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INTERNATIONAL LEAD ZINC RESEARCH ORGANIZATION, INC.

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July 30, 1970

To: All Members of the Environmental Health Committee

From: J. F. Cole, Sc. D.

Gentlemen:

Enclosed are two reports for your interest:

1) LH-151 Study Atmospheric Contaminants in Animals and Man
Three Month Progress Report - This report includes initial data on the exposure of rats and monkeys in preparation for the human volunteer study.

→ 2) Toxicity of Orally - Administered Lead - Program Summary
This recently completed program was sponsored by the American Petroleum Institute at Hazelton Laboratories. Through the kind permission of Dr. H. H. Golz, Medical Director of the API, we are distributing copies to members of the ILZRO Environmental Health Committee. This report is for your personal use only. It should not be cited as a reference since it will be published in the near future by Hazelton and the API.

Sincerely,

Jerome F. Cole

J. F. Cole, Sc. D.
Manager, Environmental Health

JFC:ls
Enc.

TOXICITY OF ORALLY-ADMINISTERED LEAD

PROGRAM SUMMARY

Submitted to

American Petroleum Institute
New York, New York

HAZLETON LABORATORIES, INC.
A SUBSIDIARY OF TRW INC.
P.O. BOX 30, FALLS CHURCH, VIRGINIA 22046

June 26, 1970

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HAZLETON LABORATORIES, INC.
TRW LIFE SCIENCES CENTER

TOXICITY OF ORALLY-ADMINISTERED LEAD

PROGRAM SUMMARY

Introduction

This program was designed to evaluate the oral toxicity of lead when administered to laboratory animals for lengthy periods of time. In order to provide for a more extensive and meaningful evaluation, four species of animals have been used; and a number of special studies have been performed.

In addition to the standard chronic toxicity studies, reproduction, teratology, carcinogenicity, behavioral, and metabolic studies were also performed. Special enzyme and electron microscope studies were incorporated into the chronic studies.

Studies Performed

The following separate studies were performed:

1. Two-Year (22-Month) Oral Toxicity Study in Rats
2. Two-Year (22-Month) Oral Toxicity Study in Dogs
3. Two-Year (22-Month) Oral Toxicity Study in Monkeys
4. Three-Generation Reproduction Study in Rats
5. Teratology Study in Rabbits
6. One-Year Behavioral Study in Rats
7. One-Year Behavioral Study in Monkeys
8. One-Year Newborn Rat Carcinogenesis Study
9. Tissue Enzyme Studies in Rat and Dog
10. Electron Microscopy Study
11. Metabolism Studies in Rat

Individual Study Summaries

1. 22-Month Oral Toxicity Study - Rats

This study was conducted to evaluate and characterize the effects of long-term dietary administration of lead acetate in male and female albino rats. Lead acetate was administered to the rats for 22 months at dietary levels of 10, 50, 100, and 1000 ppm (calculated on the basis of 54.6% lead content).

Criteria evaluated for a toxic effect were physical appearance, behavior, growth, food consumption, survival, clinical laboratory results, organ weights, and gross and microscopic pathology.

No compound effect was demonstrated at any test level with regard to physical appearance, behavior, growth, food consumption, survival, and clinical laboratory results.

During the early part of the study, body weight gains and food consumption in the 100 ppm and 1000 ppm male groups were somewhat lower than the control; however, after this initial lag, growth and food consumption for these groups were comparable with the control.

The clinical values for the test groups showed no significant differences from those for the controls. Hemoglobin determinations for the 1000 ppm male group were somewhat lower than the controls but within normal range for laboratory rats. At 12 months, the glucose-6-phosphatase determinations for the 100 and 1000 ppm groups were lower than

the controls; however, the terminal glucose-6-phosphatase values for all test groups were comparable with those for the controls.

The blood lead content determined at 12 and 22 months for the high level rats was somewhat elevated compared with the control values. There was also an increase of delta-aminolevulinic acid in the urine of the high level animals at each determination and at 22 months, an increase of coproporphyrin in the urine.

Statistical evaluation of organ weight data revealed significantly increased kidney/body weight ratios for the 1000 ppm male groups sacrificed at six and 12 months and significantly increased kidney weights and ratios for the males and females in the 1000 ppm group sacrificed after 22 months. Grossly, the kidneys of the 1000 ppm rats which died late in the study or were sacrificed at termination presented more alterations than those from rats in the control and other test groups.

Histopathological evaluation of tissue sections revealed a definite compound effect in the kidneys of the rats at each test level and in the livers of the rats in the 1000 ppm test group. Pathologic changes in the kidneys were characterized mainly by alterations of individual renal epithelial cells including regenerative forms, hyperplasia, and neoplasia of the tubular epithelium with development of renal adenoma or adenocarcinoma. Both the incidence and the degree of regenerative

epithelium in the cortical tubules were greater in the test rats with a definite dosage relationship. Neoplastic renal alterations were observed in male rats only. Compound-related, histopathological alterations in the liver consisted of nodular hyperplasia with vacuolated hepatocytes observed primarily in the 1000 ppm females.

2. 22-Month Oral Toxicity Study - Dogs

Lead acetate was fed to four groups of six male and six female beagles each at dietary levels of 10, 50, 100, and 1000 ppm. Dosage levels were calculated on the basis of the lead content (54.6% by weight) of the lead acetate. One dog of each sex and group, except for one male dog at the 100 ppm level which died at 45 weeks because of urolithiasis (an incidental condition), was sacrificed after one year of compound feeding. The remaining animals were continued on study for 10 more months, or a total of 22 months, and then sacrificed. A comparable group of male and female beagles served as negative controls.

The dogs were observed daily for signs of compound effect. Clinical laboratory studies were performed initially and at selected intervals during the study, and lead concentration was determined in blood and urine. Gross necropsies were performed in each sacrificed animal, and selected tissues were preserved for histopathological evaluation.

Lead concentration was slightly increased in the blood of the dogs at the 1000 ppm level, particularly at 22 months, in comparison with

control values. 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 clinical values were within accepted limits and comparable between the groups.

The compound-treated dogs were comparable with the controls regarding appearance, behavior, appetite, elimination, body weight changes, organ weights, and gross pathology.

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

3. 22-Month Oral Toxicity Study - Monkeys

Lead acetate was administered orally for 22 months to three groups of young rhesus monkeys. This was a limited toxicity study in that only two test groups containing two male and two female monkeys each were used for comparison with a control group of equal size. Dose levels administered were 0, 1.25, and 25 mg/kg calculated as lead.

Daily observations for appearance, behavior, and pharmacotoxic signs were made. Body weights were taken weekly. Clinical laboratory studies were performed initially and at intervals during the study.

Gross necropsies were performed on each animal sacrificed at 22 months, and selected tissues were preserved for histopathological evaluation. No gross changes in appearance, behavior, or body weights were observed. Only incidental variations in hematological and biochemical parameters occurred.

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 degeneration of the epithelial cells of the proximal convoluted tubules and the presence of inclusion bodies in the epithelial cells of the tubules. Liver changes consisted of the presence of intranuclear inclusion bodies in the hepatocytes. Bone marrow in high level monkeys showed an increased activity.

4. Three-Generation Reproduction Study - Rats

This study was conducted to evaluate the effects of long-term ingestion of lead acetate on reproduction in albino rats. The test material at dietary levels of 10, 100, and 1000 ppm was fed to the rats through three parental and three two-litter filial generations.

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.

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 conducted to evaluate the potential of lead acetate for embryotoxicity and/or teratogenic effects in albino rabbits. Dosage levels used in dietary administration of the test material were 0 ppm (control), 54.6 ppm (low level), and 546 ppm (high level).

Two control females died within the first week following mating. One pregnant control female died on Day 29. One abortion in the control group and one apparent abortion in the high level group were experienced 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 postdelivery incubation period exceeded 80% in all groups and was comparable among the control and test groups. A total of 29 of the 33 does used in this study (excluding the two control females that died within the first week following mating) became pregnant (10 control, nine low level, and 10 high level).

Evaluation of the following criteria revealed no evidence of compound effect: maternal survival, behavior, fertility, body weights, 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. One-Year Behavioral Study - Rats

To evaluate the potential of lead acetate to adversely affect behavior patterns, groups of 10 animals from the control group and at dose levels of 10, 50, 100, and 1000 ppm were derived from the chronic toxicity study in rats. Prior to treatment, each group was trained in a number of 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.

7. One-Year Behavioral Study - Monkeys

Using established techniques for evaluating changing behavior patterns, the monkeys from the toxicity study 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.

8. One-Year Newborn Rat Carcinogenicity Study

In order to evaluate the carcinogenic potential of lead acetate, the test material was injected subcutaneously and intraperitoneally in four divided doses in neonatal rats at a level of 110 mg/kg.

At the end of one year, gross necropsies revealed that the lobes of the liver were thickened, misshaped, and adhering to other internal organs. Histopathological examination revealed chronic inflammation, foci of mineralization, slight increase in bile duct proliferation, and minimal to moderate pericholangitis.

No evidence that neoplastic changes occurred could be determined.

9. Tissue Enzyme Studies in Rats and Dogs

Selected target organs, obtained at sacrifice from the rat and dog chronic toxicity studies, were examined to evaluate the effect of the administration of lead acetate on specific enzymes in these tissues. The adenosine triphosphatase activity of the mitochondria was determined; and in the microsomal fraction, glucose-6-phosphatase activity and the DPWH cytochrome c reductase activity were determined.

Tissue enzyme studies performed on liver and kidney tissue from rats after 12 months treatment at 1000 ppm of lead in the diet demonstrated a reduction in kidney glucose-6-phosphatase concentration. Liver extracts gave no evidence of change in concentration of either glucose-6-phosphatase or adenosine triphosphatase.

10. Electron Microscopy Studies in Rats and Dogs

A limited study of tissues derived from the chronic toxicity studies at the six- and 12-month sacrifice was performed by utilizing the electron microscope.

Electron microscope studies of rat and dog tissues at 12 months indicated only the presence of nuclear inclusion bodies within the nuclei of the proximal convoluted tubules of the kidney.

11. Metabolism Studies in Rats

Radiotracer studies were performed in rats 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.

Metabolic studies utilizing radiotracer techniques were applied to tissues from rats which had received 0 and 1000 ppm lead for one month prior to the administration of ^{210}lead acetate. Seventy-two hours after administration of the radioactive material, some 90% of the radioactivity was detected in the feces of the control animals and 92% of it in the feces of the animals primed with 1000 ppm lead. Approximately 5.6% and 4.6% of the administered radioactivity was detected in the tissues of the control and pretreated rats, respectively.

General Comment

The data derived from the studies on lead acetate which were designed to evaluate the hazards attendant with the oral administration of lead to animals have shown that a dose level of 10 ppm of lead in the diet is a "no-effect" level. Minimal, equivocal effects were apparent in some animals at 50 ppm; but frank

toxicity, as demonstrated by the presence of histological lesions in certain target organs, occurred at levels of 500 ppm and 1000 ppm. At these levels; the histological changes had no apparent effect on organ functions.

Submitted by D. C. Jessup

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