INTERNATIONAL LEAD ZINC RESEARCH ORGANIZATION

292 MADISON AVENUE, NEW YORK 17, N. Y. ORegon 9-6060

EDWARD P. HASTIE
DIRECTOR

October 10, 1963

To Members of the L.I.A.-I.L.Z.R.O. Health and Safety Committee:

Subject: 1964 Research Budget - Proposals and Work Statements for Consideration at the 1964 Budget Meeting

Attached is the 1964 Catalog of Health and Safety Research for your review in preparation for the Health and Safety Committee Budget Meeting to be held October 25, 1963 at the 60 East 42nd Street Club, 27th floor, Lincoln Building, 60 East 42nd Street, New York 17, N. Y. at 9:30 a.m.

This material is designed to be used as a guide in our discussions and contains besides a list of items to be discussed, review and comments on past performance of some of the projects.

Please review these items carefully and be prepared to make your decisions at our meeting so that our recommendations may be completed at that time. We suggest that you bring the Catalog to the meeting to serve as reference.

Very truly yours,

Don G. Fowler

Don G. Fowler, Secretary
Health and Safety Committee

DGF:md
Enc.

N 205

1964

CATALOG OF HEALTH AND SAFETY
RESEARCH - LEAD

LIA-ILZRO
HEALTH AND SAFETY COMMITTEE

I. Review of Existing Projects and Extension Proposals

II. Work Statements

III. Extension Proposals

I. REVIEW OF EXISTING PROJECTS AND EXTENSION PROPOSALS

LC-75 - Human Exposure to Ambient Air Concentrations of Particulate Lead -- The Kettering Laboratory

Work on this project continues as planned with a possible future program extending over the next two years. The time extension will depend, of course, on whether significant and satisfactory results are obtained before the end of a two-year term exposure. If satisfactory and significant results are obtained sooner, the work will, of course, be terminated sooner.

Budget Recommendation $35,000

LC-69 - Characteristics of Atmospheric Lead Aerosols - Stanford Research Institute

This project has successfully completed a field sampling program in Los Angeles and San Francisco with an accumulation of ambient air data on total amounts of lead plus particle size determinations. The project has not been successful in its attempt to measure and characterize the various compounds of lead due to inability to identify compounds in the small particle sizes found in the atmosphere.

The recommended extension will allow the use of the sampling techniques already developed in other types of urban atmospheres where coal and oil fuel are used extensively for heating purposes. It is probable that the distribution of lead in these latter atmospheres will be different as to total amount and particle size from that which was found in the California cities' sampling.

Budget Recommendation $22,800

LC-76 - The Testing of Organolead Compounds for Possible Endocrinological and Anti-Mammary Tumor Activity - The Worcester Foundation

Since this is essentially a long-range medical research project into uncharted fields of medicine, for example, cancer and arthritis, it would be presumptuous to expect results after one year of study. The plan for next year's work will be a continuation of that which has already been performed.

Budget Recommendation $11,250

II WORK STATEMENTS

ULC-12 Lead Stabilized P.V.C. Pipe
(Prepared by National Sanitation Foundation and ILZRO Staff)

ULC-13 Lead Glazes for Dinnerware
(Prepared by ILZRO Staff)

ULC-14 The Radioprotective Effects of Inorganic Lead
(Prepared by the New York Medical College)

ULC-15 Toxicity Study of Ingested Paint Film
(Prepared by Industrial Bio-Test Laboratories, Inc. and Industrial Hygiene Foundation of America, Inc.

III EXTENSION PROPOSALS

LC-75 Human Exposure to Ambient Air Concentrations of Particulate Lead; The Kettering Laboratory

LC-69 Characteristics of Atmospheric Lead Aerosols; Stanford Research Institute

LC-76 The Testing of Organolead Compounds for Possible Endrocrinological and Anti-Mammary Tumor Activity; The Worcester Foundation

PART I

PROJECTS FOR CONSIDERATION AT THE
LEAD HEALTH AND SAFETY COMMITTEE
1964 BUDGET MEETING

Project	Title and Contractor	Funds		Decision	
		Requested	Approved	For	Against
LC-79	Toxicity Testing For Organo-Lead Compounds -- The Nine Laboratories, Inc. and Industrial Bio-Test Laboratories, Inc.	$10,000	10,000	✓	
	TOTAL				

10,000
93,017
[illegible]
113,317

PART II

WORK STATEMENTS FOR CONSIDERATION AT THE
LEAD HEALTH AND SAFETY COMMITTEE
1964 BUDGET MEETING

Work Statement	Title	Funds Requested	Funds Approved	Decision For	Decision Against
ULC-14	The Radioprotective Effects of Inorganic Lead	$10,054	10054	✓	
ULC-12	Lead Stabilized P.V.C. Pipe	16,500	16500	✓	in principal
ULC-13	Lead Glazes for Dinnerware	25,000	12500	✓	in principal
ULC-15	Toxicity Study of Ingested Paint Film	20,000	10000	✓	in principal
	TOTAL		49,054		

PART III

EXTENSION PROPOSALS FOR CONSIDERATION AT THE
LEAD HEALTH AND SAFETY COMMITTEE
1964 BUDGET MEETING

Project	Title and Contractor	Funds		Decision	
		Requested	Approved	For	Against
LC-75	Human Exposure to Ambient Air Concentrations of Particulate Lead	$35,000	35,000	✓	
LC-69	Characteristics of Atmospheric Lead Aerosols	22,800	8,650	✓	
LC-76	The Testing of Organolead Compounds for Possible Endocrinological and Anti-Mammary Tumor Activity	11,250	11,250	✓	
	TOTAL		54,900		

A LEAD

HEALTH AND SAFETY COMMUNICATIONS PROGRAM

October 1963

Submitted by: O. S. Tyson and Company, Inc. • 230 Park Avenue
New York 17, N. Y.

A Statement of the Problem

Basically the problem faced by LIA in the field of health is one of pervasive ignorance about contemporary lead toxicology. And this ignorance is not by any means confined to itinerant journalists. Medical health officials, industrial hygienists, municipal health boards and educators are similarly misinformed. Last month the State of Pennsylvania Medical Association published a piece on lead poisoning which was egregiously inaccurate. Since then another state medical association has picked up and reprinted the same fallacious article. The current (October) issue of Consumer Bulletin carries a major scare feature on lead poisoning. The LIA clipping service annually relays several thousand references to lead, almost invariably adverse, generally inaccurate. For the most part these go unanswered. No literature exists to provide any of the lead publics, lay or professional, with a brief summation of facts which that public should know about lead.

The effect of this general ignorance while difficult to judge precisely is considerable. It is reflected in scores of research programs now being conducted which are intended to design lead out of a variety of products -- gasoline, paints, cars, components. It is reflected in restrictive labor legislation. It is reflected in diagnostic error and treatment in many industrial accidents. It is finally a kind of ignorance which, if not combated, may jeopardize the acceptance of many of the new compounds and products which lead research programs aim to bring on the market.

Objectives of LIA Communications Program in Health

1. To inform relevant publics of the facts of lead toxicology.
2. To counteract misleading statements in the professional as well as popular press.

Publics for the Program

1. Industrial hygienists
2. Municipal and governmental health officials
3. Medical profession
4. Editors
5. Educators
6. Management and employees of firms handling or processing lead and lead products

Action to Initiate a Lead Health Education Program

1. Prepare 3-4 units of literature summing up the state of technical knowledge regarding lead toxicology and air pollution, slanting them to the need of our different publics.
2. Secure maximum circulation for above through publicity and direct mail.
3. Produce a series of Lead Health Newsletters reporting on new developments and researches in lead. This would be sent to LIA mailing list of men in public health and hygiene, industrial hygiene, etc.

4. Publish on a regular basis (in cooperation with the Kettering Laboratory) an annotated bibliography of articles appearing both here and abroad concerned with lead as a public hazard.

5. Automate the process of responding to editors whose publication carry inaccurate articles about lead. These would be answered with an appropriate cover letter and copy of our new bulletin "Facts about Lead - for editors and educators." The object of this exchange would not be to enter into controversy with the editor but to inform and educate him so that he will take a more skeptical attitude toward future scare stories which may cross his desk.

6. Continue and accelerate our current program of contact with medical and health associations.

Cost Estimate

1.	Preparation and production of four basic information bulletins.	$6,000
2.	Developing appropriate mailing list and preparing addressograph plates.	1,500
3.	Lettering bibliography program.	10,000
4.	Mailing of Lead Health Newsletter (six mailings projected).	2,000
5.	Mailing and production costs.	1,000
6.	Publicity counsel and assistance in instrumenting the entire communications effort.	15,000
7.	Contingency	5,000
	Total	$40,500

THE WORCESTER FOUNDATION FOR EXPERIMENTAL BIOLOGY

Shrewsbury, Massachusetts

THE TESTING OF ORGANOLEAD COMPOUNDS FOR POSSIBLE ENDOCRINOLOGICAL AND ANTI-MAMMARY TUMOR ACTIVITY

PROJECT No. LC-76

Report No. 2
For Period September 1, 1962 to August 31, 1963

Principal Investigator

Ralph I. Dorfman

Sponsored by

International Lead Zinc Research Organization
292 Madison Avenue
New York 17, N. Y.

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Table 1
Worcester Foundation for Experimental Biology
Shrewsbury, Massachusetts

ASSAY REPORT

Estrogen Assay
(Subcutaneous Injection)

Submitted by: Dr. Henry
Samples Tested: As listed
Test Animal: Mouse, female, 19-21-day-old.
Vehicle: Sesame oil, 0.1 ml. per injection.
Procedure: Injected once daily for 3 consecutive days and autopsy on 4th day.

Material Administered	Total Dose µg.	No. of Mice	Mean Uterine Ratio ± S.E.
0	0	10	0.89 ± 0.045
Estrone	0.05	10	2.47 ± 0.260
	0.15	10	3.80 ± 0.228
	0.45	10	5.52 ± 0.223
HP-23	100	7	1.13 ± 0.088
HP-24	100	6	1.57 ± 0.060
HP-25	100	7	1.07 ± 0.058
HP-26	100	7	1.65 ± 0.080

Table 2

Worcester Foundation For Experimental Biology
Shrewsbury, Massachusetts

ASSAY REPORT

Estrogen Assay
(Subcutaneous Injection)

Submitted by: Dr. Henry
Samples Tested: As listed
Test Animal: Mouse, female, 19-21-day-old
Vehicle: Sesame oil, 0.1 ml. per injection
Procedure: Injected once daily for 3 consecutive days and autopsy on 4th day.

Material Administered	Total Dose µg.	No.of Mice	Mean Uterine Ratio ± S.E.
0	0	10	0.91 ± 0.056
Estrone	0.05	9	1.87 ± 0.137
	0.15	10	4.65 ± 0.198
	0.45	10	5.15 ± 0.207
HP-24	100	10	0.86 ± 0.032
	200	9	0.97 ± 0.072
	400	10	0.75 ± 0.049
HP-26	100	8	0.96 ± 0.047
	200	9	0.87 ± 0.031
	400	9	0.90 ± 0.033

Table 3

Worcester Foundation For Experimental Biology
Shrewsbury, Massachusetts

ASSAY REPORT

Anti-Estrogen Assay
(Injection)

Submitted by:
Samples Tested:
Test Animal: Mouse, female, 19-21-day-old
Vehicles: Estrone, sesame oil, 0.1 ml. per injection. Test material, CMC, 0.1 ml. per injection.
Procedure: Administer treatments at separate sites once daily for 3 consecutive days and autopsy on 4th day.

TEST MATERIAL Designation	Total Dose µg.	Estrone Total Dose µg.	No. of Mice	Mean Uterine Ratio ± S.E.
0	0	0	9	1.02 ± 0.137
0	0	0.4	9	4.85 ± 0.236
17-Methylene-Δ^5-3β-ol	2000	0.4	9	4.54 ± 0.141
HP-27	50	0.4	9	4.23 ± 0.205
	3000	0.4	0	DEAD

Table 4

Worcester Foundation for Experimental Biology
Shrewsbury, Massachusetts

ASSAY REPORT

Anti-Estrogen Assay
(Injection)

Submitted by:
Samples Tested:
Test Animal: Mouse, female, 19-21-day-old
Vehicles: Estrone, sesame oil, 0.1 ml. per injection. Test material, CMC, 0.1 ml. per injection.
Procedure: Administer treatments at separate sites once daily for 3 consecutive days and autopsy on 4th day.

TEST MATERIAL Designation	Total Dose µg.	Estrone Total Dose µg.	No.of Mice	Mean Uterine Ratio ± S.E.
0	0	0	10	0.87 ± 0.054
0	0	0.4	9	4.30 ± 0.208
HP-23	1	0.4	10	6.18 ± 0.260
HP-24	1	0.4	10	5.47 ± 0.125
HP-26	1	0.4	10	4.99 ± 0.214

Table 5

Worcester Foundation For Experimental Biology
Shrewsbury, Massachusetts

ASSAY REPORT

Anti-Estrogen Assay
(Injection)

Submitted by:
Samples Tested:
Test Animal: Mouse, female, 19-21-day-old
Vehicles: Estrone, sesame oil, 0.1 ml. per injection. Test material, CMC, 0.1 ml. per injection.
Procedure: Administer treatments at separate sites once daily for 3 consecutive days and autopsy on 4th day.

TEST MATERIAL Designation	Total Dose µg.	Estrone Total Dose µg.	No.of Mice	Mean Uterine Ratio ± S.E.
0	0	0	10	1.10 ± 0.064
0	0	0.4	10	5.63 ± 0.165
EP-27	5	0.4	10	5.55 ± 0.221
	20	0.4	10	5.32 ± 0.280
	60	0.4	10	5.42 ± 0.254
	240	0.4	10	5.28 ± 0.214

Table 6

Worcester Foundation For Experimental Biology
Shrewsbury, Massachusetts

ASSAY REPORT

Anti-Estrogen Assay
(Injection)

Submitted by:
Samples Tested:
Test Animal: Mouse, female, 19-21-day-old
Vehicles: Estrone, sesame oil, 0.1 ml. per injection. Test material, CMC, 0.1 ml. per injection.
Procedure: Administer treatments at separate sites once daily for 3 consecutive days and autopsy on 4th day.

TEST MATERIAL Designation	Total Dose µg.	Estrone Total Dose µg.	No. of Mice	Mean Uterine Ratio ± S.E.
0	0	0	10	1.01 ± 0.083
0	0	0.4	10	5.36 ± 0.225
EP 28	50	0.4	10	5.44 ± 0.177
	500	0.4	10	5.28 ± 0.156
EP 29	50	0.4	10	5.58 ± 0.291
	500	0.4	ALL DEAD	
EP 30	50	0.4	10	5.94 ± 0.280
	500	0.4	10	6.33 ± 0.234
EP 31	50	0.4	10	5.85 ± 0.207
	500	0.4	10	5.01 ± 0.117

Table 7

Worcester Foundation For Experimental Biology
Shrewsbury, Massachusetts

ASSAY REPORT

Chick Androgen Assay

Submitted by:
Samples Tested:
Test Animal: Day-old male white Leghorn Chick
Vehicle: CMC, 0.05 ml. absolute alcohol per inunction.
Procedure: Inunct once daily for 7 days and autopsy on 8th day.

Material Inuncted	Total Dose µg.	Mean Body Wt.	No. of Chicks	Mean Comb Ratio ± S.E.
0	0	47	12	0.30 ± 0.019
Testosterone	1	51	9	0.33 ± 0.025
	3	47	10	0.33 ± 0.018
	9	52	10	0.47 ± 0.025
	27	50	9	0.51 ± 0.055
	81	47	7	0.67 ± 0.110
HP-25	300	49	10	0.27 ± 0.019
	600	50	9	0.31 ± 0.014
	1200	50	8	0.28 ± 0.019

Table 8

Worcester Foundation For Experimental Biology
Shrewsbury, Massachusetts

ASSAY REPORT

Chick Androgen Assay

Submitted by:
Samples Tested:
Test Animal: Day-old male white Leghorn Chick
Vehicle: CMC, 0.05 ml. absolute alcohol per inunction.
Procedure: Inunct once daily for 7 days and autopsy on 8th day.

Material Inuncted	Total Dose µg.	No. of Chicks	Mean Comb Ratio ± S.E.
0	0	9	0.36 ± 0.020
Testosterone	2	9	0.43 ± 0.028
	8	9	0.62 ± 0.051
	32	9	0.80 ± 0.076
HP-23	300	8	0.41 ± 0.036
HP-24	300	10	0.40 ± 0.050
HP-25	300	10	0.46 ± 0.040
HP-26	300	8	0.40 ± 0.029

Worcester Foundation for Experimental Biology
Shrewsbury, Massachusetts

Table 9

ASSAY REPORT

Chick Anti-Androgen Assay

Submitted by: Dr. Emery
Samples Tested: As Listed
Test Animal: Day-old male white Leghorn Chick.
Vehicle: Testosterone enanthate, sesame oil, 0.5 ml. Test material, sesame oil, 0.05 ml. per inunction.
Procedure: On first day chicks are injected once with testosterone enanthate. Beginning on same day inunct test material once daily for 7 days and autopsy on 8th day.

TEST MATERIAL Designation	Total Dose mg.	Testosterone Enanthate Total Dose mg.	No. of Chicks	Mean Body Wt. g.	Mean Comb Ratio ± S.E.
0	0	0	9	57	0.44 ± 0.039
0	0	0.5	12	54	1.24 ± 0.084
EP23	3	0.5	8	60	1.41 ± 0.048
EP25	3	0.5	8	53	1.46 ± 0.118
EP26	3	0.5	8	44	1.38 ± 0.087

Worcester Foundation for Experimental Biology
Shrewsbury, Massachusetts

Table 10
ASSAY REPORT

Chick Anti-Androgen Assay

Submitted by:
Samples Tested:
Test Animal: Day-old male white Leghorn Chick.
Vehicle: Testosterone enanthate, sesame oil, 0.5 ml. Test material, sesame oil, 0.05 ml. per inunction.
Procedure: On first day chicks are injected once with testosterone enanthate. Beginning on same day inunct test material once daily for 7 days and autopsy on 8th day.

TEST MATERIAL Designation	Total Dose mg.	Testosterone Enanthate Total Dose mg.	No. of Chicks	Mean Body Wt. g.	Mean Comb Ratio ± S.E.
0	0	0	12	50	0.39 ± 0.020
0	0	0.5	11	48	0.79 ± 0.077
Ro 2-7239	0.5	0.5	10	51	0.71 ± 0.043
	1	0.5	10	56	0.63 ± 0.059
HP-1	3	0.5	11	54	0.97 ± 0.071
HP-7	3	0.5	12	62	1.09 ± 0.055
HP-15	0.5	0.5	12	59	0.91 ± 0.066
	1	0.5	11	59	0.91 ± 0.072
	2	0.5	11	61	0.88 ± 0.059
	3	0.5	11	61	1.01 ± 0.060

LIA11441

Table 11

Worcester Foundation for Experimental Biology
Shrewsbury, Massachusetts

Rat Anti-Androgen Assay
(Injection)

Submitted by: Dr. Henry
Samples Tested: As listed
Test Animal: Rat, male, 21-day-old.
Vehicle: Testosterone, CMC, 0.5 ml. per injection
Test material, CMC, 0.5 ml. per injection
Procedure: Castrate 21st - 28th day of life. Beginning on same day, administer treatments at separate sites once daily for 7 days and autopsy on 8th day.

TEST MATERIAL Designation	Total Dose mg.	Testosterone Total Dose mg.	No. of Rats	Mean Body Wt. g.	TISSUE RATIO ± S. E. Prostate	Seminal Vesicles	Levator Ani
0	0	0	10	94	0.07±0.005	0.15±0.011	0.19±0.024
Testosterone		2	10	90	0.55±0.125	1.28±0.076	0.35±0.033
Progesterone	150	2	10	94	1.27±0.055	1.24±0.051	0.63±0.029
	75	2	10	95	1.06±0.072	1.22±0.104	0.68±0.033
HP-15	2	2	10	85	1.53±0.068	1.54±0.055	0.80±0.039
	4	2	8	79	1.76±0.107	1.63±0.094	0.82±0.032
	8	2	10	76	1.65±0.136	1.66±0.094	0.83±0.039
HP-1	2	2	10	84	1.70±0.147	1.67±0.125	0.88±0.029
	8	2	10	87	1.50±0.078	1.55±0.081	0.85±0.034
HP-7	2	2	9	91	1.34±0.070	1.40±0.137	0.77±0.040
	8	2	10	95	1.22±0.046	1.39±0.046	0.79±0.015

LIA11442

Worcester Foundation for Experimental Biology
Shrewsbury, Massachusetts

Table 12
ASSAY REPORT

Mouse
Anti-Androgen Assay
Injection

Submitted by:
Samples Submitted:
Test Animal: Mice, male, 21-23 days
Vehicle: Sesame Oil - 0.1 ml. per injection - Testosterone
CMC - 0.2 ml. per injection - Test Compound
Procedure: Castration - 21-23 days of age. Injections started day of surgery and continued once daily for 7 days. Autopsy on 8th day.

TEST COMPOUND Designation	Total Dose mg.	Total Dose of Testosterone mg.	No.of Mice	Mean Body Wt. g.	TISSUE RATIO ± S. E. Prostate	Seminal Vesicles
0	0	0	7	16	0.10 ± 0.005	0.21 ± 0.039
0	0	0.8	9	18	0.28 ± 0.029	1.08 ± 0.087
Progesterone	20	0.8	9	17	0.26 ± 0.019	0.65 ± 0.052
HP-31	5	0.8	ALL DEAD			
	15	0.8	ALL DEAD			
HP-30	5	0.8	ALL DEAD			
	15	0.8	ALL DEAD			

LIA11443

Worcester Foundation for Experimental Biology
Shrewsbury, Massachusetts

Table 13
ASSAY REPORT

Mouse
Anti-Androgen Assay
Injection

Submitted by:
Samples Submitted:
Test Animal: Mice, male, 21-23 days
Vehicle: Sesame Oil - 0.1 ml. per injection - Testosterone
CMC - 0.2 ml. per injection - Test Compound
Procedure: Castration - 21-23 days of age. Injections started day of surgery and continued once daily for 7 days. Autopsy on 8th day.

TEST COMPOUND Designation	Total Dose mg.	Total Dose of Testosterone mg.	No.of Mice	Mean Body Wt. g.	TISSUE RATIO ± S. E. Prostate	Seminal Vesicles
0	0	0	11	20	0.05 ± 0.005	0.18 ± 0.010
0	0	0	11	17	0.19 ± 0.016	0.97 ± 0.163
Progesterone	10	0.8	12	20	0.17 ± 0.009	0.70 ± 0.095
	20	0.8	12	20	0.17 ± 0.012	0.51 ± 0.171
	40	0.8	9	18	0.18 ± 0.013	0.56 ± 0.052
NP-27	5	0.8	ALL DEAD			

LIA11444

Worcester Foundation for Experimental Biology
Shrewsbury, Massachusetts

Table 14

ASSAY REPORT

Mouse
Anti-Androgen Assay
Injection

Submitted by:
Samples Submitted:
Test Animals: Mice, male, 21-23 days
Vehicle: Sesame Oil - 0.1 ml. per injection - Testosterone
CMC - 0.2 ml. per injection - Test Compound
Procedure: Castration - 21-23 days of age. Injections started day of surgery and continued once daily for 7 days. Autopsy on 8th day.

TEST COMPOUND Designation	Total Dose mg.	Total Dose of Testosterone mg.	No.of Mice	Mean Body Wt. g.	TISSUE RATIO ± S. E. Prostate	Seminal Vesicles
0	0	0	12	19	0.09 ± 0.011	0.17 ± 0.011
0	0	0.8	12	20	0.20 ± 0.022	0.80 ± 0.058
Progesterone	20	0.8	12	22	0.15 ± 0.012	0.45 ± 0.049
	40	0.8	12	21	0.18 ± 0.016	0.40 ± 0.046
HP-27	10	0.8	ALL DEAD			
HP-28	10	0.8	ALL DEAD			

LIA11445

Table 15

Wordester Foundation for Experimental Biology
Shrewsbury, Massachusetts

ASSAY REPORT

Anti-Androgen Assay-Mouse
(Injection)

Material Administered	Test Compound Total dose mg.	Testosterone Total Dose mg.	No. of Mice	Mean Body Wt. g.	TISSUE RATIO ± S. E. Prostate	Seminal Vesicles
0	0	0	0	21	0.13±0.032	0.21±0.026
		0.8	9	20	0.17±0.014	0.86±0.080
19-Norprogesterone	20	0.8	8	20	0.19±0.018	1.05±0.063
	40	0.8	8	24	0.15±0.018	0.51±0.046
HP23	40	0.8	6	17	0.21±0.027	1.05±0.156
HP26	40	0.8	5	15	0.29±0.033	1.16±0.198
HP27	40	0.8	9	22	0.22±0.013	1.08±0.104

Worcester Foundation for Experimental Biology
Shrewsbury, Massachusetts

Table 16

ASSAY REPORT

Mouse
Anti-Androgen Assay
Injection

Submitted by: Dr. Malcolm Henry
Samples Submitted: As Listed
Test Animal: Mice, male, 21-23 days
Vehicle: Sesame Oil - 0.1 ml. per injection - Testosterone
CMC - 0.2 ml. per injection - Test Compound
Procedure: Castration - 21-23 days of age. Injections started day of surgery and continued once daily for 7 days. Autopsy on 8th day.

TEST COMPOUND Designation	Total Dose mg.	Total Dose of Testosterone mg.	No. of Mice	Mean Body Wt. g.	TISSUE RATIO ± S. E. Prostate	Seminal Vesicles
0	0	0	10	16	0.08 ± 0.005	0.19 ± 0.024
0	0	0.8	10	19	0.19 ± 0.019	0.93 ± 0.074
Progesterone	20	0.8	6	19	0.25 ± 0.018	0.87 ± 0.186
	40	0.8	9	19	0.21 ± 0.031	0.74 ± 0.038
RP-27	10	0.8	DEAD			
	40	0.8	DEAD			

LIA11447

Worcester Foundation for Experimental Biology
Shrewsbury, Massachusetts

Table 17

ASSAY REPORT

Mouse
Anti-Androgen Assay
Injection

Submitted by:
Samples Submitted:

Test Animals: Mice, male, 21-23 days
Sesame Oil - 0.1 ml. per injection - Testosterone
CMC - 0.2 ml. per injection - Test Compound

Procedure: Castration - 21-23 days of age. Injections started day of surgery and continued once daily for 7 days. Autopsy on 8th day.

TEST COMPOUND Designation	Total Dose mg.	Total Dose of Testosterone mg.	No. of Mice	Mean Body Wt. g.	TISSUE RATIO ± S. E. Prostate	Seminal Vesicles
0	0	0	7	17	0.02 ± 0.010	0.17 ± 0.018
0	0	0.8	4	17	0.17 ± 0.012	1.01 ± 0.151
Progesterone	2.5	0.8	9	16	0.10 ± 0.022	0.90 ± 0.111
	5	0.8	9	19	0.11 ± 0.016	0.96 ± 0.163
	10	0.8	8	16	0.09 ± 0.015	0.84 ± 0.147
HP 32	5	0.8	Dead			
HP 33	5	0.8	Dead			

Table 18

Worcester Foundation for Experimental Biology
Shrewsbury, Massachusetts

ASSAY REPORT

Parabiosis Assay
(Injection)

Submitted by:
Samples Tested:
Test Animals: Male and Female rats 21-day-old
Vehicle: CMC, 0.2 ml. per injection
Procedure: Place a castrated male (29-31 days of age) in parabiosis with an intact female (29-31 days of age). Begin injection of test material on day of surgery and continue once daily for 10 days and autopsy on 11th day.

			MALE - CASTRATE				INTACT FEMALE	
Material Administered	Total Dose mg.	No. of Pairs	Mean Body Wt. g.	Mean Prostate Wt. mg ± S.E.	Mean Seminal Vesicles Wt. mg ± S.E.	Mean Levator Ani Wt. mg ± S.E.	Mean Body Wt. g.	Mean Ovarian Wt. mg ± S.E.
0	0	5	101	11.5±1.080	11.8±1.021	16.0±2.362	93	124.0±21.884
Testosterone	0.8	5	103	55.1±11.003	52.4±11.327	26.2±3.989	93	61.5±27.204
HP-21	10	5	95	12.0±17.008	10.7±1.144	11.9±1.508	85	96.5±27.950

Worcester Foundation for Experimental Biology
Shrewsbury, Massachusetts

Table 19 — Exper. No.
ASSAY REPORT — Autopsy Date:

Anti-Mammary Tumor Assay

Submitted by: Dr. Malcolm Henry
Samples Tested:
Test Animal: Sprague-Dawley rat, female, 45 days old on delivery.
Vehicle: CMC, 0.5 ml per injection.
Procedure: Tumor seeds were transplanted into the rats at 52 days of age. Beginning 5 weeks later, these animals having growing tumors were injected once daily for 14 days. On the 14th day, each rat received 5 μc/10 μ mole glycine--2-C^{14} in 0.5 ml 0.9% saline followed by autopsy 24 hours later.

Material Administered	Total Dose mg.	No. of Rats	Mean Body Wt. g.	No. of Tumors	Mean Tumor Wt. mg.	Mean Specific Activity cpm/mg Protein ± S.E.
0	0	11	233	20	2648	102.3 ± 3.99
2α-Methyl-5α--dehydrotestosterone	7.0	11	252	20	1990	89.0 ± 2.98 (P<0.01)
HP-28	21.0**	7	239	11	3701	108.2 ± 2.88
HP-29	21.0**	10	240	19	2497	117.2 ± 2.68 (P<0.01)
HP-30	21.0	8	233	14	5438	122.8 ± 4.45 (P<0.01)
HP-31	21.0+	10	220	17	2041	105.7 ± 2.58

** Considerable inflammation at site of injection.
+ Slight inflammation at site of injection.

Table 20

Worcester Foundation for Experimental Biology
Shrewsbury, Massachusetts

ASSAY REPORT

THYMOLYTIC AND ANTI-INFLAMMATORY ASSAY
(Injection)

Submitted by:
Samples Tested: 100-125 g
Test Animal: Male rat 21-23 days old at start.
Vehicle: CMC, 0.5 ml. per day by injection.
Procedure: Bilaterally adrenalectomize rats on 23rd-26th day of life and insert two cotton pellets subcutaneously. Beginning on same day, administer treatment once daily for 6 consecutive days and autopsy on 7th day. Remove granulomas (cotton pellets), weigh, dry for 24 hours at 100°C and weigh again. Also remove and weigh, after blotting dry, each thymus gland.

Material Administered	Total Dose mg.	Mean Body Wt. g.	THYMUS TEST No. of Rats	Mean Thymus Ratio ± S.E.	ANTI-INFLAMMATORY TEST No. of Obs.	Mean Dry Tissue Wt. mg. ± S.E.
0	0	153	7	2.74 ± 0.144	8	129.2 ± 18.972
Cortisol	0.5	147	7	2.65 ± 0.180	8	121.9 ± 12.827
	1.0	152	8	2.41 ± 0.147	8	163.2 ± 23.409
	2.0	150	8	2.52 ± 0.111	8	96.6 ± 3.863
	4.0	155	8	1.30 ± 0.092	8	73.1 ± 8.914
HP 32	5	137	4	3.71 ± 0.269	4	104.2 ± 9.354
HP 33	5	139	7	3.14 ± 0.188	8	103.9 ± 15.518

Groups HP 32 - HP 33 - Compound Under Skin

Worcester Foundation for Experimental Biology
Shrewsbury, Massachusetts

Table 21

ASSAY REPORT

THYMOLYTIC AND ANTI-INFLAMMATORY ASSAY
(Injection)

Submitted by:
Samples Tested:
Test Animal: Male rat 21-23 days old at start.
Vehicle: CMC, 0.5 ml. per injection.
Procedure: Bilaterally adrenalectomize rats on 23rd-26th day of life and insert two cotton pellets subcutaneously. Beginning on same day, administer treatment once daily for 6 consecutive days and autopsy on 7th day. Remove granulomas (cotton pellets), weigh, dry for 24 hours at 100°C and weigh again. Also remove and weigh, after blotting dry, each thymus gland.

Material Administered	Total Dose mg.	Mean Weight THYMUS TEST No. of Rats		Mean Thymus Ratio ± S.E.	ANTI-INFLAMMATORY No. of Obs.	Mean Dry Tissue Wt. mg. ± S. E.
0	0	8	67	3.48 ± 0.331	8	24.7 ± 2.230
Cortisol	0.5	11	75	3.43 ± 0.177	11	17.4 ± 1.389
	1.5	12	75	3.08 ± 0.118	12	17.7 ± 1.539
	4.5	12	72	1.35 ± 0.107	12	15.3 ± 1.212
HP-23	8	5	80	3.79 ± 0.337	5	17.6 ± 2.132
HP-24	8	4	80	4.28 ± 0.728	4	22.8 ± 5.874
HP-25	8	DEAD →				
HP-26	8	3	75	4.13 ± 0.424	3	21.0 ± 4.362
	8	5	70	3.47 ± 0.522	5	17.7 ± 2.650

Table 22

Worcester Foundation for Experimental Biology
Shrewsbury, Massachusetts

ASSAY REPORT

THYMOLYTIC AND ANTI-INFLAMMATORY ASSAY
(Injection)

Submitted by: Dr. Henry
Samples Tested: HP-13
Test Animal: Male rat 21-23 days old at start.
Vehicle: CMC, 0.5 ml. per injection.
Procedure: Bilaterally adrenalectomize rats on 23rd-26th day of life and insert two cotton pellets subcutaneously. Beginning on same day, administer treatment once daily for 6 consecutive days and autopsy on 7th day. Remove granulomas (cotton pellets), weigh, dry for 24 hours at 100°C and weigh again. Also remove and weigh, after blotting dry, each thymus gland.

Material Administered	Total Dose mg.	Mean Body Wt.	THYMUS TEST No. of Rats	THYMUS TEST Mean Thymus Ratio ± S. E.	ANTI-INFLAMMATORY No. of Obs.	ANTI-INFLAMMATORY Mean Dry Tissue Wt. mg. ± S. E.
0	0	85	8	3.81 ± 0.274	8	23.5 ± 2.600
Cortisol	1.0	82	10	3.04 ± 0.129	10	17.6 ± 1.169
	4.0	81	10	1.52 ± 0.136	10	12.8 ± 1.127
HP-13	1	72	7	3.65 ± 0.288	7	16.8 ± 1.845
	2	78	8	3.81 ± 0.255	8	20.7 ± 1.515

Table 23

Worcester Foundation for Experimental Biology
Shrewsbury, Massachusetts

ASSAY REPORT

THYMOLYTIC AND ANTI-INFLAMMATORY ASSAY
(Injection)

Submitted by:
Samples Tested:
Test Animal: Male rat 21-23 days old at start.
Vehicle: CMC, 0.5 ml. per injection.
Procedure: Bilaterally adrenalectomize rats on 23rd-26th day of life and insert two cotton pellets subcutaneously. Beginning on same day, administer treatment once daily for 6 consecutive days and autopsy on 7th day. Remove granulomas (cotton pellets), weigh, dry for 24 hours at 100°C and weigh again. Also remove and weigh, after blotting dry, each thymus gland.

Material Administered	Total Dose mg.	Mean Body Wt.	THYMUS TEST No. of Rats	THYMUS TEST Mean Thymus Ratio ± S.E.	ANTI-INFLAMMATORY No. of Obs.	ANTI-INFLAMMATORY Mean Dry Tissue Wt. mg. ± S. E.
0	0	70	9	4.10 ± 0.111	9	29.5 ± 4.229
Cortisol	0.5	70	9	3.18 ± 0.238	9	19.3 ± 1.700
	1.5	74	10	2.93 ± 0.140	10	19.0 ± 1.288
	4.5	74	9	1.90 ± 0.167	9	11.5 ± 1.972
HP-13	4	57	5	4.63 ± 0.694	5	10.2 ± 2.631
	8	75	2	2.68 ± 0.704	2	18.0 ± 0.606
HP-14	4	61	3	3.82 ± 0.250	3	16.3 ± 0.836
	8	62	2	3.86 ± 0.773	2	19.7 ± 2.575

Worcester Foundation for Experimental Biology
Shrewsbury, Massachusetts

Table 24
ASSAY REPORT

Mouse
Anti-Androgen Assay
Injection

Submitted by: Dr. O. M. can der Want, T.N.O. (Lead Compounds)
Samples Submitted: As listed
Test Animal: Mice, male, 21-23 days
Vehicle: Sesame Oil - 0.1 ml. per injection - Testosterone
CMC - 0.2 ml. per injection - Test Compound
Procedure: Castration - 21-23 days of age. Injections started day of surgery and continued once daily for 7 days. Autopsy on 8th day.

TEST COMPOUND Designation	Total Dose mg.	Total Dose of Testosterone mg.	No.of Mice	Mean Body Wt. g.	TISSUE RATIO ± S. E. Prostate	Seminal Vesicles
0	0	0	6	15	0.05 ± 0.013	0.20 ± 0.033
Kb149(TNO)	20	0.8	DEAD			
G 93(TNO)	20	0.8	DEAD			
S 82(TNO)	20	0.8	DEAD			
S 129(TNO)	20	0.8	9	16	0.17 ± 0.021	0.75 ± 0.046
Progesterone	2.5	0.8	9	16	0.19 ± 0.015	0.93 ± 0.080
	5	0.8	8	18	0.19 ± 0.014	0.88 ± 0.066
	10	0.8	8	17	0.20 ± 0.017	0.76 ± 0.053
	20	0.8	9	17	0.14 ± 0.015	0.46 ± 0.041
0	0	0.8	6	15	0.22 ± 0.029	1.20 ± 0.156

Worcester Foundation for Experimental Biology
Shrewsbury, Massachusetts

Table 25

ASSAY REPORT

Mouse
Anti-Androgen Assay
Injection

Submitted by:
Samples Submitted:
Test Animal: Mice, male, 21-23 days
Vehicle: Sesame Oil - 0.1 ml. per injection - Testosterone
CMC - 0.2 ml. per injection - Test Compound
Procedure: Castration - 21-23 days of age. Injections started day of surgery and continued once daily for 7 days. Autopsy on 8th day.

TEST COMPOUND Designation	Total Dose mg.	Total Dose of Testosterone mg.	No.of Mice	Mean Body Wt. g.	TISSUE RATIO ± S. E. Prostate	Seminal Vesicles
0	0	0	8	20	0.08 ± 0.014	0.25 ± 0.021
0	0	0.8	8	21	0.23 ± 0.017	0.91 ± 0.118
Progesterone	10	0.8	7	19	0.20 ± 0.012	0.65 ± 0.053
	20	0.8	8	19	0.21 ± 0.017	0.71 ± 0.082
SK-116 (TMO)	0.1	0.8	8	19	0.17 ± 0.012	0.77 ± 0.046
SK-74 (TMO)	0.1	0.8	8	18	0.18 ± 0.027	0.91 ± 0.124
WI-488 (TMO)	0.1	0.8	6	18	0.21 ± 0.025	0.82 ± 0.096
SK-79 (TMO)	0.1	0.8	8	18	0.23 ± 0.023	1.03 ± 0.118
MG-22 (TMO)	0.1	0.8	8	18	0.21 ± 0.019	0.89 ± 0.097

Worcester Foundation for Experimental Biology
Shrewsbury, Massachusetts

ASSAY REPORT
Table 26

Estrogen Assay
(Subcutaneous Injection)

Submitted by:
Samples Tested:
Test Animal: Mouse, female, 19-21-day-old.
Vehicle: Sesame oil, 0.1 ml. per injection.
Procedure: Injected once daily for 3 consecutive days and autopsy on 4th day.

Material Administered	Total Dose µg.	No. of Mice	Mean Uterine Ratio ± S.E.
0	0	10	1.08 ± 0.059
Estrone	0.05	10	2.11 ± 0.090
	0.1	10	3.27 ± 0.297
	0.2	9	4.85 ± 0.267
	0.4	8	5.26 ± 0.277
SK-116 TMO	100	6	3.41 ± 0.375
SK-74 TMO	100	5	0.87 ± 0.060
WI-488 TMO	100	6	1.08 ± 0.095
SK-79 TMO	100	6	0.88 ± 0.073
Ms-22 TMO	100	6	1.01 ± 0.071
Kb-149 TMO	100	6	1.13 ± 0.068
SK-83 TMO	100	6	0.86 ± 0.072
SK-82 TMO	100	6	1.09 ± 0.076
SK-129 TMO	100	6	1.02 ± 0.080

Table 27

Worcester Foundation For Experimental Biology
Shrewsbury, Massachusetts

ASSAY REPORT

Anti-Estrogen Assay
(Injection)

Submitted by: Dr. van der Want
Samples Tested:
Test Animal: Mouse, female, 19-21-day-old
Vehicles: Estrone, sesame oil, 0.1 ml. per injection. Test material, CMC, 0.1 ml. per injection.
Procedure: Administer treatments at separate sites once daily for 3 consecutive days and autopsy on 4th day.

TEST MATERIAL Designation	Total Dose µg.	Estrone Total Dose µg.	No.of Mice	Mean Uterine Ratio ± S.E.
0	0	0	12	0.97 ± 0.088
0	0	0.4	12	5.75 ± 0.208
SK82 (TWO)	1000	0.4	DEAD	
SK83 (TWO)	1000	0.4	DEAD	
Mb149 (TWO)	1000	0.4	DEAD	
SK79 (TWO)	1000	0.4	DEAD	
SK116 (TWO)	1000	0.4	2	3.79 ± 0.074
W188 (TWO)	1000	0.4	DEAD	
M22 (TWO)	1000	0.4	DEAD	
SK74 (TWO)	1000	0.4	DEAD	
SK129 (TWO)	1000	0.4	12	4.59 ± 0.210

TABLE 28

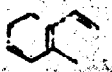
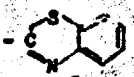
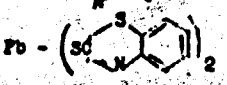
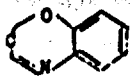
SUMMARY

Possible Significant Activities Requiring Further Confirmation

Table No.	Compound	Significant Activity
20	HP 32	Anti-Inflammatory Activity
	HP 33	
21	HP 23	Anti-Inflammatory Activity
	HP 26	
22	HP 13	Anti-Inflammatory Activity
	HP 14	
24	SK 129 (TMO)	Anti-Androgen
25	SK-116 (TMO)	Anti-Androgen
26	SK-116 (TMO)	Estrogen

TABLE 29

COMPOUNDS STUDIED

Code	Structure
Sk 116	Phenyllead triacetate
Sk 74	Dibutyllead diacetate
Kb 149	Triethyllead acetate
Sk 79	N(tributylplumblyl)-imidazole
Sk 82	Trimethyllead acetate
Sk 83	Tributyllead acetate
Ma 22	Diethyllead diacetate
Wi 488	Trimethyllead cyanamide
Sk 129	Tetrabutyllead
HP 1	Tetraphenyllead
HP 7	Bis-triphenyllead sulphide
HP 13	Thiobenzyltriphenyllead
HP 14	Thiophenyltriphenyllead
HP 15	Thioacetyltriphenyllead
HP 21	Lead Benzyl Mercaptide
HP 23	Lead dithiobenzoate
HP 24	Lead thiophenolate zinc acetate complex
HP 25	Triphenyllead dimethylarsinate
HP 26	Bis-diphenylchlorolead methyl arsinate
HP 27	ϕ_3 $PbSCH_2COOEt$
HP 28	$(\phi)_3$ Pb - S - 
HP 29	$(\phi)_3$ PbS - 
HP 30	$(\phi)_2$ Pb - 
HP 31	$(\phi)_3$ Pb - S - 

STANFORD RESEARCH INSTITUTE

Menlo Park, California

October 1, 1963

Proposal for Research

SRI Proposal No. PA 63-312 Project # LC-69

CHARACTERISTICS OF ATMOSPHERIC LEAD AEROSOLS

by Elmer Robinson

Prepared for

International Lead Zinc Research Organization
292 Madison Avenue
New York 17, New York

Attention: Dr. Schrade F. Radtke

The objectives of this research program are:

1. To extend the exploratory sampling of atmospheric lead aerosols to environments where atmospheric emissions are not dominated by automobile exhausts
2. To extend the size range of the sampling system for a single sampling period
3. To characterize more completely the nature of the lead aerosol.

Background Discussion

The proposed research program is an extension of our study on "Characteristics of Atmospheric Lead Aerosols," SRI Project 4211. This study has successfully developed a sampling and analysis procedure whereby the size distribution of the lead aerosol can be obtained; the method has been used in exploratory sampling of ambient lead aerosols in several places in the San Francisco Bay Area and in Los Angeles. Considerable effort has been expended to determine the lead compounds which make up the atmospheric lead aerosol. While the compounds cannot yet be completely delineated, provisional information has been obtained.

From the atmospheric sampling which has been carried out thus far, a typical size distribution might be one having a mass median equivalent diameter of 0.3 μ, 25% of the mass in particles larger than 1.0 μ, and 30% of the mass in particles less than 0.1; the total size range might extend from 0.05 to 3 μ. To obtain a size distribution with the techniques used to date requires a sampling period of 4 to 8 hours; in any one test period the size distribution can be determined in only a portion of the full range which the instrument can cover. Thus, any attempt to describe the complete size distribution of the lead aerosol requires either extrapolating single test results beyond the limits of the sampling system or combining the results of several test periods into one average distribution. Neither procedure is entirely satisfactory without more detailed knowledge of the characteristics of ambient aerosol size distributions. Hence, an extension of the present sampling method to permit a more complete coverage of the full size distribution of the lead aerosol is a logical continuation of the present research program.

Test data which, in a single sampling period, would describe more nearly the full size range of the lead aerosol is especially important in developing methods for interpreting data from simpler and more routine sampling techniques.

The exploratory lead aerosol field sampling to date was carried out at various locations in California during the summer of 1963. It seems clear that the principal source of lead in these samples was automobile exhaust. Thus, the results are obviously biased toward TEL effluents. Cholak and co-workers* have pointed out a variety of sources

*Cholak, J., L. J. Schaver, and T.D. Sterling. The lead content of the atmosphere. J. Air Poll. Cont. Assn. 11 (6) 261 (June 1961).

for lead aerosols in urban areas but they emphasized the combustion of coal and dust from high-lead soils. They further state that 90% of the lead in coal will be discharged to the atmosphere and that increased levels of lead in the atmosphere in certain cities during the winter may be due to the increased combustion of coal.

Because there are urban sources of lead in addition to the automobile, some data should be gathered on the nature of the atmospheric aerosol in these other environments. The purpose of this sampling would be to examine the aerosol size characteristics in some areas where the automobile may not necessarily be the major source of the lead aerosol. Places which would most likely come within this category would be some eastern or mid-western areas during the winter when coal and fuel oil were being used in relatively large amounts. Some effort should also be made to obtain measurements on background dust in some remote areas. There are many remote areas in California suitable for measurements of natural atmospheric lead concentration.

In addition to obtaining data on lead aerosols in these other areas, some attention should be given to identifying the reasons for and the significance of apparent variations in lead aerosol size distributions. For example, during the first sampling program in Los Angeles, it was noted that afternoon analyses showed generally a smaller sized aerosol than morning tests. Further studies of aerosol characteristics should attempt to determine to what extent such variations represent real changes in source characteristics of atmospheric processes and to what extent they might be due to random effects.

The nature of the lead aerosols is of interest because exposure to excessive concentrations can be toxic. Particle size and concentration are only two factors which have an important bearing on the toxicity of the aerosol in any given situation. Dr. Kehoe mentions that another factor is the chemical composition of the atmospheric aerosol.* Some lead compounds are much less troublesome than others. Thus, information on the chemical nature of the atmospheric lead aerosol could have important applications to community problems. The research program to date has included considerable effort to identify the lead compounds making up the aerosol. Electron and x-ray diffraction techniques were the principal methods used, but the experiments were, in general, unsuccessful. The lack of success can be traced to the small size of most of the aerosol particles, the low concentration of lead in the total atmospheric dust, serious interferences from other atmospheric aerosol materials, and the lead aerosol itself being probably not a single compound but a complex mixture of compounds. Some characteristics of the lead aerosol, in particular its solubility, have been obtained from studies of bulk samples obtained with high-volume samplers. A more careful delineation of the physical characteristics of the lead aerosol would provide some bases for estimating the nature of the aerosol in the absence of direct compound identification.

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*Kehoe, R. A. The hygienic aspects of the production and use of lead antiknock compounds. Journ. APCA 13 (7) 314 (July 1963).

Method of Approach

The proposed continuation of the studies of atmospheric lead will extend and complement the program which is being concluded; much of the methodology has already been established.

The extension of size range which can be covered in a single sampling period can doubtlessly be done by operating two Goetz Aerosol Spectrometers in parallel (one unit operating in the large size range, the other unit in the small size range). A second Aerosol Spectrometer cannot be added to the present mobile sampling system without making some changes. At present, there is not enough power for a second system. The largest single power user is the refrigeration system which maintains the Aerosol Spectrometer at a constant temperature. Several possible solutions to this problem are (a) installing special refrigeration with lower power requirements, (b) operating two spectrometers from a single refrigeration system and (c) adding additional generating capacity. The final choice would depend on which scheme could be most readily adapted to field service. Weight, space, complexity, and dependability are the most important criteria.

Consideration should also be given to the design of a single aerosol spectrometer with a greater range. This could be obtained by increasing the size of the collector cone and thus providing an increased path length within the unit. An analysis of the present design indicates that if the present cone were increased from 8 to 14 cm, the path length would increase from the present 44 cm to more than 100 cm. This would markedly increase the size range of the unit. However, whether the single large unit would perform better than two standard units can only be determined by careful calculation using the presently available data on the lead aerosol characteristics.

A decision on the best way to expand the size range should be made early in the program because, if at all possible, the expanded sampling system should be used in the extended field program which has been proposed. It should also be tested in the Los Angeles area and the results compared with tests made in 1963.

The proposed field program will be designed to explore the size distribution as well as some of the physical and chemical characteristics of atmospheric lead aerosols in areas where automobile exhaust may not necessarily be the major source. Sampling should be carried out in the winter or early spring in order to obtain the maximum influence of space-heating effluents. It is planned to carry out this sampling in two cities and to spend about three weeks in each city. In selecting the two cities, one of the chief criteria will be the fuel used. Attempts will be made to select one city where coal is the major fuel and another where fuel oil predominates.

Samples will also be taken in various rural areas to determine the lead content of natural dust aerosols. This probably can be done most conveniently by making stopovers in rural areas during trips between urban sampling locations. Special trips to some remote California areas would also be made.

During the field work, samples will be taken and reserved for the compound identification research. High-volume sampling will probably

be the basic technique used. The laboratory program will investigate characteristics such as solubility, melting points, vaporization temperatures, etc., of the lead compounds found in bulk samples as a means of general characterization. Emphasis will be placed initially on those compounds previously identified in automobile exhaust. Further work on single particle identification will not be emphasized, but exploratory experiments will be carried out on techniques that might overcome the known difficulties facing this type of analysis. Some analytical effort might be directed to segregating the high-volume sample so that sufficient concentration of the lead compounds is attained for x-ray diffraction. This lead enrichment might be accomplished directly in the collector; additionally, there may be need for selective extraction and/or evaporative procedures in order to provide suitable samples for diffraction.

The three research phases outlined above -- size range extension, non-automobile environmental sampling, and compound characterization -- can be integrated into a 12-month research program. The following tentative time-table for the research program seems practical. Field sampling in coal- and oil-burning areas should be carried out during the period of January-March, 1964 using either the extended range system if it could be assembled in time or the present unit. The spring and summer program would include calibration work, rural and local Bay Area sampling, and about one month of sampling in Los Angeles using the extended range unit. The analytical compound identification program will continue as a steady effort.

Data analysis and report preparation will conclude the contract. In addition to the final report it would seem desirable to spend some time preparing one or two comprehensive technical papers describing the program and its results. These could be given at the spring meeting of a technical society such as the American Geophysical Union or the American Chemical Society. Pertinent discussion and criticism during such a technical presentation could be considered in planning the latter part of the research program.

II INSTITUTE QUALIFICATIONS

This research, like the present program, will be carried out in the Department of Atmospheric Sciences with considerable assistance from the Analytical Research Department. The facilities to be used will include not only the specialized project equipment, but also the extensive laboratory facilities of the Institute. Those specialized facilities which are expected to be used during the research program include aerosol generating and analysis equipment, the Hitachi electron microscope, and the x-ray diffraction equipment.

Personnel who have been the major contributors to the present research are expected to continue their association with the program: Mr. Elmer Robinson, Project Leader; Dr. John E. DeVries, Manager, Analytical Services; Dr. T. Hopkins, Chemist (x-ray diffraction); Dr. S. Chaikin, Chemist (electron microscopy); Mr. F. L. Ludwig, Meteorologist (field sampling and calibration studies). In addition, other Institute staff members will be consulted when their specialized knowledge could be useful in solving research problems that arise.

1. Exploratory field sampling studies will be made in environments where emissions are not necessarily dominated by automobile exhaust, and, in particular, sampling will be carried out during the heating season in two cities where coal and oil are used extensively and in some remote rural areas.

2. The size range of the sampling method for a given sampling interval will be extended to more nearly cover the range of the atmospheric lead aerosol. Field sampling in Los Angeles will be used to show the application of the expanded range technique.

3. Studies will be carried out to more completely characterize the physical and chemical nature of the atmospheric lead compounds.

4. A final report and at least one technical scientific paper will be prepared describing the research results.

Reporting Schedule

The reporting schedule of the International Lead Zinc Research Organization will be followed.

Estimated Time and Charges

It is anticipated that the proposed research can be completed in a 12-month period for an estimated cost of $68,400. This amount is based on the assumption that the size range can be extended by adding a second Gcets Aerosol Spectrometer to the system. Should this not be the case, the cost of the most reasonable alternative will be determined and if it exceeds the budgeted amount, specific authorization will be obtained prior to any purchase commitment.

The estimated costs include 11 man-months of senior meteorologist and meteorologist time, 8 man-months of senior chemist and chemist time and 5 man-months of clerical and technician time. Also included is the purchase of a suitable Aerosol Spectrometer.

Contract Form

The Institute is a not-for-profit, non-endowed organization. In view of this status, the Institute requests that each research client make an advance deposit to cover working capital requirements for the project being undertaken. The Institute's invoicing procedure is described in the attached standard research agreement. The usual advance deposit on a project of this size is $11,400. If the research client

wishes to eliminate payment against periodic invoices, the advance deposit may be increased to 100% of authorized funds. Upon completion of a project, any unexpended project funds are returned to the client.

This proposal can be established as a project by signing and returning two copies of the enclosed standard agreement, which are incorporated herein and made a part of this proposal, along with a check for the advance payment.

The substituted paragraph 10 and the added paragraph 11 in our present contract with Lead Industries Association, Inc. dated 1 August 1962 are quite satisfactory for inclusion in this proposed contract if desired.

Performance dates covering a period of 12 months can be inserted on page 3 of the Standard Agreement Form to suit International Lead Zinc Research Organization contracting and funding schedules.

Acceptance Period

This proposal will remain in effect until 31 January 1964. After that date, we will gladly consider an extension of time if desired.

We will be able to commence work immediately after authorization.

Respectfully submitted,

Signed: Elmer Robinson

Elmer Robinson, Senior Meteorologist
Department of Atmospheric Sciences

Approved:

Signed: Charles J. Cook

Charles J. Cook, Director
Chemical Physics Division

ER:ej

THE WORCESTER FOUNDATION FOR EXPERIMENTAL BIOLOGY

Project # LC-76

Shrewsbury, Massachusetts

September 11, 1963

RESEARCH PROPOSAL

To: International Lead Zinc Research Organization
292 Madison Avenue
New York 17, N. Y.

Title of Research: THE TESTING OF ORGANOLEAD COMPOUNDS FOR POSSIBLE ENDOCRINOLOGICAL AND ANTI-MAMMARY TUMOR ACTIVITY.

General Statement: Dr. Malcolm C. Henry of Natick, Massachusetts, has given and intends to continue to give us various organolead compounds for testing. We have also received compounds from Professor van der Want. We found some compounds with possible important utilities. We wish to continue these studies. Our tests will include androgenic activity (male sex hormone), estrogenic activity (female sex hormone), anti-estrogenic (possible anti-tumor activity), anti-androgenic activity (possible treatment of prostatic cancer), and anti-inflammatory activity (possible treatment for rheumatoid arthritis and allergic diseases).

We should also be pleased to study organolead compounds from other sources.

Budget: December 1, 1963 to November 30, 1964.

1 Bioassay Technician	$ 5,000
Rats, Mice and Chicks	3,000
Consumable Supplies	1,000
Direct Costs	$ 9,000
Indirect Costs 25%	2,250
TOTAL	$11,250

Signed: Ralph I. Dorfman

RID/s

Ralph I. Dorfman

HUDSON HOAGLAND, Ph.D., Sc.D. Executive Director
GREGORY PINCUS, Sc.D. Research Director
RALPH I. DORFMAN, Ph.D. Director of Laboratories
BRUCE CRAWFORD Business Manager

N 205.05

LC 75

PROPOSAL TO INTERNATIONAL LEAD ZINC RESEARCH ORGANIZATION FOR THE CONTINUATION, THROUGH THE YEAR 1964, OF THE FINANCIAL SUPPORT OF THE PROGRAM OF RESEARCH OF THE KETTERING LABORATORY, IN THE DEPARTMENT OF PREVENTIVE MEDICINE AND INDUSTRIAL HEALTH OF THE COLLEGE OF MEDICINE OF THE UNIVERSITY OF CINCINNATI, ON THE PROJECT, "HUMAN EXPOSURE TO AMBIENT AIR CONCENTRATIONS OF PARTICULATE LEAD"

Reference is made herein to a proposal made on October 3, 1962, in which an experiment then underway was described in some detail, as a part of a systematic investigation of the metabolism of lead in the human body which had been in progress since 1937. Since October(1962), the experiment then referred to has been completed, with respect to the scheduled exposure of the two subjects within the respiratory chambers, and the final period of observation of the subjects (for a period, after the termination of the experimental exposure, sufficient to permit the elimination of most of the lead that was absorbed during the experimental exposure). The data obtained in this experiment to the present time have been charted and will be employed in the formulation of a progress report covering the details of the work, for presentation to the sponsors of the experimental program.

The following paragraphs are concerned with continuation of the program through 1964. While the prior experiment was in the stage in which the experimental subjects were scheduled within the chamber, a further experiment, involving two new subjects, was designed and initiated. The two subjects were located and engaged after a thorough clinical and physiological survey to establish their suitability for the task ahead of them, and the preliminary observations, which are an important part of any such experiment, were initiated on March 19th, 1963. These observations have continued without interruption to the present time, and this initial period, prior to the experimental exposure, will be terminated on September 8th, when the crucial part of the experiment begins as of 8:00 a.m., September 9th. The second stage of the experiment is scheduled to continue throughout (and beyond) December 31, 1964, and is therefore the subject of this proposal.

As predicted (third paragraph on page 5 of the proposal of October 3, 1963), the successful conclusion of the previous experiment made it necessary, in determining the threshold value (for public safety) of finely divided lead dispersed in the ambient atmosphere of the community, "to establish the order of magnitude of the increase in the concentration of lead in the air in the respiratory chamber (above that in the ambient air), which is required to bring about a barely demonstrable response (in terms of the excretory rate and the concentration in the blood), on the part of the human subject, to essentially continuous respiratory exposure to lead." With this idea in mind, the present experiment has been designed in the manner described below.

Two men in similar respiratory chambers will be subjected to parallel experimental conditions. Their exposure to lead in the air will begin at the lowest of three projected levels of concentration (0.010 mg. per cubic meter), and will recur every other day, for 3 hours per day, over the period of about 65 days (this is the approximate length of time required in previous experiments to reach a constant level of lead in the body of the subject, as revealed by a constant daily output of lead in the urine, and a constant concentration in the blood).

The concentration of lead in the air will then be doubled and the same regimen will continue for another 65 days, after which the concentration will be tripled, during another period of 65 days.

N 205.06

be observed in association with 3 hours of exposure, every other day, in this initial three-stage experiment. This, however, remains to be seen.)

Dependent upon the outcome of the observations described thus far, two similar and successive series of observations will be made, in which the duration of the exposure of the subjects on every other day will be increased to 6 hours and 9 hours, respectively.

If the entire series of observations are required to answer the primary question, this experiment probably will extend over the period of two years. It is entirely possible, however, that this question can be answered satisfactorily in a shorter period of time, not less, however than a full year. The issue, in our view, must be that of providing a definitive answer, as a sound physiological basis for a standard of the quality of ambient air in this regard. This experiment, as those which have preceded it, is time-consuming and costly, but this is not the time, in view of the seeming nearness of this goal, to "cut corners," or to settle for less than adequate information. It is in this spirit that the continuing support for this work is sought.

The budget for the continuation of this work will remain essentially unchanged in 1964, and in consequence of this situation, the split between the four present sponsoring groups - namely Ethyl Corporation, E. I. duPont de Nemours and Company, the U. S. Public Health Service and the International Lead Zinc Research Organization may well and properly continue as in 1963. Should there be any alteration in this arrangement, the parties concerned will be informed promptly.

Signed: Robert A. Kehoe

Robert A. Kehoe, M. D., Director
Kettering Laboratory

DATE: September 4, 1963

INDUSTRIAL BIO-TEST LABORATORIES, INC.
1810 Frontage Road
Northbrook, Illinois
Telephone CHestwood 2-3-3-

Project ULC-15

February 4, 1963

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

Dear Mr. Fowler:

We certainly enjoyed your visit last Friday and look forward to working with you in the future.

Considerable thought has been given to the problem of the toxicity of lead in paint. We would like to suggest the following method of study.

If you could provide us with five powdered pigments containing these levels of lead, 1, 8, 16, 23 and 30 per cent, we would conduct 90-day subacute oral feeding studies on rats. We would presume that these would be suitably weathered samples.

Each different pigment would be fed to rats at dietary levels of 0.01, 0.05, 0.20, 1.00 and 5.00 per cent. These five samples would be fed concurrently utilizing two common control groups. Each group would consist of ten male and ten female albino rats. The following parameters would be studied during the feeding period:

growth, recorded weekly
complete blood counts, initially and at 45 and 90 days
urinalyses, initially and at 45 and 90 days
daily observation of all animals for reactions and mortality

At the end of the 90-day feeding period, all animals will be necropsied. Weights of these organs will be recorded: liver, kidney, heart, gonads, spleen and brain. The ratios of these organs' weights to both brain and body weights will be determined. All of these measurement data will be subjected to suitable statistical treatment.

Tissue sections of twenty different organs will be prepared for examination by a pathologist. All control animals and all animals from each 5 per cent dietary level will be so examined. If unusual histopathologic changes are noted in some animals from the highest feeding level of any pigment, animals on the next lowest level will also be examined. If it is necessary, animals from all test levels will be examined.

Since you are interested in the possible interaction of lead with other ingredients of the paints during the weathering process and the effect of this interaction on toxicity, we shall perform some additional statistical evaluations on all the combined data. This is an attempt to elucidate the statistically significant effects of some of the variables being studied.

The analysis of variance contemplated will be set up in this manner:

Analysis of Variance

Variables	Degrees of Freedom
Per Cent Lead in Pigments	4
Dietary Level of Pigments	5
Sex	1
Interactions	
Per Cent Lead x Dietary Level of Pigment	20
Per Cent Lead x Sex	4
Dietary Level of Pigment x Sex	5
Per Cent Lead x Dietary Level of Pigment x Sex	20
Rats (Error)	480
	539

It is obvious that the main variables listed above will be statistically significant, but it is the interactions in which we are interested. We plan to subject measurements from four of the parameters to this particular Analysis of Variance, namely: body weight gain, hemoglobin concentration, liver: body weight ratio and kidney: body weight ratio. If parameters other than these show up as significant in our preliminary statistical evaluations, we will substitute those values.

We will report the feeding study on a particular pigment individually. A supplementary report will be written for the additional statistical analyses on the pooled data. The cost of this study will be $20,000.

For your information, we are enclosing a copy of our client list.

Thank you for giving us the opportunity to discuss these studies with you.

Sincerely,

Signed: J. C. Calandra

J. C. Calandra
Director

JCC:jp
Enclosure

INDUSTRIAL HYGIENE FOUNDATION OF AMERICA, INC.

Mellon Institute, 4400 Fifth Avenue

Pittsburgh 13, Pa.

PROJECT # ULC-15

December 17, 1962

PROSPECTUS FOR TOXICITY STUDIES FOR LEAD INDUSTRIES ASSOCIATION

Introduction

The present allowable level of lead pigment in certain paints has been set at 1%. There are no data available to indicate whether or not a higher level of lead pigment in these paints may be associated, for practical purposes, with no significantly greater health hazard than the 1% level.

In order to obtain information on this question, the investigation outlined below has been designed.

However, before setting out to design the investigation, it is advisable to consider its possible pitfalls and to define the criteria according to which the data will be evaluated.

In general, the plan of the investigation is to compare the toxicity of a given, higher level of lead pigment in paint with the toxicity inherent in the 1% level of lead pigment.

As an initial step, this investigation accordingly proposes to demonstrate a harmful effect by feeding a toxic amount of the paint film from paint containing 1% lead pigment. This needs to be done in order to establish a base line against which the harmful effects of a paint film containing a higher percentage of lead pigment will be compared.

Whether or not the effects produced by the ingestion of a greater amount of lead pigment (because of a higher percentage of lead in the paint film) are significantly increased would depend upon the nature of the effect-versus-dose response. If the response is one-for-one, that is, a straight-line response; then for every significant increase in dose there is a significant increase in effect. If this be the case, then any increase in the percentage of lead pigment in the paint would be associated with a corresponding increase in the health hazard. If, however, the dose-effect response is a curved line with the concavity upward; then, at a low dosage level, a significant increase in dosage is reflected by an insignificant increase in effect. Under these circumstances, it is only at high lead-intake levels that added dosage results in an accelerated increase of effect.

Another question which must be answered is how the toxic effects are to be measured. One of the most sensitive measuring sticks, and commonly used in the determination of health hazard in new drugs or food additives, is the effect of the unknown chemical, when added to the food of young rats, upon their growth curve (gain in weight). Another measure applicable in this investigation would be the total accumulation of lead in a critical tissue as determined by chemical analysis. A third, less sensitive, measure might be the effect upon bone marrow function.

N 205.08

It is obvious that, rather than the percentage of lead in the food, it is the weight of lead absorbed into the system which is the factor determining the development of toxic effects. In the case of the proposed investigation, the magnitude of the effect produced by feeding lead-containing paint film will depend upon a number of conditions:

1. The weight of lead ingested
2. The chemical nature of the lead pigment (which determines its solubility and absorbability).
3. The physical and chemical character of the film in which the lead pigment is incorporated. This also influences the solubility and absorbability of the lead pigment.
4. The fineness with which the lead-containing film is ground for incorporation in the animal food. This has an influence on the solubility of the suspended lead pigment, and hence, its absorbability.

Proposal

The investigation will, of necessity, consist of a preliminary phase for the determination of the "base line" followed by the main study. This preliminary phase will involve three groups of 15 young rats each. One group, fed a standard basal diet, will serve as control. A second group, fed the same diet plus a pre-determined dosage of paint film (from paint containing 1% lead pigment) is intended to demonstrate a minimal toxic effect. The third group of rats will be fed the same diet plus twice the amount of paint film from paint containing 1% lead pigment as the second group.

The dosage level of paint film to be used on the rats is critical. It will depend upon the chemical nature of the lead pigment, its solubility, and known toxicity. The extent to which the other ingredients of the paint film may modify the toxicity hazard can only be surmised. Therefore, the choice of dosage level becomes an educated guess and the possibility of guessing wrong must be taken into account together with the consequences of guessing wrong; namely, of having to repeat the study.

The Lead Industries Association is expected to supply the paint film(s) in a form suitable for incorporation into the diet food, ground uniformly to a reproducible size. Pertinent information regarding the composition of the film material, particularly in regard to the lead pigment, its composition, physical properties, and percentage. The dosage level(s) of the paint film(s) to be used in the animal feed will be fixed by agreement between the representatives of the Lead Industries Association and the investigators.

The feeding of the animals will continue for three months. During this time, the food consumption (and therefore the lead intake) as well as weekly body weights will be recorded and charted. After this period, the rats will be killed with ether and autopsies will be performed. The livers and kidneys will be weighed and the ratios of organ weight/body weight will be determined. Blocks of liver and kidney from each animal will be embedded in paraffin and sections prepared for microscopic examination. A chemical analysis of a tissue for lead content will be done on each animal.

Assuming that a minimal dosage of paint film from paint containing 1% lead pigment has been found which produces a measurable effect upon the rats, then another study (the main study) can be begun in which a group of rats is fed a higher (predetermined) percentage of lead pigment contained in the same amount of paint film as is given the group in which the paint film from paint containing 1% lead. The latter group will serve as the base line against which the effects obtained in the first group will be compared. A third group of rats fed only the basal diet will serve as negative controls. If desired, other groups of rats may be added, to be studied simultaneously, with intakes of ground-up paint film containing larger percentages of lead pigment, differing from one another by a factor of two or more.

Cost

The outline of the investigation is given in tabular form below and the cost is indicated.

Preliminary Phase (with film from paint containing 1% lead)

Group	No. of Rats	Diet	Duration	Cost
1	15	Basal (control)	3 mo.	
2	15	Basal plus paint film (weight to be agreed upon)	3 mo.	$8,000
3	15	Basal plus twice the weight of paint film as in Group 2.	3 mo.	

Second Phase (main study with increased % lead in film)

Group	No. of Rats	Diet	Duration	Cost
1	15	Basal (control)	3 mo.	
2	15	Basal plus paint film (weight to be agreed upon)	3 mo.	$8,000
3	15	Basal plus same weight paint film as in Group 2 but with X% lead in paint.	3 mo.	
4	15	Basal plus same weight paint film as above but with Y% lead in paint.	3 mo.	$2,500
5	15	Same as Group 4 but with Z% lead in paint.	3 mo.	$2,500

LC 79

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 205.09

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.

N 205.1

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 techniques of Weil**, Thompson***, and Thompson 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.

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

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

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**, Thompcon***, and Thompcon 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.

LC 79

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.

N 205.12

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	

7/12/63
Project # ULC-12

WORK STATEMENT

LEAD STABILIZED P.V.C. PIPE

INTRODUCTION

Rigid polyvinyl chloride pipe is important in today's market and should assume greater importance in the future. Various compounds of lead have been and are used successfully as stabilizers for rigid P.V.C. formulations and the efficiency, effectiveness and economy involved in the use of lead stabilizers is not an issue, but is accepted by manufacturers of plastics. What is at issue is the amount of lead which can leach from piping when it is used for potable water supplies. Since the use of rigid P.V.C. pipe has a good future potential as a replacement for metallic pipe, it will be well for us to measure the lead release or leachability from the pipe when it is used in standard household water supply systems.

Producers of P.V.C. pipe appear to hesitate to use lead as a stabilizer because of the possibility of such pipe being inadvertently used in a water supply system. If, however, we could reassure the plastics industry with appropriate measurements of lead leachability (assuming that the lead release is low enough) then such producers would feel free to use these highly desirable lead compounds.

SCOPE

The purpose of this proposed program is to obtain lead release data from standard formulations of rigid P.V.C. piping in order to promote the use of such lead stabilizers in the industry. The program will be carried on in two consecutive phases, the first of which will examine in preliminary fashion whether or not excessive amounts of lead will be released from standard piping systems. If the lead release is satisfactorily low then the second phase of the program will be carried on which will involve obtaining tests and approval of such piping systems for use in potable water supplies. Of course, if the first phase of the program shows that we are not in the right ball park and that lead leaches from the piping systems in excessive amounts, we will go no further.

ECONOMIC INCENTIVE

Rigid vinyl pipe is presently being produced in the amount of about 20,000,000 pounds per year of which only 20% is presently stabilized with lead compounds. We estimate that this provides a market of not more than 80 tons of lead per year at the present time. If all of the rigid P.V.C. pipe were stabilized with lead compounds, the present market would be about 400 tons of lead per year. However, this is a rapidly expanding market and the future potential looks good, such that in 1967 it is estimated that 100,000,000 pounds of rigid P.V.C. pipe will be produced.

Therefore, the present situation is of less importance to us than the future and, since it will take some time to carry on a testing and approval program, and since the results of the testing and approval program should be available before the real market potential can be realized, it would be incumbent upon us to carry out such a program.

NATIONAL SANITATION FOUNDATION TESTING LABORATORY, INC.

Headquarters * School of Public Health * University of Michigan

Dr. Henry F. Vaughan, President
Charles A. Parish, Exec. Director
Walter F. Snyder, Sec'y-Treasurer

Normandy 3-1511
Ext. 2378

ANN ARBOR, MICHIGAN
48104

August 2, 1963

Mr. Don C. Fowler, Project Manager
International Lead Zinc Research Organization
292 Madison Avenue
New York 17, New York 10017

Re: Plastics Misc.
(Project # ULC-12)

Dear Mr. Fowler:

From your letter of July 30th we have considered the minimum testing that we feel should be included and this matter has been discussed with Doctor McCord and he concurs that this would be a minimum that should be considered at this time. We would propose, therefore, that the preliminary testing program to determine lead leachability be on the following basis:

1. That at least three levels of lead concentrates be used in the PVC pipe.
2. That pipe be produced from at least 2 PVC pipe manufacturers.
3. The tests would be performed on two types of waters to be agreed upon before the testing is undertaken.
4. That the lead stabilizer compounds that are being used in Europe be the ones considered in the test. This would assume that one stabilizer would be considered in this initial testing program. If you feel that more than one type of lead compound should be included, then this would double the amount of testing outlined under 1 and 2 above.
5. The cost of conducting the testing over a minimum of a one year period on the basis of the outline above would be $16,500. This would cover the performance of all the tests as outlined in the above breakdown on the basis of one lead compound.

If you wish to proceed with the program, we believe that it would be necessary to have some further discussions at which time we could get into more detail regarding the specific procedures.

Yours very truly,

Signed: Charles A. Parish

Charles A. Parish
Executive Director

CAP/el

N 205.14

10/4/63
Project # ULC-13

WORK STATEMENT

LEAD GLAZES FOR DINNERWARE

INTRODUCTION

In 1960 two cases of lead poisoning were reported as being caused by the use of dinnerware made by a pottery in California. Some four million pieces of this ware had been distributed throughout the United States by this pottery and the California Health Department insisted on the return and confiscation of all of this dinnerware. The resulting economic loss to the pottery caused it to go out of business. The U. S. Potters Association requested the Lead Industries Association to join with it in an investigation of the lead release from standard U. S. products of potteries in various parts of the United States. We soon found that there was not even a standard method for testing lead and heavy metal release from glazed pottery and this became our first order of business. In a joint project the U. S. Potters Association and the Lead Industries Association engaged the Kettering Laboratory to develop a test method and to use this method on representative products to determine its suitability.

The test method was successfully developed and has been accepted by A.S.T.M. as a tentative standard. This phase of the project having been completed, it now appears necessary to investigate other factors which have been brought to light by the operation at the Kettering Laboratory.

The Kettering Laboratory tests were run on many different types of glazes from many different manufacturers. Since the results of the tests were spread over a rather wide range of values, it became of interest to us to inquire as to the reasons for the variations. Briefly, variations in lead release from glazed dinnerware appear to be caused by (1) glaze composition, (2) firing temperature and certain kiln conditions, (3) composition of the bisque, (4) other variables not adequately understood.

Lead is a standard constituent of glaze compositions for most of the pottery dinnerware made and sold in this country and abroad. It is desirable that glazes contain lead for many technical and economic reasons; for instance, the plastic properties imparted to the glaze by its lead composition allow a wider latitude in firing temperature and this is important in production potteries.

SCOPE

The purpose of our proposed investigation is to inquire into the parameters that it is necessary to control in production potteries to make use of lead in a safe and economic manner. We are here defending a market and also proposing to expand this market by publishing guidelines

which will allow production potteries to operate with certainty in a field where up to now there has been a great deal of empirical and guesswork operation.

By using our standard test method referred to in the Introduction, we will be able to investigate and to enumerate in a quantitative manner for the use of production potteries the various parameters that are necessary to ensure a smoothly operating pottery.

BOOK MIC INCENTIVE

As evidenced by the preceding discussion, the economic justification of this research is primarily defensive. Even isolated cases of defective glazes produce adverse publicity which is harmful to the lead industry in other areas. It is therefore important to initiate research which will identify the requisites needed to produce safe lead glazes and to determine optimum manufacturing procedures.

Marketwise, the total consumption of lead in ceramics in the U.S.A. was about 16,000 tons in 1962, plus an additional 5,000 tons imported from Mexico. What percentage of this quantity goes into glazed dinnerware is not known, although a rough estimate of 5,000 to 10,000 tons seems reasonable.

PROJECT # ULC-14

NEW YORK MEDICAL COLLEGE
Flower and Fifth Avenue Hospitals
Fifth Avenue at One Hundred and Sixth Street
New York 29, New York

Department of Pathology
Laboratory of Experimental Pathology

July 15, 1963

Frank Princi, M. D.
Kettering Laboratory of Applied Physiology
University of Cincinnati
College of Medicine -- Eden Avenue
Cincinnati 19, Ohio

Dear Frank:

Enclosed is the final protocol of our proposed series of experiments on rats designed to produce data concerning the radioprotective effects of inorganic lead. Incorporated are the suggestions of Dr. Donald R. Biggs. The budget is for the work as outlined and in the event it is necessary to pursue unexpected results, supplemental funds will be requested with appropriate justifications.

If the protocol is accepted, we would like to start work not later than October 1, 1963 if possible. After January 1, 1964 we will be moving our laboratories into a new research building and new projects may be delayed. The principal investigator will be the contracting officer for the institution.

Sincerely yours,

Signed: Bernie

Bernard M. Wagner, M. D.
Professor and Chairman
Department of Pathology

BMW/emp

N 205.16

Title of Project:

THE RADIOPROTECTIVE EFFECTS OF INORGANIC LEAD

SPECIFIC AIMS:

Chemical agents utilized in the radiation protection of tissues, organs and whole animals vary as to structure, mode of action and species studied. Trace metals play a vital role as intracellular co-factors. Zinc, cobalt, iron, magnesium, manganese and copper are some of the metallic ions with established functions in various enzyme and transport systems.[1] Not well understood is the high concentration of cadmium in the renal cortex and the relationship of selenium[2] to muscle metabolism. It is conceivable that in the complex, inter-related systems that control cell function, radiation induces rapid ionization, formation of free radicals and disrupts molecular spatial arrangements. In such situations the various metallic ions would undergo severe alterations in their valence shells. The possible introduction of appropriate ions to stabilize the early changes produced by radiation is worthy of study.

This laboratory has an unusual competence in cytochemistry and histochemistry. In addition, a major group of investigators are studying the structure and function of the cardiovascular system. These include:

	Field	Formerly
Dr. Sara Schiller	Biochemistry	University of Chicago
Dr. William Outstein	Biophysics	New York University
Dr. Ralph Strebel	Exp. Path.	Institute of Research, University of Montreal (Dr. Selye)
Dr. S. Mehos	Exp. Path.	Downstate Medical Center, N.Y.
Harold Appleton, M.S.	Clinical Chemistry	National Institute of Health
Dr. Bernard Wagner	Exp. Path.	University of Washington

Working with this group on a part-time basis is an electronics expert and a physicist.

The tissues from the animals in the various experimental groups will be prepared by special techniques developed in this laboratory to evaluate intracellular, extracellular and vascular changes. Vascular changes include fixation for observations of constriction or dilatation.[3-6]

Preliminary studies employing inorganic, ionized lead in the intact animal has indicated that the vascular changes consequent to lethal whole-body X-irradiation may be modified. The purpose of this investigation is to explore in detail the following questions:

1. Radiation projection of lead as a function of dose, time and tissue levels.
2. Influence of animal age, sex and diet.
3. Mechanisms of protection at a histochemical, cytological level.
4. Blood and urine changes during experiments.

METHODS:

Experiment I			Validity of Previous Observations		
Group	IA	$Pb(Ac)_2$	200 mg/kg	6 rats	400 r
	IB	NaCl	200 mg/kg	6 rats	400 r
	IC	$Pb(Ac)_2$	200 mg/kg	6 rats	400 r •
	ID	$Pb(Ac)_2$	250 mg/kg	6 rats	400 r
	IE	$Pb(Ac)_2$	250 mg/kg	6 rats	400 r •
	IF	$AgNO_3$	200 mg/kg	6 rats	400 r
	IG	$AgNO_3$	250 mg/kg	6 rats	400 r

Note: Doses 50 mg/kg/day. X-ray: 24 hrs. after last dose except Groups IC and IE where irradiation precedes salt administration. Group IB is a sham treated radiation exposure control. Wistar rats 100 gms.

EXPERIMENT II — Effect of Dose and Time

Days		1	2	3	4	5	7	10	14	21
Group	2A (6)	+	+	+	-	+	R	-	-	-
	2B (6)	+	+	-	+	R	+	-	-	-
	2C (6)	+	-	+	+	+	-	R	-	-
	2D (6)	+	+	-	-	+	-	+	R	-
	2E (6)	+	-	-	-	+	-	+	+	R
	2F (6)	+	+	+	-	-	R	+	-	-

\+ 50 mg PB $(AC)_2$ I.P.
\- No injection
R 400 r X-ray

The experimental design is to relate dose schedule as a fixed variable to time. In each group of 6 animals there are 3 males and 3 females of 100 gms. initial weight. Note that Group 2B and 2F will receive Pb in the post-irradiation period.

Experiment III — Enhanced Diet Study

Repeat of Experiment I with the following exceptions

Group	III-IA	Ad libitum diet
	III-ID	Enriched protein diet 25% including 8E amino acids
	III-IF	Ad lib
	III-IG	Enriched

The 8E rich amino acids will be administered by gavage and will constitute a 25% inc. in protein N over the standard daily consumption of food.

Throughout all experiments, blood and urine samples will be obtained at judicious intervals for the following determinations:

BLOOD	Hemoglobin Hematocrit Peripheral WBC and differential Na,K,Ca, electropherogram Lead B U N
URINE	Lead Proteins Casts Cells, Sp. Gravity

Tissues obtained at autopsy will include:
Brain, Lungs, Heart, Liver, Spleen, Stomach, Kidneys, Adrenals, Gonads, Skin, Bone, Bone Marrow.

In addition to routine staining, where indicated the following histochemical reactions will be analyzed.
1. Oxidative enzymes
2. Phosphatases
3. Basic proteins
4. Connective tissue polysaccharides

The experiments will be modified during the course of the investigation at the discretion of the investigators. Promising leads will be followed at no additional expense. The experimental design will yield statistically significant information which will answer in large part the original problem listed under title of project. The project will require 6-8 months for completion.

CURRICULUM VITAE

Bernard M. Wagner, M. D. - Professor and Chairman, Dept. of Pathology (Princ. Invest.)
Ralph Strebel, Ph.D. Assistant Professor of Experimental Pathology
Lillian Bedell, B.A. Chief, Histochemistry Laboratory

FACILITIES AVAILABLE

Animal Facilities
Histochemistry Laboratory
Cytochemistry Laboratory
Histopathology Laboratory
X-ray machine (supervoltage available)

REFERENCES:

1. Enzymes: Dixon, M. and Webb, E.C., Academic Press Inc., New York, 1958
2. Schubert, J.R., Muth, O.H., Oldfield, J.E. and Remmert, L.F.: Experimental Results with Selenium in White Muscle Disease of Lambs and Calves, Fed. Proc., 20: 689, 1961
3. Wagner, B.M.: Studies of Vasoconstriction, Bull. N.Y. Academy of Medicine, 38: 66, 1962
4. Van Citters, R., Wagner, B. M. and Rushmer, R.: Architecture of Small Arteries during Vasoconstriction, Circ. Res., 10: 668, 1962.

5. Van Citters, R., Wagner, B.M. and Rushmer, R.: Arterial Walls during Vasoconstriction, Cor et Vasa, 1:4, 1962 (text in Russian)

6. Wagner, B.M.: Aleutian Disease of Mink, Arthritis and Rheumatism, August, 1963

BUDGET

PERSONNEL		
Principal Investigator - Dr. B. Wagner		------
Co-Investigator - Dr. Ralph Strebel		3,000
Technician, part-time		2,500
Animal Care-taker, part-time		1,440
EQUIPMENT		
NONE		-----
CONSUMABLE SUPPLIES		
Animals - Wistar Rats (100) - Animal Food, bedding		200
Histochemical reagents, supplies		1,000
Histology supplies		300
Blood and Urine tests		500
OTHER EXPENSES		
X-ray technician, part-time and use of equipment		200
TOTAL DIRECT EXPENSES	$9,140	
Overhead 10%	914	$10,054 TOTAL