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MEDICAL RESEARCH REPORT #EA7102

THE CHRONIC TOXICITY OF LEAD

The American Petroleum Institute
Environmental Affairs
1801 K Street, N.W.
Washington, D.C. 20006

PREFACE

This publication describes in brief summary the results of one of the most comprehensive toxicological studies of lead that has ever been undertaken. The investigation spanned a period of about three years during which lead acetate was fed for prolonged periods to four species of experimental animals. The study consisted of ten separate but related projects which were reported individually.

The entire report is so voluminous as to preclude wide distribution on a routine basis. The purpose of this highly abbreviated summary is to call attention to the existence of these reports and to advise interested persons of their availability. The Institute feels that research of this scope and significance should be in the public domain. For this reason the entire report has been filed with:

The Clearinghouse for Federal Scientific and Technical Information
National Technical Information Services
5285 Port Royal Road
Springfield, Virginia 22151

Requests for copies of the reports on any or all of the individual projects should be directed to the clearinghouse.

This research was performed under contract with the American Petroleum Institute by:

Toxicology – Biosciences Laboratory
Hazleton Laboratories, Inc.
Falls Church, Virginia 22046

under the direction of the Senior Investigator, D. Clifford Jessup, Ph.D. The conclusions contained in the reports are those of the investigators. Inquiries concerning the research should be addressed to Dr. Jessup.

Copies of this summary report may be purchased from the American Petroleum Institute at $1.00 per copy. Requests from non-profit institutions for complementary copies will be considered.

American Petroleum Institute

April 1971
Washington, D.C.

INTRODUCTION

Although medical-toxicological literature contains what may be considered a plethora of information on clinical lead poisoning in man and on the effects of short term exposures of experimental animals to large amounts of lead, little information is available on the toxic effect of prolonged exposures to small amounts of lead.

This research was designed to evaluate the effects of ingestion of small amounts of lead by laboratory animals over a substantial part of their lifespan. Four mammalian species, rats, dogs, monkeys and rabbits, were studied. In addition to the common chronic toxicity tests, the program included studies on reproduction, teratology, behavior, carcinogenicity, and metabolism as well as special enzyme and electron microscopy studies.

Lead was administered perorally to the animals as lead acetate, $Pb(C_2H_3O_2)_2 \cdot 3H_2O$. Dietary levels and dosages were calculated on the basis of actual lead, this being 54.6% of weight of the acetate. The amount of lead administered to the animals was far in excess of the usual intake by man. For example, on a per body weight basis, the dogs and rats on the lowest dietary level ingested approximately 100 times the amount ingested by an average American adult. In the monkeys the intake was 300 times as high as that of the average American adult.

Where applicable, the life-table technique was used for the statistical analysis of survival and analysis of variance (F-Test) at the 5% probability level was used for the other criteria of toxic effect.

Individual reports on the ten projects comprising the investigation have been issued under the following titles:

1. 22-Month Chronic Toxicity Study – Rats
2. 22-Month Dietary Administration – Dogs
3. 22-Month Chronic Toxicity Study – Monkeys
4. Three Generation Reproduction Study – Rats
5. Teratology Study – Rabbits
6. Rat Behavior Study; Monkey Behavior Study
7. One-Year Newborn Rat Carcinogenesis Study
8. Tissue Enzyme Studies
9. Electron Microscopy Study
10. Radiotracer Metabolism Study

INDIVIDUAL STUDY SUMMARIES

1. 22-Month Chronic Toxicity Study – Rats

The purpose of this project was to characterize and evaluate the effects of long-term oral administration of lead acetate in male and female albino rats (Charles River CD).

Methods. Lead acetate was incorporated into basal laboratory diet and offered to four groups of 50 male and 50 female rats each for 22 months at lead (as lead) concentration levels of 10, 50, 100, and 1000 ppm. A control group of 100 male and 100 female rats was maintained on the basal laboratory diet.

The criteria used for evaluation of toxic effect included:

Gross Effects:
- Appearance
- Behavior
- Food consumption and growth
- Survival

Hematology:
- Complete blood count
- Hematocrit
- Coagulation and Prothrombin times

Biochemistry:
- BUN
- Glucose
- Methemoglobin
- Blood lead
- Urine coproporphyrin
- Urine ALA

Enzymes:
- SGOT
- Alkaline phosphatase
- G-6-P

Cholinesterase (red cells and plasma)
LDH

Urinalysis

These observations and tests were carried out periodically throughout the feeding period. At six-month intervals randomly selected animals from each group were sacrificed for gross and microscopic pathology and for organ weight determinations.

Results. No compound-related effect was demonstrated at any test level for the following parameters: physical appearance, behavior, growth, food consumption, survival, and clinical laboratory studies.

Although not considered indicative of adverse effects, the following responses were observed: during the early part of the study, weight gain and food consumption in the 100 ppm and 1000 ppm male groups were somewhat lower than those in the controls; however, after this initial lag, growth and food consumption for these groups were comparable with those of the controls. Hemoglobins were slightly lower in the 1000 ppm animals than in the controls but were within the normal range for laboratory rats. At 12 months, the glucose-6-phosphatase levels in the 100 ppm and 1000 ppm groups were lower than in the controls; however, the terminal glucose-6-phosphatase values in all test groups are comparable with those in the controls.

The blood lead concentrations in the high level rats were somewhat elevated at 12 and 22 months compared with the controls. There was also an increase of urinary delta-amino-levulinic acid in the high level animals at each determination and an increase in urinary coproporphyrin as well at 22 months.

Statistical evaluation of organ weight data revealed significantly increased kidney/body weight ratios for the 1000 ppm males that were sacrificed at 6 and 12 months and significantly increased kidney weights and kidney/body weight ratios for the 1000 ppm males and females that were sacrificed after 22 months.

In the 1000 ppm animals at 22 months unique and distinctive histopathologic changes were observed in the kidneys of animals of both sexes. These changes included intranuclear inclusion bodies and cytomegaly in most of the animals. Neoplasia of the cortical epithelium was observed in certain of the male animals. Atypical (nodular or adenomatous) renal epithelial hyperplasia was observed in seven of 15 males and in three of 15 females in the 1000 ppm group, in four of 14 males in the 100 ppm group, and in a single male of the 50 ppm group. Murine nephrosis was also observed in animals from all groups including the controls.

Histopathological examination of the liver also revealed a compound-related effect in the rats of the 1000 ppm test group. This consisted of nodular hyperplasia with vacuolated hepatocytes and was observed mainly in the females.

2. 22-Month Dietary Administration -- Dogs

This study was conducted to evaluate and characterize the effects of long-term dietary administration of lead acetate in male and female dogs.

Methods. Groups of six male and six female beagles were fed lead acetate in their diets at lead concentrations of 10, 50, 100, and 1000 ppm. A control group of six males and six females was maintained on the basal laboratory diet.

The criteria used for evaluation of toxic effect included:

Gross Effects:
- Appearance
- Behavior
- Appetite
- Growth
- Elimination
- Survival

Hematology:
- Complete blood count
- Hematocrit
- Coagulation and Prothrombin times

Blood Biochemistry:
- BUN
- Glucose
- Lead
- Sodium
- Potassium
- Calcium
- Phosphorus
- Magnesium
- Chloride
- Proteins (electrophoresis)

Enzymes:
- Alkaline phosphatase
- SGOT
- SGPT
- G-6-P
- LDH

Urine:
- Routine urinalysis
- Lead

Coproporphyrin
ALA

These observations and tests were carried out periodically throughout the feeding period.

After one year on test, one male and one female were sacrificed and weights of nine principal organs were determined. The organs were examined for gross and microscopic pathology.

Results. One male in the 100 ppm group died of urolithiasis after 45 weeks on test. This was considered to be an incidental condition.

All of the dogs that received lead were comparable with the controls in respect to appearance, behavior, appetite, elimination, body weight, organ weights, and gross pathology.

Lead levels in the blood of the dogs of the 1000 ppm group were slightly higher than those of the controls, particularly at 22 months. No other consistent compound-related trends or alterations were observed. Two high level dogs had moderately decreased hemograms at 22 months in comparison with initial values, and two high level dogs had slightly increased delta-aminolevulinic acid content in the urine at 22 months. The remaining parameters were within accepted limits and were comparable between the groups.

Histopathological examination revealed a very slight increase in the degree and incidence of vacuolation of the hepatocytes in the compound-treated animals and a thickening of the glomerular tufts observed mainly in the kidneys of the females at the 1000 ppm level. No other cytopathologic changes were observed.

3. 22-Month Oral Toxicity Study – Monkeys

The principal objective of this project was to evaluate the effect of lead on the behavior of monkeys. The secondary objective was to evaluate the long-term toxicity of orally administered lead acetate in the non-human primate. This report deals with the general toxicity of lead in monkeys. The sixth report in this series is concerned with the behavioral aspects of the project.

Methods. Two groups consisting of two male and two female rhesus monkeys each received daily dosages of 1.25 and 25.0 mg/kg, respectively, of lead acetate in distilled water by stomach intubation, seven days per week for 22 months.

A control group of two male and two female monkeys were intubated with distilled water, alone, and maintained under conditions similar to the test groups.

The criteria used for evaluation of toxic effect included:

Gross Effects:
Appearance
Behavior
Appetite

Elimination
Growth
Survival

Hematology:

Complete blood count
Hematocrit
Coagulation and Prothrombin times

Blood Biochemistry:

BUN
Glucose
Bilirubin
Sodium
Potassium
Chlorides
Carbon dioxide
Calcium
Phosphorus
Proteins (electrophoresis)
PBI

Enzymes:

Alkaline phosphatase
SGPT
SGOT
G-6-P
LDH

Urinalysis

These observations and tests were carried out periodically throughout the feeding period.

Gross and microscopic pathological examinations were made on all animals that died or were sacrificed at the termination of the study.

Results. One male from the 25 mg/kg group died after nine months on test. Death apparently was due to respiratory disease and was not considered attributable to the lead.

No gross changes in appearance, behavior, or body weights were observed. Only incidental variations in hematological and biochemical parameters occurred. Analyses performed on urine indicated no deviations from normal.

Histopathological examination of tissues indicated compound-related changes in sections of liver, kidney, and bone marrow from animals receiving 25 mg/kg/day for 22 months. The kidney lesions consisted of inclusion bodies and degeneration of the tubular epithelial cells. In the liver

intranuclear inclusion bodies were observed in the hepatocytes. Bone marrow in the high level monkeys showed increased activity.

4. Three-Generation Reproduction Study – Rats

The purpose of this study was to evaluate the effect of long term ingestion of lead acetate on reproduction in albino rats (Charles River CD).

Methods. The test material was fed to the rats through three parental and three two-litter filial generations at dietary levels of 10, 100, and 1000 ppm of lead.

Criteria evaluated were the indices of fertility, gestation, live birth, and lactation; the litter size; and the physical appearance and growth of the pups during the nursing and postweaning period.

Results. There was no evidence at any test level of an adverse effect on the survival, appearance, behavior, body weight gain, and food consumption of the parental generations; on the reproductive performance of the parents reflected by the various indices; or on the growth, appearance, and behavior of the offspring. The organs of representative weanlings sacrificed from control and test groups showed no compound-related, gross changes at any of the test levels.

5. Teratology Study – Rabbits

This study was to evaluate the potential of lead acetate for embryotoxicity and/or teratogenic effects in albino rabbits (New Zealand White).

Methods. Three groups each consisting of 15 male and 15 female rabbits were mated. Two groups of females were fed lead acetate in the diet at lead concentrations of 54.6 and 546 ppm from day 7 through day 16 of their gestation period. The third group of females and all of the males received the basal laboratory diet, alone.

Does were sacrificed on day 29 or day 30 of the gestation period. Caesarean sections were performed and the following observations recorded: number and placement of uterine implantation and resorption sites; number and placement of live and dead fetuses at delivery; number of live and dead fetuses following 24-hour incubation period (one-half of the live fetuses in each litter were incubated); fetal weight and length (crown - rump); external fetal anatomy and gross visceral features. All fetuses were preserved and processed for study of visceral and skeletal abnormalties. Necropsies were performed on all the does.

Results. Two control females died within the first week following mating. One pregnant control died on Day 29. One abortion in the control group and one apparent abortion in the high lead group occurred during the study. Dead fetuses were found at Caesarean section in four low level and three high level females. Survival of fetuses during the 24-hour post-delivery incubation

period exceeded 80% in all groups and was comparable with the control group. A total of 29 of the 43 does used in this study (the two control females that died within the first week following mating were excluded) became pregnant (10 control, 9 low level, and 10 high level).

By the following criteria there was no evidence of compound effect: maternal survival, behavior, fertility, body weight, and gross visceral pathology; uterine implantation and resorption sites; number, weight, and length of live and dead fetuses; and gross external visceral, and skeletal fetal anatomy.

6. Rat Behavior Study; Monkey Behavior Study

Rat Study. This project was designed to determine whether long-term administration of lead has an adverse effect on behavior patterns. It was carried out in 10 animals (5 males and 5 females) from each of the five groups (control, 10, 50, 100, and 1000 ppm feeding levels) in the 22-month chronic toxicity test. Prior to initiation of the lead feeding, these animals were trained in the following standard behavioral procedures:

Flinch-Jump Test
Rope-Climb Test
Spontaneous Locomotor Activity Test

At six-month intervals, the rats were retested to determine if compound administration had any effect. No compound-induced effect could be determined at levels of treatment up to 1000 ppm for one year.

Monkey Study. Using established techniques for evaluating changing behavior patterns, the monkeys from the chronic toxicity study (two males and two females per group, stomach-intubated with 0, 1.25, and 25.0 mg/kg/day, respectively) were evaluated prior to treatment and at intervals up to one year.

At levels up to 25 mg/kg, no consistent behavioral changes occurred which could be attributed to the compound administration.

7. One-Year Newborn Rat Carcinogenicity Study

In order to study the possible carcinogenicity of lead, the acetate was injected on the following sequential schedule: (1) subcutaneously at 48 hours of age; (2) intraperitoneally at 7 days of age; (3) subcutaneously and intraperitoneally at 23 to 25 days of age at increasing concentrations until the cumulative maximum tolerated dose (110 mg/kg) was attained.

At the end of one year, necropsies revealed that the lobes of the liver were thickened, misshapen, and adherent to other internal organs. Histological examination revealed chronic inflammation, foci of mineralization, slight increase in bile duct proliferation, and minimal to moderate pericholangitis. In kidney tissue, numerous megalocytes were seen in the proximal convoluted tubules. There was no evidence of neoplastic changes.

8. Tissue Enzyme Studies

Selected target organs, obtained at the 6- and 12-month sacrifices from the rat chronic toxicity study, were examined to evaluate the effect of the chronic administration of a diet containing 1000 ppm of lead on specific enzymes. The adenosine triphosphatase activity of the mitochondria and glucose-6-phosphatase activity in the microsomal fraction, were determined.

The studies on liver and kidney tissues from rats that had received 1000 ppm of lead in the diet for 12 months demonstrated a reduction in kidney glucose-6-phosphatase. Liver extracts gave normal values for both glucose-6-phosphatase and adenosine triphosphatase.

9. Electron Microscopy Studies

Tissues derived from the rat chronic toxicity study at the six-month sacrifice were examined with the electron microscope. Samples of liver, kidney, bone marrow, and small intestine of three males and three females from the control and 1000 ppm groups were studied.

The only finding that was unique to the rats that had received lead was the presence of inclusion bodies within the nuclei of the proximal convoluted tubules of the kidney.

10. Radiotracer Metabolism Study

The purpose of this study was to determine the extent of gastrointestinal absorption, the distribution in selected tissues, and the rate and route of excretion of ^{210}lead acetate following oral administration to rats. Metabolic studies utilizing radiotracer techniques were employed.

Two groups of rats (Charles River CD), one maintained on basal laboratory diet and the other fed a diet containing 1000 ppm of lead for 30 days, were given a mean single oral dose by stomach intubation of 0.024 microcuries/gram and 0.021 microcuries/gram respectively. Seventy-two hours after administration of ^{210}lead acetate, approximately 90% of the radioactivity was detected in the feces of the control rats and 92% in the feces of the animals primed with 1000 ppm lead. The urine of the controls contained 0.66% of the administered radioactivity and the urine of the treated rats, 0.76%. Approximately 5.6% and 4.6% of the administered radioactivity were retained in the gastrointestinal tracts, the organs, and the carcasses of the control and treated rats, respectively.

An attempt was made to locate, within selected tissues, the subcellular sites of ^{210}lead deposition employing autoradiography and electron microscopy. Tissues from four rats on the radiotracer metabolism experiment were studied. Sufficient amounts of radioactive lead were not absorbed from the gastrointestinal tract and deposited in the tissues to produce readable autoradiographs.

PROGRAM SUMMARY

These studies have shown that the administration of lead acetate to rats, dogs and monkeys for 22 months at a dietary level of 10 ppm as lead produced no effects that could be detected by any of the techniques that were employed. Thus, 10 ppm in the diet is a "no-effect" level. Minimal, equivocal effects were observed in some animals at 50 ppm. Histological changes, principally in the kidney, occurred in the rats of the 100 ppm and 1000 ppm groups. At these levels, however, functional changes were not detected.