EPA-600/1-80-032
September 1980

PESTICIDE EFFECTS ON PRENATAL CARDIOVASCULAR PHYSIOLOGY

I. An Electrocardiographic Study of Mirex-Exposed Rat Fetuses and Newborns

II. An Analysis of the Causes of Perinatal Deaths Induced by Prenatal Exposure to Mirex

by

Casimer T. Grabowski
Department of Biology
University of Miami
Coral Gables, Florida 33124

EPA Grant No. R-8053010

Project Officer

Dr. Neil Chernoff
Experimental Biology Division
Health Effects Research Laboratory
Research Triangle Park, NC 27711

HEALTH EFFECTS RESEARCH LABORATORY
OFFICE OF RESEARCH AND DEVELOPMENT
U.S.ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

DISCLAIMER

This report has been reviewed by the Health Effects Research Laboratory, U. S. Environmental Protection Agency, and approved for publication. Approval does not signify that the contents necessarily reflect the views and policies of the U. S. Environmental Protection Agency, nor does mention of trade names or commercial products constitute endorsement or recommendation for the use.

FORWARD

The many benefits of our modern, developing, industrial society are accompanied by certain hazards. Careful assessment of the relative risk of existing and new man-made environmental hazards is necessary for the establishment of sound regulatory policy. These regulations serve to enhance the quality of our environment in order to promote the public health and welfare and the productive capacity of our Nation's population.

The Health Effects Research Laboratory, Research Triangle Park, conducts a coordinated environmental health research program in toxicology, epidemiology, and clinical studies using human volunteer subjects. These studies address problems in air pollution, non-ionizing radiation, environmental carcinogenesis and the toxicology of pesticides as well as other chemical pollutants. The Laboratory participates in the development and revision of air quality criteria documents on pollutants for which national ambient air quality standards exist or are proposed, provides the data for registration of new pesticides or proposed suspension of those already in use, conducts research on hazardous and toxic materials, and is primarily responsible for providing the health basis for non-ionizing radiation standards. Direct support to the regulatory function of the Agency is provided in the form of expert testimony and preparation of affidavits as well as expert advice to the Administrator to assure the adequacy of health care and surveillance of persons having suffered imminent and substantial endangerment of their health.

The report details research into the effects of Mirex, an insecticide, on the development of the cardiovascular system of rodents. It details changes in electrocardiogram patterns of fetuses whose mothers were exposed to this insecticide during different periods of gestation.

PREFACE

One of the great problems of contemporary Teratology is the development of both criteria and tests for adequately evaluating developmental toxicity. This document describes (1) the development of a new testing procedure, electrocardiography applied to fetuses and newborns of small laboratory animals; and (2) the application of this method to a study of the developmental toxicity of the pesticide Mirex.

In Part I the results of testing 18-1/2 day rat fetuses are described. It is shown that a high incidence of cardiovascular problems are present even in healthy-appearing, Mirex exposed fetuses. In Part II this technique is applied to the perinatal period. It is shown that the cardiovascular problems which originate during the last third of gestation persist to parturition and help to explain the high incidence of perinatal death induced by Mirex. The usefulness of the ECG test for both diagnostic and analytical purposes is demonstrated by these studies.

TABLE OF CONTENTS

PART I

AN ELECTROCARDIOGRAPHIC STUDY OF CARDIOVASCULAR PROBLEMS IN MIREX-EXPOSED RAT FETUSES

ABSTRACT

Sperm-positive rats were intubated with Mirex in oil (5 to 10 mg/kg) on days 8-1/2 to 15-1/2. Controls were untreated or oil-fed. Testing was done on day 18-1/2. Fetuses were sequentially exposed and ECGs obtained with the fetus attached to the placenta and uterus. Counterparts of standard leads, I, II and II were used. Fetuses were weighed and examined afterwards. Swollen fetuses were rated on a scale of 1 (slight edema under chin) to 5 (3 mm edema across back). ECGs from 81 controls and 205 Mirex fetuses were obtained. They were evaluated for rate of heart beat, regularity of beat, PR intervals and other features. One control exhibited an abnormality, a transitory period of premature atrial contractions. Mirex-fed fetuses exhibited tachycardia, closely correlated with degree of edema. The heart rate increased from 150/min in controls to 180 in slightly swollen to 224 in swollen fetuses. Mean PR intervals increased with degree of swelling and with dose. The frequency of first degree heart block was also dose-related, ranging from 20% to 77%. Second degree heart blocks were found in 8%, 3% showed arrhythmias, and one had atrial flutter/fibrillation. These cardiovascular problems seem primarily related to the Mirex-induced edema and demonstrate that fetal edema is not innocuous. These data demonstrate the usefulness of fetal electrocardiography to detect functional teratology.

INTRODUCTION

The developmental toxicity of the pesticide Mirex (dodecachloro-octahydro - 1,3,4,-metheno-2H-cyclobuta (c,d) pentalene),also sold as a component of Ferriamicide, has been the subject of several studies. Gaines and Kimbrough ('70) reported a reduction in litter size in rats fed Mirex during gestation, and cataracts and a lowered viability in surviving neonates. Khera et al. ('76) found that feeding pregnant rats 1.5 to 12.5 mg/kg of Mirex on days 6 to 15 of gestation produced fetal death, skeletal and visual abnormalities, and fetal edema. Chernoff et al ('79) fed pregnant rats 5 to 38 mg/kg of Mirex on days 7 to 16 and found increased fetal mortality, decreased weight, and a significant incidence of enlarged cerebral ventricles, undescended testicles and skeletal abnormalities. They also found a dose-related incidence of edematous fetuses, ranging from 6 to 75%.

Khera et al ('76) did not attribute any significance to the edema. Chernoff et al ('79) commented that some of the severely edematous fetuses were dead, but others had beating hearts and responded to tactile stimulation. Embryonic and fetal edema is induced by many agents which are also teratogenic in amphibia, birds, and several species of mammals including humans (Giroud et al. '55; Grabowski, '70 and '77). Edema in the early stages of development has been traced to several types of abnormal development in a sequence of events termed the edema syndrome (Grabowski, '64, '70; Turbow, '66; Jaffee, '74). However, the consequences of edema during fetal stages are poorly understood. Mirex induces conspicuous edema in the later stages of development, therefore it seemed a good agent to study the effects of prolonged swelling on the fetus.

The initial phase of the Mirex study concentrated on the fetal heart since hypoxia-induced edema in the 3 to 5 day chick embryo has been shown to induce hypervolemia, hypertension, and changes in the rate of heart beat (Grabowski and Schroeder '68; Grabowski et al '69). The primary techniques used to study the heart was the fetal electrocardiogram. We have previously shown that anatomical and functional cardiac disorders could be prenatally detected with this method in rat fetuses exposed to Trypan blue (Grabowski and Tunstall '77). Mirex-induced edema, in the present study, was found to be closely associated with a variety of cardiovascular problems, especially a high incidence of first and second degree heart blocks. The usefulness of

the ECG method to supplement standard teratological techniques is demonstrated.

MATERIALS AND METHODS

Long-Evans rats obtained from the Blue Spruce Farms, Altamont, N. Y. were used. Animals were maintained at 24°C ± 1°; 40 to 60% relative humidity; and on a 12 hour daylight period. They were housed in polypropylene cages on Sanicel bedding and fed Purina Lab Chow and water ad libitum. Virgin females in estrus, as determined by vaginal smear, were housed overnight with males and examined the following morning for the presence of sperm in the vagina. Rats were considered 1/2 day pregnant on the morning they were found to be sperm-positive.

The Mirex used was of commercial purity and was obtained from the Mississippi State Laboratory, Mississippi State University. It was dissolved in peanut oil and fed to the rats by gastric intubation. Treated rats were intubated daily with 5,6,7 or 10 mg/kg Mirex in 1/2 cc peanut oil. Initially animals were fed on days 6-1/2 to 15-1/2 of pregnancy. Later this was reduced to days 8-1/2 to 15-1/2 because we found this was just as effective as the longer treatment period. The doses for each rat were based on their weights on the day before the feeding regimen was started. Controls were given either no treatment or 1/2 cc plain peanut oil on days 8-1/2 to 15-1/2. Daily records of maternal weight and food and water intake were kept. All testing was done on day 18-1/2 of pregnancy.

Some preliminary results of these ECG studies have been published (Grabowski and Tunstall, '77; Grabowski, '78, '79), but since this is the first full report, the equipment and procedures are described in detail. The basic unit is a Tektronix 122 preamplifier powered with a Tektronix 125 Power Supply. The voltage gain on the preamplifier was set at 1000x; the high frequency response set at 50 cycles and low frequency response at 80 cycles. The electrodes consisted of about 15mm of .007 inch pure silver wire soldered to the two leads of a miniature shielded cable (fig. 1). The terminal portion of the shielding was twisted into a ground, to which another piece of silver wire was also soldered. The output of the preamplifier was monitored on an oscilloscope for convenience, but all measurements were made on permanent records obtained on a Brush 220 recorder. One mm of deviation on this instrument is equivalent to 1 mv at full sensitivity, but it was usually used at half of this value. The recorder delivers the chart at speeds of 1, 5, 25 and 125 mm/sec with an accuracy of ± 0.25%. The preamplifier, rat and fetuses were kept in a galvanic cage which was kept warm (36°C) with an infra-red lamp placed overhead.

Various combinations of leads were tried, but the only ones routinely used in this particular study were comparable to leads I, II, III of conventional human electrocardiography. These are, respectively, shoulder to shoulder, right shoulder to left thigh, and left shoulder to left thigh (fig. 2). Electrodes were inserted into muscle to a depth of about 1 mm. The ground wire was inserted into the perineal area.

On day 18-1/2 of pregnancy the rats were anesthetized with an IP injec-

Fig. 1. An 18-1/2 day rat fetus in position for ECG recording. The electrodes are in position I. Note the umbilical cord (arrow) extending to the placenta which is still attached to the uterus.

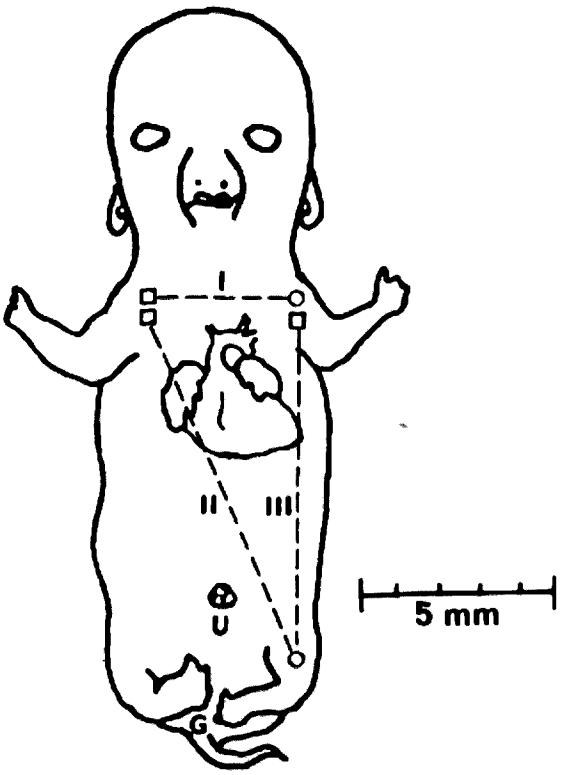

Fig. 2. Scale drawing of rat fetus of 18-1/2 days shown with drawing of the heart superimposed on the chest wall. The placement of the electrodes for positions I, II and II are indicated.

Abbreviations

G, ground electrode placement
U, umbilicus
Squares, positive electrode placement
Circles, negative electrode placement

jection of sodium pentobarbital, 30 mg/kg. Laparotomies were started about 15 minutes later. All further procedures were timed beginning with the first incision. The fetuses were removed one at a time and placed into a shaped holder attached to a micromanipulator (fig. 1). Placental attachment to the uterus was maintained and the umbilical cord arranged with minimal possible tension. This arrangement eliminates electrical noise due to maternal muscle movements. The cable, attached to another micromanipulator, was positioned over the fetus and the electrodes inserted. The chart was first run at a speed of 5 mm/sec to check the rate of heart beat. Recordings from leads I, II and III were successively made at 25 and 125 mm/sec, and then the rate of heart beat was rechecked at 5 mm/sec. The time required to prepare each fetus was 1 to 2-1/2 minutes. Tracings were taken for a period of about 1-1/2 minutes, sometimes longer if a problem was detected. It took about 20 to 30 minutes to check a full litter. Occasionally fetuses were rechecked in between litter mates or at the end of the session. Data from these were not used for the quantitative summaries because the rate of heart beat slows down considerably after the fetus is detached from the uterus. All quantitative measurements were made from lead II tracings. This study is primarily based on an analysis of ECGs from 81 control fetuses and 205 Mirex-fed fetuses.

All fetuses were weighed at the conclusion of the recording session. They were then placed in a dish of saline and examined under a stereoscopic microscope for the presence of visible abnormalities and edema. Several ways to quantitate the edema were tried. Wet weights did not correlate well with edema because treated fetuses sometimes exhibited a moderate degree of growth retardation (see e.g. the wet weight data in Table 2). A good visual estimate of subcutaneous swelling could be made by examining immersed fetuses because the skin is very transparent at this stage. A rating system was adopted which ranked fetuses on a scale of 1 to 5, defined as follows:

0 - no edema apparent;
1° - slight swelling, mostly under the chin;
2° - visible edema under chin, along belly and back;
3° - edema extends to head and upper arm, 1/2 to 1 mm of edema visible across back;
4° - 1-1/2 to 2mm of edema across back;
5° - 2-1/2 to 3mm of edema across back.

Two operators independently rating the fetuses would agree in their estimates to within 1/2 degree. Later in the study, this scale was correlated with percent water determinations by taking a series of fetuses treated with from 5 to 7 mg of Mirex/kg body wt., rating them on the edema scale, obtaining wet weights and then drying at 70°C to constant weight.

Thoracic dissections were performed under a stereomicroscope on about half of the fetuses in both control and treated groups. Three fetuses, a control, a normal-appearing treated and a swollen treated, were preserved in Bodian's solution, sectioned at 15 u and stained with Ehrlich's hematoxylin and Eosin. Significance was determined by the t-test.

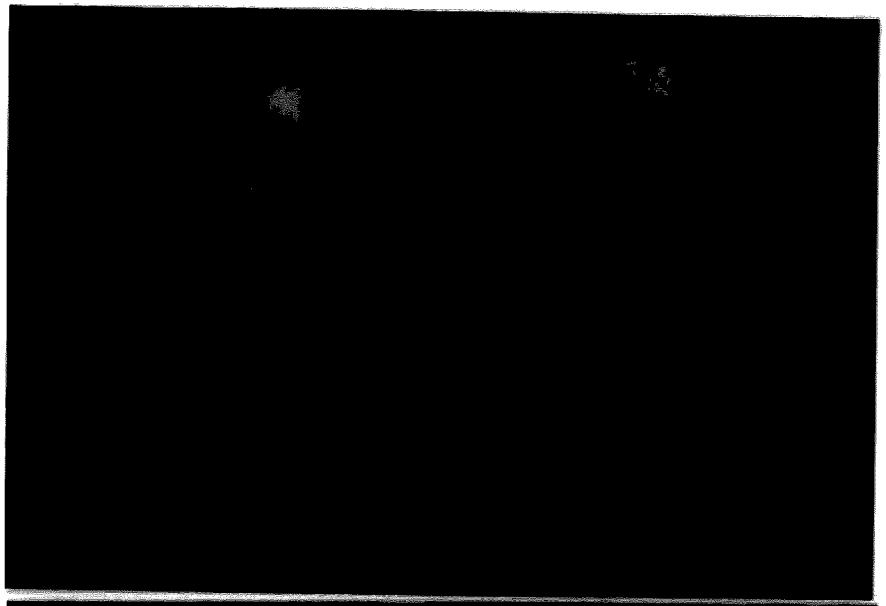

Fig. 3. A swollen 4° rat fetus, 18-1/2 days, along with a normal appearing litter mate on the left. Both came from a mother who was fed 6 mg/kg Mirex per day. The subcutaneous edema in this photo is particularly evident across the forehead, chin and neck and abdominal wall.

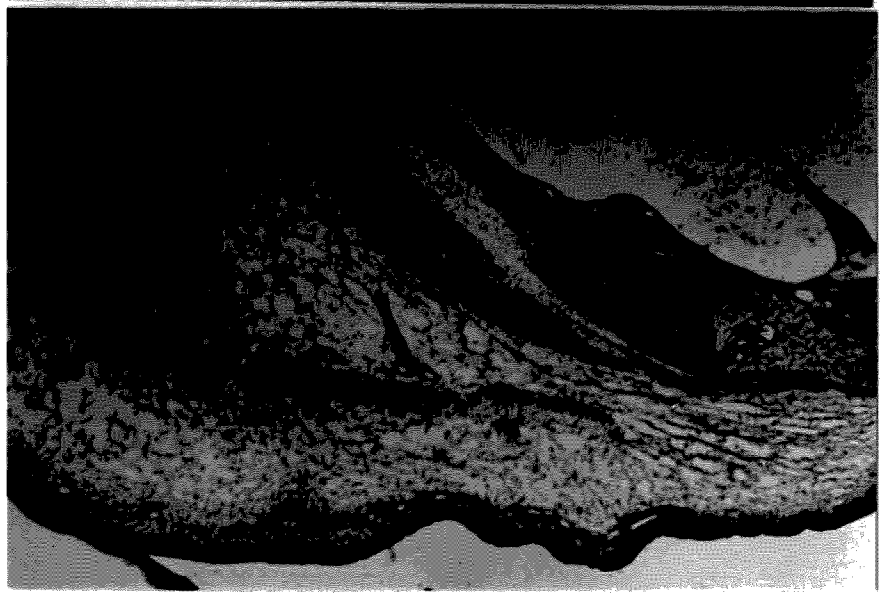

Fig. 4. Cross section through the anterior thoracic wall of an edematous fetus. Note the loose, space-filled, subcutaneous connective tissue, even though considerable shrinkage of this region has taken place from the histological treatment. Note also that the edematous tissue extends between some of the superficial muscles.

RESULTS

Lethal and Teratogenic Effects of Mirex

The death rate was only slightly above normal in the 5 and 6 mg/kg groups but quite high in the 7 and 10 mg/kg group (Table 1). Some treated fetuses appeared undersized and retarded. Seven fetuses (out of 205) were microphthalmic. No other externally visible abnormalities were noted.

Mirex-induced edema

The degree of edema observed in 18-1/2 day fetuses never reached the extremes observed in near term fetuses exposed to Mirex, when some individuals appear swollen to twice normal size (Khera et al. '76; Chernoff et al. '79). For the most part the edema observed would be considered slight and could be readily missed without careful examination under saline (fig. 3). About half of the controls exhibited 1^{o} edema, never more, but 85% of the treated fetuses were appreciably edematous. Half of the treated and control fetuses were dissected after ECG testing. There were no grossly discernible differences in the size of the thorax, abdomen or viscera between normal and edematous fetuses. The edema seemed largely confined to subcutaneous layers. This was confirmed by examination of the serial sections. The edematous areas were extensive areas of very loose connective tissue, sometimes extending into and between the superficial musculature (fig. 4).

As the investigation progressed, it became apparent that even a slight degree of edema could have a detectable physiological effect on the fetus. It became desirable to correlate the edema scale with total water determinations. The mean water content of 18-1/2 day controls was 89.09% (Table 2). Edematous fetuses from Mirex-treated mothers showed regular increases to 89.77% water at level 2 edema, up to 91.17% at level 4. The increments are not great, but this is not surprising since the controls are already 89% water. The difference between controls and level 1 treated fetuses was not significent, but that between controls and level 2, 3 and 4 fetuses was ($P < .001$). Similarly the difference between treated fetuses of levels 2 and 3 was also significant ($P < .001$) as was the difference between treated fetuses of levels 3 and 4. In effect, the water content data of Table 2 represent a calibration of the edema scale.

Characteristics of the ECG of normal rat fetuses

The ECG of the 18-1/2 day rat fetus is mature and clearly shows P, QRS, and T components. The tracings look somewhat different than those of the human adult, presumably because of the small size of the heart and the fact that the electrodes are placed very close to it (fig. 2). The QRS interval in adult humans is about 0.08 seconds versus 0.02 sec. in the rat fetus. Consequently the individual components cannot always be easily visualized at a chart speed of 25 mm/sec, which is the standard clinical rate. The record for every fetus contains segments run at 3 different speeds, 5, 25 and 125mm/sec. Records obtained at the 5 mm/sec rate are convenient for measuring rate of heart beat and visualizing arrhythmias (fig. 7a). Those at the 25 mm/sec rate are good for visualizing the T wave and for detecting some abnormalities (fig. 7b). Records obtained at the 125 mm/sec rate are the only ones in which the P and QRS complexes are sufficiently spread out to be examined with

Table 1

Lethal and Teratogenic Effects of Mirex Treatment

Treatment	Number attempted	Maternal deaths	Number litters	Living fetuses	Resorptions	Microphthalmos
None	3	0	3	34	1	0
Oil only	5	0	4	47	5	
MIREX-5mg/kg						
on days 6-1/2 to 15-1/2	2	0	2	23	1	0
on days 8-1/2 to 15-1/2	9	0	8	70	7	3
MIREX-6mg/kg						
on days 8-1/2 to 15-1/2	8	0	6	60	7	3
MIREX-7mg/kg						
on days 6-1/2 to 15-1/2	10	2	8	0	80	0
on days 8-1/2 to 15-1/2	11	2	9	49	33	0
MIREX-10mg/kg						
on days 6-1/2 to 15-1/2	10	1	3	13	48	1

Table 2

Wet Weights and Water Content of 18-1/2 Day Fetuses

	No.	Wet Weight Mean ± SEM	% H_2O Mean ± SEM
Controls	23	1.320g ± .015	89.09 ± .096
Treated*, 1^o Edema	3	1.373g ± .065	89.53 ± .353
Treated, 2^o Edema	39	1.242 ± .034	89.77** ± .087
Treated, 3^o Edema	31	1.278 ± .033	90.52** ± .108
Treated, 4^o Edema	30	1.357 ± .030	91.17** ± .088

* Treated with Mirex, 5 to 7 mg/kg/day on days 8-1/2 to 15-1/2 of gestation.

** Significantly higher than controls ($P<.001$). The increments between 2^o - 3^o and 3^o - 4^o were also significant ($P<.001$).

clarity (fig. 7c).

The rate of heart beat was obtained from the ECG records taken as soon as possible after the fetus was exposed and at the conclusion of the recording session. The heart rate usually starts to slow down gradually after fetal exposure, but sometimes remains constant or even speeds up slightly. The before and after measurement of heart rate almost always agreed within ± 10% The mean rate of heart beat of 18-1/2 day fetuses (untreated controls) was 149 ± 4.95 (SEM) beats per minute.

The ECGs obtained from 81 control fetuses of this study were normal with but a single exception. One untreated control fetus developed a transient episode of premature atrial contractions, most of which were blocked. The abnormality started 30 seconds after recording was started. Two minutes later the beat returned to normal. This fetus was rechecked 10, 20 and 40 minutes after being removed from the mother. The rate of beat was reduced at these times, but the beat was regular and ECG normal. This particular abnormality was not found in any treated fetus of this series. It was also the only abnormality found in over 300 ECGs of control fetuses taken in this and other experimental series.

ECGs of Mirex-treated fetuses

Edema and the rate of heart beat. The heart rate in treated fetuses was more rapid than normal and this increase was related to the degree of visible edema (fig. 5). The mean rate in both treated and untreated controls is 150 beats/min. It jumps to 181 beats/min. in the slightly swollen (2^o) and 224 beats/min. in the grossly swollen (4^o) treated fetuses. There was a drop in mean rate of heart beat in severely swollen fetuses (5^o) due to a very low rate (down to 144 beats/min.) in a few individuals. All the differences in the rates of heart beat between fetuses with 1^o edema or higher and treated controls were significant ($P < .001$), but that between treated fetuses without visible edema and controls was not.

First degree heart block in treated fetuses. The PR interval in normal humans varies somewhat with age and rate of heart beat. The range for newborns is 0.07 to 0.14 seconds with an average of 0.11 sec (Schaffer and Avery, '77). The range for adults is 0.14 to 0.18 seconds (Litman, '72). A prolongation of the PR intervals beyond 0.20 seconds in adults indicates a delay in impulse conduction from the sinus nodes to the ventricles and is called a 1^o heart block.

The mean PR interval in untreated control rat fetuses was .069 ± .008 (SD) seconds and in oil-fed controls was .065 ± .006 seconds. The difference between the two is probably due to subtle improvements in technique as the study progressed. The mean PR interval of all controls was .067 ± .007 sec., about 8 mm on the records taken at a chart speed of 125 mm/sec (fig. 7c). A 1^o heart block in 18-1/2 day rat fetuses was defined as any PR interval greater than the mean interval in controls (.067 sec) plus 3 standard deviation intervals (.021 sec), i.e. 0.088 seconds. This is a convenient interval to measure because it is 11 mm on the 125 mm/sec records.

The PR interval in Mirex-treated fetuses was considerably more variable

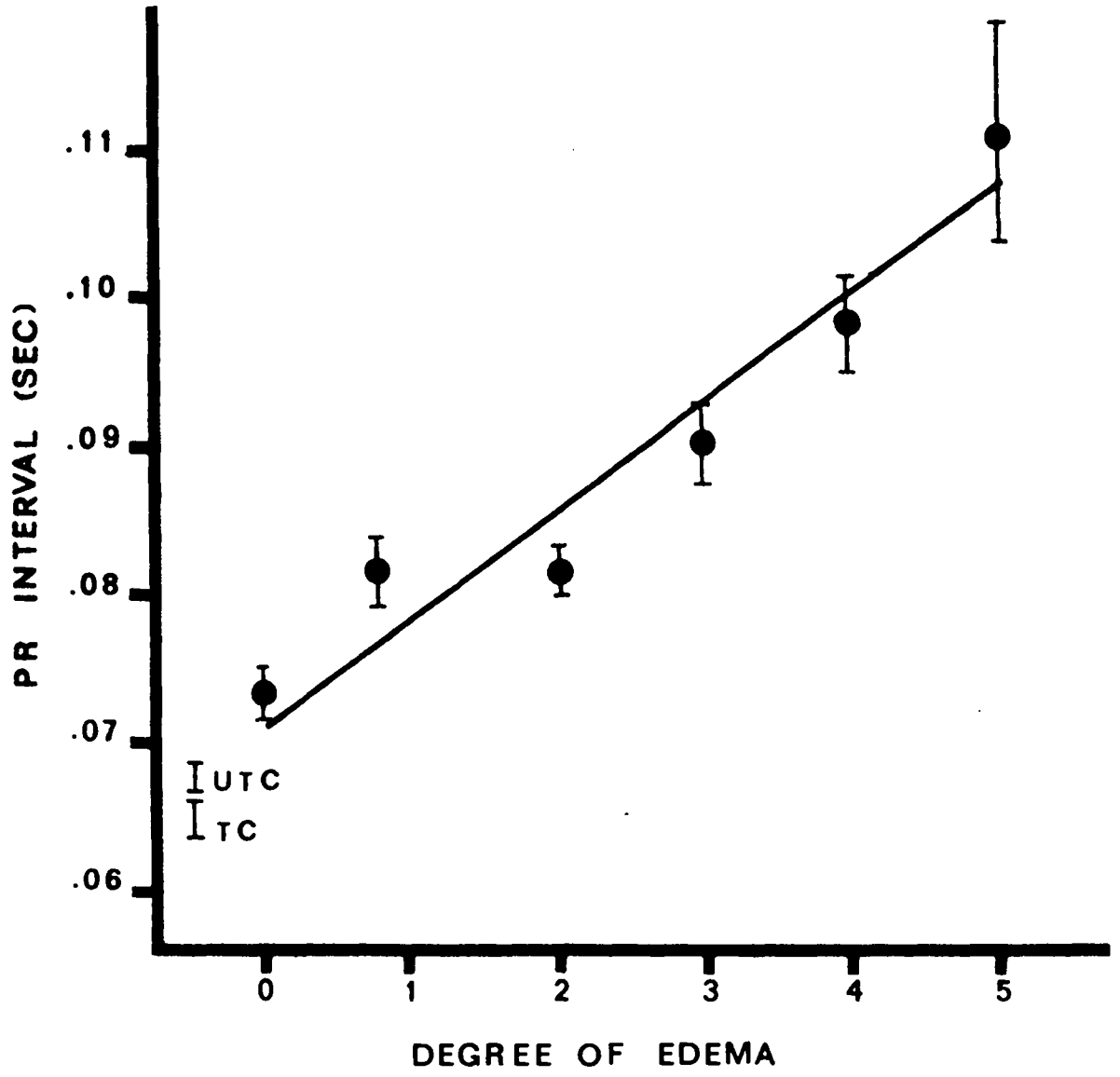

Fig. 6. Mean PR intervals (± SEM) in the ECG's of Mirex-treated fetuses with different degrees of edema.

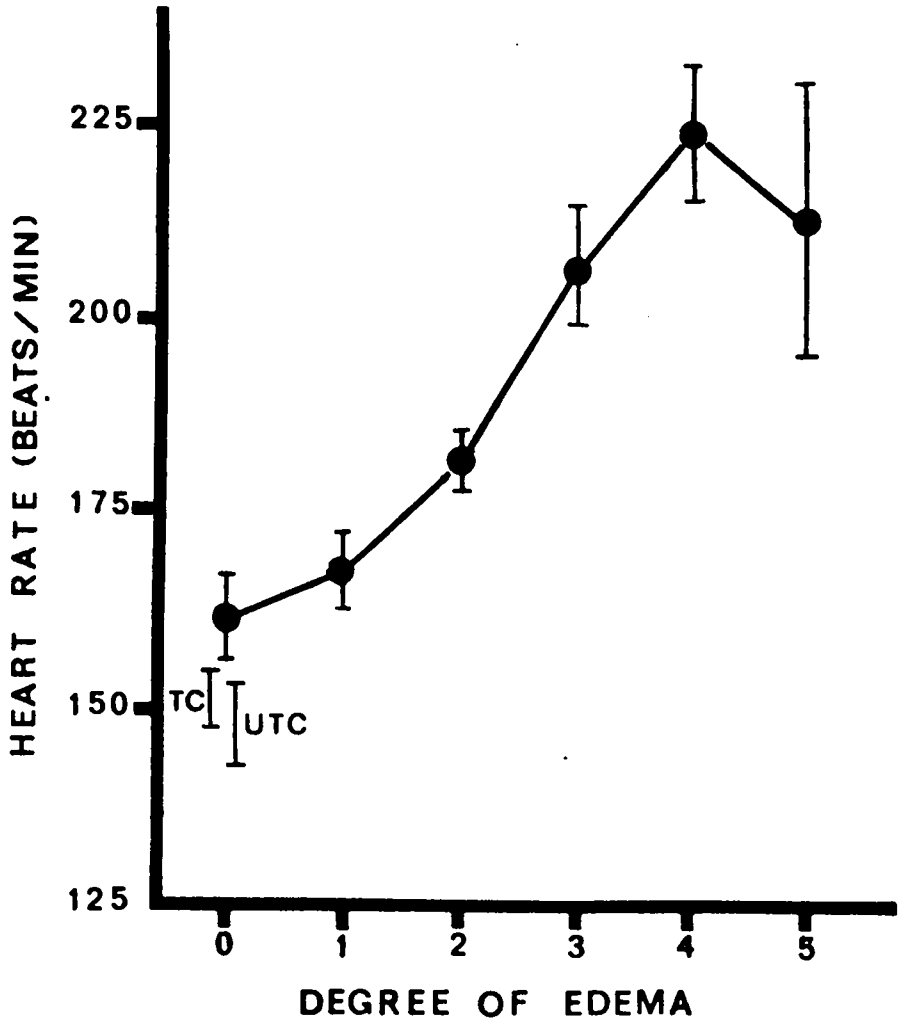

Fig. 5. The rate of heart beat (mean ± SEM) in Mirex-treated fetuses with different degrees of edema.

Abbreviations

TC, Treated controls

UTC, Untreated controls

than that of controls. Though in many of them the PR interval fell within the normal range, in others it was prolonged (figs. 8 and 9), sometimes up to 3-1/2 times normal. First degree heart blocks, using the criterion defined above, were common and related to both dose and degree of edema. The frequency of 1° heart block ranged from 20% in the 5 mg/kg group to 77% in the 10 mg/kg group (Table 3). An increase in mean PR interval was also dose-related (Table 3). There was a proportional relationship between PR intervals and the degree of edema present in the fetuses (fig. 6). The differences between the mean PR intervals of the ECGs of all edematous fetuses (1° through 5°) were significantly greater ($P < .001$) than those of oil fed controls.

Second degree heart blocks and other abnormalities. Some of the 1° heart blocks apparently led to a 2° block, in which a P wave is not followed by a QRS complex. This indicates that an atrial contraction has not been followed by a ventricular contraction. Such cardiac pathology occurred in from 4 to 10% of the groups of Mirex-treated fetuses. All of the cases of 2° heart blocks were Type I (Wenckebach), in which the missing beat is preceded by progressively longer PR intervals (fig. 9). Fetuses with 2° heart blocks were often rechecked for periods of up to 20 minutes after being removed from the uterus. The irregularities inevitably persisted.

A single case of atrial flutter or fibrillation was found in one of the 8 severely swollen (5°) fetuses (fig. 10). Several kinds of arrhythmias, presumably pacemaker problems, were found in the ECGs of 5 individuals scattered through the dosage groups (figs. 11a and b).

Morphological Observations. No gross differences were apparent in the size and proportion of the thoracic cavity in the dissected fetuses of treated and control groups. There were no grossly visible differences in the appearance of the heart. The serial sections through the control and two treated fetuses did not show any obvious differences between their hearts.

DISCUSSION

Mirex toxicity

The developmental toxicity of Mirex fed to pregnant rats has been well documented (Gaines and Kimbrough, '70; Khera et al. '76; and Chernoff et al. '79). Lowered pre- and postnatal viability, prenatal edema as well as a few anatomical abnormalities have been reported. Mirex-induced microphthalmos (4% in this study) had not been observed before. The major new observations of this study have been (1) a description of the Mirex-induced cardiovascular changes; and (2) a quantitation of the occurrence of edema. The scale of edema ratings, which fortuitously was adopted early in the study, eventually made feasible a number of correlations between edema and other problems. The maximum amount of edema which we observed in 18-1/2 day fetuses was never as extreme as that shown by Chernoff et al. ('79). This is due to the fact that the latter made observations on the 20th day of gestation and affected fetuses apparently continue to swell throughout gestation. We have noted a much higher incidence of edematous fetuses than either Khera et al. ('76) or Chernoff et al. ('79) even though our dosage was in the moderate range. This

Table 3

PR intervals and heart block in Mirex-fed rat fetuses

	Number	Mean PR interval in seconds ± SEM	1^{o} heart block	2^{o} heart block
Untreated Controls	34	.069 ± .0014		
Treated Controls	47	.065 ± .0009		
5 mg Mirex	93	.079 ± .0019	20%	4%
6 mg Mirex	60	.091 ± .0023	50%	10%
7 mg Mirex	49	.086 ± .0036	27%	4%
10 mg Mirex	13	.110 ± .0069	77%	8%

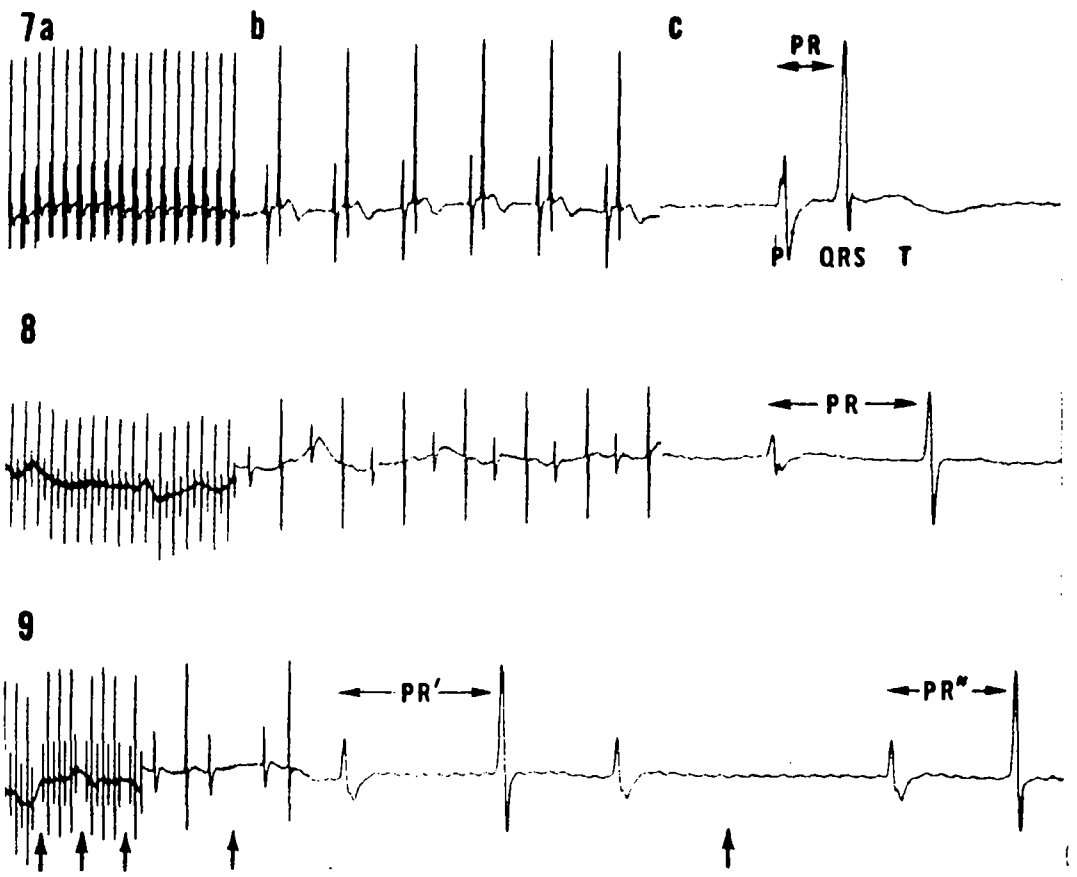

Fig. 7. Electrocardiogram of a normal 18-1/2 day rat fetus, taken at chart speeds of (a) 5 mm/sec; (b) 25 mm/sec; and (c) 125 mm/sec. The rate of heart beat was 172/min. The P, QRS, and T components are labeled in 7c, along with the PR interval (.064 sec). Actual size.

Fig. 8. Electrocardiogram of a Mirex-treated fetus (5mg/kg per day) showing a pronounced 1^{o} heart block. Note the extremely long PR interval (0.16 sec). Segments of the 5, 25 and 125mm/sec record are shown. The waviness in the base line is due to drift in the amplifier. Edema 2^{o}. Heart rate 168/min.

Fig. 9. Electrocardiogram of a Mirex-treated fetus (6 mg/kg per day) showing a 2^{o} heart block. The missing QRS complexes are indicated by arrows. Note that the two PR intervals, PR' and PR", are of different duration. Edema 4^{o}. Heart rate 210/min. Segments of the 5, 25, 125 mm/sec record are shown.

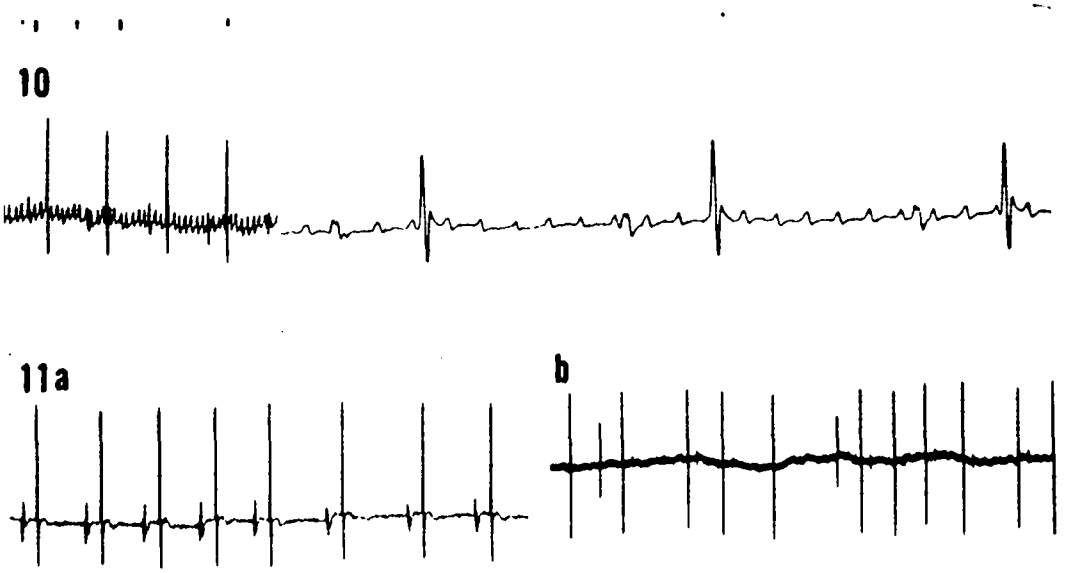

Fig. 10. Electrocardiogram of a Mirex-treated fetus (6 mg/kg) with either atrial flutter or fibrillation. The P waves are occurring at a rate of 36/second. Edema 5°. Heart rate 204/min. Segments of the 25 and 125mm/sec record are shown.

Fig. 11. Electrocardiograms of two treated fetuses with arrhythmia. (a) A mild irregularity in a fetus exposed to 6 mg/kg/day. Note the varied intervals between successive QRS waves. Edema 2°. Heart rate 228. Chart speed 25 mm/sec. (b) A very irregular beat due to an arrhythmia along with a 2° heart block in a fetus exposed to 10 mg/kg/day. Edema 5°. Heart rate 120. Chart speed 5 mm/sec. These ECGs illustrate the variety of conditions detectable by this method.

probably reflects our emphasis on edema as well as method of observation.

In addition to the edema, several kinds of cardiovascular problems were detected in Mirex-fed fetuses. Some of these, such as the arrhythmias, are probably benign. Others, such as 2° heart block and atrial flutter/fibrillation, are more serious and apparently are responsible for at least some of the high incidence of perinatal mortality induced by Mirex treatment (Grabowski, '79). These cardiovascular disturbances appear to be a significant facet of Mirex toxicity in the prenatal period.

The physiological basis for the Mirex-induced cardiovascular changes.

The most prominent cardiovascular symptoms observed were tachycardia and 1° and 2° heart blocks. It is difficult to ascribe a possible cause to these symptoms on the basis of human pathology. Among the many causes of tachycardia which could possibly be operative in this situation are anoxia, hypotension, and cardiac failure (Marriott and Myerburg, '70). There is no reason to suspect the first two, but the latter is a possibility. Chernoff et al. ('79) observed enlarged cerebral ventricles in Mirex-fed fetuses, and this could possibly result in increased intracranial pressure. However, increased intracranial pressure leads to bradycardia in adults rather than tachycardia. The list of agents which can induce heart blocks is also long and includes vagal stimulation, anoxia, ischemic heart disease, and hyperkalemia (Marriott and Myerburg, '70). Though rat fetuses are very susceptible to hyperkalemia, especially after exposure to hypoxia, (Chernoff & Grabowski, '71, Grabowski, '73), we have found only a slight elevation in serum potassium in Mirex-treated fetuses (5.1 mEq/l $\pm$ 1.4 in 18-1/2 day controls; 6.2 $\pm$ 1.6 in treated). This would seem to eliminate either hyperkalemia or anoxia as possible explanations of the observed data. Measurements of fetal blood pressure, P_{O_2} and blood volume will be necessary to decide between the remaining alternatives. Such studies are underway. The only clue available at the moment is that the correlation between the degree of visible edema observed and degree of tachycardia and prolonged PR intervals is too close to be totally spurious.

Prenatal edema and its consequences

Mirex was the primary subject of this investigation, but also, this was a study of fetal edema and its physiological effects. Embryonic and fetal edema can be induced by numerous agents in several groups of vertebrates as well as by genetic factors. Edema in the embryonic stage can have lethal and teratogenic effects due either to the distention of hollow organs during a critical period of development or to the mechanical effects of persistent hematomas near or within developing structures (Jost, '53; Giroud et al, '55; Grabowski, '70 and '77, and Jaffee, '74).

The significance of edema produced in the fetal stages is, in contrast, poorly understood and usually dismissed without comment. The present study shows that fetal edema is associated with significant cardiovascular effects. The enlarged cerebral ventricles in Mirex-fed fetuses (Chernoff et al. '79) are probably another manifestation of fetal edema. Such enlargements could affect the function of the central nervous system. Other hollow organs could

be similarly affected. Posner and Darr ('70) have suggested that the edema induced by the antihistamine, chlorcyclizine, is responsible for the high incidence of cleft palate produced by this agent because the swelling of facial tissues interferes with their morphogenetic movements.

Fetal edema in mammals is not uncommon. Some of the agents which induce conspicuous edema include chlorcyclizine (King et al. '65); thalidomide (Khera, '75); clamping of uterine blood vessels (Leist and Grauwiler, '74); carbon monoxide and vasopressin (Grabowski, unpublished). It is occasionally observed in human babies (Potter '61, Schaffer and Avery '77). Studies on the physical and chemical properties of fetal blood are under way which may help understand why mammalian fetuses are susceptible to this type of fluid disturbance.

The potential of fetal electrocardiography in developmental toxicity studies

The domain of teratology has been enlarged in recent years to include functional as well as anatomical disorders induced by various environmental factors (Wilson '73). Suitable procedures for the systematic detection and evaluation of functional disorders in experimental animals are not, as yet, common. Fetal electrocardiography could become a relatively simple, standardized procedure for detecting functional disorders of the cardiovascular system. Numerous specific disorders can be detected and measurements of the rate of heart beat, whether feeble or excessive, could give some indication of the general state of health of the fetus. It can also be applied to newborns (Grabowski, '79). The ECG equipment required is neither expensive nor esoteric. The skill required is not beyond the reach of a good technician. A litter can be tested in about half an hour, and the individuals could still be subsequently used for necropsy or skeletal analysis. This method could be a valuable adjunct to traditional procedures.

LITERATURE CITED

Chernoff, N., and Grabowski, C. T., 1971 Physiological responses of the rat fetus to maternal injections of epinephrine and vasopressin. British J. Pharmacol., 43: 270-278.

Chernoff, N., Linder, R. E., Scotti, T. M., Rogers, E. H., Carver, B. D., and Kavlock, R. J. 1979 Fetotoxicity and cataractogenicity of Mirex in rats and mice with notes on Kepone. Environmental Research, 18: 257-269.

Gaines, T. B., and Kimbrough, R. D. 1970 Oral toxicity of Mirex in adult and suckling rats. Arch. Environ. Health, 21: 7-14.

Giroud, A., Lefebvres, J., Prost, H. and Dupuis, R. 1955 Malformations des membres due a des lesions vasculaires chez le foetus de rat deficient en acide pantothenique. J. Embryol. Exp. Morphol., 3: 1-12.

Grabowski, C. T. 1964 The etiology of hypoxia-induced malformations in the chick embryo. J. Exp. Zool., 157: 307-326.

Grabowski, C. T. 1970 Embryonic oxygen deficiency -- a physiological approach to analysis of teratological mechanisms. Adv. Teratol., 4: 125-169.

_____ 1973 Fetal cardiac physiology and hypoxia-induced hyperkalemia. Teratol. 7: A16.

_____ 1977 Altered electrolyte and fluid balance. Handbook Teratol., 2: 153-170. eds. Wilson and Fraser.

_____ 1978 ECG analysis of effedts of Mirex on cardiovascular physiology of rat fetuses. Teratol., 17: 34A.

_____ 1979 An analysis of the causes of perinatal deaths induced by prenatal exposure to Mirex. Teratol., 19: 27A.

Grabowski, C. T., and Schroeder, R. E. 1968 A time-lapse photographic study of chick embryos exposed to teratogenic doses of hypoxia. J. Embryol. exp. Morph., 19: 347-362.

Grabowski, C. T., Tsai, E. N. C., and Toben, H. R. 1969 The effects of teratogenic doses of hypoxia on the blood pressure of chick embryos. Teratol., 2: 67-76.

Grabowski, C. T., and Tunstall, A. C. 1977 An electrocardiographic study of rat fetuses treated with Trypan blue. Teratol., 15: 32A.

Jaffee, O. C. 1974 The effects of moderate hypoxia and moderate hypoxia plus hypercapnea on cardiac development in chick embryos. Teratol., 10: 275-282.

Jost, A. 1953 La degenerescense des extremites du foetus de rat sous des actions hormonales (acroblapsie experimentale) et la theorie des bulles myelencephaliques de Bonnevie. Arch franc. Pediat., 10: 865-870.

Khera, K. S. 1975 Fetal cardiovascular and other defects induced by Thalidomide in cats. Teratol., 11: 65-72.

Khera, K. S., Villeneuve, D. C., Terry, G., Panopio, L., Nash, L., and Trivett, G. 1976 Mirex: a teratogenicity, dominant lethal and tissue distribution study in rats. Fd. Cosmet. Toxicol., 14: 25-29.

King, C. T. G., Weaver, S. A., and Narrod, S. A. 1965 Antihistamines and teratogenicity in the rat. J. Pharmacol. Exp. Ther., 147: 391-398.

Leist, K. H., and Grauwiler, J., 1974 Fetal pathology in rats following uterine-vessel clamping on day 14 of gestation. Teratol. 10: 55-67.

Litman, B. 1972 Electrocardiography, sixth edition, Yearbook Medical Publishers, Inc., Chicago.

Marriott, H. J. L., and Myerburg, R. J. 1970 Recognition and Treatment of Cardiac Arrhythmias and Conduction Disturbances. Ch. 32C in The Heart, J. W. Hurst and R. B. Logue, eds. McGraw-Hill Book Co., New York, pp 489-536.

Potter, E. L. 1961 Pathology of the Fetus and Infant, second edition. Year Book Medical Publishers, Inc., Chicago.

Posner, H. S., and Darr, A. 1970 Fetal edema from benzhydrylpiperazines as a possible cause of oral-facial malformations in rats. Toxicol. Appl. Pharmacol. 17: 67-75.

Schaffer, A. J., and Avery, M. E. 1977 Diseases of the Newborn, fourth

edition. W. B. Saunders Company, Philadelphia.

Turbow, M. W. 1966 Trypan blue-induced teratogenesis of rat embryos cultivated *in vitro*. J. Embryol. Exp. Morph. 15: 387-396.

Wilson, J. G. 1973 Environment and Birth Defects, Academic Press, New York.

PART II

AN ANALYSIS OF THE CAUSES OF PERINATAL DEATHS INDUCED BY PRENATAL EXPOSURE TO MIREX

ABSTRACT

Electrocardiograms of normal and Mirex exposed rats (6 mg/kg/day of insecticide on days 8 to 15 of gestation) were obtained on day 21-1/2 of gestation, at birth and for several days afterwards. Data on 252 control fetuses and newborns were compared with that on 219 treated fetuses. On day 21-1/2, a high incidence of cardiovascular problems was found in treated fetuses, e.g. 21% first degree heart blocks 2% second degree heart blocks, and 6% third degree blocks, as well as a fair number (24%) of dead and dying individuals. Of those that were allowed to be born, every attempt was made to obtain ECGs within 5 min. of birth. Pups were then weighed and tattooed so that individuals could be followed to day 5. Of the treated pups, 27% were stillborn, 17% died within 6 hours of birth, another 15% within 48 hours. At birth 20% had first degree heart blocks, and 4% had second degree blocks. All those with second degree blocks died shortly after birth. Some of the other deaths were also correlated with cardiovascular problems. Respiratory problems were also common and 4% died from failure to breath. Cardiovascular and respiratory problems are responsible for the majority of the high incidence of perinatal deaths induced by prenatal exposure to a moderate dose of Mirex.

INTRODUCTION

Mirex is not a potent teratogen in the classic sense, since even high doses induce only a low incidence of visible malformations. However, this pesticide does induce a very high rate of perinatal mortality (Gaines and Kimbrough, 1970; Khera et al. 1976; Chernoff et al. 1979). Grabowski and Payne (1980) found a high incidence of cardiovascular problems, diagnosed by electrocardiogram, in 18-1/2 day fetuses exposed to Mirex. In the present study, an attempt was made to determine if these problems persisted into the perinatal period and to see if they help to explain the deaths induced by exposure to a moderate dose of Mirex (6mg/kg). A positive correlation was found.

MATERIAL AND METHODS

Long-Evans rats obtained from Blue Spruce Farms, Altamont, New York were used. Animals were maintained at 24°C $\pm$ 1°, 40-60% relative humidity and on a 12 hour daylight period. They were housed in polypropylene cages on Sanicel bedding and fed Purina Lab Chow and water ad libitum. Virgin females in estrus, as determined by vaginal smear, were housed overnight with males and examined the following morning for the presence of sperm in the vagina. Rats were considered a half-day pregnant on the morning they were found to be sperm-positive.

The Mirex used was of commercial purity and was obtained through the Mississippi State Laboratory, Mississippi State University. It was dissolved in peanut oil and fed to the rats by gastric intubation. Treated rats were intubated daily with 6mg per kg Mirex on 1/2 cc peanut oil. This treatment was given on days 8-1/2 and 15-1/2. The dosage for each rat was based on their weight the day before the feeding regimen was started. Controls were given either no treatment or 1/2 cc plain peanut oil on days 8-1/2 - 15-1/2. Daily records of weight and food intake were kept.

On day 21-1/2 of pregnancy, that is the morning before the pups were supposed to be born, some control and treated rats were subjected to laparotomy and pups tested as previously described (Part I; Grabowski and Payne, 1980).

Every attempt was made to get an ECG record on pups as soon as possible after birth and also to get an accurate record of stillborns before maternal cannibalism could possibly occur. This was accomplished by frequently observing the mothers on day 21-1/2 until birth began. Occasionally we would take a full term rat home at night and bring her back to the laboratory after parturition had started. One or two hours might elapse before the first pups were checked in those cases, but most of the time the pups were checked within 5 min. of delivery.

Initially, we felt the only feasible way to get the electrocardiograms from active newborns was to anesthetize them with a small amount of sodium pentathol injected subcutaneously. This automatically became a terminal experiment because the mother would refuse to re-accept the babies and would cannibalize them. The anesthetized pups, however, made it possible to get quality records with all three leads, I, II, III. Midway through the study we found that ECGs could be obtained from unanesthetized pups by placing the electrodes across their backs while they were in a prone position on their abdomens, making skin contact with the aid of electrode gel, and working rapidly during brief periods of inactivity. The records obtained in this manner are not as neat and clean as those from anesthetized rats because the movement of the animal interferes, but they are adequate for analysis. Only recordings from Lead II were attempted. Electrocardiograms of the newborn were obtained shortly after the mother had separated the placenta, which usually occurred with 2-5 minutes after birth. The pups were weighed after the recordings were made, tattooed in a standardized pattern on the hips, and then returned to the female after the whole litter was checked. Electrocardiograms and weights were also checked on days 1, 2 and 5. Weights were checked on days 15 and 30. The tattoo marks lasted several days and before they were covered by fur, the saddle markings became apparent. Sketches were made of these so that throughout the observation period each specific individual could be identified.

This study is based on observations of 161 live newborns obtained from control litters, 116 treated newborns (Table 1), and 128 fetuses at day 21-1/2 (78 controls and 50 treated) (Table 6). Differences between untreated and oil fed controls were negligible and hence all data from controls were treated as a single group. Statistical comparisons were made using the Student "t" test.

RESULTS

Newborns. The mean rate of heart beat in the 161 controls (both groups) was 256 beats per minutes and the mean PR interval as obtained from the ECG tracings was 0.061 seconds (Fig. 2; Table 2). Two types of arrhythmia were found. In one type, the one or two beats associated with the peak of a breathing effort were displaced, usually occurring somewhat prematurely (Fig. 1a). These anomalous beats could be readily correlated with breathing activity because respiratory activity shows up clearly on the ECG record. In the other type of arrhythmia, the rate of heart beat would

Table 1

Summary of Newborn Data

	Untreated Controls	Oil-fed Controls	Mirex Treated
Mothers			
No. of litters attempted	10	6	24
Not pregnant	2	0	5
Died during treatment	0	0	4
No. of litters delivered	8	6	15
Newborns			
No. Alive	86	75	116
No. Stillborn	3	0	32

Table 2

Comparison of control and Mirex-treated newborns during the first 5 postnatal days

		Newborn	Day 1	Day 2	Day 5
Weighing grams	(Control)	5.71±.53	6.44±1.15	7.34+.75	10.57±1.19
	(Treated)	5.6±.63	6.1±.69	6.5±.78	10.7±1.88
Heart rate beats/min	(Control)	256.42±37	330±30	359±25	345±49
	(Treated)	221±38	302±27	341±26	342±19
PR interval seconds	(Control)	.061±.0078	.053±.0048	.050±.0048	.051±.0080
	(Treated)	.078±.0192	.054+.0032	.049±.0046	.047±.0014

Means ± S.D. The number of newborns was 161 in the control groups (both untreated and oil fed) and 116 in the treated. There were between 40 and 60 individuals in each of the day 1 to 5 groups.

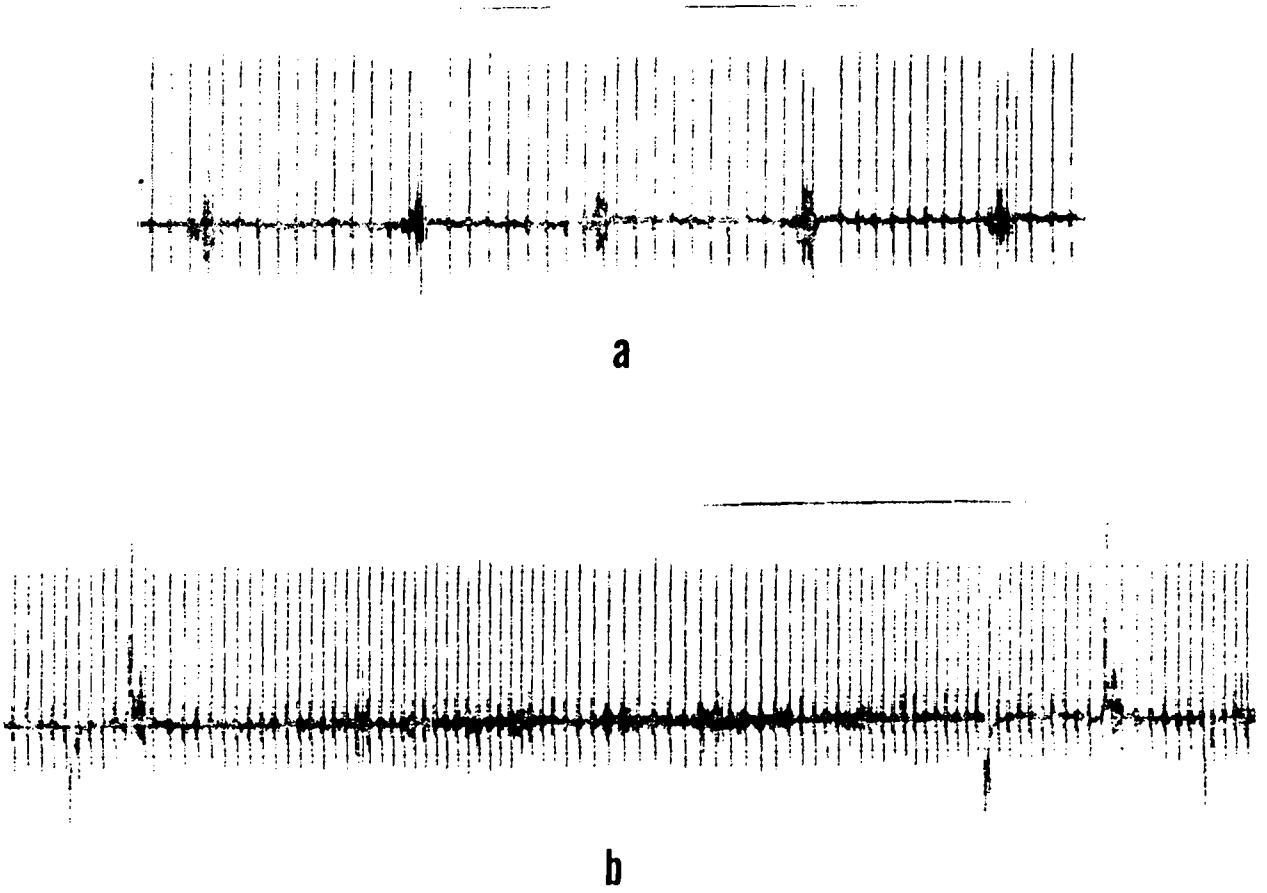

Figure 1. Arrhythmias found in newborn control rats. (a) Misplaced beats with breathing efforts. Note also that breathing movements (arrows) are clearly evident in the ECG tracing. (b) Changes in rate of heart beat at 5 to 15 second intervals. The rates vary in this segment from 144 to 216 beats per min. Both segments taken at 5mm/sec chart rate.

speed up or slow down somewhat for periods of 15 to 45 seconds (Fig. 1b). These arrhythmias occurred in about 15% of the control animals. Whenever the neonates were rechecked, it was found that a spontaneous remission would occur 15 to 40 minutes after birth.

Two untreated controls were obviously in distress when born. The heartbeat was 40 beats per minute in one, and 27 and irregular in the other. PR intervals of these individuals were, respectively, 0.16 sec and 0.27 sec, easily classified as severe first degree heartblocks. The first died within 30 minutes of birth and the other within 34 minutes of birth. Data on these two individuals were not included with the other controls in the calculations of mean heart rate and PR intervals, since one of the functions of those particular calculations was to determine the norm of healthy individuals. These two were obviously very unhealthy.

Changes in heart rate and ECGs during the first 5 days. The heart rate and the ECG characteristics of controls become more stable within the first 12 to 24 hours after birth. The mean heart rate in the controls increased to 330 beats per min on the day after birth. The PR interval is much shorter than that of newborns and the standard deviation is narrower. All these features indicate a stabilization from the trauma of being born. No deaths occurred and no abnormalities of ECG whatever were found in members of this group. Neither heart rate nor PR intervals changed in any appreciable fashion between days 1 to 5 (Table 2).

Electrocardiograms of Mirex-Treated Individuals

There were 24 females treated with Mirex but 5 turned out to be not pregnant and 4 died during the treatment. A total of 148 individuals were born to the remaining 15 mothers (Table 4). Thirty-two of these were stillborn. All of them apparently died within less than a day before birth. In some of these the heart could still be stimulated to contract by pinching it with tweezers, indicating a very recent death. The mean weight at birth of the 116 born alive was just slightly lower than that of controls (Table 2). The mean rate of heartbeat was 220 beats per min., significantly lower than that of the controls. The mean of the PR intervals of the treated was .078 sec., appreciably greater than that of controls. The range of variation was also much greater. The criteria for first degree heart block were calculated by the same method as that used for 18-1/2 day fetuses (Grabowski and Payne 1980; Part 1 of this report). I.e. any individual with a PR interval greater than 0.084 sec, which is the mean of the healthy controls, 0.061 sec, plus 3 standard deviations of 0.0078 sec., was considered to have a first degree heart block. This interval, 0.084 sec., was a convenient one to measure because it is 10.5 mm on the ECG tracing taken at 125 mm/sec. None of the PR intervals of the controls (except for the two dying newborns) exceeded this figure but 23 of the Mirex-treated newborns did (Figs. 3 and 5; Table 3).

Seven newborns had second degree heart blocks (Fig. 4). None of

Table 3

Cardiac Abnormalities in Mirex-treated Newborns at Birth

Conditions	No.	%
First degree heart block*	23**	20.0
Second degree heart block	4**	3.5
Premature atrial contractions	1**	0.9

* PR interval of .084 sec or more, i.e. mean of controls plus 3 standard deviations.

** Out of total of 116 live newborns.

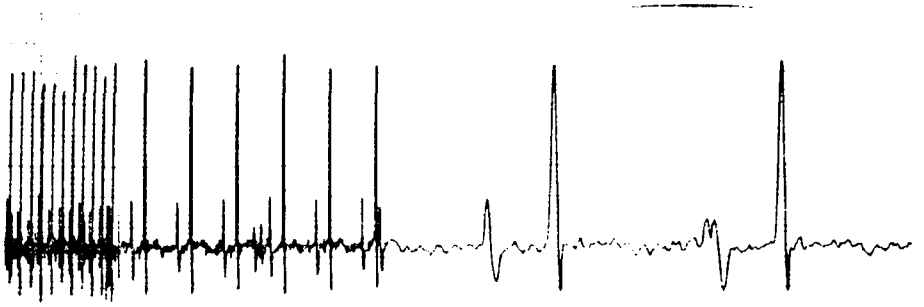

Figure 2. Electrocardiogram of a control newborn, 5 min. old. Rate of heart beat, 228. Segments of the ECG were taken at (a) 5, (b) 25, and (c) 125mm/sec.

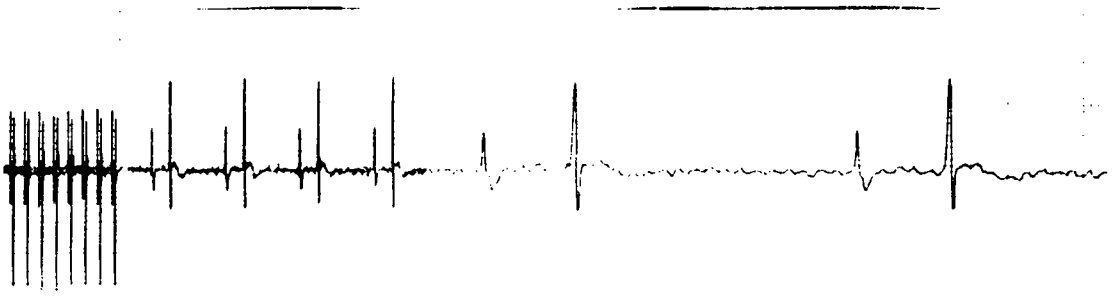

Figure 3. ECG of a Mirex-treated newborn 4 min. old, showing a firse degree heart block. HR, 162 beats/min. Segments taken at different speeds as in Fig. 2.

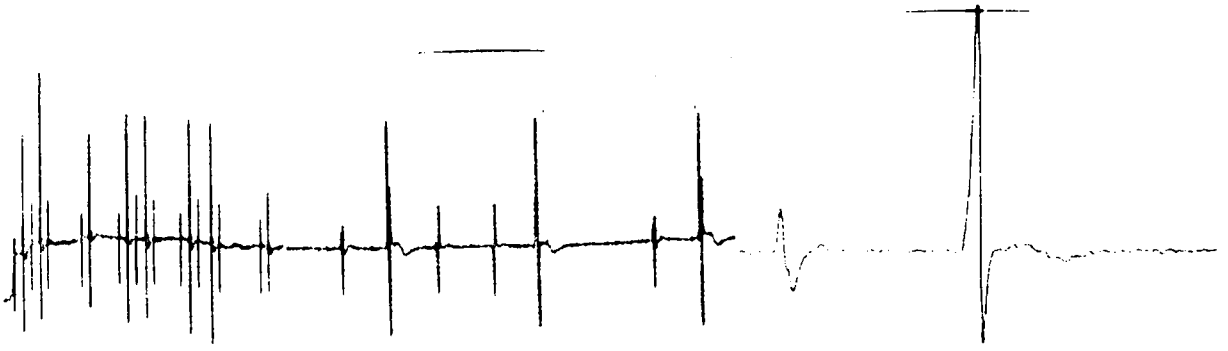

Figure 4. ECG of a Mirex-treated newborn, 5 min. old, showing severe second degree heart block. HR, 84-114 beats/min. Note the greatly prolonged PR interval. The missing QRS complexes are indicated by arrows. The block persisted until the pups died, 35 min. after birth. Segments taken at different speeds as in Fig. 2.

these seven recovered and all died within 60 minutes after birth. One individual was born with premature atrial contractions which disappeared within 30 minutes after birth. Four individuals had inverted QRS complexes (Fig. 5). Two of these had a normal ECG by 15 minutes after birth, but the other two died without recovery.

Observations on treated neonates for several days after birth. Just as with the controls, the majority of newborns stabilized by day one and the heart rates and PR intervals remained more or less constant through day five. The ECG characteristics were very close to those of the control group. The mean weights of the treated group were not significantly different than those of controls over the five-day period (Table 2).

Two individuals, both from the same litter, developed classic hydrocephaly after birth. This was first noted when they were two weeks old. The condition progressively became more prominent and both were sacrificed and autopsied on day 45. The cranium was greatly enlarged in both and the cerebral cortex was reduced to a very thin layer, only a millimeter thick. In keeping over 1,000 individuals to approximately 2 months of age, no control individual ever displayed this condition. One rat, prenatally exposed to carbon monoxide, developed a comparable case of hydrocephaly.

Causes of Postnatal Death

A major goal of this study was to determine the causes of the high perinatal death rate so characteristic of Mirex treatment. A number of correlations were possible in the postnatal period. In this section, only the 65 treated newborns which were not anesthetized could be compared, because the anesthetized pups were killed by their mothers.

Eleven individuals died within a few hours of birth (Table 4). Four individuals never respired. This was evident from their ECG records as well as by visual observations. These four were obviously doomed to die. Two of these also had second degree heart blocks at birth, one had a first degree heart block and the fourth had a normal ECG at birth, although all developed progressively more severe heart blocks as the hypoxic stress increased (Fig. 6). Three other newborns who did respire had second degree heart blocks at birth (Fig. 4). None of these three gave any indication of recovery from the blocks and all died within sixty minutes after birth. One individual had a moderate first degree heart block and died later. One individual had an umbilical hernia. It's ECG was normal and the heart beat was regular and strong, but it was cannibalized by its mother a few hours after birth. It is impossible to say whether it died of natural (i.e. internal) causes or from maternal action. Two individuals died within three hours after birth without apparent cause, at least their electrocardiograms and respiratory rates were perfectly normal at the time they were checked (Table 5).

It is obvious that some of these premature deaths were associated

Table 4

Perinatal Mortality

	Controls*	Mirex Treated
Before birth	3/164 (1.8%)	32/148 (22%)**
Within 6 hours after birth	2/161 (1.2%)	11/65 (17%)***
Between 6 to 24 hours	0	6/54 (11%)
Between 1 to 2 days	0	2/48 (4%)
Between 2 to 5 days	0	0

* Both Untreated and oil fed controls
** The total pool of Mirex-treated newborns
*** Only the 65 unanesthetized pups were counted in this and subsequent groups

Table 5

Symptoms Prior to Death in 65 Treated Newborns

Day 0	Failure to begin respiration	4
	Second degree heart block	3
	First degree heart block	1
	Cannibalization (umbilical hernia)	1
	No symptoms apparent	2
Day 1	First degree block at birth	1
	No symptoms apparent	5
Day 2	Abnormally long QT interval on day 1	1
	No apparent cause	1

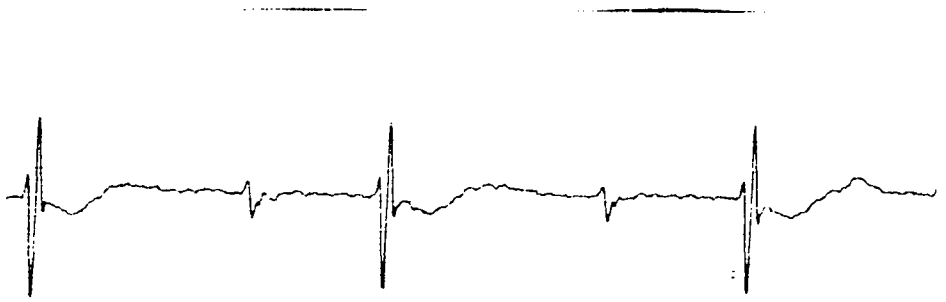

Figure 5. ECG of an Mirex-treated newborn, 30 min. old. Showing first degree heart block and inverted QRS complexes. Heart rate 96/min. Segment at 125 mm/sec. This pup died 65 minutes after birth.

Figure 6. ECG of a Mirex-treated newborn, 4 min. old. No respiration started and the heart beat is feeble and erratic. Segments at (a) 5 mm/sec and (c) 125 mm/sec.

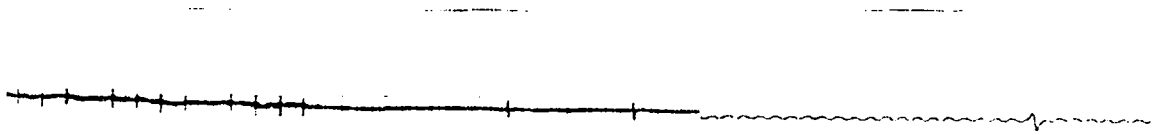

Figure 7. ECG of a Mirex-treated fetus at 21-1/2 days of gestation. Probably a third degree heart block with only feeble P waves present.

with the observed cardiovascular symptoms. The abnormal ECGs may indicate that a serious cardiovascular disorder existed in these newborns and was a primary cause of death, or simply by a secondary reflection of some other illness.

Severe edema was not associated with these postnatal deaths since grossly swollen fetuses were never born alive. Some of those that died later did have some visible edema, but other sick animals did not appear to have any.

Respiratory problems were a major cause of postnatal deaths. Four pups totally failed to begin respiration. Numerous others appeared to have difficulty initiating respiration, something rarely observed in the controls. Two individuals were observed to have a period of respiratory distress about 4 to 6 hours after birth, ie. they were visibly cyanotic and gasping for breath, but they did recover.

Lungs were preserved and sectioned from three pups that died between 4 to 24 hours after birth without any previous history of cardiovascular symptoms or respiratory distress that was observed. There was considerable hemorrhage in the tissues and the majority of the alveoli (about 75%) were collapsed. There was just a suggestion of hyaline membranes. However, the tissues displayed considerable edema and a pediatric pathologist suggested that alveolar collapse occurred due to "edemic congestion". It is possible that other pups also died from respiratory distress that did not happen to be observed.

Six individuals died between 6 to 24 hours after birth. Only one of these had a prior history of any kind. It had a 1^{o} heart block at birth. An additional two individuals died on day two. One of these had an abnormally long QT interval, about 4 times normal, the day previous to death. This may be a significant observation because an abnormal QT interval is thought to be one of the causes of premature infant death in humans. In our experience testing the ECGs of several hundred fetuses, only one other individual with such a long QT interval was found. This was in an 18-1/2 day fetus that had been prenatally treated with Trypan Blue.

Prenatal Observations

Many of the newborns looked as if they had died during or just prior to delivery. To confirm this, a number of observations were made of individuals examined on the morning of day 21-1/2 of gestation, ie. about 6 to 18 hours before birth would have occurred. The fetuses from 4 litters of untreated controls and 5 litters of oil-fed controls were compared to those from 11 Mirex-fed mothers (Table 6). Again the differences between the two control groups were inconsequential e.g the mean heart rate in untreated controls was 185 $\pm$ 33.5 SD beats per min. and 179 $\pm$ 34.3 in oil-fed controls. Therefore the data from the two control groups are considered together.

Table 6

Mortality and ECG Data in Fetuses

Examined on Day 21-1/2 of Gestation

	Controls	Mirex-treated	
	No.	No.	%
Number of fetuses	78	103	
Number dead	0	18	18%
Number alive	78	85	
First degree heart block	0	18	21%*
Second degree heart block	0	2	2%*
Third degree heart block	0	5	6%*

* out of the 85 living

There were no dead or dying within the group of the 78 control fetuses. Their mean PR interval was .067 sec $\pm$ .0079 (SD). Of the 93 fetuses recovered from treated mothers, 18 were dead. All nine members of one litter were dead, the other nine were from two other litters. None of the 18 appeared morphologically retarded compared to their litter mates, hence could not have been dead for more than 1-2 days. Though early resorptions were counted, they are not being cited here because the aim of this experiment is to determine perinatal death rate.

The incidence of cardiovascular problems detected in the ECGs was high. Eighteen of the 85 living fetuses had a first degree heart block, i.e. the PR interval was greater than .090 seconds (the mean of the controls, 0.067 sec + 3 S.D.s of .0079 sec). Two fetuses had a second degree heart block. An additional 5 fetuses seemed to be on the verge of death. Their heart rates were very slow (10 to 80 beats per min.), feeble, irregular, and typically consisted of only one component, presumably P waves (Fig. 7). This could be either a third degree heart block or an advanced stage of heart failure. It is doubtful that this latter group in particular would have survived the rigors of birth.

DISCUSSION

Cardiovascular Problems and Perinatal Death

This study was an attempt to analyze the factors which are directly responsible for the high rate of perinatal mortality induced by the pesticide Mirex. It is also a study in functional teratology.

Gaines and Kimbrough (1970) found high levels of pre- and post-natal deaths in rats after extended exposure to low levels of Mirex. Litter size was reduced by about 25% in females fed 25 ppm Mirex mixed with their food (about 1.8 to 2.8 mg/kg/day) throughout gestation and lactation. Of those born alive, only 53 to 61% survived to weaning. Khera et al. (1976) found prenatal death rates of 8.4% after rats were fed 6.0 mg/kg/day Mirex on days 6 to 15 of gestation (compared to 3.7% in controls) and 40% after 12.5 mg/kg/day. Chernoff et al. (1979) found prenatal death rates ranging from 16.8% after exposure of rats to 7 mg/kg/day to 100% after exposure to 38 mg/kg/day. In the present study of rats prenatally exposed to 6 mg/kg/day of Mirex, 19% were dead just before birth and another 6% appeared on the verge of death. This is in agreement with a stillborn rate of 22% of those allowed to go to birth.

Of those born alive, 17% died within a few hours after birth and an additional 15% by 2 days after birth. A scattering of morphological abnormalities have been described in all of these studies, but, apart from edema, these are not sufficiently frequent or severe to account for these death rates.

A clue to a possible cause of these late prenatal and early postnatal deaths was found in the studies described in Part I of this report (see

also Grabowski and Payne, 1980). In 18-1/2 day fetuses exposed to moderate doses of Mirex (5 to 7 mg/kg/day) the incidence of first degree heart block ranged from 20 to 50% and the incidence of second degree heart block was 4 to 10%. This high incidence of cardiovascular problems, which was very closely correlated with degree of visible edema, could very well affect perinatal health and vitality. The ECG study of fetuses at day 21-1/2 showed that these cardiovascular symptoms persist up to birth at about the same frequency. In addition, by day 21, there were significant numbers of dead and dying fetuses.

Most of the newborns (20%) who were born with first degree heart block recovered during the first day after birth. Some of these did die, indicating that this disorder, which is not necessarily serious in itself, was at least indicatory of a health problem. A second degree block is more serious, and all pups who were born with this problem died within an hour of birth. Therefore, it is apparent that the cardiovascular problems which were evident at 18-1/2 days do persist into the perinatal period, and are correlated with some perinatal death.

The cause of the first and second degree heart blocks induced by Mirex is problematical. None of the usual explanations for these problems in adults, such as hyperkalemia and hypoxia, seem to apply (see Discussion, part 1). Though the mean PR interval at 18-1/2 days was very closely correlated with degree of edema, this is not quite as apparent during the perinatal period. This is partly due to the fact that the grossly swollen individuals die before or during birth. Many of the newborns which showed moderate and severe cardiovascular symptoms had little subcutaneous swelling evident.

Respiratory Distress

A surprising discovery was that treated newborns exhibited a fairly high incidence of respiratory distress. Four pups (4%) failed to begin respiration. It is perhaps significant that two of these four also had second degree heart blocks immediately after birth, evident before the respiratory failure could have induced sufficient hypoxia to account for this symptom. Numerous other treated newborns had difficulty in initiating respiration. Two others recovered from classic symptoms of Respiratory Distress Syndrome (RDS) several hours after birth. Three others that died between 4 to 24 hours after birth without any symptoms that were observed showed lung collapse due to edemic congestion. Some of the others which died during the first day after birth may have done so from RDS which was detected.

It would appear that one of the consequences of fetal edema is pulmonary congestion which can lead to respiratory distress at birth or shortly thereafter.

The ECG Technique and Perinatal Studies

This technique was very useful in this Mirex study. Objective evaluations of cardiac activity, ie. rate of heart beat, PR intervals, heart blocks etc., were readily obtained in permanent record form which could be examined at leisure, and re-examined if desired. A record of breathing activity was also obtained in the newborns with no extra effort. Both sets of observations yielded valuable data in evaluating the developmental toxicity of Mirex. The only difficult phase of this procedure was waiting for the dams to give birth, which usually occurred at night. Keeping the animals in a room in which the duirnal cycle was reversed, a relatively simple procedure, would help to ensure that most of them would go into parturition during a normal work day.

The ECG technique also provided clear documentation of functional problems induced by Mirex which do not have any readily apparent morphological basis, i.e. examples of functional teratology. Whether the physiological problems described have permanently affected the survivors has not yet been ascertained. However, it is clear that many affected individuals were killed by these functional problems and others nearly so.

The Developmental Toxicity of Mirex

This study extends further the observations of others that Mirex induces a high rate of perinatal death. The moderate dose of Mirex used, 6 mg/kg/day on days 8 to 15, induced a prenatal death rate of 22-25% and a postnatal rate of 32%. It is noteworthy that many of these deaths would normally be missed in standard studies. Most necropsy studies on rats are performed on day 20-1/2 of gestation. These would miss the prenatal mortality that occurs just before and during birth, as well as the postnatal deaths. Even routine postnatal studies would miss some dead pups because of maternal cannibalism (see e.g. Khera and Ruddick 1973.

This study also provides evidence that most of these perinatal deaths are related to either (1) cardiovascular problems, or (2) respiratory failure. The evidence that the cardiovascular problems are associated with the observed edema is circumstanial, but strong. The respiratory failure seems also to be associated with pulmonary edema, it would appear, then that knowing the basis for the development of fetal edema will be necessary to more fully understand the developmental toxicity of Mirex and the numerous other agents which induce this problem (Grabowski 1977).

LITERATURE CITED

Chernoff, N., Linder, R. E., Scotti, T. M., Rogers, E. H., Carver, B. D., and Kavlock, R. J. 1979 Fetotoxicity and cataractogenicity of Mirex in rats and mice with notes on Kepone. Environmental Research, 18: 257-269.

Gaines, T. R., and Kimbrough, R. D. 1970. Oral toxicity of Mirex in adult and suckling rats. Arch. Environ, 21: 7-14.

Grabowski, C. T. 1977 Altered electrolyte and fluid balance. Handbook Teratol. 2: 153-170, eds: Wilson & Fraser.

Grabowski, C. T., and Payne, D. B. 1980 An Electrocardiographic study of cardiovascular problems in Mirex-fed rat fetuses. Teratol. (in press)

Khera, K. S., and Ruddick, J. A. 1973 Polychlorodibenzo-p-dioxins: Perinatal Effects and the Dominant Lethal Test in Wistar Rats. In: Chlorodioxins-Origin and Fate, E. A. Blair, ed. Adv. in Chemistry Series, No. 120 pp. 70-84.

TECHNICAL REPORT DATA

(Please read Instructions on the reverse before completing)

1. REPORT NO.	2.	3. RECIPIENT'S ACCESSION NO.
EPA-600/1-80-032		PB81 104739

4. TITLE AND SUBTITLE
Pesticide Effects on Prenatal Cardiovascular Physiology.

5. REPORT DATE
SEPT. 1980 Issuing Date.

6. PERFORMING ORGANIZATION CODE

7. AUTHOR(S)
Casimer T. Grabowski, Ph.D., Professor of Biology,
Dept. of Biology, Univ. of Miami, Coral Gables, Fla.

8. PERFORMING ORGANIZATION REPORT NO.

9. PERFORMING ORGANIZATION NAME AND ADDRESS
Department of Biology
University of Miami
Coral Gables, Florida 33124

10. PROGRAM ELEMENT NO.
1LA629

11. CONTRACT/GRANT NO.
R-805301010

12. SPONSORING AGENCY NAME AND ADDRESS
Environmental Protection Agency (Neil Chernoff)
Health Effects Research Laboratory
Environmental Research Center
Research Triangle Park, NC 27711

13. TYPE OF REPORT AND PERIOD COVERED

14. SPONSORING AGENCY CODE
600/11

15. SUPPLEMENTARY NOTES
Project Officer: Neil Chernoff

16. ABSTRACT

Sperm-positive rats were intubated with Mirex in oil (5 to 10 mg/kg) on days 8-1/2 to 15-1/2. Controls were untreated or oil-fed. Testing was done on day 18-1/2. Fetuses were sequentially exposed and ECG's obtained with the fetus attached to the placenta and uterus. Counterparts of standard leads, I, II and II were used. Fetuses were weighed and examined afterwards. Swollen fetuses were rated on a scale of 1 (slight edema under chin) to 5 (3 mm edema across back). ECG's from 81 controls and 205 Mirex fetuses were obtained. They were evaluated for rate of heart beat, regularity of beat, PR intervals and other features. One control exhibited an abnormality, a transitory period of premature atrial contractions. Mirex-fed fetuses exhibited tachycardia, closely correlated with degree of edema. The heart rate increased from 150/min in controls to 180 in slightly swollen to 224 in swollen fetuses. Mean PR intervals increased with degree of swelling and with dose. The frequency of first degree heart block was also dose-related, ranging from 20% to 77%. Second degree heart blocks were found in 8%, 3% showed arrhythmias, and one had atrial flutter/fibrillation. These cardiovascular problems seem primarily related to the Mirex-induced edema and demonstrate that fetal edema is not innocuous. These data demonstrate the usefulness of fetal electrocardiography to detect functional teratology.

17. KEY WORDS AND DOCUMENT ANALYSIS

a. DESCRIPTORS	b. IDENTIFIERS/OPEN ENDED TERMS	c. COSATI Field/Group
Rat fetuses Mirex Electrocardiography		06T

18. DISTRIBUTION STATEMENT	19. SECURITY CLASS (*This Report*)	21. NO. OF PAGES
RELEASE TO PUBLIC	UNCLASSIFIED	46
	20. SECURITY CLASS (*This page*)	22. PRICE
	UNCLASSIFIED	