mirex treatment (Grabowski and Payne, '80). However, we have seen similar conduction delays in fetuses exposed to trypan blue (unpublished observation). Rajala et al. ('83, '84) reported that epinephrine caused ventricular conduction blocks in chicks, and Abe and Kawai ('83) observed that hornet venom produced conduction delays in adult rats almost identical with those shown in Figure 2A,B. In the latter study, the conduction blocks were transient. Whether they represent a potentially lethal effect in fetal rats is uncertain, but we found that mice did not live beyond 40 days of age when their mothers were treated with 250 mg/kg EGME on days 7–16 of gestation (unpublished observation). Robinson and Cameron ('84) found that neonatal rats with multiple EKG changes induced by prenatal Nitrofen exposure did not survive to 70 days of age. Likewise, Grabowski and Payne ('83) reported that neonates with second- and third-degree heart blocks died within an hour of birth. Based on EKG observations alone, it is not unreasonable to predict that postnatal survival would be reduced in the EGME-exposed fetal rats.

The EKG proved to be a sensitive tool for identifying maternal treatments of EGME that affect heart function in offspring. QRS changes were evident not only from subjective analysis of EKG's but from quantitative measure of QRS duration. A considerable number of P waves that appeared abnormal were found in control and exposed fetuses and were apparently not due to EGME exposure. Grabowski and Payne ('80, '82, '83), who have examined a large number of fetal-rat EKG's, have not made note of such P wave variations. Arrhythmias occurred too infrequently to be of any significance in the present study.

LITERATURE CITED

Abe, T. and N. Kawai (1983) Cardioactive effects of hornet venom, *Vespa mandarinia*. Comp Biochem. Pysiol., *76*:221–225.

Andrew, F.D., and B.D. Hardin (1984) Developmental effects of inhalation exposure of gravid rabbits and rats to ethylene glycol monoethyl ether. Environ. Health perspect., *57*:13–23.

Detweiler, D.K. (1981) The use of electrocardiography in toxicological studies with rats. In: The Rat Electrocardiogram in Pharmacology and Toxicology. R. Budden, D.K. Detweiler, and G. Zbinden, eds. Pergamon Press, New York, pp. 83–115.

Dubin, D. (1974) Rapid Interpretation of EKG's 3rd ed. Cover, Tampa, Florida.

Grabowski, C.T., and A.C. Tunstall (1977) An electrocardiographic study of fetuses treated with trypan blue. Teratology, *15*:32A.

Grabowski, C.T., and D.B. Payne (1980) An electrocardiographic study of cardiovascular problems in Mirex-fed rat fetuses. Teratology, *22*:167–177.

Grabowski, C.T., and D.B. Payne (1982) The causes of perinatal death induced by prenatal exposure of rats to the pesticide, Mirex. Part I: Preparturition observations of the cardiovascular system. Teratology *27*:7–11.

Grabowski, C.T., and D.B. Payne (1983) The causes of perinatal death induced by prenatal exposure of rats to the pesticide, Mirex. Part II: Postnatal observations. J. Toxicol. Environ. Health, *11*:301–315.

Hardin, B.D. (1983) Reproductive toxicity of the glycol ethers. Toxicology, *27*:91–102.

Hardin, B.D., P.T. Goad, and J.R. Burg (1984) Developmental toxicity of four glycol ethers applied cutaneously to rats. Environ. Health Perspect., *57*:69–74.

Hardin, B.D., R.W. Niemeier, R.J. Smith, M.H. Kuczuk, P.R. Mathinos, and T.E. Weaver (1982) Teratogenicity of 2-ethoxyethanol by dermal application. Drug Chem. Toxicol. *5*:277–94.

Hollander, M., and D. Wolfe (1973) Non Parametric Statistical Methods. John Wiley and Sons, New York.

Kameyama, Y., T. Tanimura, and M. Yasuda. (1980) Spontaneous malformations in laboratory animals–Photographic atlas and reference data. Cong. Anom., *20*:25–106.

Milliken, G.A., and D.E. Johnson (1984) Analysis of Messy Data. Lifetime Learning Publications, Belmont, California.

Nagano, K., E. Nakayama, H. Oobayashi, T. Yamada, H. Adachi, T. Nishizawa, H. Ozawa, M. Nakaichi, H. Okuda, K. Minami, and K. Yamazaki (1981) Embryotoxic effects of ethylene glycol monomethyl ether in mice. Toxicology, *20:*335–343.

Nelson, B.K., J.V. Setzer, W.S. Brightwell, P.R. Mathinos, M.H. Kuczak, and T.E. Weaver (1982) Comparative inhalation teratogenicity of four industrial glycol ether solvents in rats. Teratology, *25:*64A.

NIOSH (1983) The glycol ethers, with particular reference to 2-methoxyethanol and 2-ethoxyethanol: Evidence of adverse reproductive effects. DHHS (NIOSH) Publication No. 83–112.

Palmer, A.K. (1972) Sporadic malformations in laboratory animals and their influence on drug testing, Adv. Exper. Med. Biol., *27:*45–60.

Rajala, G.M. (1980) Cardiac dysfunction in epinephrine-treated chick embryos. Teratology, *21:*63A.

Rajala, G.M., R.S. Kuhlmann, and G.L. Kolesari (1983) EKG changes in epinephrine-treated chick embryos. Teratology, *27:*70A.

Rajala, G.M., R.S. Kuhlman, and G.L. Kolesari (1984) The role of dysrhythmic heart function during cardiovascular teratogens in epinephrine-treated chick embryos. Teratology, *30:*385–392.

Robinson, K.S., and A.M. Cameron (1984) In utero exposure to nitrofen induces postnatal changes in rat electrocardiograms. Teratology, *29:*54A.

Siegel, S. (1956) Non Parametric Statistics. McGraw Hill, New York.

Watkinson, W.P., E.H. Hoke, D.A. Whithouse, and E.H. Rogers (1983) Electrocardiographic studies of developing rodents exposed *in utero* to trypan blue. In: Abnormal Functional Development of the Heart, Lungs and Kidneys: Approaches to Teratology. R.K. Kavlock and C.T. Grabowski, eds. Alan R. Liss Inc., New York, pp. 208–224.

Wilson, J.G. (1965) Embryological considerations in teratology. In: Teratology—Principles and Techniques. J.G. Wilson and J. Warkany, eds. The University of Chicago Press, Chicago, pp. 251–261.

Wilson, J.G. (1973) Environment and birth defects. Academic Press, New York, p. 19.