Problem: What happens to tetraethyllead (TEL) in the body ?

Work of previous investigators:

In the four previous investigations (Norris & Gettler '25, Kehoe & Thamann '31, Bihovskaya '48, Kröller '49), only a dozen analyses of tissues for TEL have been reported. These analyses were determinations of lead volatilized with steam. Boiling various decomposition products of TEL in water actually regenerates TEL, as Calingaert et al. ('48) recently demonstrated. This suggests that methods other than steam distillation might be useful. The paucity of data also suggests the difficulty of the problem.

Plan of attack:

At the beginning two main objectives are evident:
(1) distribution of TEL among tissues under various conditions, and
(2) reactions of TEL in vivo, - their products, rates and mechanisms.

To approach these objectives, analytical methods must be developed, (a) methods of measuring TEL and (b) methods of detecting and of measuring its decomposition products. For example, there are no published methods for TEL in water and no proven methods for TEL in tissues. Until analytical tools are developed, the problems of more physiological interest serve principally to direct and to set requirements for the analytical development.

Progress:

In 1953 analytical methods were developed in this laboratory for measurement of TEL in air and in water. These were applied to the fundamental problem of the distribution of TEL between air and water at equilibrium. The finding, of preliminary nature, was that about 0.4 mgm. of TEL dissolve in a liter of water at room temperature. About 100 times this much had previously been reported to dissolve (Ethyl Corp. memorandum no. 45-72). Other work of exploratory character, both analytical and synthetic was also done that year, as given in my annual report of January 18th, 1954.

Progress (continued):

During 1954 attention has been directed to biological problems. I summarize our findings here, with some interpretation of their significance and implications.

For the first time, direct evidence on the following 9 points has been obtained:

(1) Persistence of TEL in live animals for at least 3 days

The continuing presence of TEL in the body for days has long been suspected. Its presence is a fact of considerable importance in planning prophylactic and therapeutic regimes. Methods should be devised to hasten elimination of TEL from the body before it decomposes. Specific proposals for this are included in the paragraphs that follow. Measures should be taken to afford the body continuous help in detoxification of the decomposition products of TEL as they are continually produced in vivo. Also, continuing help should be given the metabolic systems being attacked by these decomposition products before they are detoxified. Both aid in detoxification and protection for metabolic systems can be sought with improved rationale as we increase our knowledge of TEL metabolism and of its effects. Note the underlying assumption here that the TEL molecule is intrinsically less toxic than its products.

Progress (continued):

(2) Distribution of TEL among 16 different tissues

Now we have evidence of where TEL goes in the body; we are no longer solely dependent upon analyses for lead, per se. The data are basic to understanding the mechanism of TEL poisoning. Any explanation must be reconcilable with this information. A new limit is thus set to speculation. At the same time, the physiological problems of TEL poisoning are seen in a new light.

Briefly, concentrations of pentane-soluble lead (in milligrams per kilo of fresh tissue) were found to be, in ascending order:

blood serum	none
stomach & contents	0.4
caecum & contents	0.5
lungs	1.3
large bowel & contents	1.5
leg muscle	1.7
abdominal muscle	2.0
hide	2.1
small bowel & contents	2.6
brain	2.7
spleen	3.4
carcass	3.7
whole blood	3.7
heart	4.8
fat	5.3
kidneys	6.2
liver	23.

These data are from a 193 gram rat that was exposed for 2 hours to 2.3 milligrams of TEL vapor, then killed with carbon monoxide.

Progress (continued):

(3) Localization of TEL in liver

The high degree of localization of TEL in liver suggests that this organ is acting as the body's principal (defense.) Here is, chiefly, where the body combats TEL poisoning. What assistance can be given to the liver ? What prophylaxis ? Answers to these questions would be more rational if we knew what defensive metabolic mechanisms are involved.

Perhaps it is to the liver that we may best look for signs of slight exposure to TEL. For example, is serum cholinesterase depressed after slight repeated exposures to TEL ? This might occur as a result of interference with synthesis of this enzyme by the liver. If it does occur, might it serve as a clinical aid in detecting and following TEL poisoning ?

one cannot say that this is not the case, but, if so, it is probably only a side effect.

It should be very easy to ask Wallenof to test a few samples of blood from animal given slight exposure.

If any cholinesterase action occurs cholinesterase of the erythrocytes would be the more likely one to be affected. It more nearly reflects alterations in psychic function.

Progress (continued):

(4) Excretion of TEL via bile

The concentrations of TEL found in various parts of the G-I tract point to biliary excretion of TEL. Administration of cathartics may thus have a rational basis in hastening disposal of TEL excreted by the liver. If bile provides one of the major excretory routes for TEL, steps to prevent recirculation of TEL from bile are imperative. Its absorption from the bowel must be minimized in one way or another. Catharsis is one way. Also, consider the possibility of absorbing TEL into the intestinal contents. Perhaps a wax or indigestible oil might absorb and hold TEL, possibly with the aid of some bulky material such as agar or methyl cellulose. Or adsopption on a fatty resin might prove practicable. Destruction of TEL in the bowel is theoretically possible through processes of oxidation or, less likely, of acid hydrolysis; however, disposal of the decomposition products might be more troublesome than disposal of TEL as such from the body.

Or, looking at this finding in another way, it would seem appropriate to increase the rate of formation of bile and to hasten excretion of TEL from the liver. This might be pictured as a "flushing out" of the liver. Presumably, procedures are available for stimulation of these two functions.

Progress (continued):

(5) Formation of triethyllead ion in vivo

This ion may prove to be one of the initial products of decomposition of TEL in vivo. If so, one may expect a prompt lowering of cholinesterase activity in tissues where it appears. Reversal of effects of TEL by drugs antagonistic to such a process may then be anticipated. For instance, injection of atropine and magnesium salts or of cholinesterase might reverse effects of TEL.

Presence of the positive triethyllead ion suggests other possibilities, too. Positive ions, in general, are known to move slowly in and out of living cells. Therefore, movement of triethyllead ion is probably slow and quite possibly is much slower than movement of TEL. Here again one sees the importance of ridding the body of TEL before it decomposes in vivo, that is, before movement of the lead atom through the tissues is slowed down.

In this finding one may also see reason for expecting different physiological responses to poisoning by TEL and by ionic lead compounds. Cell interiors are probably more easily accessible to TEL than to lead in ionic form. Also, one may predict on this basis that higher concentrations of lead will occur in brain after poisoning by TEL than after poisoning by triethyllead ion. By further extension of the same idea, higher concentrations of lead will occur in brain after poisoning by TEL than after poisoning by inorganic lead ion. This speculation is substantiated in part by Kehoe's data on fatal cases of lead poisoning.

Progress (continued):

(6) Absorption spectrum of diethyllead dithizonate

In the search for methods of detecting decomposition products of TEL in tissues, it is advisable to seek certain classes of facts about the behavior of known lead compounds. Particularly is this true of compounds that may occur with decomposition of TEL in the body. Thus, one needs solubility information to aid in separating compounds from tissues and from each other. Chemical reactions of various kinds may, likewise, be of analytical utility. With such ends in view, absorption spectra of triethyllead dithizonate and of diethyllead dithizonate were measured. (We had, of course, first synthesized the lead compounds.) These spectra differed from each other and from the spectrum of inorganic lead dithizonate. The spectrum of diethyllead dithizonate lies between those of the other two dithizonates. Thus, the reaction with dithizone serves to distinguish among these three types of lead compounds. Furthermore, these three types of compounds can be distinguished from TEL by its failure to react with dithizone. Differences among these spectra are small. They may prove most useful after separation of the lead compounds has first been effected.

Progress (continued):

(7) Detection of inorganic lead in pentane extracts of tissues

The presence of inorganic lead in these pentane extracts is an unexpected and annoying finding. Discovery of the presence of this lead is so recent that the obvious methods of dealing with it are only partially explored. Fortunately, the inorganic lead appears under some circumstances but not under all circumstances. It does, of course, complicate the analytical procedures.

(8) Regeneration of TEL at room temperature from products of its decomposition with air and water

When TEL, air and water are shaken together, I find that a white precipitate forms. If the mixture is allowed to remain at room temperature and the water is tested from day to day for pentane-soluble lead, a progressive increase is found. Thus, regeneration of TEL is probably occurring. This process may occur wherever such decomposition products of TEL are in contact with air and water. Might this be of interest where spills of TEL are cleaned up ? Or, might it explain why concrete continues to give off TEL vapor long after contamination with TEL ? The nature of the decomposition products and the reactions involved remain to be determined.

Progress (continued):

(9) Stability of triethylllead chloride in water at room temperature and at refrigerator temperature.

Because there was no information on this subject, and because triethyllead compounds may be expected in the body among the decomposition products of TEL, we synthesized triethyllead chloride and observed its stability. Shaking triethyllead chloride with air and water produced less than 0.1% of conversion of 270 micrograms into pentane-soluble lead compounds. Nor was any pentane-soluble lead found in the solution after standing 5 days at room temperature. Likewise, a refrigerated aqueous solution of triethyllead chloride yielded no pentane-soluble lead after standing for 6 months.

This kind of information is important if one is to search the tissues for such a compound. It gives hope that the compound will not decompose before one has time to extract, isolate, identify and measure it. It complements the information about stability in boiling water of this and other compounds published by Calingaert et al. ('48).

Progress (continued):

Other work of the year is less interesting, in part because its promise is yet to be fully realized. For example:

(1) Development of a technic for exposing rats to TEL vapor for hours at a time with minimal loss of TEL on fur

(2) Changes in technic of measuring TEL in tissues, modifications of extraction procedures, testing of extracts in various ways, etc.

(3) Attempts to isolate decomposition products of TEL from tissues by use of various solvents (particularly methylene chloride), by reaction with dithizone, and in other ways

(4) Synthesis of several triethyllead and diethyllead compounds that may be formed in vivo, and observation of certain of their properties of analytical interest such as partitioning between aqueous and non-aqueous solvents and reaction with sulfide

(5) Experiments in detection of triethyllead and diethyllead compounds after their addition to tissues, with and without added TEL

Proposal for continuation:

I suggest the work proceed along these two lines:

(1) Improvement of technic for measuring TEL in tissues

There are various reasons for pursuing this problem:

(a) Need of a standardized and reliable technic for study of both the two main objectives, - distribution of TEL & reactions of TEL

(b) Insufficiencies and uncertainties in present technics, - contamination of tissue extracts with inorganic lead, lack of adequate ancillary evidence for estimation of accuracy

(c) Advantages of getting rid of TEL from tissue preparations before attempting to extract other organolead compounds

(2) Extraction, isolation, identification and measurement of organolead derivatives of TEL from tissues

Extraction of TEL from tissues should preferably be accomplished by a technic that leaves its lead-conbaining derivatives unchanged. One could then attempt to draw them into solution in one or another solvent. Perhaps addition of reagents to the tissue may be helpful in altering sclubilities and partitibns between solvents. Or, one may resort to addition of known organolead compounds to tissues to obtain suggestions of how the unknown derivatives of TEL may behave under such conditions.

Proposal for continuation (continued):

Specifically in immediate continuation of this work, I propose to examine series of pentane extracts of liver, blood and, possibly, urine of rats after they have inhaled TEL. I propose to fractionate the lead compounds in those extracts and to measure the quantities of lead present in those fractions, with further characterization of the nature of the lead present (ionic, inorganic etc.) where practicable.

If it appears that the fractionation technic used at first is impracticable, I will try others. Perhaps the problem will yield more readily to different treatment of the tissue than the simple homogenization with water that I am currently using. Other treatments will be tried. It may be there is no simple satisfactory solution to this problem. Then I will of necessity proceed to the extraction of organolead derivatives of TEL burdened with a complex preliminary treatment of tissues for removal of TEL

I anticipate that work along these two lines will take more than a year.

As a budget I suggest

Stevens	$8500	7,800.00
Feldhake (part time). . . .	2000 ✓	2000.00
College graduate (full time)	3200	
	$ 13,700	9,800.00
Supplies	$6000	4540.00
Equipment (including Toledo scale & Evelyn colorimeter)	$1000	1000.00
		Contingency 1,000
	$ 20,700	16,340.00
	2330	

Regarded as maximum even though open to question pk

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