MR. ROBERT L. ZIEGFELD ✓

AZI-LIA EXPANDED RESEARCH PROGRAM

AMERICAN ZINC INSTITUTE, INC.

LEAD INDUSTRIES ASSOCIATION, INC.

292 MADISON AVENUE
NEW YORK 17, N. Y.

July 2, 1963

SCHRADE F. RADTKE
Director of Research

IN REPLY REFER TO
LC999-83(40)

REPLY REQUESTED

To: All Members of the Health and Safety Committee

From: Don G. Fowler, ILZRO

Subject: Toxicity testing for organo-lead compounds.

Enclosures: For your review, comments and vote are the following:

(1) Proposal No. 1 - The Hine Laboratories Inc., San Francisco, California. Dated June 17, 1963.
(2) Proposal No. 2 - Industrial Bio-Test Laboratories, Inc., Northbrook, Illinois. Dated June 12, 1963.
(3) Proposal No. 3 - Industrial Hygiene Foundation of America, Inc. Pittsburgh, Pennsylvania. Dated December 17, 1962.
(4) Proposal No. 4 - Woodard Research Corporation, Herndon, Virginia. Dated April 25, 1963

At the Health and Safety Committee meeting on February 28, 1963, you will recall we discussed the screening of our new organo-lead compounds for toxicity. At that time we had proposals from two laboratories covering this work and it was felt by the membership that additional laboratories should be contacted and invited to send in proposals for the conduct of these tests. Accordingly, I solicited three other laboratories and obtained two additional proposals. Each of the four laboratories submitting proposals has been visited and investigated by me as to competency, capacity, cost, and standing in the field.

Summary of the Proposals:

	Price Per Compound
1. The Hine Laboratories Inc., San Francisco, California: Screening tests including ingestion and skin absorption. (10% reduction for 6 or more compounds submitted at the same time.)	$250.
2. Industrial Bio-Test Laboratories, Northbrook, Illinois: Range-finding toxicity tests for ingestion and skin absorption.	$275.

re: LC 79-63.

	Price Per Compound
3. Industrial Hygiene Foundation of America, Pittsburgh, Pennsylvania: Range-finding tests of ingestion and skin absorption.	$400.
4. Woodard Research Corporation, Herndon, Virginia: Range-finding tests of ingestion and skin absorption.	$180.

Recommendation: We recommend your favorable consideration of proposal No. 1 from the Hine Laboratories and proposal No. 2 from the Industrial Bio-Test Laboratories, Inc., for the following reasons:

1. Both laboratories are directed by competent individuals and staffed with experienced and competent help.

2. Their prices are in line with what you would expect to pay for this service.

3. The Directors of both Laboratories are engaged in daily activities which include a wide range of lead analyses and evaluations on the industrial scene. This is not true of the other two laboratories. For instance, the Woodard Laboratory has no lead work going on at the present time and when questioned were obviously unfamiliar with the details of lead analyses, and we have no desire to assist in the education at our expense of a commercial laboratory.

4. Preliminary screening of these materials is only the first step in any evaluation of compounds which may have commercial application and it will be well to select laboratories who have the capability to carry on subsequent testing such as vapor inhalation and eye irritation when these evaluations are needed. Both of the laboratories we recommend have such capabilities.

5. When our organo-lead compounds have developed a commercial feasibility, it will be necessary to generate certain toxicity data to be submitted to various government agencies to satisfy the requirements of such agencies. Both of the laboratories we recommend have the know-how to generate the necessary data and to guide us in obtaining the necessary government approval.

Funds and Recommendations: We recommend your approval of Proposals No. 1 and No. 2 so that either or both may be used in present and subsequent screening and toxicity testing. Funding will be as necessary to cover the number of compounds to be tested.

Don G. Fowler

Don G. Fowler
Project Manager

BALLOT

Mr. Dom G. Fowler
International Lead-Zinc Research Organization
292 Madison Avenue
New York 17, New York

I approve (do not approve) the use of The Kline Laboratories and the Industrial Bio-Test Laboratories on Project LC 79-63, "Toxicity Testing for Organo-Lead Compounds."

Name: _____

Company: _____

Date: _____

NOTE:
PLEASE RETURN THIS BALLOT BY JULY 29, 1963.

N 191.01

PROPOSAL NO. 1

THE HINE LABORATORIES INC.
Research and Development
1099 Folsom Street - San Francisco 3, California

June 17, 1963

Mr. Don G. Fowler
Lead Industries Association
292 Madison Avenue
New York 17

Dear Don:

It was a great pleasure to visit with you last week and to renew our friendship. Our previous work in the Air Pollution field was most enjoyable. I trust that we may find a field of joint effort in the matters of lead and the Lead Industries. I have always admired the work of these industrial associations such as API and Lead Industries, and have felt that they are fine examples of the American tradition of both competition and cooperation.

In regard to the matter of developing information for the safe handling of new lead products, our Laboratory would be most happy to work in this area with your group and would be pleased to make a proposal for your consideration relative to the extent of work which should be done and the costs involved. As I understand your immediate problem, there are a number of new lead compounds in varying degrees of development whose toxicities are in general unknown. Because of your long experience with organic leads and knowledge as to the much higher toxicity of these compounds as compared with different inorganic leads, it does seem appropriate for you to have determined the proper order of magnitude of toxicity so that suitable handling precautions as have been developed for both types of compounds could be applied. I would suggest, therefore, that consideration be given to determining toxicity by oral and percutaneous routes for all compounds, and that of toxicity following inhalation by compounds which may have a significant vapor pressure. At this stage only order of magnitude figures need be obtained, and we propose to use our modification of the standard method of Deichmann, in which the LD_0 and LD_{100} would be bracketed by administration of the compounds over a range of doses or concentrations. At such time as further information is needed concerning the compound, more precise values should be obtained in order to better define the toxicity and other tests would have to be performed to conform with the requirements of the Hazardous Substance Labeling Act.

We are appending our recommendations for accomplishing this preliminary toxicity evaluation and the costs relative to these. We should like to point out that our Laboratory has had considerable experience in the handling of dangerous and highly toxic compounds, including explosive materials, and that our staff, therefore, is competent to handle compounds of any degree of toxicity and potential hazard.

It is always more convenient and less expensive to work with a series of compounds, and for that reason our Laboratory gives a 10% discount on six or more samples submitted at a single time. We are in a position to start work on any toxicity test within approximately ten days after receipt of the compound or notification of its shipment.

We would be most pleased also to be considered in connection with other studies in which your laboratory might solicit the aid of a professional group such as ours, including the evaluation of the toxicity of paints.

I am looking forward to further pleasant associations.

Very truly yours,

Signed: Charley

C. H. Hine, M. D., Ph.D.
Director
The Hine Laboratories, Inc.

PROPOSAL FOR THE LEAD INDUSTRIES RELATIVE TO PRELIMINARY TOXICITY SCREENING PROJECTS

I. Intragastric toxicity: Groups of two rats (either sex, 100 gms. or more in weight) are given suitable dose levels with an increment of 1.5 in order to bracket LD_0 and LD_{100}. Cumulative mortalities are determined at 24, 48 and 168 hours. Total cost $100.00

II. Percutaneous toxicity: Groups of two rabbits (either sex, 2½ kgs. or more in weight) are given suitable dose levels with an increment of 1.5 in order to bracket the LD_0 and LD_{100}. Cumulative mortalities are determined at 24, 48 and 168 hours. Total cost 150.00

III. Vapor Toxicity: Groups of two rats (either sex, 100 or more gms. in weight) are exposed for one hour to suitable concentrations with an increment of 1.5 in order to bracket the LD_0 and LD_{100}. Cumulative mortalities are determined at 24, 48 and 168 hours. Total cost 200.00

Cost for all three tests, per compound 450.00

Cost for the oral screen and percutaneous, per compound 250.00

10% reduction for six or more compounds submitted at the same time

PROPOSAL NO. 2

INDUSTRIAL BIO-TEST LABORATORIES, INC.
1810 Frontage Road
Northbrook, Illinois
Telephone CHestwood 2-3030

June 12, 1963

Mr. Don G. Fowler
Project Manager
Lead Industries Association
292 Madison Avenue
New York 17, New York

Dear Don:

We will conduct acute oral and acute percutaneous toxicity studies as per our proposal of February 11, 1963.

The only change will be in the acute percutaneous test in which two rabbits will be used at each of 3 dose levels.

The eye irritation and acute inhalation tests are not of prime importance initially. These tests may be done later if further interest in a particular compound develops.

The cost for these tests will be $275.00 per material if a minimum of five materials are submitted at the same time.

Sincerely yours,

Signed: Joe

J. C. Calandra
Director

JCC:par

N 191.03

INDUSTRIAL BIO-TEST LABORATORIES, INC.
1810 Frontage Road
Northbrook, Illinois
Telephone CRestwood 2-3030

February 11, 1963

Mr. Don G. Fowler, Project Manager
Lead Industries Association
292 Madison Avenue
New York 17, New York

Dear Mr. Fowler:

We are enclosing our proposal for range-finding toxicity tests on materials in which your association is interested. The cost of these tests will be $600.00 per material tested.

If you have any questions on these procedures please do not hesitate to call us. Thank you again for permitting us to submit our proposals.

Sincerely,

Signed: J. C. Calandra

J. C. Calandra
Director

JCC:par
Encl.

RANGE-FINDING TOXICITY STUDIES

I. Range-Finding Acute Oral Toxicity - Albino Rats

A. Acute Oral Toxicity - Albino Rats

Healthy, young albino rats of the Sprague-Dawley strain with an average body weight of approximately 100 grams are used as test animals. The rats are divided into groups of four animals each for dosing purposes.

All animals used are kept under observation for five days prior to experimental use, during which period they are checked for general physical well-being and homogeneity. The animals are housed individually in stock cages and permitted a standard laboratory rat diet* plus water ad libitum until 16 hours immediately prior to oral intubation.

On the morning of the first test day, after a 16-hour fast (water permitted), the selected dose groups of four rats each are intubated with previously calculated doses of the test material. All doses are administered directly into the stomachs of the test rats using a hypodermic syringe equipped with a ball-pointed intubating needle.

Following oral administration of the test material, the rats are returned to their stock cages and observed for the succeeding 14 days. All significant reactions and mortalities which occur are recorded. Particular attention is given to the observation during the first four hours after intubation.

At the end of the observation period, all data are collected and the range-finding acute oral mean lethal dose ($RFLD_{50}$) of the test material is calculated using the technique of Weil**, Thompcom***, and Thompcom and Weil****.

* Rockland Rat Diet, Rockland Farms, New City, New York.

** Weil, Carrol S.: Tables for Convenient Calculation of Median-Effective Dose (LD_{50} or ED_{50}) and Instructions in Their Use. Biometrics, Sept. 1952.

*** Thompcom, William R.: Use of Moving Averages and Interpolation to Estimate Median-Effective Dose. Fact. Rev., Nov. 1947.

**** Thompcom, William R. and Weil, Carrol S.: On the Construction of Tables for Moving Average Interpolation, Biometrics, March 1952.

II. Range-Finding Acute Percutaneous Toxicity - Albino Rabbits

Young adult, New Zealand strain male albino rabbits are employed as test animals. The body weights of the rabbits used range from two to three kilograms. All rabbits are maintained under observation in the laboratory for five days prior to testing. During the pre-test period the animals are examined with respect to their general health and suitability as test animals. The rabbits are housed in stock cages and maintained on the standard laboratory rabbit ration*. Food and water are permitted ad libitum.

Twenty-four hours prior to the dermal applications, the backs of the rabbits are shaved free of hair with electric clippers. The animals are then returned to their stock cages to await testing on the following day. The 24-hour waiting period allowed recovery of the stratum corneum from the disturbance which accompanies the clipping procedure and also permits healing of any microscopic abrasions possibly produced during the process.

On the testing day, the dermal dose for each test rabbit is weighed accurately on an analytical balance. Applications of the test material are made in solid form on the exposure sites of the rabbits. Three dose levels employing four rabbits each are used. After each application, the exposure site is covered by wrapping the trunk of the animal with an impervious plastic sheeting which is securely taped in place. This plastic wrap insures intimate contact of epidermis and test material.

The test material remains in contact with the skin for 24 hours. Behavioral reactions are observed and recorded during the contact period, after which the plastic sheeting is removed from each test rabbit. The exposure sites are examined for local reactions and the animals returned to their stock cages. At this time it will also be possible to obtain an estimate of the primary skin irritating properties of the test materials. Observations for mortality, local reactions, and behavioral abnormalities are continued for a total of 14 days following the skin applications.

At the end of the observation period, all data were collected and the range-finding acute percutaneous mean lethal dose ($RFLD_{50}$) of the test material is calculated using the techniques of Weil**, Thompson***, and Thompson and Weil****.

* Rockland Rabbit Diet, Rockland Farms, New City, New York
** See footnote**, page 1
*** See footnote***, page 1
**** See footnote****, page 1

III. Range-Finding Acute Inhalation Toxicity - Albino Rats

Young, adult albino rats of the Sprague-Dawley strain were employed as the test animals. Pre- and post-test care and observation of these rats are essentially the same as that already described for rats used in the range-finding acute oral toxicity test (see p. 1). However, animals used for inhalation exposure were not fasted prior to testing.

Five groups of four rats each are utilized in the study of the test material. The average body weight of the test animals are approximately 250 grams. The inhalation exposures are conducted in a multiple inhalation chamber designed for simultaneous exposure to atmosphere saturated and fractionally saturated with the test material, e. g. 1/2 saturated, 1/4 saturated, 1/8 saturated and 1/16 saturated at room temperature (25°C). The test is designed to run for a four-hour period.

At the end of the exposure period, the rats are observed for the succeeding 14 days.

IV. Range-Finding Eye Irritation Test - Albino Rabbits

A group of five albino rabbits are used to evaluate the eye irritating properties of the test material. Young adult, New Zealand strain animals are selected for the test.

The test method employed is patterned after that of Draize et al*. Exactly 50 mg of solid test material is instilled into the conjunctival sac of the right eye of each test rabbit in a group of five animals. The left eye of each animal serves as a scoring control.

One, 24, 48, 72, 96 hours, and 7 days following the initial instillations, the cornea, iris, and palpebral conjunctiva are examined individually and graded for irritation and injury according to a standard scoring system*. The maximum possible score at any one examination and scoring period is 110 points which indicates maximal irritation and damage to all three ocular tissues. Zero score indicates no irritation whatever.

After the completion of the test, the scores are analyzed and a descriptive eye irritation rating is assigned to the test material. The criteria used for assignment of the descriptive rating are the frequency, the extent, and the persistence of irritation or damage which occurred to the three ocular tissues. In the classification system used, special emphasis is placed upon irritation or damage to the cornea. Correspondingly less stress is placed upon conjunctival and iridial effects.

The descriptive rating assigned are one of the following, each of which characterizes a particular level of ophthalmic irritation and damage:

Non-Irritating
Practically Non-Irritating
Minimally Irritating
Mildly Irritating
Moderately Irritating
Severely Irritating
Extremely Irritating
Maximally Irritating

* "Methods for the Study of Irritation and Toxicity of Substances Applied Topically to the Skin and Mucous Membranes", Draize, John H., Woodard Geoffrey, and Calvery, Herbert O., J. Pharm. & Exp. Ther. 82,4, December 1944.

PROPOSAL NO. 3

INDUSTRIAL HYGIENE FOUNDATION OF AMERICA, INC.
Mellon Institute, 4400 Fifth Avenue
Pittsburgh 13, Pa.

December 17, 1962

Mr. Louis Cappelli, Project Manager
Expanded Research Program
Lead Industries Association -
American Zinc Institute, Inc.
292 Madison Avenue
New York 17, New York

Dear Mr. Cappelli:

In accordance with our discussion when you visited us here in Pittsburgh, the following is an outline of the range-finding tests, information we should like to have on the compound, and the time required for such studies. The charges are listed for a single test on a single compound and the total charge when all tests are made on a single compound.

The charge for the complete range-finding study, which includes (individual test charges in parentheses):

1. The determination of the single dose oral administration LD50 for male, albino rats ($175),
2. The determination of the skin penetration LD50 for male, albino rabbits based upon a single continuous 24-hour contact with the intact and abraded skin ($225),
3. A single continuous inhalation exposure of male, albino rats to a single concentration for a period not exceeding eight hours ($175),
4. The determination of the primary skin irritation score which is an evaluation of the effect of the continuous exposure of the intact and abraded skin of male, albino rabbits for a maximum period of 24 hours ($75), and
5. The evaluation of the eye irritation produced in male, albino rabbits ($75), is $700.

It would expedite our testing if you could supply as much of the following data as possible: the structural formula, the molecular weight, the specific gravity, the boiling point, the vapor pressure, the stability at room temperature, and the solubility especially in water, organic solvents, and corn oil.

The usual time period required from the time of the receipt of the chemical to the date of the submitting of the final report is seven to eight weeks, which includes a one-to-two week period for the laboratory acclimatization of the animals and a two-week post-exposure period for the evaluation of the possible delayed effects or the recovery.

Sincerely yours,

Signed: HHS

H. H. Schrenk, Ph.S.
Managing Director

HHS:ds

cc: Mr. Don G. Fowler

PROPOSAL NO. 4.

WOODARD RESEARCH CORPORATION
Laboratory and Consulting Service
P. O. Box 405, Herndon, Virginia

April 25, 1963

Dr. Don Fowler
Project Manager
Lead Industries Association
292 Madison Avenue
New York 17, New York

Dear Doctor Fowler:

First let me apologize for the delay in sending you a research proposal as discussed when you visited with us last month. I put aside the notes taken at our conference for preparation of a proposal and they were then mislaid. After that, the whole project slipped from my mind until your phone call.

We are enclosing three copies of a proposed protocol for range finding toxicity tests on organo-lead compounds.

The estimated costs per compound for conducting the range finding tests are as follows:

Oral range finding in rats	$ 45.00
Dermal range finding in rabbits	75.00
Eye irritation	45.00
Inhalation, acute	115.00

We will be sending along a protocol for the lead limit paint study at a later date.

Sincerely yours,

Signed: Geoffrey Woodard

Geoffrey Woodard, Ph.D.
Pharmacologist

GW:sv

Enclosure

WOODARD RESEARCH CORPORATION

LEAD INDUSTRIES ASSOCIATION

ORGANO-LEAD COMPOUNDS

RANGE FINDING TOXICITY TESTS

ORAL-RATS: Rats weighing 150 to 250 grams, fasted overnight, will be given single oral doses of the compound under test in a suitable vehicle (corn oil, polyethylene glycol 300, water, or water suspension) as follows:

Number of Rats	Dose - mg/kg
2	1 X
2	3.16X
2	10. X
2	31.6 X
2	100 X
2	316 X

Where X is a suitable power of 10. If necessary, this dosage schedule will be expanded in order to yield both surviving and non-surviving animals. Signs of pharmacological and toxicological effects will be recorded. Representative survivors and non-survivors will be subjected to necropsy. Survivors will be held for 14 days after dosage.

DERMAL-RABBITS: The fur will be removed from the trunk of five young adult rabbits by close clipping. The compound under test either undiluted if a liquid or a suspension or solution in a suitable vehicle will be applied to the skin with gentle rubbing with a glass rod until the compound appears to have penetrated the skin or to have been spread uniformly. The trunk will then be wrapped with a protective covering and the rabbit placed in its cage. After 24 hours, the covering will be removed and any excess compound will be removed from the skin. Readings for irritation (erythema, edema) will be made at that time and again at two, three, four, and seven days. Signs of chemically induced effects will be recorded. At 14 days all survivors and any non-survivors in the interim will be subjected to necropsy. The dosage schedule will be the same as for the rat oral excepting that one animal at each dose will be used and no dose over 3160 mg/kg will be given.

EYE IRRITATION-RABBITS: The material under test will be placed in one eye of each of three rabbits, the other eye remaining as a control. If a liquid, 0.1 ml will be used per eye. If a solid, 100 mg will be ground in an agate mortar with 0.1 ml of polyethylene glycol 300 and the suspension placed in the eye. Readings for erythema, swelling, iritis, and corneal damage will be made at 2, 24, 48, 72 hours and at 5 and 7 days after application.

ACUTE INHALATION: Ten rats or alternatively five rats, three guinea pigs, and five mice will be placed in a suitable inhalation chamber. The compound under study will be introduced so as to yield practical atmospheric levels by means of a nebuliser for relatively non volatile or a bubbling device for volatile compounds. Exposure would continue for six to seven hours. Measured amounts of the inhalation atmosphere would be removed several times during the course of the exposure for chemical determination of the level in the chamber. Non-survivors and all surviving 14 days would be subjected to necropsy with special attention being given to the lungs, respiratory pathways, livers, kidneys, and hearts.

Inhalation studies would be made only when the compound appeared to present a possible hazard by this route because of its physical properties or because of high toxicity by other routes of administration.

Signed: Geoffrey Woodard

Geoffrey Woodard, Ph.D.
Pharmacologist

Submitted: April 23, 1963