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# Fetal rat development as influenced by maternal lead exposure

by

Charles David Miller

A Thesis Submitted to the
Graduate Faculty in Partial Fulfillment of
The Requirements for the Degree of
MASTER OF SCIENCE

Department: Veterinary Anatomy, Pharmacology and Physiology
Major: Veterinary Physiology

Signatures have been redacted for privacy

Iowa State University
Ames, Iowa

1977

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# INTRODUCTION

Research on the effects of lead has been conducted in domestic and laboratory animals, examining absorption, metabolism, excretion, and toxic effects. Lead has been administered in various chemical forms via a number of routes of exposure, with particular emphasis on efforts to establish levels which may adversely affect neonatal development. However, conflicting evidence has been reported in the literature concerning lead's fetotoxic and teratogenic effects in hamsters, mice, and rats. This research project was designed to investigate the effects of lead acetate on fetal development of rats. Specific attention was directed towards teratogenicity, fetal toxicity, and neuro-DNA synthesis. These experiments differ from most previously reported research in that lead exposure and blood values were established and maintained throughout breeding and gestation. Lead dosing simulated a more natural route of exposure through oral gavage in constrast to some experimental methods involving intravenous, intra-amnion, or intraperitoneal injection (Kruckenberg _et al._, 1976; McClain and Becker, 1972; McClain and Becker, 1975).

Lead has been reported to adversely affect learning in sheep and children exposed to chronic low levels. Carson _et al._ (1974) demonstrated visuomotor deficits in lambs

prenatally exposed to lead. These observations raise a question--does lead exposure during fetal development cause a reduction in total brain cell numbers? In this experiment total brain DNA was measured as an indication of total cell numbers.

# LITERATURE REVIEW

## General Considerations of Lead

Lead is considered a metallic poison that primarily enters the body through the gastrointestinal tract and respiratory system. The major route of absorption of lead salts is the gastrointestinal tract. Most cases of lead intoxication in children and farm animals result from oral lead consumption (Allcroft and Blaxter, 1950; Chisolm and Kaplan, 1968). From the gastrointestinal tract lead is either passed into the blood or is excreted in fecal matter. Therefore, the rate of excretion through the bowel appears to be the governing factor influencing the absorption of lead by this route. The respiratory tract is the second major route of lead absorption. Early work with lead by Blumgart (1923) and Minot (1924) demonstrated that lead can be absorbed from all portions of the respiratory tract, including the nasal passages. Absorption via the respiratory system is more rapid and complete than through the gut, perhaps due to the high carbon dioxide tension present which creates an acid medium aiding solubility of the lead particles (Harvey, 1975).

Inorganic lead is very slightly, if at all, absorbed through the intact skin (Aub et al., 1924). However, organic lead (tetraethyl lead) is readily absorbed dermally (Kehoe et al., 1935).

Many cases of lead poisoning are induced by the absorption of large quantities of lead over relatively short periods of time. This is classified as acute lead poisoning, which contrasts with chronic lead poisoning. Acute poisoning often occurs if the amount of lead ingested is so great that the excretion capacity of the renal system is exceeded. When this point is reached, lead is absorbed into the body tissues (Landown et al., 1974).

Absorbed lead is first deposited in the blood and soft tissue and then eventually in bone. Deposition into body tissues is partly governed by the chemical structure of the specific lead compounds, with deposition being more rapid in the case of organic than with inorganic compounds. The deposition of lead in tissues appears to be regulated by the same physiologic mechanisms that control the metabolism of calcium and phosphorus (Shields and Mitchell, 1941; Six and Goyer, 1970). Absorbed lead follows calcium to the bone, where over 90 percent is deposited in a nondiffusible form as a tri-lead phosphate (Schroeder and Tipton, 1968). Only a small percentage remains in the soft tissues and blood. Since lead is tightly bound in bone, it leaves the skeleton very slowly.

Other physiopathologic effects of lead have been reported in several recent books, theses and review articles that provide detailed information concerning the biologic

influence of lead in hemesynthesis, erythrocyte morphology and function, immune response and renal function (National Academy of Sciences, 1972; DeBruin, 1971; Goyer and Rhyme, 1973; Hemphill, 1973; and Lassen, 1976).

Elimination of lead occurs through urine, feces, and milk. It is also minimally excreted in sweat, the concentration being similar to that found in urine (Berman, 1966). Kehoe et al. (1935) reported that in man a daily lead excretion greater than 0.6 mg represents abnormal exposure for humans. After absorption has ceased, the rate of excretion of lead depends mainly on the rate of its mobilization from skeleton deposits. At best, lead is slowly removed from the body (Kehoe and Thamann, 1929). Absorbed lead is normally excreted in bile and eliminated in the feces (Klaassen and Shoeman, 1974). Under excessive exposure, or when chelation therapy is employed, urinary excretion of lead is increased. Lead is excreted in the milk of both man and animals. The range of normal values of lead in milk of humans and cattle is below 0.05 mg/l and 0.01 mg/l, respectively (Hammond and Aronson, 1964; Kehoe et al., 1940). Figure 1 is a modified schematic flowchart as presented by Goyer and Chisolm (1972), which summarizes lead exposure, absorption, excretion, and biologic effects.

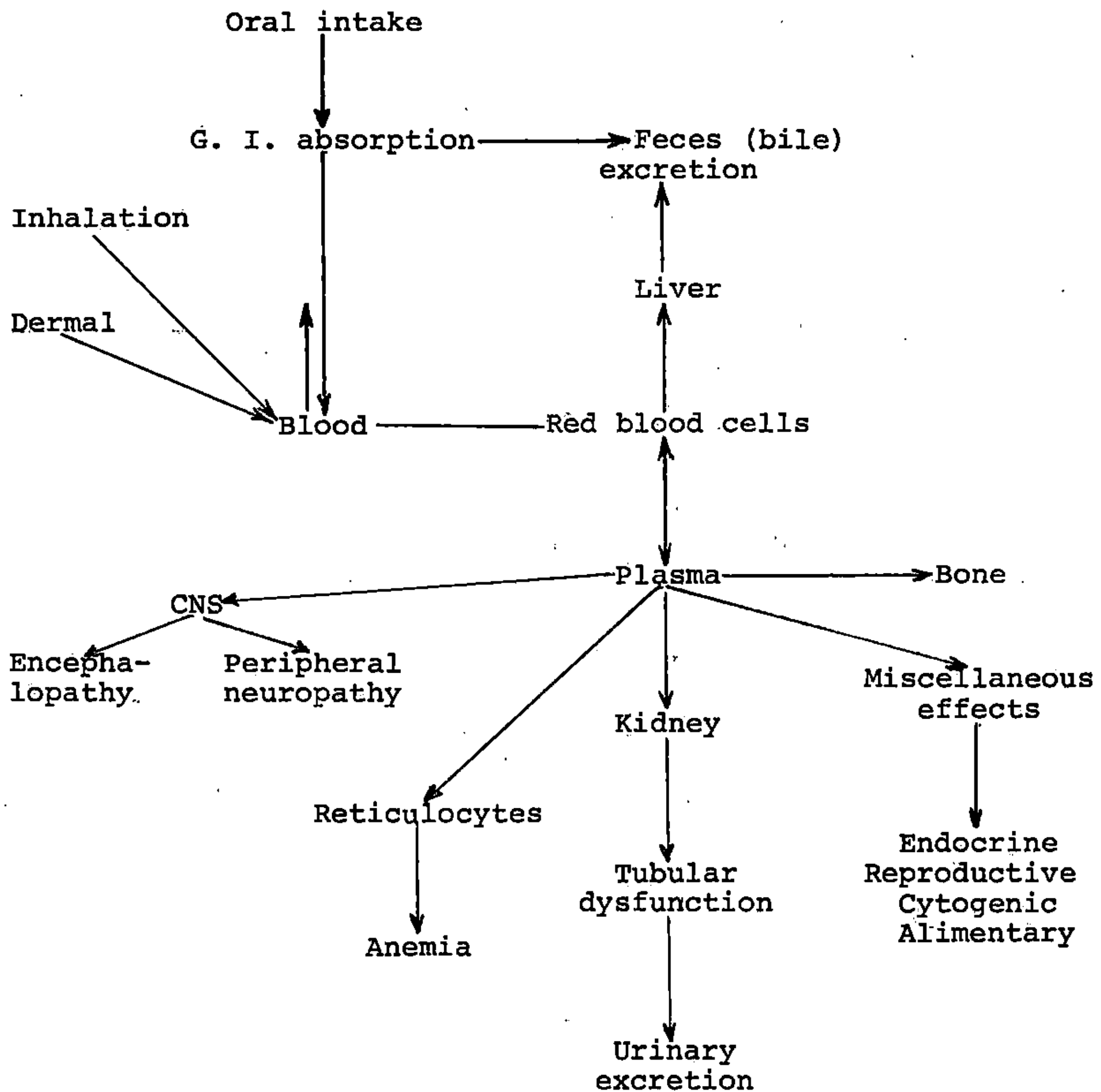


Figure 1. Schematic of lead exposure, absorption, excretion, and biologic effects (Modified from Goyer and Chisolm, 1972)

## Neuropathologic Effects of Lead Exposure

The need for understanding the neurotoxic effects of lead exposure became salient with the apparent connection to mental retardation and plumbism of lead exposed children (Chisolm and Harrison, 1956; Landrigan *et al*., 1975; Smith *et al*., 1963). Researchers have used a variety of measurement techniques in appropriate animal models to assess the different neuropathologic effects. These techniques have ranged from macro and microscopic anatomical evaluation to DNA measurement and cellular enumeration.

Pentschew and Garro (1966) first introduced an experimental model of lead encephalopathy. They reported that when neonatal rats were poisoned through suckling mothers which were fed a diet containing 4 percent lead carbonate, neurologic changes were characterized by capillary dilation, glial proliferation, areas of transudation and spotty hermorrhages. The resultant neurologic damage produced was considered secondary to the cerebral edema observed.

Thomas *et al*. (1971) also produced encephalopathy in suckling rats by adding 4.5 percent lead acetate to the feed and 1.0 percent lead acetate to the drinking water. Using light and electron microscopic techniques the researchers observed edema, endothelial swelling, and abnormalities in Purkinje cells. Similarly, Rosenblum and Johnson (1968)

produced encephalopathic lesions in mice, consisting of abnormally large numbers of fibrous intercapillary strands in several cerebral loci and astrocytosis of the hippocampus.

Thomas and Thomas (1974) reported lead induced encephalopathic lesions in the brains of newborn rats. The lesions were distinctly observed in the cerebellum, and consisted of alterations in capillary endothelial cells, the formation of platelet thrombi and scattered areas of hemorrhage and edema.

Krigman *et al*. (1974) induced lead encephalopathy in rats by adding 4 percent lead carbonate to the diet of nursing mothers. They reported that lead intoxication was associated with retardation of neural tissue growth without reduction of cell populations, alteration of myelin formation and a size reduction in neuronal axons. The number of myelin lamellae in the sheaths, however, was reduced.

Michaelson and Sauerhoff (1974) indicated there may be a possible link between malnutrition and lead exposure which causes paraplegia and cerebellar vascular damage in the suckling rat. They proposed an improved model for differentiation between encephalopathy caused by malnutrition and that caused by lead exposure.

Michaelson (1973) intoxicated newborn rats by including lead acetate in the diet of nursing animals on the

day of birth and further reported that within the cerebellum there was extensive capillary breakdown and hemorrhage. Arrested body growth, ataxia, and paraplegia were also reported. Additionally, a 15 to 20 percent deficit in new cell formation in the cerebellum was found as measured by DNA content at three weeks of age. DNA content has been reported to be constant within a single diploid cell in any species (Hotchkiss, 1955; Vendrely, 1955). It is possible to calculate the number of diploid cells in an organ by analyzing for total organ DNA and dividing by DNA per cell (Enesco and Leblond, 1962; Winick and Noble (1965). Using this technique, DNA has come to be used as a reference standard for calculating the number of cells present.

## Placental Transfer and Fetal Tissue Absorption

The transfer of lead across the human placenta and its potential threat to the conceptus have been recognized for over a century. Early evidence of this problem resulted from reports that women working in lead industries commonly exhibited unusually high rates of sterility, spontaneous abortion, and stillbirths (Carpenter, 1974). Some early work clearly demonstrated the presence of lead in fetal tissues and left little doubt that the human placenta was

permeable to this toxic metal (Karlog and Moller, 1958; Kehoe et al., 1935).

A more recent study by Barltrop (1969) was conducted on apparently normal humans and indicated that placental transfer of lead began early in gestation and that the total lead content in fetal tissues increased throughout pregnancy. A typical adult distribution of lead was found in fetuses with the highest concentrations in bone and liver, but there were also significant amounts in kidney, brain, and placenta. Barltrop further noted that the location of lead within the fetus at different stages of gestation is probably of far greater importance than the total amount present at term. Work that has been conducted on transplacental movement of lead in humans indicates that the lead content of the umbilical cord is approximately equal to the maternal blood lead level of 10 to 20 μg/100 ml (Gershanik et al., 1974; Harris and Holley, 1972).

Early work by Baumann (1933) demonstrated the first placental transfer of lead in a mammal. Pregnant rats were injected with very small doses (0.01 μg) of radioactive lead ($^{212}Pb$) and its distribution in the mother and fetus was observed via autoradiography. Lead rapidly crossed the placenta and localized within the fetal tissues, primarily developing bone. Another early study in dogs by Calvery

et al. (1938) confirmed the permeability of mammalian placenta to lead.

Green and Gruener (1974) studied the kinetics of maternal-fetal transfer of lead in the rat. They reported that lead was transferred at different stages of gestation and its transport was so rapid that the fetus was in equilibrium with the dam 24 hours after injection.

Hubermont et al. (1976) administered lead nitrate to female rats in drinking water at 0.1, 1, and 10 ppm for three weeks before mating, during pregnancy, and three weeks after delivery. In maternal animals a significant increase in blood lead concentration and lead concentration in kidney was found in the 10 ppm group. In newborns, lead concentrations in blood and in kidney were also significantly increased in the 10 ppm group.

Barltrop et al. (1971) examined the subcellular distribution of lead in four fetal soft tissues: heart, liver, spleen and kidney. Labeled lead chloride was intraperitoneally administered to 6 rats and tissue was collected at intervals between 2 hours and 14 days following administration. The spleen and kidney contained considerable amounts of lead; the liver contained relatively less; and the heart contained very little.

Singh et al. (1976) fed rats 100 and 200 mg of $Pb(NO_3)_2$ per kilogram body weight before and during pregnancy. Mean

blood lead values for pregnant female rats prior to delivery were 65 and 75 μg/100 ml, respectively. Lead content in pups just after birth was higher in kidney than in the liver, with brain unusually high (910 μg per 100 g of brain). Singh further hypothesized that since the brain is exceptionally sensitive to the effects of lead poisoning, as stated in Goyer and Rhyme's review (1973), this high amount of lead found within the brain suggests the possibility of brain abnormalities being developed during gestation.

## Reproductive Effects of Lead

Lead has been incriminated in reduced fertility, abortion, and neonatal death in man and animals. Flury (1934) reported disturbances in the menstrual cycle in women poisoned with lead and observed development of transitory sterility in women lead workers. Normal fertility returned after cessation of exposure to lead.

Pindborg (1945) observed in Danish women that lead oxide, when orally ingested, was an abortifacient. He reported 60 percent of the pregnancies ended in abortion in the first trimester. Wilson (1966) more recently reported human abortion to be associated with lead in drinking water and high fetal tissue lead levels. James

*et al*. (1966) fed lead acetate at a rate of 9 mg/kg for 45 days of gestation, causing abortion and subsequent maternal death in sheep. Sharma (1971) also reported abortion, resorption, and failure to breed when sheep were fed an average of 11.8 mg lead/kg/day prior to and during gestation.

Stowe and Goyer (1971) found that either paternal-only or maternal-only lead exposure resulted in a reduction in the number of rat pups per litter, a retardation in mean pup birth weight, and a reduction in pup survival. These effects were magnified in offspring whose parents both had been exposed to lead.

Hilderbrand *et al*. (1973) monitored the reproductive estrus cycles of 60 sexually mature rats exposed to 5 μg and 100 μg lead acetate per day for 35 days. The females treated with 5 μg experienced irregularity of the estrus cycle, while the group treated with 100 μg had persistent vaginal estrus after a period of normal estrus and the development of ovarian follicular cysts with a reduction in the number of corpora lutea also noted.

Vermande-van Eck and Meigs (1960) have also reported that the ovaries of lead burdened rhesus monkeys had a reduced number of developing follicles.

## Teratogenic Effects of Lead

Lead has been administered in various forms, via a number of routes in efforts to establish exposure levels which may adversely affect mammalian embryonic development.

Ferm and Carpenter (1967) administered lead nitrate, lead chloride, and lead acetate in 50 mg/kg intravenous doses to hamsters on either day 7, 8, or 9 of pregnancy. Fetal examination between day 12 and 15 of the 16-day gestation period found specific congenital skeletal malformations localized within the developing sacral and coccygeal vertebra. In similar studies, Carpenter et al. (1973) and Ferm and Ferm (1971) administered lead nitrate intravenously at doses of 25 and 50 mg/kg to maternal hamsters during 5 equal time periods of the eighth and ninth days of gestation. Teratogenic effects of lead were restricted to the developing tail bud. Increased incidence of embryonic resorption and malformation proportionately paralleled increased lead exposure.

Schroeder and Mitchener (1971) examined the toxic effect of several trace elements on reproduction in mice and rats in a multi-generation study. Lead, continuously administered at 25 ppm in the drinking water was found to severely depress reproduction in mice. Most of the lead

exposed mice died and consequently were incapable of reproduction. From the five lead exposed mice originally allowed to breed, only eight first generation, and two viable second generation litters were produced in a 6-month period. Of the 72 live offspring in the first generation, 69 were runts, 9 died, and 4 matured to breeding age. Lead was also found to be toxic to breeding rats, but less so than to mice. In the first generation of rat offspring, there were 12 deaths, and 40 runts in 19 litters containing 173 progeny. The second and third generation animals also produced litters in which runts and deaths were common.

McClain and Becker (1972) reported that tetraethyl lead (TEL), tetramethyl lead (TML), and tri-methyl lead chloride (Tri ML) were found to be essentially nonteratogenic in Sprague-Dawley rats. Orally administered doses of TEL (7.5, 15, or 30 mg/kg), TML (40, 80, 112, or 160 mg/kg), and Tri ML (15, 30, and 38 mg/kg) were given as three divided doses during early organogenesis or late organogenesis of gestation. The highest doses of each compound resulted in maternal death, and lower doses produced a dose-related fetal growth retardation and delayed ossification of bone.

In a similar study, Kennedy et al. (1975) treated pregnant albino mice and rats by gavage with doses of lead

acetate up to 714 mg/kg or tetraethyl lead (TEL) at 10 mg/kg. The compounds were administered daily during the period of rapid organogenesis. Maternal toxicity was observed and fetal resorption, as well as retardation of development, were encountered at higher dose levels. Fetuses from lead exposed females were examined grossly for internal structural and skeletal development, but no teratogenic responses were evident.

McClain and Becker (1975) intravenously injected lead nitrate into pregnant rats in single doses of 25, 30, 50, or 70 mg/kg on each of days 8 through 17 of gestation. Treatment on day 9 of gestation resulted in grossly deformed fetuses with malformations including absent or shortened tail, sirenoform monsters, absence of posterior portions of the body, urorectal defects, soft tissue anomalies of the urogenital system, and axial skeletal defects. Administration of lead on day 16 produced hydrocephalus and hemorrhage of the central nervous system.

# MATERIALS AND METHODS

## Prestudy

A prestudy composed of 25 rats was conducted to establish some basic information concerning lead dosage as related to blood lead levels, effects of the gavage technique of dosing on reproduction, and to determine whether sufficient maternal and fetal tissues were available for lead and DNA analyses. Additionally, the prestudy provided experience with analytical procedures and familiarized the experimenter with the potentially fatal blood collecting and dosing techniques.

The oral dosage levels used in the prestudy were 0, 35, 50, 75, and 100 mg/kg. Following lead exposure from two weeks to two months, the virgin females were bred to unexposed males, resulting in the pregnancy of twenty-one of twenty-five animals. Those that did not conceive were in the 50 and 75 mg/kg dosage groups. The average number of pups per litter was 9. Weekly blood lead analyses resulted in levels between 40 and 120 μg/dl in animals given 50, 75, and 100 mg/kg lead as lead acetate. Dissection and weighing of maternal and fetal tissues indicated that lead and DNA analyses were feasible.

## Experimental Animals, Facilities and Maintenance

Ten virgin male and forty virgin female hooded rats[1] were used as subjects. Upon receipt, each animal was randomly assigned to an individual cage[2] and allowed a two-month acclimation period. All animals were maintained on a commercial laboratory ration[3] and water, both of which were available *ad libitum*. The average background levels of lead in feed and water were found to be 2.3 ppm and 6.0 ppb, respectively. The colony room utilized throughout the experiment was adequately ventilated and concealed from outside light. The ambient temperature of this room ranged between 21.1-23.9°C. Standard fluorescent laboratory lighting was utilized in a constant 10-hour light, 14-hour dark cycle. Each animal was handled daily beginning on day one of the experiment for daily oral gavage.

## Experimental Design

The following design was developed to accommodate three replicates with four levels of exposure per replicate. On day 0 of the experimental period following acclimation, all

---

[1] Long-Evans strain; Blue Spruce Farms, Altamont, NY.

[2] Cage dimensions: 8"W x 7"D x 10"H.

[3] Wayne Lab-Blox Shorts; Allied Mills, Inc., Chicago, IL.

40 animals were individually weighed. Thirty-six females were then randomly assigned to an exposure level within a replicate. Each tier of the cage rack[1] housed four animals, alternating an empty cage, thereby minimizing cross-contamination and eliminating coprophagy.

## Lead Exposure

Blood lead levels and desired length of exposure were determined by the prestudy. Each experimental animal was randomly assigned to one of four groups which received daily dosages of lead as acetate at either 0, 50, 75, or 100 mg/kg body weight. Care was taken to control for dosage volume across exposure groups. All rats within each replicate were weighed daily. The highest body weight in the 100 mg/kg body weight treatment level was used to calculate the daily administered volume. This daily dose volume was determined by calculating the required amount of lead based on a weekly prepared stock solution of lead acetate. Dose volumes given to each rat within a replicate were made equal by adding double distilled water to the calculated lead solution volume for levels 0, 50, and 75 mg/kg body weight. The total daily dose volume of lead solution and water never exceeded 2 ml per dose.

[1]Becton-Dickinson and Co., Rutherford, NJ.

The measured doses of lead acetate solution were administered by gavage. This insured that precise amounts of lead were administered. Gavage intubation was achieved with an 18-gauge curved ball-tipped feeding needle (2 1/4 mm ball)[1] attached to sterile disposable 3 ml syringes. Dosage was calculated following a weekly chemical analysis of 1,000 ml of lead acetate stock solution. To prevent possible transfer of disease organisms or residual lead accumulation, each replicate of the test had its own gavage apparatus which was cleaned daily following dosing, with a dilute nitric acid solution.

## Estrous Cycle and Breeding

Vaginal smears were taken daily from female rats beginning seven days prior to lead exposure and continuing until day 6 of pregnancy. Individual records were kept for each animal to establish its estrous cycle. After lead exposure for three weeks, females showing pro-estrus were individually introduced overnight to one male.

Sperm or vaginal plugs present the following morning determined day zero of pregnancy. Vaginal smears indicating diestrous and mid-ventral laparotomies on day 6 further substantiated pregnancy.

---

[1]Popper & Sons, Inc., New Hyde Park, NY.

## Maternal Tissue Examination and Preparation

A blood sample was collected prior to initial exposure and subsequent blood samples were collected every two weeks. Each animal was anesthetized with ether and the blood collected from the orbital sinus utilizing a 100 µl capillary tube. Approximately 1.5 ml of blood was taken and placed in heparinized lead-free vacutainers.[1] The samples were immediately shaken and refrigerated at 43°C until analyzed for lead.

On day 20 of gestation, each female rat was euthanized with ether and 2 ml of whole blood was taken for lead analysis. The maternal liver and kidneys were excised, weighed and stored frozen for subsequent lead analysis. The reproductive tract was removed, including ovaries, and weighed. The number and position of live and resorbed fetuses were recorded.

## Fetal Examination and Preparation

Each fetus and placenta was removed from the uterus and dried on absorbent paper, weighed and examined for external malformations. The brain, liver and both kidneys

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[1]Becton-Dickinson and Co., Rutherford, NJ.

were removed from each fetus. The composite collection of each fetal organ (except brain) constituted a pooled sample which was weighed and analyzed for lead.

Immediately following fetal decapitation, the brain was quickly placed in pre-weighed and labeled scintillation vials and frozen on dry ice. The brain samples were then transferred to a storage freezer and maintained frozen until DNA analysis.

The rat fetuses harvested on day 20 of gestation were examined under a dissecting microscope for developmental and skeletal abnormalities according to the categories of anomalies presented in Appendices B and C. Prior to categorizing, the fetuses were fixed, eviscerated and stained in accordance with the procedures outlined in Appendix A.

## Tissue Analyses

A representative sample of fetal and maternal livers and both kidneys of all animals were oven-dried overnight, and ashed in a muffle oven at about 500°C. The lead in these samples was dissolved with 2 N hydrochloric acid and analyzed using a Perkin-Elmer Model 303 atomic absorption spectrophotometer.[1] Using known samples, a standard curve

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[1]Perkin-Elmer, Norwalk, CT.

was constructed and the amount of lead in each experimental sample was calculated based on this curve (Hyde, et al., 1977)

A similar atomic absorption technique was used in whole blood lead analysis (Hessel, 1968). The blood lead was chelated with ammonium pyrrolidine dithiocarbamate and extracted into methyl isobutyl ketone for atomic absorption analysis using the spectrophotometer.

The brain samples maintained in a frozen state were lyophilized and reweighed. The remaining dried tissue was defatted using two 12-hour extractions with chloroform : methanol (2:1), followed by two 12-hour ether extractions. The dried fat-free tissue (DFFT) was weighed and ground to a fine powder in a Wiley mill. Samples were then stored in a desiccator until analyzed for DNA. DNA was extracted with TCA by the method of Schneider (1945). DNA content in the DFFT was determined using Burton's diphenylamine method (1956). These procedures for preparation and analysis are found in Appendix D.

## Data Handling

Rats which failed to conceive were not included in the statistical analysis with rats having viable fetuses. Each rat or litter was considered an experimental unit receiving

one of the four experimental treatments. A two-way analysis of variance, with treatments and replications as main effects, and treatment by replication as the appropriate interaction term, was used to compare the four different treatments. The critical value of $\alpha$ was set at .025 *a priori* to insure against chance production of a significant test due to the large number of analyses.

To allow for an equal number of subjects per replicate within each treatment level, the four lightest animals were removed from the experimental design prior to treatment and replication assignment. Animals were then randomly assigned to one of the twelve cells formed in the treatment by replication matrix so that all cells contained an equal number of subjects (N=3).

# RESULTS

Gross physical examination and dissecting microscope examination using skeletal staining revealed no fetal teratogenic malformations. Small developmental differences were noted across pups as shown in Figure 2. These differences were in degree of ossification in caudal vertebrae and sternebrae. The numbers of ossified caudal vertebrae varied between 2 and 3 present and the number of ossified sternebrae varied between 2 and 5 present. Fetuses from two dams, control animal 10, and 100 mg/kg treatment animal 24, appeared more advanced than others as the cranial bones (frontal, parietal and intraparietal) were starting to fuse together and sternebrae were more completely ossified as seen in Figure 2. All other exposed and control fetuses examined, using the criterion found in Appendices C and D, appeared to be at day 20 of their gestational period.

Tables 1 and 2 present the means (±S.E.) for each treatment group over each of the dependent measures taken for analysis, and includes the significance level for each respective analysis of variance. All significant differences pertain to effects of treatment only. That is, no significant effects of replication or the treatment by replication interaction were obtained from any sets of data analyzed.

Figure 2. Fetal Skeletal Staining

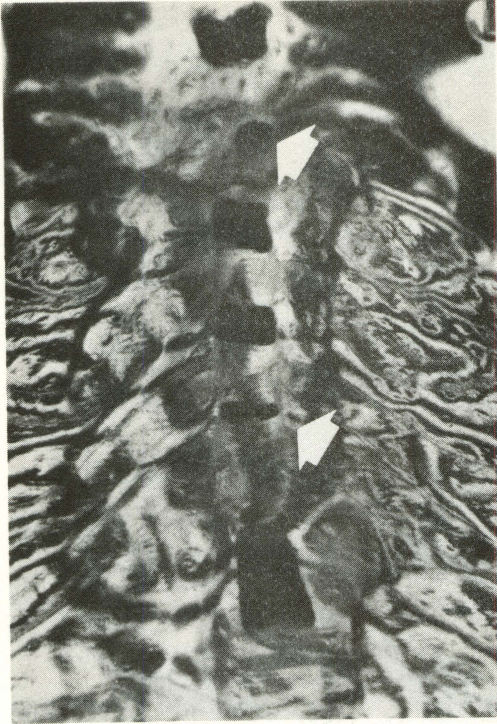
A. Varied degrees of sternebrae ossification viewed in most fetal rats at day 20 of gestation and depicted by animal 2.

B. Advanced sternebrae ossification as seen in animals 10 and 24 and exemplified by animal 24.

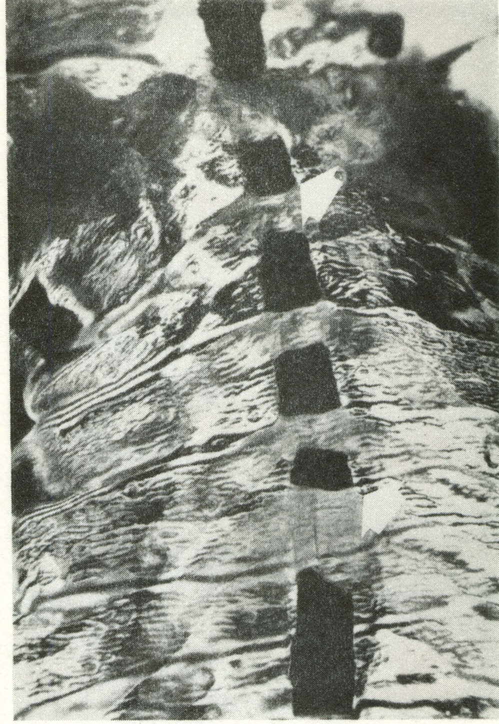
C. Cranial bones as typically seen in most fetal rats at day 20 of gestation and shown here by animal 2.

D. Cranial bones in advanced ossification as seen in animals 10 and 24 and depicted by animal 10.

Arrows indicate sites for comparison.

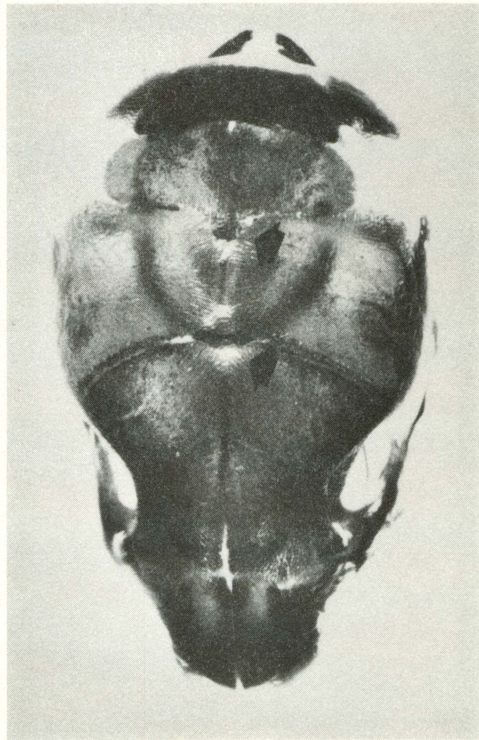


A



B

C



D

Table 1. Kidney and liver weights and lead concentrations (ppm) in lead exposed and control rats

| | Treatment level | | | | |
|---|---|---|---|---|---|
| | Control 0 (mg/kg) | 50 (mg/kg) | 75 (mg/kg) | 100 (mg/kg) | Significance |
| Maternal kidney lead[a] (ppm) | 0.78±0.1 | 18.99±1.71 | 22.59±2.04 | 26.21±2.80 | P=.0001 |
| Maternal liver lead[a] (ppm) | 0.90±0.12 | 2.09±0.20 | 2.57±0.32 | 3.63±0.27 | P=.0001 |
| Fetal kidney lead[a,b] (ppm) | 0.6 ±0.26 | 5.93±0.35 | 5.9 ±0.46 | 5.63±0.07 | P=.0007 |
| Fetal liver lead[a,b] (ppm) | 1.01±0.17 | 1.49±0.31 | 1.67±0.21 | 2.33±0.27 | P=.006 |
| Maternal kidney weight[a,c] (g) | 2.0 ±0.07 | 2.22±0.11 | 2.35±0.51 | 2.35±0.09 | NS |
| Maternal liver weight[a,c] (g) | 13.13±0.40 | 14.31±0.35 | 14.31±0.53 | 15.77±1.38 | NS |

| | | | | | |
|---|---|---|---|---|---|
| Fetal kidney weight[a,b,c] (mg) | 23.73±0.00 | 22.24±0.00 | 27.95±0.00 | 23.64±0.00 | NS |
| Fetal liver weight[a,b,c] (mg) | 272.00±0.05 | 237.52±0.02 | 231.71±0.12 | 237.29±0.02 | NS |

[a]Mean and S.E.

[b]Pooled fetal sample

[c]Wet weight

Table 2. Tissue weights, breeding attempts, DNA analysis and number of fetuses in control and lead exposed rats

| | Treatment level | | | | |
|---|---|---|---|---|---|
| | Control 0 (mg/kg) | 50 (mg/kg) | 75 (mg/kg) | 100 (mg/kg) | Significance |
| Uterus and conceptus weight[b] (per pup) (g) | 4.71±0.13 | 3.83±0.26 | 4.19±0.29 | 4.25±0.39 | NS |
| Ovarian weight[a,b] (mg) | 11.19±0.00 | 11.24±0.00 | 11.71±0.00 | 15.23±0.05 | NS |
| Breeding attempts | 1.56±0.18 | 2.13±0.40 | 2.89±0.54 | 3.14±0.40 | NS |
| Placental weight[a,b] (mg) | 68.35±0.02 | 63.93±0.02 | 70.73±0.03 | 65.69±0.02 | NS |
| Conceptus weight[a,b] (g) | 3.35±0.11 | 3.13±0.13 | 3.52±0.16 | 2.73±0.24 | P=.002 |
| Number of viable pups[a] | 9.0 ±0.88 | 9.13±1.20 | 8.22±1.41 | 8.57±1.23 | NS |
| Number of non-viable pups[a] | 1.1 ±0.2 | 2.25±0.60 | 1.78±0.55 | 2.42±0.65 | NS |

| | | | | | |
|---|---|---|---|---|---|
| Fetal brain weight[a,b,c] (mg) | 178.40±0.04 | 148.47±0.01 | 156.81±0.22 | 156.39±0.16 | NS |
| Fetal brain DNA[a,c] (mg/g DFFT) | 170.78±2.61 | 169.5 ±3.23 | 168.33±8.94 | 166.0 ±8.19 | NS |

[a]Mean and S.E.

[b]Wet weight

[c]Pooled fetal sample

An examination of Tables 1 and 2 indicates that all measures of lead content (ppm) (maternal liver and kidney, and pooled fetal liver and kidney per dam) showed treatment differences while only one measure of weight (average fetal weight per dam) displayed similar differences. Generally, the lead exposure at all of the three levels used (50, 75, and 100 mg/kg) did not appear to affect DNA content in brain or any measure outside those pertaining to lead content.

A posteriori mean comparisons, i.e., Scheffe tests (Kirk, 1968), were conducted for each group on each of the five significant treatment-related effects mentioned above:

1. Maternal kidney lead content - all exposed groups were found to be equivalent in terms of ppm lead; therefore, their values were pooled and compared against the control group, i.e., exposed v. unexposed. The exposed animals contained significantly more lead in their kidneys than unexposed controls ($p<.01$).
2. Maternal liver lead content - differences were found between all groups in a dose response fashion. That is, the 100 mg/kg group contained significantly higher lead levels than both the 75 and 50 mg/kg groups ($p<.05$ and $p<.01$,

respectively) which were, however, equivalent. All three lead exposed groups contained significantly greater amounts of lead than the control group ($p<.01$ in each case).

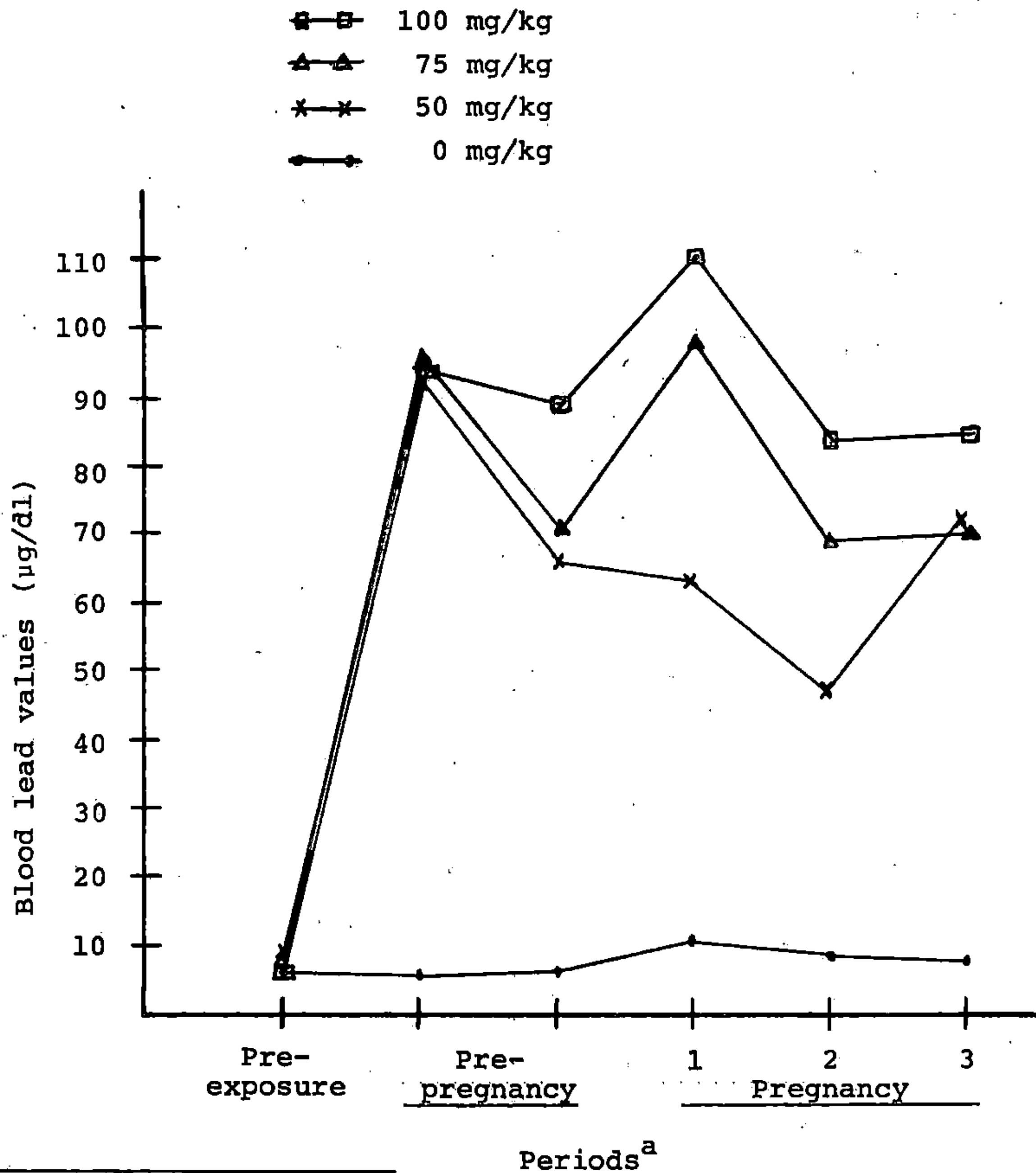
3. Fetal kidney lead content - as with the maternal kidney lead content data, all three exposed groups were equivalent and, therefore, pooled to compare against the control group. Results indicated that the average fetal kidney lead content per dam for an exposed dam was significantly greater than for an unexposed dam ($p<.01$).
4. Fetal liver lead content - between-group comparison showed only the 100 mg/kg v. 0 mg/kg comparison to be significantly different ($p<.01$). The average fetal liver lead content per dam was in a dose response fashion but the 50 and 75 mg/kg groups were equivalent to both the 0 and 100 mg/kg groups.
5. Conceptus weight - this variable, measured as an average weight per dam, was not consistent across groups. That is, the control and 75 mg/kg exposed dams had higher average conceptus weights than the 100 mg/kg dams ($p<.05$ and $p<.01$,

respectively); however, the average conceptus weights for the 50 mg/kg exposed dams were equivalent to those of all other group dams.

The maternal blood lead values sampled at pre-exposure, pre-pregnancy, and pregnancy, indicate that three blood lead levels and a control level were maintained throughout pregnancy (Figure 2). Statistical tests were not conducted on the data due to the large number of missing and spurious values found throughout the replicates (Appendix Table 6) resulting in an uneven number of values per animal. Mean values presented in Table 3 represent values based on differing numbers of subjects per cell. The data from Table 3, graphically presented in Figure 3, present two distinct points: first, a uniform background exposure level was present in all animals during pre-exposure conditions and was maintained in the control group through the remainder of the experiment; and, second, by the onset of pregnancy, blood lead levels varied across treatment levels and were maintained throughout pregnancy.

Table 3. Blood lead values (mean ± standard error) for each treatment at pre-exposure, pre-pregnancy exposure, and pregnancy exposure. (Numbers in parentheses indicate number of samples per cell on which the mean is based)

| Treatment level (mg/kg) | Pre-exposure (µg/dl) | Pre-pregnancy (µg/dl) | Pre-pregnancy (µg/dl) | Pregnancy (µg/dl) week 1 | Pregnancy (µg/dl) week 2 | Pregnancy (µg/dl) week 3 |
|---|---|---|---|---|---|---|
| | (9) | (4) | (9) | (4) | (6) | (3) |
| 100 | 6 ± 0 | 94 ± 15 | 89 ± 14 | 110 ± 14 | 83 ± 17 | 84 ± 17 |
| | (9) | (5) | (9) | (5) | (8) | (8) |
| 75 | 8 ± 1 | 95 ± 10 | 71 ± 9 | 98 ± 12 | 69 ± 13 | 71 ± 12 |
| | (9) | (3) | (9) | (7) | (6) | (6) |
| 50 | 10 ± 2 | 93 ± 33 | 66 ± 14 | 63 ± 16 | 47 ± 44 | 72 ± 23 |
| | (8) | (5) | (9) | (7) | (4) | (9) |
| 0 | 6 ± 1 | 7 ± 2 | 8 ± 11 | 12 ± 1 | 9 ± 2 | 8 ± 2 |




[a]Pre-exposure, pre-pregnancy, and pregnancy periods are in weeks. Between pre-pregnancy and pregnancy number of weeks varies due to breeding attempts.

Figure 3. Blood lead values v. blood sampling periods

# DISCUSSION AND CONCLUSIONS

In the present investigation, thirty-six female rats were randomly assigned to one of four treatment groups, with three replicates each, and subjected to an oral intake of lead (0, 50, 75, and 100 mg/kg, respectively) to determine the effects on fetal survival and development. At day 20 of gestation, having been orally exposed to lead for a minimum of five weeks, pregnancy was terminated. Analyses were subsequently conducted on maternal blood, as well as maternal and fetal soft tissues, for lead content. Fetal brains were analyzed for total DNA; fetal skeletal examination for malformations was conducted; and reproductive tract and conceptus weights were measured.

The interpretation of the blood lead data is made somewhat difficult by occasional missing or erratic values. Although not statistically analyzed, two points were visually apparent. First, with the exception of occasional high values, a relatively stable control blood lead level was maintained throughout the experiment. This indicated that background lead exposure from the environment, although ever present, was relatively low. Second, the three treatment levels showed trends toward three distinct blood lead levels. Considering the blood values from each animal individually, one can see a rapid rise in blood lead levels for the first

two weeks of exposure, with all animals tending to reach a plateau in blood lead concentration by the onset of pregnancy. Since we have no additional information regarding the blood levels between pre-exposure and pre-pregnancy, one explanation of the nearly identical rise in blood lead values for all treatment groups is that this is a nonlinear rise. This could be demonstrated by increased sampling over this week-duration, possibly showing a slow increase for the first few days in blood levels indicating deposition in soft tissues and bone. Then, having surpassed these absorption capacities, a rapid rise to a peak value would occur. The fact that nearly identical peak values were reached at the first sampling could be explained as blood's carrying capacity. Since approximately 90 percent of blood lead is membrane bound to red blood cells, a finite carrying capacity can be reached. These data indicate that at 50 mg/kg this capacity was saturated; therefore no subsequent rise is noted with larger doses of 75 mg/kg or 100 mg/kg. This apparent "maximum carrying capacity" if true, could be unique to the rat as higher blood lead values associated with higher exposures have been reported in other species (Lassen, 1976; Sharma, 1971). The presence of extremely erratic values across weeks can be attributed to laboratory contamination or error. For example, some fluctuation in values could have been induced or brought on because of the

application of a macro-technique of blood lead analysis to relative micro amounts of blood, i.e., ≈ 2 ml. In any event, despite the fact that there were occasional problems in the blood analyses, the results are important because they exemplify an attempt to establish a dose-response correlation between oral lead intake and blood lead values. The data prompt one to question the reliance on dosage alone as a single measure of exposure. Our data show blood lead values from one animal were consistently higher or lower when compared to others subjected to the same dosage level, indicating the need to monitor both parameters, lead exposure and blood lead values.

The significance of the liver and kidney lead analyses are two-fold. First, lead orally introduced was found in the soft tissues as well as the blood, thereby substantiating that maternal animals were indeed exposed to, and absorbed significantly different amounts of, lead (Table 1). Second, the fetal liver and kidney lead analyses demonstrate the permeability of the placenta to lead, thereby exposing the developing fetus to lead concentrations.

Nutritional and lead toxicity studies indicate that DNA values (total cell numbers) of the rat neonatal brain can be significantly reduced post parturition (Michaelson, 1973; Zamenhof *et al.*, 1971; Zeman and Stanbrough, 1969). This period is known to be a time when neural cells undergo a

great increase in total numbers, often referred to as the "brain growth spurt" (Dobbing and Sands, 1971). During gestation, later-dividing neurons, especially those in the cerebellum, would not undergo as rapid an increase in cell numbers as they do during the early postnatal period. Therefore, nutritional deficiencies and toxicoses during the prenatal phase may not significantly reduce cell numbers (Endo et al., 1974). Our DNA data support this hypothesis. However, a trend toward decreased fetal brain weights respective to lead dosing of dams was visually apparent but was not satistically significant by analysis of variance ($p = .025$).

Although lead is a reported teratogen in mice, hamsters, and rats, only slight developmental differences between animals were observed in this study. These ossification differences were attributed to individual animal variations, which supports the conjecture that teratogenic effects caused by lead do not seem to occur under natural routes of exposure. That is, those studies which do purport to obtain skeletal teratogenic effects from lead exposure may in reality be measuring an artifact of experimental technique, or, at best, a teratogenic response occurring from an unnatural or surgical route of exposure.

The apparent effects of lead on the reproductive system included a significant reduction in conceptus weight

in the high dosage (100 mg/kg) treatment rats, as well as a trend, which approached significance ($p = .043$), toward a greater number of breeding attempts in order to attain conception for lead exposed animals. The lack of effect on organ weights and litter size further substantiates that oral lead exposure failed to greatly influence fetal survival and development in rats.

## SUMMARY

This experiment is a unique, multifaceted study that attempted to evaluate and correlate lead dosage v. blood lead values and fetal rat development.

Female Long-Evans strain hooded rats were exposed to 0, 50, 75, and 100 mg/kg of lead via oral gavage from two weeks prior to breeding until day 20 of gestation. Blood lead values were calculated from weekly collected blood samples. Lead analyses were also conducted on the maternal and fetal kidneys and livers. Fetal and maternal organ weights were recorded along with reproductive tract data. Fetal brains were analyzed for DNA content as an indication of total cell numbers.

This study confirms other reports in the literature that oral exposure to lead does not cause skeletal teratogenic effects in rats. This study further indicates that in the rat, oral exposure of up to 100 mg/kg does not cause fetal death or abnormalities. The experimental data obtained in this study showed no lead induced reduction in total cell numbers in the fetal rat brain.

Our data also indicate that it is highly desirable to monitor levels of exposure and blood lead concentrations to obtain a more complete indication of lead exposure in the rat.

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# APPENDIX A

## Fetal Skeletal Staining Procedure

Solutions

| | | |
|---|---|---|
| A. | Potassium hydroxide 1% aqueous | |
| B. | Alizarin Red S. | 0.1 gram |
| | Potassium hydroxide | 10 grams |
| | Distilled water | 1 liter |
| C. | MALL's solution: | |
| | Glycerin | 20 ml |
| | Distilled water | 79 ml |
| | Potassium hydroxide | 1 gram |

Technique

1. Fix whole specimens in 95% alcohol for at least 3 days.
2. Transfer to acetone and leave for several days to dissolve out the fats which would otherwise stain intensely and obscure the view of the bony structures.
3. Wash well with 95% alcohol; then immerse in 95% alcohol for 24 hours.
4. Immerse in solution A from 1 to 7 days, according to the size of the specimen, until the bones are clearly visible through the muscle.
5. Transfer to solution B until the bones are stained the desired depth of color. This takes from 1 to 7 days

and the solution should be changed on the fourth day.

6. Clear in solution C until no more color comes out.
7. Pass into a mixture of equal parts of glycerin and water. Gradually increase the glycerin percentage through 3-4 passes until concentration is pure glycerin.
8. Store in pure glycerin.

Bones are stained red; soft tissue is transparent and unstained.

Notes

If the initial cleaning in potassium hydroxide solution has progressed to the proper stage, only the bone will be stained, but otherwise soft tissue will also be stained.

The prolonged preliminary fixation in alcohol renders the tissue less liable to maceration in the potassium hydroxide solution.

Objects fixed in liquids other than alcohol may be stained by this method provided they are soaked in 90% alcohol for at least 3 days.

The technique is particularly suitable for demonstrating developing bone.

# APPENDIX B

## Categories of Anomalies

Developmental

1. Developmental Failure - Primordium fails to appear or does not develop to a significant degree as a whole. Agenesis designates this condition. Examples: absence of whole arm (or of radius or hand); absence of kidney.
2. Developmental Arrest - Progressive development falls short of normal condition: cleft palate; double uterus; umbilical hernia. Normal growth halts; dwarfism.
3. Developmental Excess - Growth is exaggerated: gigantism. Normal numbers are increased: extra digits; double penis; true twin. Processes exceed normalities; extensive hairy coat; thick epidermis (ichthyosis).
4. Fusion or Splitting - Examples: horseshoe kidney; cleft ureter.
5. Failure to Subdivide - Retention of common, primitive fields; fused eyes (cyclopia), fingers (syndactyl) or thighs (sympodia).
6. Failure to Atrophy - Normally, the temporary features remain; anal membrane; cervical fistula; double vena cava.

7. Failure to Consolidate - Examples: lobed or accessory spleen, pancreas or suprarenal gland.
8. Incorrect Migration - Normal shift fails wholly or in part; undescended testis; unascended kidney; fused ears. Normal shift is exceeded; ovary in external genitalia.
9. Misplacement - Organs occupy unexpected locations; transposed viscera; palatine teeth.
10. Atypical Differentiation - The developmental course and end product are unlike that in the normal fetus; congenital tumors osteogenesis.
11. Atavism - Ancestral recurrences: azygos lobed lung, elevator muscle of clavicle (as in climbing primates).

(From Developmental Anatomy, 6th Ed., by Leslie Arey; pp. 183-184).

# APPENDIX C

## Check List of Bones

### Ossified and Incomplete

| Bone | Description |
|---|---|
| Nasal | Nearly complete, pale center area |
| Premaxilla | Nearly complete, pale anterior lateral area |
| Maxilla | Fusion of 3 segments |
| Zygoma | Well defined, not fused |
| Palatine | Not colored |
| Vomer | Not present |
| Lacrimal | Not present |
| Frontal | (Dorsally) meets anterior, incomplete more posterior |
| Parietal | Nearly complete except a small portion dorsally |
| Interparietal | Present |
| Basisphenoid | Flat plate + Pterygoid Processes also lateral posterior processes ) alisphenoids present |
| Presphenoid | Beginning |
| Occipital | 4 parts - 1 ventral, 2 posterior, 1 dorsal |

| Bone | Description |
| --- | --- |
| Squamosal | Present with zygomatic process |
| Periotic capsules | Semicircle anterior - medial - posterior |
| Tympanic bulla | Not present |
| Ossicles | Not present |
| Turbinates | Not present |
| Hyoid | Present ventral basisphenoid |
| Mandible | Well defined |
| Cervical | No centra |
| Thoracic | Centra present - each vertebra of 3 unfused parts |
| Lumbar | 6 present + unfused centra |
| Sacral | 4 present + unfused centra |
| Caudal | 3 present |
| Sternebra | 2 to 5 present |
| Clavicle | Present |
| Scapula | Spine even more defined |
| Humerus | Present |
| Radius and ulna | Trochlear notch becoming visible |
| Carpals | Not present |
| Metacarpals | 3 most lateral |
| Phalanges | Not present |
| Ilium, ischium, pubis | Well defined |

| Bone | Description |
| --- | --- |
| Femur, fibia, fibula | Assuming normal characteristics |
| Tarsals | Not present |
| Metatarsals | 4 most lateral |

# APPENDIX D

## Preparation of Dried Fat-free Tissue (DFFT)

(Schneider, 1945)

1. Approximately 2 g of brain tissue was thawed, minced and weighed into a tared sample jar.
2. The samples were freeze-dried for about 24-36 hours.
3. They were then weighed and the loss due to water was determined.
4. The tissue was extracted twice with 50 ml of chloroform: methanol (2:1) for 12 hours each time under constant agitation with a wrist action shaker.
5. The tissue was then extracted twice with diethyl ether for 12 hours while under agitation.
6. The solvent was poured off and the tissue allowed to air dry for 12 hours before oven drying it at 50°C for 2 days.
7. The DFFT was weighed and the loss due to lipid was determined.
8. The tissue was ground to a fine powder in a Wiley mill equipped with a No. 40 sieve, oven-dried at 50°C for 2 days, and placed in a desiccator until ready for use.

## Extraction of Total Nucleic Acids
## (Schneider, 1945)

Procedure

1. Samples of DFFT (25 mg) were placed in centrifuge tubes. To each tube was added 4.5 ml of 5% TCA.
2. The material was allowed to stand for about 2 hours until all the tissue was saturated.
3. The tubes were placed in a 90°C water bath for 15 minutes, then cooled and centrifuged for 10 minutes at 3,500 x g at 4°C. This treatment quantitatively dissociates the DNA and RNA from the tissue proteins and leaves the protein in an insoluble residue which is removed by centrifugation.
4. The supernatant was poured into a graduated conical centrifuge tube.
5. The residue was resuspended in 4.5 ml of 5% TCA, placed in a 90°C water bath for 15 minutes, cooled and centrifuged as before.
6. The decant from the second digest was combined with that from the first extraction.
7. The combined extracts which constitute the nucleic acid fraction of the brain tissue were diluted to a total volume of 10 ml with 5% TCA.

# Determination of DNA Prepared from DFFT
(Burton, 1956)

## Reagents

1. Diphenylamine reagent: 1.5 g of diphenylamine was dissolved into 100 ml of glacial acetic acid. This reagent was prepared on the day it was to be used. Just before use, 0.5 ml of aqueous acetaldehyde (16 mg/ml) was added to the reagent.
2. 1 N perchloric acid: Concentrated (70%) perchloric acid (9 ml) was added to 91 ml of distilled water.
3. Standard: The standard was prepared from calf thymus DNA. Dilutions ranged from 100 µg to 400 µg DNA.

## Procedure

1. Aliquots of standard and nucleic acid extract were diluted to 1 ml with 5% TCA.
2. To each tube was added 1 N perchloric (1 ml) acid and diphenylamine reagent (4 ml). The solution was mixed.
3. Color development occurred by incubating the samples in a 30°C water bath for 16-20 hours.
4. Absorbance was determined at 600 nm on a Gilford Model 2000 recording spectrophotometer.

# APPENDIX E

## Additional Tables of Raw Data

Table 4. Maternal kidney and liver weights and lead concentrations (ppm) in lead exposed and control rats

| Animal Number | Treatment level (mg/kg) | Maternal kidney weight (g) | Maternal liver weight (g) | Maternal kidney lead (ppm) | Maternal liver lead (ppm) |
|---|---|---|---|---|---|
| 5 | 100 | 2.63 | 25.58 | 11.1 | 3.7 |
| 9 | 100 | 2.20 | 15.29 | 32.8 | 3.0 |
| 2 | 100 | 1.87 | 17.02 | 27.0 | 3.4 |
| 39 | 100 | 2.47 | 16.94 | 22.8 | 2.7 |
| 26 | 100 | 2.00 | 13.21 | 23.6 | 3.2 |
| 3 | 100 | 2.65 | 13.15 | - | 3.4 |
| 37 | 100 | 2.43 | 14.61 | 28.9 | 2.9 |
| 12 | 100 | 2.42 | 10.97 | 25.5 | 5.4 |
| 29 | 100 | 2.51 | 15.15 | 38.0 | 4.1 |
| 24 | 75 | 2.63 | 14.63 | 32.6 | 2.5 |
| 13 | 75 | 1.63 | 12.29 | 22.1 | 2.5 |
| 11 | 75 | 1.97 | 13.97 | 28.9 | 2.5 |
| 34 | 75 | 2.70 | 16.60 | 23.0 | 2.2 |
| 14 | 75 | 2.71 | 14.50 | 25.9 | 3.0 |
| 4 | 75 | 2.14 | 11.98 | 14.5 | 4.9 |
| 38 | 75 | 3.08 | 16.19 | 23.7 | 2.1 |
| 35 | 75 | 2.17 | 13.46 | 15.6 | 1.7 |
| 33 | 75 | 2.15 | 15.18 | 17.0 | 1.7 |
| 32 | 50 | 1.98 | 13.69 | 20.0 | 2.4 |
| 23 | 50 | 1.81 | 14.86 | 22.6 | 1.5 |
| 21 | 50 | 1.93 | 13.74 | 24.3 | 3.0 |
| 22 | 50 | 2.84 | 14.18 | 25.9 | 2.8 |
| 25 | 50 | 2.45 | 12.83 | 14.9 | 2.0 |
| 8 | 50 | 2.17 | 15.78 | 21.5 | 2.4 |
| 15 | 50 | 2.09 | 16.04 | 15.9 | 1.8 |
| 1 | 50 | 2.32 | 13.64 | 15.5 | 1.7 |
| 30 | 50 | 2.39 | 14.04 | 10.3 | 1.2 |
| 20 | 0 | 1.68 | 12.31 | 0.7 | 1.0 |
| 36 | 0 | 1.84 | 12.13 | 1.3 | 1.5 |
| 27 | 0 | 2.32 | 14.36 | 0.7 | 0.5 |
| 16 | 0 | 1.82 | 13.76 | 1.1 | 0.8 |
| 7 | 0 | 1.99 | 11.58 | 0.8 | 0.7 |
| 10 | 0 | 2.12 | 13.20 | 0.4 | 1.4 |
| 31 | 0 | 1.94 | 14.72 | 0.4 | 0.5 |
| 17 | 0 | 2.30 | 11.85 | 0.8 | 0.7 |
| 19 | 0 | 1.98 | 14.24 | 0.8 | 1.0 |

Table 5. Total fetal liver and kidney weights and lead concentrations (ppm) in lead exposed and control rats

| Animal Number | Treatment level (mg/kg) | Fetal liver weight (g) | Fetal kidney weight (g) | Fetal liver lead (g) | Fetal kidney lead (g)[a] |
|---|---|---|---|---|---|
| 5 | 100 | - | - | - | |
| 9 | 100 | 1.70 | 0.188 | 3.0 | 5.8 |
| 2 | 100 | 2.07 | 0.259 | 2.4 | |
| 39 | 100 | 2.05 | 0.162 | 2.8 | |
| 26 | 100 | 1.49 | 0.136 | 2.6 | 5.5 |
| 3 | 100 | - | - | - | |
| 37 | 100 | 1.87 | 0.231 | 2.8 | |
| 12 | 100 | 0.28 | 0.252 | 1.1 | 5.6 |
| 29 | 100 | 2.36 | 0.184 | 1.6 | |
| 24 | 75 | 0.95 | 0.115 | 1.4 | |
| 13 | 75 | 1.96 | 0.212 | 2.0 | 3.3 |
| 11 | 75 | 1.67 | 0.189 | 3.2 | |
| 34 | 75 | 0.21 | 0.047 | 1.6 | |
| 14 | 75 | 1.93 | 0.257 | 1.1 | 7.2 |
| 4 | 75 | 0.68 | 0.068 | 1.5 | |
| 38 | 75 | 1.85 | 0.231 | 1.6 | |
| 35 | 75 | 1.78 | 0.165 | 1.3 | 5.2 |
| 33 | 75 | 2.70 | 0.215 | 1.4 | |
| 32 | 50 | 1.77 | 0.171 | 2.2 | |
| 23 | 50 | 1.52 | 0.201 | 1.2 | 6.5 |
| 21 | 50 | 1.10 | 0.116 | 2.4 | |
| 22 | 50 | - | - | - | |
| 25 | 50 | 0.57 | 0.046 | 0.5 | 7.5 |
| 8 | 50 | 2.37 | 0.202 | 0.9 | |
| 15 | 50 | 2.27 | 0.191 | 2.4 | |
| 1 | 50 | 2.75 | 0.251 | 0.4 | 4.1 |
| 30 | 50 | 2.39 | 0.201 | 1.6 | |
| 20 | 0 | 1.25 | 0.116 | 2.0 | |
| 36 | 0 | 1.36 | 0.118 | 0.8 | 0.5 |
| 27 | 0 | 1.93 | 0.172 | 0.5 | |
| 16 | 0 | 1.08 | 0.104 | 1.2 | |
| 7 | 0 | 2.29 | 0.208 | 0.7 | 1.0 |
| 10 | 0 | 2.55 | 0.199 | 0.9 | |
| 31 | 0 | 1.42 | 0.200 | 1.0 | |
| 17 | 0 | 1.44 | 0.100 | 1.6 | 0.3 |
| 19 | 0 | 1.48 | 0.126 | 0.4 | |

[a]Values for pooled samples in each replicate

Table 6. Blood lead values of the respective rats

| Animal Number | Treatment level (mg/kg) | Pre-exposure Week 1 | Pre-gestation Week 2 | Pre-gestation Week 3 | Gestation Week 4 | Gestation Week 5 | Gestation Week 6 |
|---|---|---|---|---|---|---|---|
| | | (μg/1 ml) | | | | | |
| 5 | 100 | 0.05 | 0.71 | 0.74 | ng[a] | ng | ng |
| 9 | 100 | 0.05 | 0.74 | 0.91 | 0.75 | 0.60 | le[b] |
| 2 | 100 | 0.05 | 1.34 | 1.69 | 1.02 | 0.83 | 0.70 |
| 39 | 100 | 0.08 | 4.32[c] | 0.88 | le | 0.72 | le |
| 26 | 100 | 0.07 | 0.97 | 0.57 | le | 0.80 | le |
| 3 | 100 | 0.06 | 1.63[c] | 1.18 | ng | ng | ng |
| 37 | 100 | 0.06 | le | 0.36 | 1.27 | 1.48 | le |
| 12 | 100 | 0.05 | le | 1.23 | 1.36 | le | 1.18 |
| 29 | 100 | 0.05 | le | 0.49 | le | 0.54 | 0.63 |
| 24 | 75 | 0.11 | 0.79 | 0.90 | 0.93 | 0.88 | 0.89 |
| 13 | 75 | 0.08 | 1.06 | 1.13 | 0.77 | 0.51 | 0.48 |
| 11 | 75 | 0.06 | 1.21 | 0.82 | 1.32 | 0.87 | 0.85 |
| 34 | 75 | 0.09 | 0.65 | 0.67 | 0.67 | le | 0.61 |
| 14 | 75 | 0.14 | 1.02 | 0.99 | 1.21 | 1.46 | 1.44 |
| 4 | 75 | 0.10 | 0.03[c] | 0.66 | le | 0.42 | le |
| 38 | 75 | 0.03 | le | 0.44 | le | 0.52 | 0.52 |
| 35 | 75 | 0.05 | le | 0.47 | le | 0.50 | 0.49 |
| 33 | 75 | 0.06 | le | 0.35 | le | 0.33 | 0.36 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 32 | 50 | 0.09 | 0.55 | 0.78 | 0.55 | 0.56 | le |
| 23 | 50 | 0.11 | 1.51[c] | 1.01 | 0.90 | 0.40 | 0.61 |
| 21 | 50 | 0.05 | 0.65 | 0.72 | 0.59 | 0.59 | 0.70 |
| 22 | 50 | 0.07 | 0.87[c] | 0.51 | ng | ng | ng |
| 25 | 50 | 0.15 | 1.22[c] | 0.51 | 0.23 | le | 1.13[c] |
| 8 | 50 | 0.27 | 1.60 | 1.57 | 1.44 | le | 1.83 |
| 15 | 50 | 0.03 | le | 0.45 | le | 0.55 | 0.49 |
| 1 | 50 | 0.06 | le | 0.29 | 0.43 | 0.34 | 0.37 |
| 30 | 50 | 0.08 | le | 0.13 | 0.30 | 0.39 | 0.37 |
| 20 | 0 | 0.05 | 0.07 | 0.07 | 0.07 | le | 0.07 |
| 36 | 0 | 0.03 | 0.11 | 0.11 | 0.10 | 0.08 | 0.06 |
| 27 | 0 | 0.05 | 0.11 | 0.06 | 0.14 | 0.05 | 0.04 |
| 10 | 0 | 0.06 | 0.37[c] | 0.06 | le | le | 0.02 |
| 7 | 0 | 0.27[c] | 0.04 | 0.04 | 0.12 | le | 0.13 |
| 16 | 0 | 0.15 | 0.02 | 0.13 | 0.10 | le | 0.15 |
| 31 | 0 | 0.05 | le | 0.04 | 0.15 | 0.12 | 0.03 |
| 17 | 0 | 0.06 | le | 0.13 | 0.14 | 0.25[c] | 0.08 |
| 19 | 0 | 0.05 | le | 0.07 | le | 0.10 | 0.12 |

[a]ng = No gestation

[b]le = Laboratory analysis error

[c]Value not included in statistical analysis

Table 7. Maternal reproductive tract and fetal data in lead exposed and control rats

| Animal Number | Treatment level (mg/kg) | Number of breeding attempts | Ovarian wet weight (mg) | Uterus and conceptus wet weight (g) | Viable[a] conceptus wet weight (g) |
|---|---|---|---|---|---|
| 5 | 100 | 10[c] | 521.3 | - | - |
| 9 | 100 | 3 | 116.2 | 53.28 | 2.98 |
| 2 | 100 | 2 | 101.6 | 56.65 | 3.45 |
| 39 | 100 | 3 | 128.4 | 60.44 | 3.06 |
| 26 | 100 | 2 | 117.1 | 38.66 | 3.13 |
| 3 | 100 | 10[c] | 51.8 | - | - |
| 37 | 100 | 3 | 120.0 | 37.92 | 2.66 |
| 12 | 100 | 4 | 95.7 | 11.80 | 1.86 |
| 29 | 100 | 5 | 118.7 | 50.29 | 2.00 |
| 24 | 75 | 6 | 121.7 | 43.42 | 3.23 |
| 13 | 75 | 2 | 95.8 | 50.47 | 3.43 |
| 11 | 75 | 2 | 115.6 | 52.88 | 3.38 |
| 34 | 75 | 3 | 113.1 | 15.50 | 4.53 |
| 14 | 75 | 5 | 117.2 | 47.25 | 3.18 |
| 4 | 75 | 2 | 119.8 | 23.25 | 4.09 |
| 38 | 75 | 3 | 144.0 | 41.54 | 3.45 |
| 35 | 75 | 2 | 112.8 | 41.96 | 3.06 |
| 33 | 75 | 1 | 113.5 | 66.98 | 3.35 |
| 32 | 50 | 2 | 120.0 | 41.84 | 3.62 |
| 23 | 50 | 3 | 99.8 | 42.94 | 2.65 |
| 21 | 50 | 1 | 115.7 | 28.71 | 3.13 |
| 22 | 50 | 10[c] | 52.6 | - | - |
| 25 | 50 | 3 | 135.4 | 18.04 | 2.58 |
| 8 | 50 | 4 | 137.5 | 55.50 | 3.20 |
| 15 | 50 | 2 | 113.8 | 49.13 | 3.19 |
| 1 | 50 | 1 | 124.9 | 65.77 | 3.26 |
| 30 | 50 | 1 | 112.4 | 52.84 | 3.45 |

[a]Mean

[b]Total pooled value

[c]Not included in statistical test

| Viable[a] placental wet weight (mg) | Number of viable pups | Number of non-viable pups | Total[b] fetal brain wet weight (g) | Fetal[b] brain DNA (mg/g DFFT) |
|---|---|---|---|---|
| - | - | - | - | - |
| 628.8 | 8 | 4 | 0.93 | 174 |
| 675.5 | 11 | 2 | 1.58 | 164 |
| 673.0 | 10 | 2 | 1.41 | 185 |
| 676.3 | 8 | 5 | 1.00 | 166 |
| - | - | - | - | - |
| 744.4 | 9 | 3 | 1.36 | 121 |
| 595.0 | 2 | 0 | 0.13 | 167 |
| 625.8 | 12 | 1 | 1.62 | 185 |
| 726.7 | 6 | 4 | 1.06 | 109 |
| 649.0 | 10 | 2 | 1.34 | 176 |
| 614.6 | 11 | 2 | 1.54 | 164 |
| 860.0 | 1 | 4 | 0.17 | 169 |
| 721.7 | 12 | 0 | 1.14 | 201 |
| 823.3 | 3 | 3 | 0.05 | 196 |
| 731.2 | 8 | 0 | 1.05 | 157 |
| 596.7 | 9 | 1 | 1.14 | 162 |
| 642.9 | 14 | 0 | 2.03 | 181 |
| 725.0 | 8 | 2 | 1.02 | 174 |
| 539.0 | 10 | 1 | 1.19 | 186 |
| 616.7 | 6 | 5 | 0.60 | 158 |
| - | - | - | - | - |
| 563.3 | 3 | 3 | 0.32 | 169 |
| 691.8 | 11 | 1 | 1.47 | 167 |
| 683.0 | 10 | 4 | 1.29 | 177 |
| 607.1 | 14 | 1 | 1.96 | 163 |
| 608.2 | 11 | 1 | 1.57 | 162 |

Table 7 (continued)

| Animal Number | Treatment level (mg/kg) | Number of breeding attempts | Ovarian wet weight (mg) | Uterus and conceptus wet weight (g) | Viable[a] conceptus wet weight (g) |
|---|---|---|---|---|---|
| 20 | 0 | 1 | 107.1 | 38.12 | 3.50 |
| 36 | 0 | 2 | 100.9 | 53.20 | 3.10 |
| 27 | 0 | 2 | 111.5 | 50.74 | 3.85 |
| 16 | 0 | 1 | 121.9 | 61.70 | 3.15 |
| 7 | 0 | 1 | 110.1 | 58.82 | 2.84 |
| 10 | 0 | 2 | 136.5 | 35.92 | 3.36 |
| 31 | 0 | 2 | 104.9 | 47.22 | 3.22 |
| 17 | 0 | 2 | 106.0 | 31.87 | 3.41 |
| 19 | 0 | 1 | 107.9 | 48.31 | 3.73 |

| Viable[a] placental wet weight (mg) | Number of viable pups | Number of non-viable pups | Total[b] fetal brain wet weight (g) | Fetal[b] brain DNA (mg/g DFFT) |
|---|---|---|---|---|
| 657.1 | 7 | 2 | 0.71 | 171 |
| 604.4 | 9 | 2 | 1.16 | 162 |
| 752.2 | 9 | 1 | 1.23 | 182 |
| 633.1 | 13 | 1 | 1.79 | 174 |
| 672.3 | 13 | 0 | 1.51 | 164 |
| 725.0 | 6 | 1 | 0.70 | 183 |
| 635.0 | 10 | 1 | 0.99 | 170 |
| 760.0 | 6 | 1 | 0.68 | 162 |
| 712.5 | 8 | 1 | 1.08 | 169 |