STUDIES ON THE ABSORPTION OF LEAD FROM ETHYL GASOLINE

by

Robt. A. Kehoe, M. D., Frederick Thamann and Lester Sanders

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INTRODUCTION

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NOTE:

Attention is called to an error on page 18 of this report. Monkeys No. 3, No. 4 and No. 5 are called Ringtail Monkeys, they having been so designated by the dealer from whom they were obtained. Investigation shows that they are Macacus Monkeys, of the type ordinarily called "Common Macacus." This is a matter of some importance, since cross breeding of Macacus Rhesus and Ringtail Monkeys may be regarded as a practical impossibility.

elsewhere, in so far as animal experimentation might be capable of yielding information. Thus there was time and opportunity for two types of investigation, without duplication of effort. 1. Observations could be made on men actually exposed to all the practical conditions resultant upon the distribution and use of Ethyl Gasoline. And, 2, animal experimentation dealing with the various types of exposure to the leaded gasoline under the various conditions associated with the commercial handling of such gasoline could be carried out.

The first of these has been made the object of one study by this laboratory, on which report was made in June 1925.* This study was checked and verified in detail by an independent study carried out by a com-

* Report of First Field Investigation made to Board of Directors of The Ethyl Gasoline Corp. Kehoe and Edgar, 1925.

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INTRODUCTION

In anticipation of the general distribution of Ethyl Gasoline for use in automobiles, this laboratory began a series of investigations in 1924, with the idea of determining to what extent certain dangers which suggested themselves as possibilities in connection with this commerce might be of actual importance. Evidence that no acute danger existed was to be had at the outset, by reason of the fact that the gasoline had been on the market for some months, without the occurrence of untoward results. The problem of possible dangers in connection with finely divided lead dusts in exhaust gases, was being carefully and adequately investigated elsewhere, in so far as animal experimentation might be capable of yielding information. Thus there was time and opportunity for two types of investigation, without duplication of effort. 1. Observations could be made on men actually exposed to all the practical conditions resultant upon the distribution and use of Ethyl Gasoline. And, 2, animal experimentation dealing with the various types of exposure to the leaded gasoline under the various conditions associated with the commercial handling of such gasoline could be carried out.

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mittee appointed in 1925 by the Surgeon General for Public Health.* A second study on our part is now in progress, on which report will be made at a later date. Since this problem is concerned principally with accumulation of lead, time is the important factor, so that it may not be completed until several years have elapsed.

The second type of study has been carried on continually and has been interwoven with the first to the extent that the observation of human exposure has given a more complete understanding of the details of experimental conditions which must needs be duplicated in animal experimentation. In the light of careful observation of the methods in general use for handling gasolines the hazards which may exist in connection with the handling and use of Ethyl Gasoline are capable of much more accurate statement than was possible at the time the experimental program was undertaken. The only health hazard which might arise from the commercial usage of Ethyl Gasoline is that, after long exposure, persons might eventually accumulate in their bodies sufficient lead to produce the symptoms and signs of lead poisoning of a chronic type. Exposure may occur in several ways, and may best be illustrated by examples from practical life.

The garage mechanic may have his hands bathed in gasoline for considerable periods of time in the course of repairing and adjusting carburetors and various other parts of a motor. He may use the gasoline for cleaning tools, etc., as well. If such a thing is possible he may absorb some lead through his skin out of the gasoline. In addition he may handle tobacco or other articles that go into his mouth, with unwashed hands, and so ingest lead. He may allow the gasoline to dry on his hands and may thus be exposed to small quantities of more concentrated Ethyl

* Investigation of Health Hazard from Tetraethyl Lead Gasoline - J. P. Leake - U.S.Public Health Service, 1926. U.S.Treasury Dept.

Fluid, which being less volatile than the gasoline will remain on his skin longer than the gasoline. The gasoline may spill on the floor of the garage, and if allowed to remain will evaporate, leaving behind some of the less volatile tetraethyl lead. This will vaporize to some extent, and will be decomposed in part to tri-ethyl lead compounds which will remain to make up part of the dust of the garage. In the above situations the mechanic may inhale vapors arising from the gasoline, and if the vapor contains lead he may absorb it through the lungs. After the gasoline has evaporated, he may still be exposed to some vapor of the residual tetraethyl lead, and, finally, he may inhale lead dust from the air as it is picked up by eddies and stirred up by movements in the garage. In tearing down motors the mechanic comes in contact with lubricating oil containing minute quantities of tetraethyl lead and a greater or lesser quantity of decomposition and combustion products of tetraethyl lead. He will scarcely be exposed to any important danger of skin absorption here, but he may ingest lead as a result of eating, chewing, and drinking materials contaminated by unclean hands.

The above constitutes the entire picture of the possibilities for the absorption of lead as a result of the use of leaded gasoline, with the exception of the exhaust gas exposure. The latter is completely omitted from this discussion because the experimental work herein presented has no application to this matter.

The above methods of exposure are present to a greater or lesser extent in the case of all handlers and users of Ethyl Gasoline. In different situations, however, one or the other type is of importance while the others are less important or negligible.

The service station employee is exposed to vapors arising from

gasoline, to skin contact with gasoline, to vapors and dusts from the street or area around his station, which may contain lead, or to skin contact with oil in draining crank cases. Of these, however, the skin contact with gasoline is undoubtedly the most important. However, the possibility of ingestion of lead because of unclean hands must not be forgotten, in the case of this man.

The loading rack handler of gasoline is exposed to vaporizing gasoline, to skin contact with it in filling cars, sampling, and in draining the cars by removing the drain cap. There may be occasional bad spills here in which a large body surface is covered with gasoline. There may be vaporizing tetraethyl lead and lead dusts as a result of frequent spillage over the same area of ground or loading platform. Lead vapors may be inhaled in sweeping and cleaning cars which have carried Ethyl Gasoline and lead may be ingested because of unclean hands. Here again probably the most important exposure, at present, lies in the skin contact with gasoline.

The tank wagon and bulk gasoline handler is exposed to exactly the same situations, and in case of bad fittings, careless handling or in case bucketing is done, his skin contact with gasoline may be considerable.

The owner and handler of a car is presented with the same possibility of exposure, to just the extent of his taking over the work carried out generally by the above workmen. Questions arise, therefore, as to the relative importance of these types of exposure, and as to whether any of them are sufficient in quantity to cause trouble. Experience, only, will fully answer these questions, but experimentation may give some predictions, which when taken with the proper reservations, are of considerable importance.

The facts which are of the utmost importance to determine are the

answers to the following questions.

1. Under conditions which allow for spillage, evaporation of spilled gasoline leaving residual tetraethyl lead and dust of decomposed lead, skin contact, and ingestion from contaminated hands, is the quantity of absorption sufficient to produce accumulation of lead and lead poisoning?

2. Can tetraethyl lead be absorbed out of gasoline by the skin, as long as the concentrations of lead and gasoline remain at their normal commercial level?

3. If so, can it be absorbed in sufficient quantity to accumulate in toxic amounts in the tissues?

4. Can tetraethyl lead be inhaled in the vapor arising from Ethyl Gasoline, in sufficient quantity to produce accumulation of lead?

The following experiments undertake to answer some of the above questions. Some are answered completely and decisively. Some are answered tentatively, because of the care which must be taken against a too literal interpretation of animal experimentation in terms of human beings.

(Note: Animal experiments dealing with the above subject were reported at a Conference called by the U. S. Public Health service May 20, 1925. The following studies are a continuation of experiments reported at that time, and in some cases the same animals have been used. In as much as the results in certain aspects of the problem are not conclusive as yet, some of the experiments are being continued at the present time. The data included herein, therefore, represent observations up to the present, inclusive of those previously reported.)

EXPERIMENTAL METHODS

Guinea Pigs, Rabbits, and Monkeys were treated with Ethyl Gasoline in a variety of ways, so as to study the possible means of absorption of lead contained in gasoline in various concentrations. Exposure to the gasoline was arranged in such a way as to duplicate as nearly as possible the conditions of human exposure.

I. In order to provide a situation in which animals might be exposed simultaneously to skin contact with Ethyl Gasoline, inhalation of gasoline vapor, inhalation of residual tetraethyl lead vapor, and dust of decomposition products, and to ingestion of lead from contaminated skin and food, rabbits were treated with Ethyl Gasoline in the following way:- The hair was clipped from a skin area without injury to the skin, and an accurately measured quantity of gasoline containing tetraethyl lead was dropped on and allowed to remain until absorbed or evaporated. No attempt was made to prevent inhalation of volatile materials after treatment. No precaution was taken to prevent the animal from licking himself or his companions and thus ingesting the lead. It was observed, however, that there was very little tendency shown to lick off the gasoline. In this way all the tetraethyl lead put on a given animal remained on him, or near him, in the form of vapor or dust. It even contaminated his food to some extent. In case he excreted lead his excretion remained for a time to contaminate his food and the air he breathed. In this way it was hoped to reproduce the worst conditions of commercial handling. Observations were made, and at various intervals animals were killed for analysis.

II. In order to determine whether or not tetraethyl lead might be ab-

sorbed out of gasoline the following methods were used. The hair was clipped off the fore leg and first one and then the other, on alternate days of treatment, was dipped in the gasoline and kept so immersed for a period of half an hour, after which the treated leg was carefully washed with kerosene, soap and water and then coated with cold cream (to prevent drying and cracking of the skin). In this way absorption was confined to the period of actual exposure to the gasoline. After considerable periods of exposure the animals were killed and analysis for lead was made.

III. In order to determine whether tetraethyl lead may be inhaled along with gasoline vapor from exposed surfaces of Ethyl Gasoline, experiments were arranged as follows: Air was bubbled through gasoline containing tetraethyl lead at a rate sufficient to volatilize 30 to 50% of the gasoline in six hours. The gasoline vapor was mixed with air sufficient to maintain a concentration of gasoline vapor just endurable by rabbits over a period of six hours. Preliminary experiments with the gasoline base determined what concentration could be borne and this concentration was maintained. Rabbits were exposed to these conditions daily over a period of several months. The residual gasoline after the vaporization of the 30 to 50% fraction was analyzed for its lead content, in order to determine just what amount, if any, of lead was given off in the vapor passing through the cage. The animals were killed after a period of exposure, and analyses made to determine what quantity of lead, if any, had been absorbed and accumulated.

EXPERIMENTS

I. Skin Application of Gasoline Containing Tetraethyl Lead:

1. A. Guinea Pigs:

Healthy full grown guinea pigs were selected and divided into four pens. At first they were kept in board and wire pens in a large storage room at the Dayton Plant. The pens were separated by tight board partitions so as to avoid any possibility of contamination of one pen by fecal material or dust and vapor from an adjoining pen. The front and top sides were of wire to allow ventilation and sunlight. On April 2nd the animals were moved to the Laboratory in Cincinnati where they were put in cages of concrete and galvanized iron grating, the partitions being of solid concrete.

All the guinea pigs were fed on a mixed diet which has been found to be quite satisfactory after use for a long time. The Ethyl Gasoline used for treatment was made by mixing three parts of tetraethyl lead with two parts of ethylene dibromide, and mixing this in gasoline in the proportion of 0.25 cc. to 200 cc. gasoline as needed. This corresponds to the use of the 3 E Mix in gasoline representing approximately 1 part tetraethyl lead to 1300 parts gasoline. The same gasoline base was used on the control animals.

Observations on reproductive activity were desired, so that males and females were kept together, except in the case of Pen 2. Here a male from Pen 4 was introduced periodically. It will be observed that this animal was not being treated with any lead, so that he had no influence upon this factor.

The experiment was continued over a period of eight months, during which time the oldest of the test animals received 69 treatments, at the

rate of two per week. Three animals each from Pen 1, 2 and 3 were killed for analysis March 3rd, and one each from Pen 1 and 2 were killed for analysis March 31st. The animals killed on these dates had received 22 doses and 28 doses respectively. Two pigs were stolen from the cages just prior to the time of moving to Cincinnati, one each from Pen 1 and 2. One pig from Pen 2 was badly injured in a fight, dying two days later. The remainder of the animals were killed at the end of 8 months of experimentation, on September 1st, and analyses were made then.

No illness was observable at any time during the experiment in any of the animals, and no deaths occurred from any cause except in the case of one animal in the control group, whose death was accidental. The following tables indicate the experimental findings and the analytical results.

TABLE I

Experimental History

Treatment	Pen One 1 cc. Ethyl Gasoline	Pen Two None	Pen Three 0.2 cc.Ethyl Gasoline	Pen Four 1 cc.Ordinary Gasoline
Males	7	0	5	1
Females	4	11	6	2
Deaths	None	1 (Injured in fight)	None	None
Young born	17	57	31	12
Young died	4	9	9	2
Premature births	2	3	4	1
Illness	None	None	None	None
Blood changes	None	None	None	None

TABLE II

Analytical Results

Date	Total Pb. found in Mgs. Pen 1	Pen 2	Pen 3	Pen 4
Mar. 3	2.10	2.00	1.60	
	2.10	2.00	2.00	
	2.55	2.10	Lost	
Mar. 31	2.55	2.10		
*Sept. 2	*0.90	*1.00	*0.70	*2.50
	0.90	0.82	0.63	0.50
	0.80	0.70	1.30	0.67
	0.85	0.90	1.05	
	1.20	0.80	1.15	
	1.00		2.62	
			1.56	
			1.03	

* All animals above the double line were exposed to lead dusts in Dayton plant, because of storage of lead containers in experimental quarters. All below that line were kept after such exposure over a period of five months in the Cincinnati Laboratory, where there was no exposure to lead beyond that brought about by the experimental conditions, the experiment continuing as before up to Aug. 28, 1925.

It is observed that all the animals in the group contain lead in their carcasses. Much of this lead is due, undoubtedly, to the inhalation and ingestion of lead dust in the room at the Dayton plant. (Note reference to this in Public Health Bulletin, No. 158.) The significant feature of the results is seen in that with two exceptions, the results of analyses show a lead content at the end of eight months treatment, which is decidedly lower than that at the end of three months. Obviously, during the last five months of experimental study in Cincinnati, these animals were not accumulating more lead, but were losing some which they had previously taken in. It is plain, therefore, that absorption, if any occurred, did not keep pace with secretion. This is especially significant when the test group Pen 1, is compared with control groups 2 and 4.

In the case of the latter two groups there is no possibility of further lead intake, except in so far as the food and water may have contained a normal minute amount. There was no dust, nor were there any compounds of lead in the rooms occupied by the animal cages. In spite of this fact these animals did not lose lead from their bodies at a rate in excess of that shown by the test group. The only conclusion one can arrive at, therefore, is that the test group were not absorbing appreciable quantities of lead.

B. Guinea Pigs:

Because of the exposure of the guinea pigs to extra-experimental sources of lead in the previous experiment, and because information was desired on the possibility of lead absorption from lower concentrations of tetraethyl lead in gasoline, a further study was made in the following manner.

A fresh supply of full grown guinea pigs was obtained. These were divided into two groups, Pens No. 5 and No. 6. The animals were fed in the standard manner and were kept carefully separated from each other, using all the precautions previously described to prevent contamination of control animals by dusts and vapors from the pen containing test animals. The animals in the control pen (No. 5) received no treatment and were handled only in weighing and feeding. The animals in the test pen (No. 6) were treated three times weekly with 1 cc. of gasoline containing half the standard quantity of Ethyl Fluid. This was prepared by mixing three parts of tetraethyl lead with two parts of ethylene dibromide and mixing this in gasoline in the proportion of 0.25 cc. to 400 cc. gasoline.

The same type of observations were made as in the case of the animals previously studied. The experiment began in August, 1925 and continued without interruption until the latter part of January, 1926. During this time the test animals received sixty-two treatments, with the exception of one female which died after the seventeenth treatment. This animal received an injury from a fall, resulting in a ruptured liver and death from haemorrhage into the peritoneal cavity. In the control pen three of the four animals died during the five months, one female on October 20, after the birth of two young, one female on October 27 of pneumonia, and one male on November 19 of abscesses of the liver. All of these animals were analyzed for lead content. The other survived and was killed for analysis at the end of the experiment. The following table summarizes the results of the experiment.

TABLE III

Pen No.	Treatment Type	Treatment No.	Animal No.	Sex	Date of Death	Young Born	Young Died	Premature Birth	Pb. Content in Mgs.
5	None	None	46	F.	Oct. 20	2	2	None	0.32
			Young of 46 Analyzed						0.13
			48	F.	Oct. 27	3	0	None	0.24
			49	M.	Nov. 19				0.08
			50	M.	Jan. 26 (killed)				0.35
6	1 cc. ½ conc. Ethyl Gasoline 3 x week	17	45	F.	Oct. 3 *	0	0	None	0.64
			Unborn young of No. 45 Analyzed						0.32
		62	51	M.	Jan. 26 (killed)				0.28
		62	52	M.	Jan. 26 (killed)				0.38
		62	53	F.	Jan. 26 (killed)	6	0	None	0.45

* Pregnant - 4 young almost at term.

A study of the above data indicates that there is little evidence of the accumulation of lead in the animals treated with half concentration Ethyl Gasoline. The amount of lead found in the entire carcasses of the treated animals never exceeds a fraction of one milligram, and although the average quantity found is slightly higher in test animals than in controls, there is not a sufficient difference to be significant. It is important to note that all the animals show some small quantity of lead in their bodies. However, there is no relationship between the amounts so found and either the period of time spent by the animal in our laboratory, or the period of actual treatments. For this reason one must conclude that either the animals are not absorbing appreciable quantities of lead, or that they are excreting it as rapidly as they are absorbing it.

2. A. Rabbits:

Full grown rabbits were obtained from stock raised outside the laboratory, so as to avoid using any of the offspring of leaded animals, which might by chance contain abnormal quantities of lead. Six animals were used, of which two were used as controls. The controls were weighed once a week and observed. The four test animals were treated on the skin of the belly from which the hair was clipped, with 2 cc. of one half of that concentration of Ethyl Gasoline which had been the standard experimental gasoline previously. This was prepared as previously described for the treatment of guinea pigs in Pens No. 5 and No. 6. Test animals were weighed weekly and observed. Controls and test animals were kept separated but were fed on a standard diet in the same manner.

The experiment was begun August 19, 1925, and concluded January 28, 1926. During this time no illnesses or deaths resulted from the treatment, but three of the animals including one of the controls sickened, and were either killed or died. The carcasses of all were analyzed for total lead content, exclusive of the skin. Rabbit No. 107 developed a paralysis and was killed October 14th. A necropsy showed large haemorrhages into the musculature of both sides of the body, both inside and outside the peritoneum, which were obviously the result of injury on the animal board. (It occasionally happens, in handling a considerable number of animals, that an animal becomes frightened and begins struggling violently. At such times an injury is very likely to result in the case of delicate animals as rabbits and guinea pigs.) Rabbit No. 110 developed a parasitic infection of the ear (common among rabbits) which extended into the middle ear with abscess formation. It was considered humane to kill the animal. This was done December 8th. Rabbit #112, a control, developed a pneumonia and died January 10th.

TABLE IV

No. of Rabbit	Sex	No. of Treatments	Time Period	Weight in Lbs. Initial	Weight in Lbs. Final	Offspring Living	Offspring Dead	Date of Death	Lead content in Mgs.
107	F.	20	2 mo.	3-3/4	3-1/2	0	0	Oct.14	1.40
108	F.	58	5 mo.	5-7/16	4-7/8	4	0	Jan.28*	0.11
109	F.	55	5 mo.	5-9/16	5-3/4	0	0	Jan.28*	0.35
110	F.	39	3 mo.	5-3/16	4-1/4	2	2	Dec. 8*	3.30
111	F.	Control	5 mo.	6-1/4	6-1/4	6	1	Jan.28*	0.29
112	F.	Control	5 mo.	5-3/4	5-1/4	0	0	Jan.10	0.20

* Killed for analysis.

It will be noted that all of these animals contained small quantities of lead in their carcasses. However, there are wide variations in the lead content. Two of the treated animals contain considerably larger amounts than either of the controls. On the other hand the other two test animals, treated for longer periods of time, show no greater quantity than is found in the control animals. There is, therefore, no relationship between quantity of lead found and the period and quantity of treatment. This fact alone makes one doubt if the treatment was responsible for the relatively high lead content of Rabbits No. 107 and 110. It suggests one of two possibilities. Either the relatively high lead values are accidental, i.e., they represent variations such as may be found in control animals; or, the rate of excretion in these two animals did not keep pace with that shown by normal animals, because of their complicating illnesses. The latter can hardly be true in the case of Rabbit No. 107, for his injury occurred abruptly and he was killed soon after the injury. This illness could hardly have had any influence either on the rate of absorption or the rate of excretion of lead. Furthermore, this animal was killed early in the experiment having been exposed to only twenty treatments. In the case of Rabbit No. 110 a lingering infection

might be responsible for a somewhat decreased rate of secretion, for it produces general injurious effects to all the physiological processes of the animal. However, it has been our experience previously, that such infections have a tendency to increase the rate of excretion of lead.

The evidence, therefore, points to the incidental character of the high lead content of these two animals, rather than the resultant effect of the experimental treatment. That this is likely is further indicated by the analysis for lead of two animals taken at random from the hutches of the Physiological Laboratory. One of these P_1, contained 1.10 mgs. Pb., and the other P_{11} contained 0.50 mgs. Pb. This indicates that animals kept under standard laboratory conditions are exposed to sources of lead, and normally show lead in their tissues.

In any case the results of these experiments are not fully conclusive except in so far as they point out that lead poisoning did not result from the treatment, and that rabbits do not, of necessity, accumulate lead in their bodies as a result of prolonged skin contact with gasoline containing the experimental concentration of tetraethyl lead.

3. Monkeys:

Seven monkeys have been used in this study. Two Rhesus monkeys originally intended for controls have died, one of pneumonia, shortly after being received, and the other of exposure, he having escaped during cold weather. The first experiments were conducted on two Rhesus monkeys, one male and one female, without controls. Some months later three Ringtail monkeys, one male, and two females, were procured, one of these being used since as a control.

After being obtained the monkeys were kept under observation for some weeks in order to insure their good health at the outset. A diet

of oranges, bananas, carrots, apples, eggs, nuts and milk have served to maintain an excellent state of nourishment.

Observation has been made of temperature, weight and blood findings over the entire period of pre-experimental and experimental period, for test and control animals. These observations are shown in the charts below.

The following experimental treatment was carried out. Monkey No. 1, a Rhesus male, has been treated daily (excepting Sundays and holidays), since January 19, 1925, with 2 cc. Gasoline prepared by adding 0.4 cc. Tetraethyl lead to 500 cc. gasoline. An area on the back was clipped free of hair and kept so, the gasoline being dropped on with a pipette, without any further procedure.

Up to November 18, 1926, this animal has had 515 such treatments. The chart below shows the observations as to weight, etc. This animal has never been ill at any time. He is small, and has been so since being obtained, and in addition is restless and excitable, having never been tamed to the extent shown by the other monkeys, especially the females. His sexual development is apparently not normal, since at no time does he regard the females with any interest, other than to betray a loneliness and anxiety if left alone. In as much as he was apparently of adult age* when the experiment began these things may be disregarded in all probability, so far as any experimental significance is concerned.

Monkey No. 2, a Rhesus female, weighing $6\frac{1}{4}$ lbs. at the beginning of the experiment was treated just as Monkey No. 1, up to May 20, 1926, she having received 375 treatments of Ethyl Gasoline, 2 cc. at a dose,

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* The age of these animals is problematical, since there is so little information available on matters of this kind.

since January 19, 1925. On the former date, somewhat unexpectedly, this monkey gave birth to a strong, fine young male. Treatments were discontinued at once in order to avoid any possibility of injury to the offspring from gasoline and lead which might be taken into the mouth from the skin of the mother. The young one has thrived and is healthy. He is very active. His sire is a large, strong Ringtail male, Monkey No. 5. The mother has not been ill at any time, though she was in a condition of poor nourishment when obtained. At the time of the birth of the young one, the mother showed no apparent illness, and she has continued through the suckling period up to the present to have every appearance of excellent health, with sleek hair, bright eyes, and generally good appearance.

Three new monkeys, healthy examples of the Ringtail type, were obtained in September, 1925.

Monkey No. 3, a Ringtail female, was kept as a control, observations being made as in the case of the test monkeys. This animal has been healthy and has done well until recent months. She has always had a tendency to stay away from the other monkeys, and is apparently sterile. Her occupation consists in picking ceaselessly at objects real and imaginary in her surroundings, so that she keeps her nails worn away. She looks old and has lost some weight recently. She was apparently the healthiest of the females when the experiment began, and for this reason was chosen as the control.

Monkey No. 4, a Ringtail female, was treated with 4 cc. Ethyl Gasoline daily, from November 5, 1925 to February 13, 1926, 75 treatments in all. At this time it was noted that the animal was pregnant. Not knowing the time at which the pregnancy would be terminated, but thinking it to be near at hand, the treatments were discontinued in order not to take even

the slightest chance of injuring the young through ingesting lead from the skin of the mother while suckling.

The animal delivered a normal, healthy male young one on April 3rd. The mother was not ill and both she and the young one have continued to thrive. Treatments of the mother are now about to begin, since the young one is apparently able to care for himself.

Monkey No. 5, a large ~~Ringtail~~ Common Macaque male has been treated with 4 cc. of Ethyl Gasoline daily, since February 25, 1926. Up to November 18, 1926 he has received 214 such treatments. At no time has he shown any evidence of illness. This animal is the sire of the two baby monkeys now in the laboratory.

TABLE VI

Chart of Experimental Observations
MONKEY NO. 1

Date	Weight Lbs.	Temp.F.	Thousand Erythrocytes	Leucocytes	Reading Hb. (Dare)	Stippling per 50 fields
Jan. 19, 1925	Treatment with 2 cc. Ethyl Gasoline begun.					
Jan. 19, 1925	5-1/8	102.8	4,144	13,800	70	0
Feb. 7	4-9/16	102.4	-	-	-	-
13	4-14/16	102.2	-	-	-	-
20	2-11/16	102.8	-	-	-	-
27	4-6/16	103.0	-	-	-	-
Mar. 31	4-11/16	102.6	-	-	-	-
Apr. 21	-	-	4,437	16,050	65	0
28	4-12/16	102.0	-	-	-	-
May 5	4-13/16	102.6	4,932	16,700	60	0
14	4-11/16	102.4	5,252	10,500	60	0
21	4-13/16	102.3	5,368	11,700	65	0
30	-	-	5,512	12,400	50	0
June 5	4-3/4	103.4	5,360	13,400	50	0
11	5	102.4	5,844	11,700	52	0
18	5	102.8	5,768	11,050	50	3
25	5-1/16	102.6	5,584	11,200	-	0
July 2	5-1/8	103.2	-	-	-	2
9	5-1/16	103	5,088	12,500	-	3
16	4-14/16	102.8	5,672	11,150	55	0
23	-	-	5,440	12,000	53	2
30	4-14/16	102.5	5,728	16,350	55	0
Aug. 7	5-5/16	102	4,653	12,500	55	7
14	5-3/16	102.6	5,840	11,450	55	0
22	5-1/2	102.4	6,024	9,800	54	1
29	5-1/16	102.1	5,886	14,600	57	1
Sept. 5	5-1/2	102.0	5,928	13,100	54	2
12	-	-	6,104	12,000	57	8
19	5-13/16	102.0	5,552	13,050	58	0
26	5-11/16	102.0	6,036	11,500	57	0
Oct. 3-5-7	5-7/16	102.2	6,232	13,600	60	0
10	5-6/16	102.8	5,072	22,200	50	0
17	6	101.8	4,946	10,400	55	0
24	5-13/16	101	5,616	20,050	58	0
31	5-13/16	102.5	5,640	10,150	63	0
Nov. 7	6-1/16	102.2	-	11,400	55	0
14	5-1/2	102.4	5,436	13,500	58	0
21	5-11/16	102.0	6,088	13,500	56	0
28	5-11/16	102.0	5,008	9,400	56	0
Dec. 5	5-1/2	102.1	5,260	8,850	57	0
12	5-10/16	102.4	4,960	11,000	58	0
26	-	-	5,300	11,800	60	0
Jan. 2, 1926	5-11/16	102.9	5,080	9,600	60	0
9	5-9/16	102.4	5,208	9,800	58	0
16	5-9/16	102.8	5,048	13,850	58	0
23	-	-	5,184	10,100	60	0
30	5-11/16	102.1	5,260	14,160	60	0

MONKEY NO. 1 - (Cont'd)

Date	Weight Lbs.	Temp.F.	Erythrocytes	Leucocytes	Hb.(Dare)	Stippling
Feb. 6, 1926	5-3/4	102.5	5,392	13,750	58	0
13	5-3/4	102.1	6,032	16,600	60	0
26	5-14/16	102.1	-	-	-	-
Mar. 8	5-3/4	102.4	-	-	-	-
13	5-13/16	102.0	-	-	-	-
20	5-3/4	101.8	-	-	-	-
29	5-10/16	101.6	-	-	-	-
Apr. 7	5-11/16	101.0	-	-	-	-
19	5-14/16	102.4	-	-	-	-
May 1	5-3/16	102.1	-	-	-	-
4	5-13/16	102.1	5,456	14,050	55	0
14	6-2/16	102.2	5,240	13,900	55	1
20	5-15/16	102.0	4,896	14,450	65	0
28	5-14/16	102.2	5,192	10,800	-	0
June 5	5-15/16	102.1	4,720	14,300	50	2
11	5-15/16	102.4	4,976	9,400	55	1
17	6-1/4	102.1	5,288	12,350	55	0
24	5-14/16	102.0	4,816	13,300	50	0
July 1	6-1/16	102.4	4,984	10,150	50	0
8	6	101.8	4,352	9,050	50	0
15	6	102.0	4,688	11,500	50	0
22	6-2/16	101.6	4,680	7,850	50	0
30	6-2/16	102.6	4,672	8,650	50	1
Aug. 5	6-3/16	102.0	4,552	6,900	50	1
11	6-1/6	102.0	4,160	12,400	50	1
20	6-1/2	101.0	5,280	11,800	55	0
26	6-2/16	102.2	5,504	12,450	52	0
Sept. 2	6-2/16	102.6	5,208	13,000	60	0
25	5-6/16	101.7	6,048	12,450	50	1
Oct. 2	6-5/16	102	5,464	9,300	50	0
7	6-1/4	101.8	6,024	12,600	50	0
14	6-6/16	102.0	-	9,600	55	0
22	6-1/4	102.6	6,201	14,800	50	0
29	6-9/16	101.9	6,460	11,100	53	1
Nov. 4	6-3/4	101.6	6,090	16,550	50	0
11	6-11/16	102.4	5,920	9,050	50	0
18	6-3/4	102.1	6,590	9,350	57	0

Experiment being continued.

TABLE VII

Chart of Experimental Observation
MONKEY NO. 2

Date	Weight Lbs.	Temp.F.	Erythrocytes	Leucocytes	Hb.(Dare)	Stippling
Jan. 15, 1926	Treatment with 2 cc. Ethyl Gasoline, daily, begun.					
Jan. 19, 1925	6-1/4	102.3	5,020	16,400	75	0
Feb. 7	6-6/16	102.2	-	-	-	-
13	6-1/2	102.2	-	-	-	-
20	6-11/16	102.2	-	-	-	-
27	6-4/16	102.0	-	-	-	-
Mar. 31	6-7/16	103.0	-	-	-	-
Apr. 21	-	-	-	-	-	-
Apr. 28	7-3/16	102.0	5,284	13,500	70	0
May 5	7-6/16	102.0	5,080	12,350	70	0
14	8-2/16	101.4	5,712	8,700	70	0
21	8	102.3	4,816	10,150	75	0
30	-	-	4,816	14,900	70	0
June 5	8-1/4	103.2	4,888	11,350	72	0
11	7-3/4	101.2	5,576	11,250	70	0
18	7-1/16	102.6	5,344	11,050	-	0
25	8-6/16	102.2	4,944	12,000	-	0
July 2	8-1/2	102.2	-	-	-	0
9	8-9/16	102.6	4,112	13.000	75	0
15	8-9/16	102.4	5,280	11,450	75	0
23	-	-	4,240	11,700	75	0
30	9-2/16	102.0	4,344	12,050	75	0
Aug. 7	9	102.6	4,696	13,700	75	0
14	8-3/4	102.4	5,792	11,050	70	0
22	9-1/16	102.0	5,416	11,000	73	0
29	9-3/16	102.0	5,056	10,750	73	0
Sept. 5	9-3/8	103.0	5,304	11,450	80	0
12	-	-	5,512	15,300	75	0
19	8-13/16	102.8	5,256	14,200	78	0
26	8-3/4	102.0	5,976	17,050	76	0
Oct. 3	8-11/16	102.0	6,128	11,650	78	0
10	9-2/16	102.0	5,448	15,350	80	0
17	10	101.6	5,384	10,100	85	0
26	9-14/16	103.0	5,232	19,100	85	0
31	10	102.0	5,904	12,200	78	0
Nov. 7	9-13/16	102.5	5,024	13,250	75	0
14	9-13/16	102.4	5,576	14,300	80	0
21	9-14/16	102.0	5,952	12,050	80	0
28	9-9/16	101.9	5,240	13,300	80	0
Dec. 5	9-11/16	102.0	5,184	15,750	84	0
12	10	102.1	5,568	14,100	83	0
19	9-9/16	102.6	-	-	-	-
26	9-6/16	102.4	5,346	13,500	80	0
Jan. 2, 1926	-	-	4,624	14,500	82	0
9	9-3/16	101.4	4,824	16,750	85	0
16	9-1/2	101.0	4,224	16,850	84	0

MONKEY NO. 2 - (Cont'd)

Date	Weight Lbs.	Temp.F.	Erythrocytes	Leucocytes	Hb.(Dare)	Stippling
Jan. 23, 1926	-	-	4,504	15,250	83	0
30	9-3/4	102.1	4,584	12,950	78	0
Feb. 6	9-13/16	101.2	4,832	13,900	78	0
13	10	101.0	5,464	11,250	80	0
26	10-3/4	101.2	-	-	-	-
Mar. 8	10-3/4	101.4	-	-	-	-
13	10-14/16	101.4	-	-	-	-
20	11	101.0	-	-	-	-
27	11	102.1	-	-	-	-
Apr. 7	11	102.5	-	-	-	-
19	11-6/16	101.1	-	-	-	-
May 3	11-7/17	100.6	5,052	11,600	60	0
14	11-3/4	100.0	3,280	12,250	54	0
20	Young born. Treatments and handling discontinued on account of anxiety and rage of mother at approach of workers in laboratory.					

TABLE VIII

Chart of Experimental Observations
MONKEY NO. 3 (CONTROL)

Date	Weight Lbs.	Temp.F.	Erythrocytes	Leucocytes	Hb.(Dare)	Stippling
Sept.17, 1925	-	-	5,560	22,400	80	0
25	-	-	4,904	20,600	75	0
Oct. 2	6-10/16	101.2	4,752	26,250	75	0
9	6-11/16	101.5	5,110	23,600	75	0
16	6-5/16	101.8	4,480	21,000	70	0
23	6-6/16	102.1	5,276	24,900	70	0
31	7	101.6	5,024	21,850	73	0
Nov. 7	7	101.6	4,664	16,600	70	0
14	7	101.8	4,392	21,000	70	0
21	7	100.8	4,416	24,350	80	0
28	7-1/16	102.0	5,750	19,650	80	0
Dec. 5	7-1/2	101.0	4,936	16,700	84	0
12	7-9/16	102.0	5,280	18,500	78	0
26	-	-	5,400	17,800	80	-
Jan. 2, 1926	7-9/16	101.4	5,488	19,700	80	-
9	7-5/16	101.4	6,088	13,700	90	-
16	7-6/16	101.6	5,336	15,400	84	-
23	7-1/2	101.8	5,120	17,620	85	-
30	7-9/16	102.0	4,900	17,400	83	-
Feb. 6	7-11/16	102.1	5,272	22,150	85	-
13	7-7/16	101.5	4,944	18,500	80	-
26	7-3/4	-	-	-	-	-
Mar. 8	8	102.0	-	-	-	-
20	8-1/4	100.1	-	-	-	-
29	8-3/16	100.3	-	-	-	-
Apr. 7	8-1/4	101.4	-	-	-	-
19	8-3/16	102.0	-	-	-	-
May 4	8-7/16	101.6	4,112	17,300	90	0
14	8-10/16	102.2	4,600	19,350	90	0
20	8-9/16	101.2	4,576	19,500	80	3
28	8-8/16	101.0	4,664	18,900	-	0
June 4	8-6/16	101.0	5,040	19,000	80	-
11	8-1/2	101.0	4,504	15,700	90	0
16	8-6/16	102.0	4,440	15,100	80	26
24	7-9/16	102.0	4,112	17,700	75	22
July 1	7-15/16	102.4	4,289	19,500	65	3
8	7-9/16	101.0	4,288	13,200	60	0
15	7-1/2	101.4	4,664	15,800	70	0
22	7-5/16	100.2	4,296	16,550	75	2
29	7-1/2	101.6	4,960	13,000	80	0
Aug. 6	7-1/2	102.4	4,280	15,250	70	14
13	7-5/16	101.8	5,040	18,500	80	60
26	7-3/16	101.6	5,280	20,900	67	72
Sept. 2	7-1/2	102.4	5,680	17,000	75	-
23	7	102.6	6,960	13,650	75	153
Oct. 1	7-1/4	102.2	6,048	15,400	69	94
7	6-3/4	100.0	6,018	14,700	74	26
14	6-15/16	101.6	-	17,200	70	35
22	7	101.1	6,040	16,600	70	231

MONKEY NO. 3 (CONTROL) - (Cont'd)

D a t e	Weight Lbs.	Temp.F.	Erythrocytes	Leucocytes	Hb.(Dare)	Stippling
Oct. 29, 1926	7	102.0	6,430	15,300	75	-
Nov. 9	7	102.0	6,630	15,400	70	60
12	7	101.0	6,630	18,200	80	-
18	7	101.6	6,740	15,159	70	31

TABLE IX

Chart of Experimental Observations
MONKEY NO. 4

Date	Weight Lbs.	Temp.F.	Erythrocytes	Leucocytes	Hb.(Dare)	Stippling
Sept.17, 1925	-	-	5,872	17,250	70	-
25	-	-	5,432	15,050	85	0
Oct. 2	5-15/16	101.6	5,424	17,700	80	0
9	5-3/4	102.6	5,320	13,900	80	0
16	6-1/16	101.2	5,408	20,200	83	0
23	-	-	5,492	18,300	80	0
30	7	100.6	5,496	20,000	80	0
Nov. 5	Treatment with 4 cc. Ethyl Gasoline, daily, begun.					
7	7-2/16	101.6	5,496	16,500	85	0
14	6-3/4	100.8	5,424	15,150	83	0
21	6-15/16	100.0	5,360	16,350	85	0
28	7	100.7	5,528	21,950	86	0
Dec. 5	7-2/16	101.0	5,032	14,800	84	0
12	7-10/16	101.4	5,544	18,650	86	0
19	7-11/16	-	-	-	-	-
26	-	-	5,384	17,300	84	0
Jan. 2, 1926	7-13/16	101.3	5,304	15,800	80	0
9	7-14/16	-	5,360	15,450	85	0
16	8-3/16	101.8	4,568	14,800	80	0
23	8-3/16	101.8	5,088	10,200	80	0
30	8-3/4	101.1	5,288	10,400	80	0
Feb. 6	8-11/16	100.4	4,944	10,700	80	0
13	8-3/4	101.5	5,712	11,950	82	0

Treatment and handling discontinued because of pregnancy.

TABLE X

CHART OF EXPERIMENTAL OBSERVATIONS
MONKEY NO. 5

Date	Weight Lbs.	Temp.F.	Erythrocytes	Leucocytes	Hb.(Dare)	Stippling
Sept.17, 1925	-	-	5,312	14,100	87	0
25	-	-	5,440	19,900	87	0
Oct. 2	7	101.4	5,704	20,100	98	0
9	7-5/16	102.1	5,000	25,450	95	0
17	7-3/4	102.1	5,392	22,500	98	0
24	7-3/16	103.0	5,484	17,300	95	0
31	7-11/16	101.2	5,400	15,600	90	0
Nov. 7	7-11/16	102.6	5,379	13,800	90	0
14	7-10/16	102.6	5,280	16,000	95	0
21	7-14/16	120.7	5,648	22,600	95	0
28	7-15/16	102.4	5,136	18,850	86	0
Dec. 5	7-14/16	101.0	5,336	18,550	90	0
12	7-11/16	101.8	5,344	14,510	95	0
26	-	-	5,400	16,400	96	0
Jan. 2, 1926	8-6/16	102.3	5,400	12,450	98	0
9	8-1/2	-	5,944	18,800	95	0
16	8-1/16	101.4	5,432	13,950	95	0
23	-	-	5,294	16,300	90	0
30	8-1/16	102.0	5,384	18,550	92	0
Feb. 6	8	102.4	5,170	16,450	85	0
13	8-1/4	102.2	5,200	20,500	88	0
25	Treatments with 4 cc. Ethyl Gasoline, daily, begun.					
Mar. 8	8-2/16	101.4	-	-	-	-
13	8-1/2	102.3	-	-	-	-
20	8-7/16	102.0	-	-	-	-
29	8-2/16	102.1	-	-	-	-
Apr. 7	8-1/16	102.5	-	-	-	-
19	8-1/4	101.4	-	-	-	-
May 4	8	101.4	5,728	14,200	80	2
14	8-1/16	100.0	4,488	11,250	80	4
20	8	101.4	5,056	15,050	90	3
28	8-6/16	100.8	5,504	16,200	90	1
June 3	8-1/16	98.6	5,096	17,050	76	2
11	8-3/16	101.0	4,744	11,300	85	1
17	8-6/16	101.1	5,024	14,650	90	3
24	8-1/4	101	4,320	18,850	85	0
July 1	8-1/16	101.0	5,072	14,200	80	1
8	8-3/16	101.7	5,336	12,250	90	0
15	8-1/4	101.6	4,848	11,750	85	21
22	8-6/16	102.0	4,928	11,250	90	0
29	8-1/2	101.6	4,816	15,600	80	0
Aug. 5	8-1/4	102.0	4,848	17,100	80	0
13	8-6/16	101.8	4,976	12,500	80	12
21	8-6/16	101.4	5,856	17,250	94	0
27	8-6/16	101.2	6,184	19,550	92	6
Sept. 2	8-1/2	102.0	6,120	12,200	95	2
23	8-15/16	100.0	6,136	18,300	80	0

MONKEY NO. 5 - (Cont'd)

D a t e	Weight Lbs.	Temp.F.	Erythrocytes	Leucocytes	Hb.(Dare)	Stippling
Oct. 1 ,1926	8-13/16	102.0	5,816	14,050	80	0
7	8-10/16	101.0	5,768	18,350	80	0
14	8-15/16	101.4	-	16,800	85	5
22	8-10/16	101.4	6,369	15,050	85	2
29	8-11/16	101.5	6,060	13,050	85	8
Nov. 4	8-3/4	102.1	6,310	19,500	80	-
11	8-11/16	101.2	6,800	17,850	85	22
18	8-3/4	101.6	6,810	19,050	80	8

Experiment being continued at present.

A study of the above charts shows no evidence of the development of lead poisoning in these monkeys. The appearance of stippling in the blood of the animals, however, introduces at once the question of the absorption of small amounts of lead. Since the control monkey No. 3, shows as much as any test animal, it is fair to conclude either that the stippling is not due to lead, or that there is some source of lead absorption, other than that intentionally provided. Such a source has been found to exist, during a portion of the experimental period. Monkeys No. 1 and No. 2 were quartered in the Dayton Plant during the period from January 19, 1925 to April 2, 1925, and were exposed consequently to such amounts of lead as were found to produce some absorption in the other experimental animals during that time. After being transferred to Cincinnati, this source of lead was brought to an end. This, therefore, never influenced monkeys No. 3, 4, and 5. But another source of possible lead absorption arises out of the curiosity, destructive propensities and peculiar appetites of these animals. The monkeys have been housed at night in cages of concrete with metal doors. During the day time they have been allowed to run on the tops of their cages, being separated from each other as to prevent inhalation of vapors as a result of the treatments, and inhalation and ingestion of dusts from excreta, etc. The wall and ceiling of the room were of plaster covered with paint, and there were asbestos covered pipes running through. The animals played on the pipes and tore off the asbestos, and then began to tear off the plaster from the wall. It was noted after the lapse of some time that they were eating the plaster, and with it some of the paint, this being a lead paint. Since the means of housing them was the only one available, an attempt was made to prevent the eating of the plaster by providing clean earth for their consumption. This has served to prevent the destruction and consumption

of the wall, but not until a considerable quantity of lead covered plaster was consumed. This may account for the stippling in the blood of the control monkey.

So far as the testing of the possibility of lead poisoning from skin exposure to Ethyl Gasoline is concerned, the above variable only adds to the severity of the test conditions, and in as much as the experiment is being continued nothing of practical value has been lost. However, any analytical results which might be done at a later date in order to determine what quantity of lead may have been absorbed and stored by the monkeys under test conditions, will be valueless.

The experiment is being continued under conditions which do not allow of further extraneous lead consumption. The walls of the space in which the monkeys are permitted to run about is covered with a hard sheet asbestos.

Further observations are being carried on on all of the above monkeys. The young have been separated from their mothers, allowing for the further handling of both mothers and young. The young, too, are being used now for continuing the experiment. One of them, the offspring of Monkey No. 2, is being treated daily with 2 cc. Ethyl Gasoline, while the other, the offspring of Monkey No. 4, is remaining as a control.

II. On the Absorption of Tetraethyl Lead out of Gasoline:

The question of whether or not tetraethyl lead may be selectively absorbed out of gasoline, in the concentrations dealt with commercially, is an important one. It is known that both gasoline and tetraethyl lead may be absorbed by the skin. The question is whether there is a selective absorption of tetraethyl lead, i.e., whether in a system composed of normal skin, gasoline and $Pb(Et)_4$, the conditions favor a partition of tetraethyl in the skin, rather than in the gasoline. Upon this factor the possibility of lead poisoning from skin contact with Ethyl Gasoline may depend, because of the fact that excess gasoline which is spilled or otherwise spread upon the skin in commercial handling is likely to be wiped off. In this way most of the exposure to the lead would exist only during that period of time in which the skin is being bathed in the gasoline.

For this reason experiments were carried out in which rabbits were exposed to various concentrations of tetraethyl lead in gasoline by dipping the fore foot and leg in the leaded gasoline for half an hour at a time, over various periods of time. The animals were so treated three times weekly alternating the fore feet on successive days of exposure. Precautions as previously described were taken against allowing any Ethyl Gasoline to remain on the skin, and also against excessive removal of oil from the skin.

Control animals of two kinds were provided. One group were treated with commercial gasoline containing no tetraethyl lead, in exactly the same manner as the test animals were treated with Ethyl Gasoline. Another group received no treatment of any kind. All the animals were housed in the same manner, but separately, and all were fed and cared for in the same way.

The table below indicates the experimental procedure and the results.

TABLE XI

No. of Rabbit	Source	Sex	Treatment				Fate	Weight in Lbs.		Pt
			Type	Frequency	No.	Test Period		Initial	Final	
86	Old stock*	M	1/2 hr.1:10	Daily	3	3 days	Paralysis and Death	4-1/2	-	3(
87	same	M	same	Daily	3	3 days	same	4	-	N(
88	same	F	same	Daily	3	3 days	same	4-3/4	-	N(
89	same	M	1/2 hr.gasoline only	2 x wk.	29	5 months	Survived	4-1/16	5-7/16	
90	same	M	1/2 hr.1:100	Daily	6	6 days	Survived	4-1/2	4-1/2	
91	same	F	1/2 hr.1:100	2 x wk.	32	5 months	Survived	3-3/4	4-1/2	
92	same	M	1/2 hr.1:100	2 x wk.	28	5 months	Survivd**	4-13/16	5-7/16	
93	same	M	1/2 hr.gasoline only	2 x wk.	23	5 months	Survived	4-5/16	5-3/16	
94	same	M	1/2 hr.1:100	2 x wk.	16	2 months	Died ***	4-5/16	4-9/16	
95	same	M	1/2 hr.1:100	2 x wk.	26	5 months	Survived	4-3/16	3-10/16	
96	same	M	1/2 hr.1:100	2 x wk.	25	5 months	Survived	5-3/16	6-1/2	
98	same	M	1/2 hr.gasoline only	2 x wk.	28	5 months	Survived	3-3/16	3-3/4	
Control-1	same	M	None							
Control-2	same	M	None							
Control P_1	New stock	F	None							
100	same	M	1/2 Hr.1:1000	3 x wk.	89	8 months	Survived	4-3/4	4-1/6	
101	same	M	1/2 Hr.1:1000	3 x wk.	90	8 months	Survived	3-3/4	4-2/16	
102	same	M	1/2 Hr.1:1000	3 x wk.	89	8 months	Survived	5-1/2	5	

* "Old stock" refers to animals raised in our laboratory. "New Stock" to animals raised els

** This animal became paralyzed probably from injury on the animal board, near the end of th

*** Died 2 hours after last treatment. Cause not discovered.

From the above data several important conclusions may be drawn. It may be seen at once that tetraethyl lead may be absorbed from gasoline in immediately lethal quantities, provided the concentration of tetraethyl lead in the solution is sufficiently high. Rabbits Nos. 86, 87 and 88 exposed to a 1:10 dilution of tetraethyl lead in gasoline died after the third treatment, undoubtedly as a result of lead absorption, one of these animals containing 36.46 mgs. Pb in his carcass.

There is no evidence of a selective absorption, however, for if the concentration of tetraethyl lead is reduced to 1:100 in gasoline, the rate of absorption is even lower than the proportionality existing in the various concentrations. For example, Rabbit No. 90, exposed twice as long as Rabbit No. 86 to one tenth the concentration of tetraethyl lead in gasoline, contains only about one-tenth the quantity of lead, while Rabbits Nos. 100, 101 and 102 exposed thirty times as long to one hundredth of the concentration, show at most about one thirtieth the quantity of lead. In the case of the latter three animals, however, it must be pointed out at once, that there is no evidence that they have absorbed <u>any</u> quantity of lead, for they show no more lead in their carcasses than is found in untreated control from the same source. In fact, the same thing is true of the animals exposed to 1:100 tetraethyl lead in gasoline, with two exceptions, cf. Rabbit No. 90 and No. 94.

The conclusion of most importance to be drawn from the series of experiments is that rabbits do not accumulate lead in their bodies when exposed to tetraethyl lead in gasoline in concentrations which do not exceed one part in one thousand parts by volume in gasoline.

III. On the Inhalation of Tetraethyl Lead out of Gasoline:

Five full grown healthy rabbits were obtained for this experiment from stock animals reared outside the laboratory. One of these, No. 121, was kept in the laboratory during the entire course of the experiment, with no handling or treatment of any kind, as a control on the lead content of animals from the source. Two others, No. 119 and No. 120 were used for preliminary experimentation in order to determine what concentration of gasoline vapor could be borne by rabbits. They were exposed to gasoline only. No. 119 was kept as an additional control as to lead content, and No. 120 was used for other experiments. Rabbits No. 117 and No. 118 were used in the study of Ethyl Gasoline.

The animals were enclosed in a tight metal cage with a glass front, which was supplied with an inlet tube at the top, and an outlet tube at the top on the opposite side. They were kept there for six hours daily, during which time they received neither food nor water. At first two streams of air were supplied, one of which was bubbled through gasoline. These streams were measured by carefully calibrated flowmeters, and were mixed before being led into the cage by means of a "Y" tube. The quantity of air and gasoline vapor were regulated by adjustments of the flowmeters. Preliminary experiments determined that rabbits could endure without suffering more than a mild vertigo a mixture of air and gasoline vapor containing 4.5 liters of air, together with 2 liters of air saturated with high test gasoline vapor. At this rate 160 to 170 cc. of gasoline were vaporized out of the original 400 cc. in six hours. When a low test standard commercial gasoline was used, it was necessary to bubble the entire volume of air through the gasoline, in order to vaporize the same quantity in unit time. Accordingly the standard conditions for the experiment were

established by passing 5.6 liters of air per minute through the gasoline, beginning with 400 cc. and vaporizing from 160 to 200 cc. of the gasoline in the six hour period. (Some variation occurs because of variability of room temperature.) Under these conditions the animals occasionally showed some slight intoxication from the gasoline. The air volume was in considerable excess over their respiratory requirements. The experiment with Ethyl Gasoline was begun Sept. 2, 1925. The gasoline was prepared by adding 1.5 cc. of purified tetraethyl lead to 3785 cc. of the gasoline base. 400 cc. of this gasoline was introduced daily into a closed jar through which air was bubbled into the cage. At the end of each six hour period the residual gasoline was transferred into a gallon bottle, and notation was made of the quantity used. Two samples of the original gasoline were removed for analysis from the stock bottle each time the above quantity was prepared, and when the 3785 cc. were used up, two samples of the mixed residues were taken. Thus two analyses (as a check on each other) were made on the lead content of each quantity of stock gasoline, and on each batch of the residual gasoline from that stock. In this manner a check was maintained on the quantity of lead, if any, which actually passed over into the respiration cage. Analysis of the lead in the gasoline was made by a standard method suggested by Graham Edgar.

At the end of five months of exposure daily (except Sunday and holidays) Rabbit No. 118 began to show indication of injury from the gasoline. (Thus it may be seen that the animals were exposed continually to concentrations just bordering on immediate toxicity.) This animal lost weight and collapsed in the cage after a few hours of exposure on several successive days. He was killed, therefore, and analysis of his carcass was made in the usual way for lead. Rabbit No. 117 showed no evidence of injury, and was

continued under the same experimental conditions for three months longer. At this time, April 28, 1926, the experiment was concluded, and No. 117 was killed and analyzed.

The following tables illustrate the experimental results obtained. It is scarcely worth while to present all of the data on the daily evaporation of gasoline, and the analyses of all the samples, since they are essentially the same. For this reason the tables present sufficient data to illustrate the various points involved.

TABLE XII

Typical Experiment and Calculations

Date	Gasoline at start of experiment	Gasoline remaining after 6 hrs.	Pb.content per 100 cc. gasoline	
			Original	Residual
Dec.16	400	200 cc.		
17	"	215	0.0400	0.0754
18	"	215		
19	"	200		
21	"	300	0.0405	
29	"	315		
Jan.12	"	205		
13	"	200		
14	"	215	0.0408	
15	"	200		
18	"	215		
19	"	200		
25	"	200	0.0404	0.0748
26	"	205		
27	"	215		
Totals or Averages	6000	3300	0.0404	0.0751
Total lead at start (6000 x 0.0404)				2.424
Total lead at end (3300 x 0.0751)				2.478
Total lead in vaporized gasoline (calculated from above)				N i l

TABLE XIII

Typical Experiment and Calculations

Date	Gasoline at start of experiment	Gasoline remaining after 6 hrs.	Pb. content per 100 cc. gas	
			Original	Residual
Jan. 28	400 cc	200		
29	"	205		
30	"	270	.0400	0.0787
Feb. 1	"	210		
2	"	200		
3	"	200		
4	"	200		
5	"	200	.0402	
6	"	205		
8	"	200	.0408	
9	"	200		
10	"	220		0.0780
11	"	205		
12	"	205	.0404	
15	"	230		
16	"	220		
Total and averages	6400 cc	3370	0.0403	0.0783
Total lead at start (6400 x .0403)			2.58	
Total at end (3370 x .0783)			2.64	
Total lead vaporized with gasoline (calc. from above)			Nil	

TABLE XIV

Analytical Results on Animals

Animal No.	Treatment	Period of time	Weight Lbs.	Mgs. Pb in Carcass
121	None. Control	8 Mo.	6-3/4	2.60
119	Gasoline inhalation. Control.	8 Mo.	5-1/4	0.45
117	Ethyl Gasoline inhalation	8 mo.	6-1/2	0.77
118	Same	5 mo.	4-10/16	1.00

The temperature of the gasoline was taken by having a thermometer inserted into the jar through which the stream of air was passing. It was observed at the beginning of the experiment and at the end. Variations of from 21° C. to 30° C. were observed over the period of experimentation.

A study of the data above demonstrates in two ways that under the experimental conditions the animals were not exposed to inhalation of lead vaporized out of the gasoline. In the first case Tables XII and XIII, which are typical of the analytical results obtained throughout the experiment, show that all of the lead in the gasoline remained behind in the unvolatilized gasoline. Table XIV shows the same fact in a different manner, in that the test animals fail to show evidence of having absorbed more lead than may be found in control animals that have had no exposure to gasoline containing lead. On either of these bases it may be stated definitely that at the temperatures prevailing throughout the months of these experiments, no lead volatilizes out of gasoline when the concentration of tetraethyl lead in gasoline does not exceed 0.0787 grams per 100 cc. gasoline. This is equivalent to approximately 3 cc. of tetraethyl lead per gallon of gasoline.

CONCLUSIONS

In as much as each experimental result has been discussed in its proper place it is scarcely necessary to elaborate on discussion and conclusions. A consideration of the questions originally proposed for answer may be of some benefit, however.

1. It has been shown that under conditions which allow for spillage and evaporation of spilled gasoline leaving tetraethyl lead and dust of decomposition products of tetraethyl lead, and which provide for regular skin contact and some ingestion of lead, such experimental animals as guinea pigs and rabbits do not give evidence of accumulation of lead in their bodies. Further, these animals as well as monkeys, show no evidence

whatever of injury to any of the functions normal to such animals, including reproductive functions, as a result of such treatment.

It may be objected that the experimental conditions were not severe enough to duplicate the conditions of human exposure. It has been our purpose, however, to make the conditions more severe than those which occur in the handling of Ethyl Gasoline and in our judgment we have done so.

It is worthy of note that the above conclusions, as are the experimental data, are in complete contradiction of those of Frederick B. Flinn,* on the same problem.

2. Tetraethyl lead is absorbed out of gasoline in immediately toxic quantities when the concentration of tetraethyl lead in gasoline is of the order of magnitude of 1 part to 10 of gasoline.

3. In concentrations not exceeding 1 part of tetraethyl lead to 1000 parts gasoline, there is no evidence that rabbits absorb sufficient lead to cause accumulation of lead in their tissues. There is no selective absorption of the lead under these conditions.

4. Tetraethyl lead is not given off in the vapor of Ethyl gasoline at the temperatures normally prevailing during the seasons of the temperate zone. It cannot give rise to accumulation of lead in the tissues of persons who are exposed to volatilization of normally concentrated Ethyl gasoline.

Dated, November 18, 1926.

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* Frederick B. Flinn - Some of the Potential Health Hazards from the Use of Ethyl Gasoline - The Journal of Industrial Hygiene VIII - 51-56 Feb. 1926.