STUDY OF THE POSSIBILITY OF LEAD METHYLATION IN RIVER BEDS

Proposal
To

International Lead Zinc
Research Organization, Inc.
292 Madison Avenue
New York, New York 10017

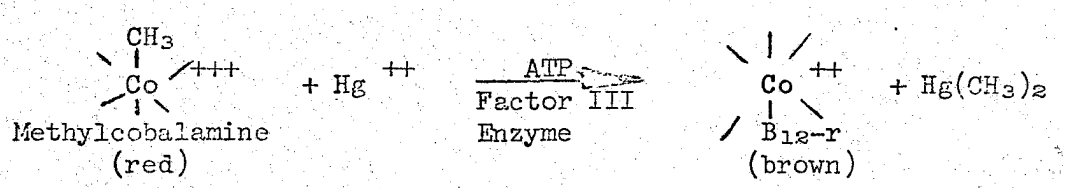
From

Ethyl Corporation
Ferndale, Michigan 48220

September 8, 1970

I. INTRODUCTION

In recent years it has been claimed by government health authorities and some other investigators that mercury discharged as an industrial waste into rivers ultimately transforms into a form which is metabolized by organisms in the river, ultimately ending up as detectable quantities in fish. This disclosure appeared very surprising in view of the supposed inertness of mercury in water and its extremely low solubility. The explanation was furnished recently by Wood, et al[1], who showed that mercury can be converted to methyl mercury and dimethyl mercury as a result of enzymatic reactions of the following type which are favored in the environment of river sludges:



Methylcobalamine (red)

(brown)

In view of the reactions which mercury undergoes, it is logical to ask whether industrial lead wastes dumped into rivers can undergo similar reactions.

Lead is most commonly discharged into rivers as the plumbic (+2) ion. If it remained in this form it would be readily available for metabolism by organisms at all levels. However, it has been shown (Allen et al[2]) that ferric hydroxide and high molecular weight organic species complex with and scavenge the lead from solution. They believe the lead is then lost quickly to the river or lake bottoms. It is therefore relevant to ask whether it can become available once more for ingestion by various organisms through a methylation reaction of the type which has been observed with mercury. If this methylation reaction did occur, it could have important implications to the lead content of all river species including edible fish. It is therefore of importance to determine whether such reactions can occur with lead.

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1. Wood, J. M., Kennedy, F.S., and Rosen, G.G. "Synthesis of Methyl-Mercury Compounds by Extracts of Methanogenic Bacterium" Nature 270 173-34 (1965).

2. Allen, H.E., Matson, W.R., and Mancy, K.H. J of Water Poll. Cont. Fed. 573-581 (1970).

II. RESEARCH PLAN

It is proposed to study the possible methylation of lead using river sludges obtained from the Mississippi River in the vicinity of the Ethyl Corporation plant at Baton Rouge. When this sludge is recovered, it will be carefully examined to determine whether any organic lead is present. Such material could conceivably be present as a result of lead being emitted from the plant in that form. If there is any detectable organic lead it will be extracted before any further studies are made using the sludge.

We will then follow the procedure of Wood[1] to see whether methylation of lead can occur under conditions where the methylation of mercury is known to occur. For convincing proof of this point in the case lead does not methylate, we will repeat the work of Wood[1] to show that mercury does methylate under these conditions.

It may be possible that the sludge as such will be difficult to work with due to factors not known to us at present. If this is the case, we would extract the methanogenic bacteria from the sludge. The methanogenic organism was isolated recently by Bryant[3]. This organism can be grown routinely on hydrogen and carbon dioxide. Cell extracts can be prepared and the activity of the mixture assayed by methane release (Wood et al[4]). Methylcobalamine is an excellent substrate for the formation of methane in the presence of the methanogenic bacteria.

Thus to conduct the experiment in the absence of sludge, we would follow the procedure of Wood[1], using a suitable concentration of soluble lead, ATP (adenosine triphosphate) the methanogenic bacteria and methylcobalamine in the reaction mixture.

Following the exposure of lead to the methanogenic environment, the mixture would be deproteinated. If organolead compounds are present, they could then be extracted using an organic solvent. Identification of the compounds could then be made using thin layer chromatography. This method of identification has been used for organomercury compounds, and we anticipate no problems with lead.

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3. Bryant, M.P., Wolin, E.A., Wolin, M.J., and Wolfe, R.S., Arch. fur Michrobiologie 59 20 (1967).

4. Wood, J.M., Allam, A.M., Brill, W. J., and Wolfe, R.S., J. Biol. Chem. 240 4564 (1965).

To complete this study, we would also examine the possible formation of alkyl lead compounds in the absence of bacteria. It is known that in a reducing environment mercury can be methylated in the presence of methylcobalamine. A parallel study will be made with lead to determine whether this reaction proceeds.

To round out the picture, it is proposed to examine the lead content of several species of fish. Fish from the upper Mississippi would be compared with those in the area near Baton Rouge. These data would be examined for trends relating to locations in the river, and also in relation to the lead contents of other foods available in the American diet, based on much information already accumulated from Ethyl Corporation and other sources.

III. EXPERIENCE

Ethyl Corporation's Research Laboratories in Detroit have been intensively studying the problem of lead in the environment for many years. Detailed studies have been made of the processes governing the incorporation of environmental lead into many food crops, and these results have been very effective in showing that lead naturally present in soils is a major contributor to lead in crops, while only in very few cases is there any measurable contribution of atmospheric lead to the edible portion of crops.

As part of its study of lead in foods, Ethyl has set up a number of greenhouses, some of which are equipped with very efficient filters so crops can be grown in comparative tests in the normal atmosphere and in the presence of carefully filtered air.

In order to support these studies, Ethyl has built up a major capability in analysis, specializing in the examination of lead and lead compounds in a variety of substrates. In addition to the very extensive studies on crops and other vegetation, we have dealt in depth with lead in such diverse materials as water, milk, fish, and seafood. Our analytical studies on milk have shown that the commonly accepted values for lead in milk are far higher than those actually occurring in practice, due to widespread analytical errors occurring in many laboratories working in this area.

Our equipment for performing these analyses covers a wide variety, from wet chemical methods through the most advanced instrumental techniques. We also have available, on a contract basis, access to electron microprobe equipment for the identification of extremely small quantities of lead compounds.

IV. STAFF

The proposed work would be under the immediate technical supervision of Dr. Gary Ter Haar, Research Associate at Ethyl Corporation's Ferndale Laboratories. For the past six years Dr. Ter Haar has been in charge of Ethyl's studies of lead in the environment as described above. He would be available for the proposed work to the extent of time needed to directly supervise the experimental work described above. It is expected that this would require one man month of his time. Dr. Ter Haar's biography is present in the Appendix to this proposal.

The major portion of the experimental work would be the responsibility of a fully trained technician reporting to Dr. Ter Haar. Selection of this technician would be made from among several individuals with substantial experience in the careful handling and study of materials containing trace quantities of lead.

Analytical work would be the responsibility of Ethyl's Analytical Section under the direction of Dr. James B. Hinkamp. Experts on Dr. Hinkamp's staff would undertake responsibilities as appropriate to their own specialized experience in wet chemical, instrumental, and microanalytical techniques.

V. FACILITIES

Ethyl Corporation's facilities at Ferndale are well known to the ILZRO group, and no detailed description is required for this proposal. Suffice it to say, that we already have on hand the facilities and equipment needed to do this work and that no additional equipment of any substantial magnitude would be required to carry out the work as described above.

VI. COST

Based on the preceding project outline, we envision that this work can be accomplished within a six month period, for a total cost of $25,791. Cost breakdown is as follows:

LABOR	
Project Personnel (6 man months) Supervision (1 man month) Analytical Support (2 man months)	$ 9194
OVERHEAD (computed at 152% of labor)	13975
Total labor + overhead	23169
SUPPLIES & TRAVEL	1000
FEE (computed at 7% of labor + overhead)	1622
Grand Total	$25791

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APPENDIX

DR. GARY TER HAAR, Research Associate, earned his B. S. (cum laude) in chemistry from Hope College in 1958 and his Ph. D. in inorganic chemistry from the University of Michigan in 1962. His doctoral dissertation was on a study of tetraboranecarbonyl and some of its derivatives. In addition, he taught laboratory classes in high-vacuum techniques and radioactive tracer studies and spent one summer doing research work using radioactive techniques.

After joining Ethyl in 1962, Dr. Ter Haar conducted research in the field of metalorganic chemistry, preparing volatile compounds of uranium and thorium under partial sponsorship of the Atomic Energy Commission. He synthesized a number of these compounds, which are highly reactive and unstable, and demonstrated a high degree of technical ability in their handling and preparation.

Dr. Ter Haar subsequently devoted 1-1/2 years to research on high-energy fuels. He then spent another 1-1/2 years as Ethyl's representative at the Argonne National Laboratory in a cooperative program on the determination of traces of lead and lead-210 in air, water, soil, and various biological specimens. Since returning to Ethyl's Detroit laboratories, Dr. Ter Haar has been concerned with the total area of environmental lead and its relationship to man.

In conducting this work, Dr. Ter Haar has been responsible for the design and experiments for the accumulation of data and for its analysis. He has worked closely with the personnel of Analytical Research and Services group in developing the techniques used to determine the trace levels of lead present in many of the samples.

The results of some of Dr. Ter Haar's work at the Argonne National Laboratories and at Ethyl's Detroit Research Laboratories were recently presented in two papers at the American Chemical Society "Symposium

on Air Conservation" held at the April, 1969 spring meeting in Minneapolis.

Bibliography of Dr. G. Ter Haar

"Dimethylamidodifluorophosphine Tetraborane-8-$F_2PN(CH_3)_2B_4H_8$ -- A Stable Adduct of B_4H_8," with M. A. Fleming and R. W. Parry, J. Am. Chem. Soc. 84, 1767 (1962).

"A Study of Tetraboranecarbonyl and Some of Its Derivatives," Dissertation Abstracts 23, 3627-28(1963).

"Amine Addition Compounds of H_3BCO and B_4H_8CO," with R. W. Parry, C. E. Mordman, and J. C. Carter, Adv. Chem. Series 42, 302-11 (1964).

"The Synthesis and Characterization of Cyclopentadienyl Derivatives of Uranium and Thorium," with M. Dubeck, Inorg. Chem. 3, 1648-50 (1964).

"Lead and Lead-210 in Rainwater," with R. B. Holtzman and H. F. Lucas, Jr., Nature 216, 353-55 (1967).

"The Sources of Lead in Perennial Rye Grass and Radishes" with R. Dedolph, R. B. Holtzman and H. Lucas, Jr. Presented at the American Chemical Society Spring Meeting, Minneapolis, Minn. April 1969.

"Air as a Source of Lead in Edible Crops" presented to the American Chemical Society in Spring Meeting, Minneapolis, Minn. April 1969.

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Ballot

UEP-27 [illegible] of Lead in River Beds.

Proposal - "Study of the Possibility of Lead Methylation in River Beds" [illegible] Corporation, September [illegible], 1970.

Amount - [illegible] ([illegible], [illegible].00, [illegible], [illegible].00)

I approve ☐

I do not approve ☐

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Name

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Company

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Date

Return by November 1, 1970
To: J. [illegible]
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New York, N. Y. 10017