6. The entire group of animals used in this experiment showed no lead line, loss of appetite, or other symptoms usually associated with lead poisoning.

7. Of the animals exposed, only guinea pigs and rabbits showed storage in the 3-hour group. Some of the animals in all species showed storage in the 6-hour group, which was more or less proportionate to the total time of exposure.

EFFECTS OF INHALATION OF VAPORS FROM ETHYL GASOLINE AND VARIOUS OTHER MOTOR FUMES

PROGRESS REPORT NO. 2, PART II.

1. The following species of animals, monkeys, dogs, rabbits, and guinea pigs, were exposed to vapors of straight gasoline and ethyl gasoline in concentration of 0.3 per cent for two hours.

2. The guinea pigs were exposed to straight gasoline, commercial ethyl gasoline, gasoline containing two and one-half, three and three-fourths, and five times the commercial amount of ethyl fluid, benzol, and benzol gasoline blends in the following concentrations:

(*a*) 1.0 per cent vapor in air for one hour.
(*b*) 0.3 per cent vapor in air for two hours.
(*c*) 0.1 per cent vapor in air for three hours.

3. The experiments were conducted in such a manner that the vapor composition in all cases was the same as that of the material evaporated.

4. A large proportion of the tetraethyl lead of gasoline containing ethyl fluid was found to remain in the residue when the liquid was evaporated in open vessels and by bubbling air through it. The first 20 to 50 per cent of the liquid evaporated at room temperatures contained relatively small amounts of lead.

5. In the first stages of the evaporation of commercial ethyl gasoline, by bubbling air through it, there is imparted to the air a very low, roughly constant concentration of tetraethyl lead.

6. Guinea pigs subjected to 114 to 125 exposures of straight gasoline showed no symptoms in low concentrations. In high concentration (1 per cent for one hour) marked intoxication occurs temporarily.

7. On similar exposure to commercial ethyl gasoline guinea pigs show no effects in the lowest concentration. Animals analyzed show lead accumulation after a number of exposures to the higher concentration (0.3 per cent for two hours). In the highest concentration the 1 per cent for one hour animals became inactive, and loss of weight was noted.

8. The animals exposed to vapors of gasoline containing two and one-half, three and three-fourths, and five times the commercial amounts of ethyl fluid all showed symptoms of intoxication, and some

developed tremors and other symptoms usually associated with lead poisoning. An accumulation of lead was found in all animals analyzed.

9. The guinea pigs exposed to motor benzol and a benzol gasoline blend showed signs of marked intoxication and irritation of the eyes and respiratory passages, and a great proportion of those exposed to high concentrations died.

10. No dogs or monkeys exposed to 0.3 per cent gasoline and 0.3 per cent commercial ethyl gasoline have shown any ill effects.

11. The guinea pigs and rabbits which died after a number of exposures to commercial ethyl gasoline showed an accumulation of lead.

TOXIC EFFECTS OF ETHYL GASOLINE WHEN APPLIED TO THE SKIN OF ANIMALS

PROGRESS REPORT NO. 2, PART III.

1. Tetraethyl lead, ethylene dibromide, gasoline containing ten times the amount of ethyl fluid used in commercial ethyl gasoline, commercial ethyl gasoline, and gasoline were applied to the skin of monkeys, dogs, rabbits, guinea pigs, and rats in amounts from one-tenth cubic centimeter to 1 cubic centimeter.

2. The summation of skin absorption, inhalation, and possible ingestion of lead is shown by a group of test animals treated with tetraethyl lead and with gasoline containing tetraethyl lead and housed together. All animals in this group showed lead accumulation on analysis of those that died or were killed.

3. A group of animals treated so as to prevent inhalation with gasoline containing ethyl fluid in commercial concentration and ten times the commercial amounts showed lead accumulation.

4. A group of animals treated so as to prevent inhalation and ingestion (by washing and blanketing) with gasoline containing ten times the commercial concentration of ethyl fluid showed lead accumulation.

5. A group of animals are being treated as given in 4, but with commercial ethyl gasoline. This work has been in progress only a comparatively short time, and no symptoms have been noted to date.

6. Animals treated with ethylene dibromide (about 95 per cent pure) showed marked intoxication and died within 24 hours after one application of 0.25 cubic centimeter.

7. Animals treated in the same manner as given in 6, but with tetraethyl lead, showed marked intoxication and died within 36 hours.

All the studies described in progress report No. 2 are being continued, and a final report will be prepared when the work is completed.

The Public Health Service, as stated by the Surgeon General, did not feel it had the funds to carry on the investigation, and the Bureau of Mines undertook a certain portion of the investigation.

More than two years ago we first took up the study on the exhaust-gases phase of the subject described in progress report No. 1. As stated, the group of animals that we exposed was composed of monkeys, chicks, guinea pigs, rabbits, and pigeons. They were exposed to exhaust gases diluted to a concentration so that they would not be affected acutely, at least, by the carbon monoxide present. It was necessary to have careful carbureter adjustment and control or we would get acute carbon monoxide poisoning, as Doctor Henderson has suggested. The carbureter was adjusted so that the exhaust gas contained instead of an average of 7 per cent carbon monoxide approximately 2 per cent. The animals actually breathed air containing 0.01 per cent or 0.02 per cent of carbon monoxide, because the average in the exhaust gas was less than one-half that usually found. This allowed the lead content of the atmosphere to be that normally found when the air contained 0.04 per cent of carbon monoxide.

No storage of lead was found in any of the animals analyzed—that is, within the experimental error of the chemical methods used—and we believe them to be as good methods as can be obtained.

The findings on exposed animals were checked by controls. Lead was injected in some unexposed animals and these killed and analyzed to obtain the lead. A report was published in December, 1924. You are acquainted with the results of this report. This report was published due to a demand for information and due to an apparent feeling among groups of people that we were holding back information at that time. As a matter of fact, we had nothing that indicated that there was lead poisoning or a possibility of lead poisoning. However, we thought best to give the information to the public and to issue progress reports as phases of the studies made it possible. Many of the animals from this first study were continued on the following, which was made to try to determine the amount of tetraethyl lead required in gasoline to cause lead poisoning or lead storage in animals exposed to engine exhaust.

In the studies described in progress report No. 2, Part I, five times the usual amount of tetraethyl lead was put in the gasoline. The same species of animals were exposed for similar periods for 206 times as in the first study.

In progress report No. 2, Part II, a study was made to try to determine the possible hazard due to the inhalation of tetraethyl

lead and gasoline containing various amounts of tetraethyl lead. In addition to unexposed controls, other controls were exposed to various motor fuels, as straight gasoline and benzol gasoline.

Attention is called to the fact that the experiments were conducted in such a manner that the vapor composition was the same at all times as the liquid evaporated; that is, all the liquid was evaporated and mixed with the air to be breathed in every case. This was done because it was found that after evaporating 20 per cent or even 50 per cent of the gasoline from the ethyl gasoline the residue contained the greater portion of the tetraethyl lead or ethyl fluid. It was found that if air is bubbled through ethyl gasoline any part of the air so treated will contain about the same amount of tetraethyl lead as any other equal portion.

The CHAIRMAN. I think Colonel Vedder has done some work along this line.

LIEUT. COL. E. B. VEDDER, M. C.

Chief, Medical Research Division

About a year ago I was requested by the Chief of the Chemical Warfare Service to make some tests of tetraethyl lead, but the whole work that we did was done with the pure compound and has no direct bearing on this present discussion, except for the fact that it does show that tetraethyl lead is absorbed by inhalation, as well as through the skin, and that it is cumulative in its action; but it bears no relation to the toxicity of its dilution in gasoline. We worked with the pure product. The report is available and will be deposited with you so you can have it for reference and get all of that data from it.

A STUDY OF THE TOXICITY OF LEAD TETRAETHYL, OCTOBER 5, 1924

By W. A. ELDRIDGE

[Supervised by D. C. Walton, lieutenant commander M. C., U. S. N.; approved by Edward B. Vedder, lieutenant colonel, M. C., U. S. A.]

I. INTRODUCTION

The object of the following tests was to determine the toxicity of lead tetraethyl upon animals and to study the symptoms produced by this compound. Four different methods were used to administer the compound, and lethal points were established by each method. The methods employed were as follows: Local application, subcutaneous injection, intravenous injection, and inhalation.

II. SUMMARY

1. The lethal point by skin application was placed at 0.6 cubic centimeter, or 0.996 gram per kilo, for guinea pigs and at 0.3 cubic centimeter, or 0.496 gram per kilo, for dogs. One cubic centimeter of pure lead tetraethyl weighs 1.659 grams.

2. The lethal point for guinea pigs by subcutaneous injection is approximately 0.08 cubic centimeter, or 0.132 gram per kilo.

3. The lethal point for dogs by intravenous injection is placed at 0.011 cubic centimeter, or 0.018 gram per kilo.

4. The lethal concentration for mice by inhalation is placed at 5.11 milligrams per liter.

5. The evidence indicates that smaller doses are cumulative in action by whatever route administered.

6. When an animal receives a lethal dose by skin application its life may be saved by washing with kerosene, followed by tincture green soap and water, provided this treatment is carried out within 30 minutes after application.

III. HISTORICAL

Although chronic lead poisoning is the commonest of all forms of metallic poisoning, true acute lead poisoning that is the effect of a sudden absorption of lead in man has been hitherto unknown. When a large quantity of some soluble salt of lead is swallowed it gives rise to the ordinary symptoms of irritant poisoning, namely, nausea, vomiting, pain in the abdomen, violent purging, weakness, and collapse.

In animals also it has been difficult hitherto to produce acute lead poisoning, because when most forms of lead are injected the proteins of the blood are precipitated, and death so produced is purely mechanical; and only local symptoms were produced by administration by mouth. However, Harnack injected salts of lead triethyl. In the frog this produced general paralysis, apparently by the action of lead on the central nervous system. In the dog the symptoms following intravenous injection were weakness and paralysis, violent diarrhea and colic, chorealike movements, tremors, and convulsions. The diarrhea was found to be due to violent contractions of the intestinal walls, which maintained a degree of contraction even when no peristaltic wave was passing. (Cushny, Pharmacology and Therapeutics, p. 653.)

Lead tetraethyl was developed by the General Motors and Du Pont corporations as an addition to motor fuels. But soon after large-scale production was commenced a number of men handling this product became ill, and several of them died. The following description of the symptoms was included in a report to the Standard Oil Co. by Doctors Thompson and Schoenleber, rendered May 18, 1924:

"The first symptoms observed are a marked fall in blood pressure, sometimes as low as 60 points below normal. There is an accompanying fall in body temperature, which has been recorded as low as 94.6° Fahrenheit, or 4° below normal. The heart action is slowed, the pulse having dropped in one case as low as 48; that is, 12 or more counts below normal. There is at first some digestive disturbance, such as loss of appetite, vomiting, and a tendency to looseness of the bowels. Vertigo may be present. The red blood corpuscles show marked changes in size and shape. The blood in one of the fatal cases failed entirely to coagulate and at autopsy showed an unusual color such as is observed in carbon-monoxide poisoning. There is no cyanosis and no shortness of breath, nor is headache or other pain complained of. The urine remains normal usually, but in one case reported, treated by Doctor Aub, of Harvard, lead was present in it. Following these symptoms in severe cases other phenomena appear indicative of profound cerebral disturbance: The victim suffers from persistent insomnia and becomes delusional, extraordinarily restless, and talkative. His gait is staggering like that of a drunken man, but there is no paralysis, and convulsions do not appear. There is exaggerated movement of

all the muscles of the body, accompanied by perspiration, and the patient finally becomes violently maniacal, shouting loudly, leaping out of bed, attempting to smash furniture or windows, and behaving like a violent case of delirium tremens. Morphine only accentuates the symptoms. The victim may finally die in exhaustion. One man smashed a window and cut his hand badly. He had delusions of vision, seeing imaginary groups of persons, and accusing the doctor of trying to cut him up. Another man saw the wall paper converted into swarms of moving flies and thought pictures of his family on the walls were alive and moving about.

"Of the two fatal cases the body temperature in one patient rose to 110° just before death. Both these men had been at work only five weeks. The elder, 53 years of age, was not very vigorous, originally having a chronic fibroid condition of the lungs. The younger man was of fine physique. Doctor McCann, who has had a very extensive general practice, stated that he never in his life had seen anyone die in such agony as these two men—'they died yelling.' One man had to be strapped in bed by an expert accustomed to restraining the insane.

"As in nearly all poisonings of industrial origin, considerable personal idiosyncracy may exist as to bodily resistance. Many of the workmen showing only slight symptoms have continued at work. In other cases quite marked symptoms have developed within from three to six hours. In one case the blood pressure dropped in only three and a half hours after beginning work—from 190 to 112—a fall commensurate with that which occurs in excessive hemorrhage. Convalescence from the severe symptoms is quite protracted and may occupy from 6 to 10 weeks."

The following table, which was furnished us by the chief surgeon of the du Pont Co., records the symptoms observed in 28 such cases:

Symptoms recorded in 28 cases of tetraethyl-lead poisoning

[The figures refer to the number of cases in which each symptom was observed]

Insomnia	28
Anorexia	18
Nausea	18
Morning vomiting	10
Abdominal cramps	12
Unaccustomed and annoying dreams	11
Bodily weakness	16
Decided loss of weight	9
Markedly diminished blood pressure	20
Slowing of pulse rate	7
Photophobia	1
Metallic taste in the mouth	6
Lead line on the gums	4
Subnormal temperature	19
Hyperacidity of the urine	18
Albumin in the urine	2
Acetone in the urine	1
Skin test for lead	12
Tremors	6
Scotoma	3
Decreased muscular reactions	7
Dilated pupils	1

Itching of the skin	4
Marked drowsiness in the daytime	1
Sluggish pupils	2
Headache	7
Vertigo	10
Irritation of mucous membranes of the nose and throat	1

The physicians employed by these companies were of the opinion that the toxicity of lead tetraethyl was caused partly by the compound as a whole and could not be ascribed entirely to lead poisoning, especially in view of such symptoms as insomnia and mental excitement. But in view of the fact that little has been known heretofore concerning acute lead poisoning, and since all the symptoms described have been previously described as occurring in cases of chronic lead poisoning, we are inclined to the opinion that the toxic effects of lead tetraethyl are simply a manifestation of acute lead poisoning. Thus Osler (Principles and Practice of Medicine, ninth edition, p. 394) says:

"*Symptoms—Acute form.*—We do not refer here to the accidental or suicidal cases, which present vomiting, pain in the abdomen, and collapse symptoms. In workers in lead there are several manifestations which follow a short time after exposure and set in acutely. There may be, in the first place, a rapidly developing anæmia. Acute neuritis has been described, and convulsions, epilepsy, and a delirium, which may be not unlike that produced by alcohol. There are cases in which the gastrointestinal symptoms are intense and rapidly prove fatal. These acute forms occur more frequently in persons recently exposed and more often in winter than in summer. Da Costa reported the onset of hemiplegia after three days' exposure to lead.

"Certain of the cases with colic may present the features of an acute intra-abdominal inflammatory condition. A case may be admitted to the surgical wards with a diagnosis of appendicitis or simulate intestinal obstruction. Localized pain, slight fever, and moderate leucocytosis may be present. The history, the presence of a blue line on the gums, and the blood changes are of importance in differential diagnosis.

"The cerebral symptoms are numerous. Seven of our cases showed marked cerebral involvement. One had delusions and maniacal excitement and had to be removed to an asylum. In other cases there occurred transient delirium, attacks of unconsciousness, and in one case convulsions. Optic neuritis or neuretinitis may occur. Hysterical symptoms occasionally occur in girls. Convulsions are not uncommon, and in an adult the possibility of lead poisoning should always be considered. True epilepsy may follow the convulsions. An acute delirium may occur, with hallucinations. The patients may have trance-like attacks, which follow or alternate with convulsions. A few cases of lead encephalopathy finally drift into lunatic asylums. Tremor is one of the commonest manifestations of lead poisoning."

IV. EXPERIMENTAL

(A) *Material.*—The sample of lead tetraethyl, $Pb(C_2H_5)_4$, used was furnished by the du Pont Co. It was a transparent, pale-amber colored, volatile liquid, purity 95 per cent. The impurities were olefines. It decomposes at 120° C. and is soluble in alcohol, ether, ethyl bromide, and gasoline. It is stable toward water but decomposes in direct sunlight, and was stored in a dark bottle in a closet.

(B) *Methods and results—Local application—Guinea pig.*—This test, whereby a definite amount of the compound is applied directly to the skin of the animal, depends upon absorption by the skin for its toxic effects. The

guinea pig's chest and abdomen were shaved and cleansed 24 hours before applying the compound. The pig was strapped to an operating board and the compound was gradually applied to the skin from a pipette. The animal was allowed to remain on the board until all trace of the compound was absorbed. The time required for absorption was 20 minutes. The animal was then removed and placed in the hospital, where symptoms were noted. The results follow:

Amount applied	Weight of pig	Result	Amount applied	Weight of pig	Result
Cubic centimeter	*Grams*		*Cubic centimeter*	*Grams*	
1	578	Died in 12 hours.	0.3	502	Died in 60 hours.
1	600	Died in 82 hours.	.3	400	Died in 24 hours.
1	625	Died in 26 hours.	.3	420	Died in 18 hours.
0.75	585	Died in 60 hours.	.2	500	Lived.
.5	520	Lived.	.2	405	Do.
.5	490	Died in 36 hours.			

The average weight of three pigs receiving 0.3 cubic centimeter was 440 grams. The lethal point by local application for guinea pigs is therefore placed at 0.6 cubic centimeter per kilo.

Symptoms: The first few hours after the application was made the pigs appeared normal. With the superlethal application (1 cubic centimeter) two hours after exposure, marked depression was noted. This was quickly followed by body tremors. In four hours' time the animal was helpless, the belly and extremities trembling violently. Death followed in six hours. At the lethal point (0.3 cubic centimeter) the symptoms were the same as described, except the onset of the symptoms and death were delayed a little longer. These guinea pigs exhibited signs of pain and tenderness on pressure over the abdomen. After death the area of application appeared normal. There were no signs of excitement noted.

Local application—Dogs: Local applications were made upon six dogs, the lead tetraethyl being applied to the previously shaved thorax and abdomen. The results follow:

Amount applied per kilo body weight:	Results
0.6 cubic centimeter	Died 24 hours.
0.5 cubic centimeter	Died 30 hours.
0.5 cubic centimeter	Died 73 hours.
0.3 cubic centimeter	Died 180 hours.
0.25 cubic centimeter	Recovered.
0.2 cubic centimeter	Recovered.

The lethal point by local application for dogs was placed at 0.3 cubic centimeter per kilo body weight.

Symptoms: The symptoms following skin application to dogs were similar to those described for guinea pigs. When the animal received a lethal dose it required about half an hour for complete absorption. The dog was then released from the board and placed in a large cage, where he could be observed. The dogs gave prompt evidence of distress and abdominal pain, getting about the cage, sitting down and at once getting up, as though it was impossible to reach a comfortable position. In several instances this period of uneasiness was followed by a profuse bowel discharge, the feces being followed by watery fluid. After this the dog appeared easier and would lie down. Later the animals were depressed and comatose, but the belly was hard and

rigid and pressure caused intense pain. On the second day the condition was much the same, except that the animal could only get on its legs with difficulty. The entire belly wall was tense and rigid and the abdomen was very tender to pressure. Later trembling of the limbs occurred, followed by convulsions, collapse, and death. In the case of sublethal doses the symptoms were all milder and gradually disappeared as the animal returned to normal.

Pathology: Autopsies were performed on four dogs dying after having skin applications of lead tetraethyl.

The external appearance and body cavities were normal, except in one case. In this case necropsy was performed immediately after death, which occurred 73 hours after the application. Gas escaped as soon as the abdominal wall was opened, and the small and large intestines were acutely inflamed, probably as the result of perforation of one of the numerous ulcers. In all four cases the heart, lungs, spleen, and liver showed no change, but there was a mild congestion in all zones of the kidney. One case had a normal intestinal tract. In three cases there were ulcers throughout the intestines. These were in the region of Payers' patches, which were swollen and prominent. The ulcers were 1 or 1.5 centimeters in diameter, punched out in appearance with clean-cut edges and a smooth base with very little necrotic tissue in connection. The ulcer extended to the muscle layer undermining the mucosa slightly at that point. There was a large amount of blood in the lumen of the intestines in the three cases. One case had a small ulcer near the pyloric end of the stomach, resembling the intestinal ulcers but much smaller in diameter. In the other cases the mucosa of the stomach was normal.

From both the symptoms observed when living and the pathological findings it is evident that when lead tetraethyl is applied locally near or over the abdomen the lead acts directly on the intestines, producing acute lead colic followed by ulceration, which may lead to peritonitis. This confirms the observations of Osler concerning cases of colic in ordinary lead poisoning which present the features of an acute intra-abdominal inflammatory condition.

Local application—Cumulative effects.—A test was made upon a dog weighing 4.08 kilos, applying 0.1 cubic centimeter every 24 hours to a shaved area upon the chest, to ascertain the cumulative effects of the compound. The first ten 0.1 cubic centimeter applications did not affect the dog appreciably. After the twelfth application the dog began to show depression and occasionally some nervousness. After the eighteenth application the dog was markedly depressed, also very unsteady upon its feet, and trembling of the body showed marked increase. The trembling gradually increased until the twenty-first application had been made, the dog dying 40 minutes after. No excitement was noted.

Subcutaneous injection—Guinea pigs.—Two tests were made by subcutaneous injection. The pure compound was drawn into a hypodermic syringe and injected under the skin of the animals. The results follow:

Amount injected	Weight of animal	Results
	Grams	
0.25 cubic centimeter	510	Died in 164 hours.
0.1 cubic centimeter	580	Died in 96 hours.

Symptoms: The symptoms were similar to those noted under local application. The area of injection after death appeared normal.

Subcutaneus injection—Cumulative effects—Guinea pigs.—0.1 cubic centimeter in pure olive oil injected every 24 hours. Three guinea pigs were

selected for this test. Care was used to select pigs of the same weight. The results follow:

Amount injected	Average weight of pig	Results	Amount injected	Average weight of pig	Results
cc.	*Grams*			*Grams*	
0.01	640	0/3	0.01	640	1/3
.01	640	0/3	.01	640	2/3
.01	640	0/3	.01	640	2/3
.01	640	0/3	.01	640	3/3

Symptoms: The first three injections did not appreciably affect the animals. Six hours after the fourth injection one pig showed marked body tremors, while the other two pigs were markedly depressed. One pig died 16 hours after the fourth injection. Six hours after the fifth injection the second pig was seized with body tremors and died after 20 hours. One pig survived for the eighth injection, dying 22 hours after. There was no marked excitement noted.

Intravenous injection—Dogs.—Five dogs were used in this test. Pure olive oil was used as diluent for the compound. The results follow:

Amount injected per kilo body weight:

Cubic centimeters	Results
0.078	Died 14 hours.
.039	Died 14 hours.
.011	Died 25 hours.
.0083	Died 336 hours (delayed).
.0055	Lived.

The lethal point for intravenous injection for dogs is placed at 0.011 cubic centimeter per kilo body weight.

Symptoms: Dogs injected intravenously showed almost immediate and marked symptoms from the compound. Five minutes after being injected there were marked twitching of the face and eyes, lachrymation of the eyes and nose, and rapid breathing, followed by marked excitement lasting for one hour to two hours. During the first hour after the injection animals vomited blood-stained mucus and diarrhea was also produced. The ejection from the bowels, however, did not appear blood stained. The excitement may have been caused partly by colic. The stage of excitement was followed by collapse, the dog becoming helpless, with marked trembling of the body and extremities until death.

Inhalation—Mice.—A number of tests were run on mice by the continuous-flow method to determine the toxicity of the compound by inhalation. Ten-minute exposures to a gas-air mixture of the compound were made, five mice being used for each concentration. The average weight of the mice was 20 grams. The construction of the mouse chamber will be found in report U. S. A. M. R. D. No. 11. The results follow:

Concentration	Acute death	Delayed death	Concentration	Acute death	Delayed death
5.598	4/5	0/5	3.617	0/5	0/5
5.11	5/5	0/5	3.425	0/5	0/5
4.958	0/5	0/5	2.812	0/5	0/5
4.166	0/5	0/5	1.615	0/5	0/5
3.679	0/5	0/5	.71	0/5	2/5

The lethal concentration for mice by inhalation is placed at 5.11 milligrams per liter.

Symptoms: The mice showed activity while being exposed to the gas-air mixture of the compound. Upon removal from the gas chamber the mice were markedly excited. In the case of lethal doses the excitement gave way to marked depression followed by collapse, trembling, and death.

Inhalation—Cumulative effects—Mice.—Mice showed the cumulative effects of the compound. Ten mice were subjected to concentrations of 0.5 milligram per liter for 10 minutes once every 24 hours, the sample being changed for each run. After the fourteenth run there was one death resulting from the compound. The fifteenth run produced two deaths, the nineteenth run one death, and the twentieth run one death. The total number of experiments made was 32, resulting in five deaths from 10 mice used. The results follow:

Date	Number of mice	Concentration	Results	Date	Number of mice	Concentration	Results
May 27, 1924	10	0.49	0/10	June 18, 1924	7	.604	0/7
May 28, 1924	10	.49	0/10	June 19, 1924	7	.564	1/7
May 29, 1924	10	.438	0/10	June 20, 1924	6	.648	1/6
June 2, 1924	10	.392	0/10	June 21, 1924	5	.534	0/5
June 3, 1924	10	.497	0/10	June 23, 1924	5	.543	0/5
June 4, 1924	10	.473	0/10	June 24, 1924	5	.479	0/5
June 5, 1924	10	.458	0/10	June 25, 1924	5	.419	0/5
June 7, 1924	10	.462	0/10	June 28, 1924	5	.5	0/5
June 9, 1924	10	.481	0/10	June 30, 1924	5	.486	0/5
June 10, 1924	10	.505	0/10	July 1, 1924	5	.437	0/5
June 11, 1924	10	.428	0/10	July 2, 1924	5	.494	0/5
June 12, 1924	10	.5	0/10	July 3, 1924	5	.563	0/5
June 13, 1924	10	.552	1/10	July 7, 1924	5	.563	0/5
June 14, 1924	9	.47	2/9	July 8, 1924	5	.521	0/5
June 16, 1924	7	.626	0/7	July 9, 1924	5	.598	0/5
June 17, 1924	7	.648	0/7	July 10, 1924	5	.584	0/5

Total number of runs made were 32. The average concentration was 0.5 milligram per liter. The total deaths were 5 out of 10 mice used.

Symptoms: The symptoms produced are the same as those recorded above for mice subjected to continuous inhalation.

Metabolism: If we assume, as is indicated by the symptoms, that the toxicity of lead tetraethyl is due to the lead atom rather than the entire molecule, a study of the metabolism is of especial interest as showing a new form of acute lead poisoning. For we are dealing with a highly liquid soluble lead compound which is capable of skin penetration and which, furthermore, is comparatively stable. It is probably the only compound where acute lead poisoning follows absorption through the skin.

In chronic lead poisoning the skeleton of the body has been shown to be the storehouse for the comparatively large amount of lead accumulated. In acute lead poisoning distribution is throughout the body, with the urine and feces as the sources of elimination. Elimination through the feces takes place even when absorption is through the respiratory tract, probably by excretion from the liver along with the bile.

A number of analyses were made on organs from both dogs and guinea pigs, of which the best example is the systematic study of dog 1682 as given below:

Dog 1682: Weight 17.7 kilograms. Given a skin application of 0.25 cubic centimeter lead tetraethyl per kilo body weight, equivalent to 4.42 cubic centimeters or 7.07 grams, on August 12, 1924.

Days after application	Urine volume	Lead content	Feces weight	Lead content	Days after application	Urine volume	Lead content	Feces weight	Lead content
	Cubic centimeters	*Milligrams*	*Grams*	*Milligrams*		*Cubic centimeters*	*Milligrams*	*Grams*	*Milligrams*
	600	2.04			5	135	.6		
1	330	1.32	35	15.5	6	165	.5		
2	340	1.09	5	3.0	7	None.			
3	150	.8			8	280	.6		
4									

The dog was killed and autopsied on the eighth day.

	Milligrams lead per 50-gram sample	Weight of organ in grams (approximate)	Total lead in milligrams (approximate)
Brain	1.8	75	2.7
Bone	5.0	2,000	200
Intestines, small	4.8	1,000	96
Intestines, large	5.3	20	2.1
Kidney	5.8	50	5.8
Liver	7.6	500	76
Lung	4.2	200	16.8
Skin, area of application	25.2	1,000	504
Spleen	3.4	50	3.4
Total			906

It is seen that approximately one-fifth of all the lead applied was found in organs and tissue representing one-quarter the total weight of the dog. The amount excreted was very small, amounting to only about 1 per cent of the total applied.

In a number of other animals that had received small doses some time before being killed, in the analysis of their organs, no trace of lead was found. This would indicate that the lead was removed from the body tissues and deposited probably in the skeleton.

Since the amount of lead excreted in the feces and urine is very small, it is easy to understand how small amounts of lead tetraethyl may have a cumulative action. The differences in individual susceptibility to this cumulative action are probably to be explained, at least partially, by the different rates of excretion of lead by different individuals. It is evident from the very small lethal dose by intravenous injection (0.011 cubic centimeter) that very small amounts of lead in the circulating blood are productive of a serious disturbance of the nervous system, and also have a direct effect upon the intestines producing lead colic or even ulceration. These results of the acute action of lead are precisely similar to the symptoms of chronic intoxication, except that they are much more violent.

Prophylaxis—Counteracting the effects produced by skin application.—Chemical agents were used to counteract the effects produced by applying lead tetraethyl to the skin. In these tests a lethal dose was applied and the chemical agents applied immediately afterwards. Hydrochloric acid in strengths of 10, 25, and 50 per cent, and C. P. were used and found to be valueless. Calcium hypochlorite was used, both in the form of a dry powder and paste, and was found to have no value.

The value of solvents in removing lead tetraethyl.—A number of solvents were used to remove the lead tetraethyl before a sufficient quantity to produce

death was absorbed by the skin. In the following experiments the animals used were guinea pigs. The amount of lead tetraethyl applied was a super-lethal dose (1 cubic centimeter). The results follow:

EXPERIMENT No. 1—*Olive oil.*—Two pigs were cleansed with olive oil one minute after application of lead tetraethyl. Both pigs died.

EXPERIMENT No. 2—*Ordinary soap and water.*—Two pigs were cleansed with ordinary soap and water one minute after the application of lead tetraethyl. Both pigs died.

EXPERIMENT No. 3—*Tincture green soap.*—Two pigs were cleansed with tincture green soap and water one minute after application of lead tetraethyl. Both pigs lived.

EXPERIMENT No. 4—*Tincture green soap.*—Two pigs were cleansed with tincture green soap and water two minutes after application of lead tetraethyl. Both pigs lived.

EXPERIMENT No. 5—*Tincture green soap.*—Two pigs were cleansed with tincture green soap and water five minutes after application of lead tetraethyl. Both pigs died.

EXPERIMENT No. 6—*Kerosene.*—Two pigs were cleansed with kerosene one minute after application of lead tetraethyl. Both pigs lived.

EXPERIMENT No. 7—*Kerosene.*—Two pigs were cleansed with kerosene five minutes after application of lead tetraethyl. Both pigs lived.

EXPERIMENT No. 8—*Kerosene followed by tincture green soap and water.*—Two pigs were cleansed with kerosene, which was followed by tincture green soap and water 10 minutes after application of lead tetraethyl. Both pigs lived.

EXPERIMENT No. 9—*Kerosene followed by tincture green soap and water.*—Two pigs were cleansed with kerosene, followed by tincture green soap and water, 15 minutes after lead tetraethyl application. Both pigs lived.

EXPERIMENT No. 10—*Kerosene followed by tincture green soap and water.*—Two pigs were cleansed with kerosene followed by tincture green soap and water 20 minutes after application of lead tetraethyl. Both pigs lived.

EXPERIMENT No. 11—*Kerosene followed by tincture green soap and water*—Two pigs were cleansed with kerosene followed by tincture green soap and water 25 minutes after application of lead tetraethyl. Both pigs lived.

EXPERIMENT No. 12—*Kerosene followed by tincture green soap and water.*—Two pigs were cleansed with kerosene followed by tincture green soap and water 30 minutes after application of lead tetraethyl. One pig lived. One pig died.

EXPERIMENT No. 13—*Kerosene followed by tincture green soap and water.*—Four pigs were cleansed with kerosene followed by tincture green soap and water. Two pigs were cleansed 35 minutes after exposure. Two pigs were cleansed 40 minutes after exposure. All four pigs died.

Conclusion: Both olive oil and common soap and water proved valueless as agents to remove the lead tetraethyl from the skin. Tincture green soap was effective if used within two minutes after the application of the lead compound. Kerosene proved to be the best agent tried; all pigs lived that were kerosene treated up to 30 minutes. It was observed that the skin of the pigs that were treated with kerosene alone became tense, interfering with the movements of the animals. The skin would also crack and tear, causing great discomfort. It was found that following the application of the kerosene by tincture green soap and water removed the kerosene, leaving the skin soft and pliable.

It is quite evident from these observations that the manufacture, handling, and distribution of lead tetraethyl involves considerable danger to operatives of acute lead poisoning in those instances where considerable material is splashed upon the skin and of chronic lead poisoning from the absorption of small amounts by inhalation, by the skin or by mouth. Tests by the Bureau of Mines appear to show that lead tetraethyl in exhaust gases of automobiles is not toxic to animals, having been changed to insoluble forms of lead.

It is not believed that this compound is of any great value as a warfare agent, because of its low toxicity as a vapor and the fact that it decomposes at 120° C. Although splashes of this liquid on the skin will produce death, the toxicity for man is similar to toxicity for dogs, it would require 21 cubic centimeters to cause the death of a man. Thus it could hardly be used as a spray for lethal effect, although possibly a certain number of casualties could be also produced.

MEMBER. May I ask Doctor Sayers just one question? That is, How long he feels he will require to go on before he can contemplate final publication of his material?

Doctor SAYERS. Do you want me to answer that now or go on with it later?

The CHAIRMAN. Whichever you prefer. I thought we would have a general discussion a little later on.

Doctor SAYERS. It might come in better a little later.

The CHAIRMAN. Would that be agreeable to you, Doctor?

MEMBER. I do not care to go into the specific point that Doctor Sayers brought up, but it seemed to me that in going into the details of this matter we should have some notion as to how long the reasonable experimentation that is now projected will continue. It would be really fundamental in considering the technique of the situation. I would be glad if Doctor Sayers can enlighten us.

Doctor SAYERS. Of course, it is very difficult to give a definite estimate or anything like a definite estimate. However, I would say it ought not to take too long, because, as I have stated in this paper here, we fully realize that animal experimentation can not be readily translated to man. We talked it over with Doctor McCoy over three years ago, and that is the opinion we came to at that time. I do not know just how much longer it will take.

MEMBER. Do you think by the first of the year?

Doctor SAYERS. I will say yes.

Doctor CLARK. I should like to correct a misapprehension. Doctor Sayers spoke of the statements by my colleagues and myself in regard to the ventilation of New York tunnel, 4 parts in 10,000 carbon monoxide. That was on the standard we worked out as something which would be entirely safe for people using the tunnel, which can not be used, as it has to some extent been used, as the

standard representing actual conditions. The concentrations of gas, therefore the concentration of other substances in exhaust gases in garages and in repair shops, run three to four times as high as that standard, so that it does not afford the background which it has seemed to give.

The CHAIRMAN. I was going to ask Prof. Frederick B. Flinn, professor of physiology at Columbia University, if he would be good enough to tell us about their work.

DR. FREDERICK B. FLINN

Professor of physiology, Columbia University, New York City

When we first started our study of the possible public-health hazard from the commercial use of tetraethyl lead at Columbia University the medical committee of the allied companies suggested that inasmuch as the Bureau of Mines had been investigating the exhaust gases from engines using ethyl gasoline for some months, we confine our attention to the other hazards. Therefore we concentrated our attention on the possible health hazards that might arise from contact with the concentrated tetraethyl lead, to the possible hazard of skin absorption through contact with the dilute ethyl gasoline, the 1,000 to 1 mixture, and to the possible hazard from breathing in the fumes of the gasoline evaporating around garages and filling stations. We were unable at the beginning of our work to collect any data as to the concentration of the gasoline fumes around garages or filling stations, although we made inquiries at the laboratories of the New York State Section of Industrial Hygiene and of the Bureau of Mines. We were therefore compelled to go ahead with that part of the study rather blindly. Before our work with the concentrated tetraethyl lead had really gotten under way the unfortunate poisoning of the men at the Bayway plant occurred. The company decided that they would not distribute the concentrated lead product to the filling stations in the future. On account of this decision, it was again suggested that we discontinue our study of this part of the problem.

We gave considerable time to a study of the men sent to the Reconstruction Hospital, with the idea in mind of gaining some knowledge of the symptoms we might expect to find in our animals. It is not necessary for me to go into that part of our study, as Dr. Gilman Thompson and others will touch on the symptoms found.

On account of the above-mentioned facts, we confined our experiments to the possible hazards that might arise from any contact with ethyl gasoline, whether by skin contact or through breathing the fumes of the evaporating gasoline. In beginning our experiments as to skin absorption we were fully aware of the habit of the garage

man of cleaning the grease off of his hands by washing them in gasoline. In order to determine how much gasoline would remain on his hands if he neglected to wipe them off on a towel or waste, a few experiments were carried on, and we came to the conclusion that it amounted to approximately 10 cubic centimeters. We therefore used this as a basis for determining the amount to be applied to our animals. Rabbits, guinea pigs, rats, pigeons, and goats were used in our experimental work. When we first started our work we were rather skeptical as to whether any skin absorption of the lead actually did take place and expressed this doubt to several persons. We felt that the lead got into the system through ingestion or inhalation of the fumes. It is very hard to keep a rabbit or guinea pig from licking its fur—something we should have taken into consideration at the beginning of our work—and we felt that possibly the gasoline evaporated and left the concentrated tetraethyl lead on the skin and that the animal in cleaning its fur ingested it. We tried to rule this error out by placing jackets on the animals, but these jackets got torn, and normal animals treated in the same way died as quickly as the dosed animals. One was led to the opinion that it interfered with the physiological activity of the animal—possibly the heat-regulating mechanism.

To determine for our own satisfaction whether absorption really did take place through the skin and not by ingestion or inhalation we anesthetized three cats at different times with ether. Tracheotomy was performed and a cannula placed in the carotid so as to observe the blood pressure and respiration. The tube from the trachea was led away from the body in such a way that there was no chance for the animal to inhale any of the fumes. To further safeguard against accidental breathing in of any tetraethyl lead fumes an exhaust fan was placed near the body to draw off any fumes from the head. As a further precaution an electric fan was placed near the head. Six cubic centimeters of concentrated tetraethyl lead were allowed to flow from a pipette onto the clipped abdomen. After the animal died the skin was quickly removed and the abdomen opened up. Blisters were observed in each case all through the abdominal region, and the intestines had a peculiar bluish hue. The average lead content of the three cats outside of the skin was found to be 0.65 gram of lead. This experiment would seem to indicate that there is no doubt that this lead compound is quickly absorbed through the skin.

Our rabbits received 0.30 cubic centimeter of ethyl gasoline daily on the skin over the various periods of time. During the passing weeks some of the guinea pigs and rabbits died, but it is not our opinion that they died of lead poisoning. In some of the animals which received

the application daily for 189 to 190 days definite signs of lead intoxication were present. One of our rabbits became absolutely paralyzed in the hind quarters. On analysis this animal was found to contain 4.9 milligrams of lead per kilo. Many of the animals showed stippled cells in the blood. All of the animals that died were examined for lead, which was found to be present in every case in varying quantities.

When we observed the tendency of the smaller animals to lick themselves we decided to use the goat in part of our work. The goat has many advantages, being a hardy animal and not so susceptible to respiratory diseases as the rabbit. The goats received 2 cubic centimeters of ethyl gasoline on the neck and other parts of the body daily, and up to date the number of exposures vary from 79 to 107. One of the goats that has received 107 applications shows a very marked muscular weakness in the hind quarters. We have another goat that is beginning to develop the same weakness. At first we were not sure of our observations on this second goat, but Dr. Gilman Thompson confirmed it during one of his recent visits. Four of our goats have aborted, and an analysis of the fetuses shows that they contained on the average 3.45 milligrams of lead. Stippled cells were not found in any of our goats for the first two months, but the number of these cells has increased with the continued exposure.

We examined the blood of the first 12 men sent to the Reconstruction Hospital and found stippled cells in 9 cases. I believe that later examination indicated that the proportion of men showing stippled cells was not so great, only 50 per cent of the 46 men examined showing this condition. The same ratio held true for our rabbits, 52 per cent of them having stippled cells. These rabbits at the beginning of the work did not show any stippled cells.

Each month the feces of our animals were collected and examined for lead. The animals were not exposed to ethyl gasoline during this period of collection. The rate of excretion for the rabbit is about 0.04 milligram of lead per 24 hours. Because of this small quantity of lead, it was found necessary to collect the feces for several days.

For the purpose of comparing the effects of the ethyl gasoline with those of the concentrated tetraethyl lead, as well as to study the distribution of the lead in the body, we dosed two goats daily with 1 cubic centimeter of tetraethyl lead. These animals have received 60 applications. As the number of applications increased the animals showed a tendency to stand in a hunched position and refuse to eat. By letting them rest a few days the animals apparently recovered and would begin to eat again. The same thing has been noticed in man when he has been removed from his industrial hazard. About two weeks ago one of these goats became suddenly paralyzed

in its hind quarters, cried all day long, and died during the night. We examined this goat and found lead in all of its organs. The brain, weighing 60 grams, contained 3.36 milligrams of lead. The bones contained about 90 per cent of the lead present, thus confirming the work done at Harvard. We also found that the muscle contained 0.67 milligram of lead per 100 grams. These findings show that the lead is distributed all over the body. I think it is commonly agreed that it is the lead distributed in the various organs and carried in the blood stream that causes lead intoxication and not that stored in the bones. We have stopped dosing the second goat with tetraethyl lead for the purpose of seeing if it would recover from the lead intoxication. It shows marked improvement at this time. The goats receiving the concentrated lead excreted approximately 0.56 milligram of lead each 24 hours. The goats receiving the dilute material give a lower rate of excretion, or about 0.10 milligram per 24 hours.

Going on to our fume experiments, we can only say that we were at a loss to determine the exposure that we should give our animals. We made various evaporation tests. We poured gasoline on to non-absorbent material in order to duplicate, if possible, the conditions around filling stations and garages. We found, of course, that the rate of evaporation varied according to the temperature, air movement, and the area over which the gasoline spreads itself. However, we did find that we could evaporate on the average about 400 cubic centimeters of gasoline in four hours. We decided from this that we would aim to duplicate this evaporation in our gas chamber. This gas chamber contains approximately 180 cubic feet of air and is so ventilated that the air is changed six times per hour. During our tests the gasoline was evaporated by bubbling air through a bottle containing it in the same manner as has already been described by Doctor Sayers. Before starting the exposure of our animals to leaded gasoline we exposed them for 17 to 20 hours a day over a period of a week in the gas chamber to the determined concentration of gasoline fumes. The animals were in as good a condition at the end of the week as at the beginning. This made us feel that we could expose them to the leaded fumes for a period of four hours a day without any ill effects from the gasoline fumes. As far as we could determine from analyzing the air and the residue left in the bottle, there was, on the average, 0.03 milligram of lead in each cubic foot of air in the chamber during the exposure to ethyl gasoline. Rabbits, rats, monkeys, guinea pigs, and goats were used in this part of our work. To our surprise we found that the rat was rather susceptible to lead exposure. I do not quite understand it. Every one of our 24 rats that were exposed died. Their exposure varied anywhere from 56 to over 220 hours. We found lead