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A STUDY OF THE HAZARDS ASSOCIATED WITH THE SALE AND DISTRIBUTION OF ETHYL GASOLINE

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I Preliminary Discussion

1. The Source of Information

An investigation of the possible hazards arising by reason of the sale and distribution of Ethyl Gasoline is obviously made most readily and adequately through the observation of human beings who have been exposed to the conditions under which the hazards may arise, for a long period of time. The hazards have been maximal, not in the streets, nor in the ordinary filling stations where Ethyl Gasoline has been dispensed, but, rather in certain garages, where mechanics, car fillers, and drivers, taken collectively, have been exposed to all the hazards which have existed. For obvious reasons, the exposure to exhaust gas has been greater in the partially closed space of the garages. Likewise the inhalation of vapor of gasoline in the course of ordinary handling, and after spillage, reaches its maximum in such garages. Of the group of men who have been exposed, the garage mechanics have probably had the best opportunity for receiving injury, inasmuch as their varied work allows of a more frequent and more prolonged

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Note: The writers beg to express their appreciation of the assistance of numerous individuals in connection with different phases of the investigation. Mr. Lester Saunders, of the University of Cincinnati, assisted with the clinical work. The analytical work was carried out by Mr. Fred Thamann, who was largely responsible for perfecting the method of analysis, and by Mr. D.M. Philippi. Other members of the staff of the Ethyl Gasoline Corporation assisted the work in many ways. The officials of the Dayton Power and Light Company did everything in their power to facilitate the investigation.

exposure to all of the hazards, than that of any other group of workmen. The above facts lead one to the conclusion that a proper investigation of the problem would be made best by studying a considerable number of mechanics, car fillers, and drivers, in some garage in which a large number of cars, all of which burn Ethyl Gasoline, have been in continual use for a long time.

2. The Type of Information.

The information to be sought, in the study of such a group of men, should be obtained by the determination of all variations from normal health, including those which are specifically due to lead poisoning, or are commonly associated with it, as well as other less definite, recognizable abnormalities, which might be produced by any injurious agent. The importance of the latter resides in the fact that there are few, if any, abnormalities which may be said to be produced by lead poisoning alone, while there are a great number and variety of injuries which may be brought about by general poisons, including lead. Information in the matter should be obtained by careful questioning, of each subject as to the exact nature and period of his exposure to lead in any form, as to any previous or present exposure to other poisons, and as to any previous or present illness or symptom. The subject should be given a very careful examination, by methods, which will determine as nearly as possible, the exact status of his present health. The question of lead absorption should be investigated by the examination of adequate quantities of the excreta.

3. The Interpretation of Information.

Since there are always variations from the normal of perfect health in any considerable number of men, it is absolutely necessary to collect data from a group of men who have had no opportunity for exposure to the hazards, the effects of which are being studied. These should be collected in the same manner, and by

the same persons who examine the exposed group. Also, since there is no considerable body of data concerning the quantity of lead which may be excreted by persons who have not been exposed to the hazards under study, it is necessary to obtain such data. The same method of analysis must be employed in the study of the excreta of exposed and unexposed groups, in order to control the variation in the sensitivity of the various methods of lead analysis, and thus obtain comparable results.

II Methods of Procedure.

1. Subjects for Observation.

The garage of the Dayton Power and Light Company of Dayton, Ohio was chosen as the site for observations on exposed men. In this garage Ethyl Gasoline has been used in all the cars and trucks, about 80 in number, since the first distribution of Ethyl Gasoline began in April, 1923. The garage is a well lighted, average well ventilated, concrete garage, the floor of which is cleansed by means of running water, at infrequent intervals. There are five mechanics, two car fillers and a large number of drivers. The drivers do no filling or repair work. The labor turnover, is very slight, indeed, so that practically all the employees have been under exposure during the entire period in which Ethyl Gasoline has been used. Several of the subjects (noted in data) under study have been exposed to Concentrated Ethyl Fluid, during a portion of the time, by reason of the early method of distribution in small containers, so that these have had much more exposure to lead than is normal under present conditions of distribution. No unusual precautions of any kind are taken against lead absorption. In fact there is no fear in the mind of management or employees, of any danger from this source. Cars are coming and going during the entire day and some at night. All types of repair work except especially difficult items, are performed by the mechanics. All cars are filled with Ethyl Gasoline inside the garage, by means of portable filling tanks and pumps. Spillage of gasoline

on the floor may be seen by any casual observer from time to time. When this occurs the gasoline is wiped up with a cloth by a bare-handed operator, who usually washes neither the cloth nor his hands thereafter.

Control subjects were obtained by going to Columbus, Ohio, a city in which Ethyl Gasoline had never been distributed at the time of the investigation. An attempt was made to find a garage similar to that of the Dayton Power and Light Company. Since that was found to be impossible, an arrangement was made with an employment agency for obtaining white men of all types, just as they appeared at the agency. They were employed by the day and released at night during the period of the observations. Among these were farmers, common laborers, skilled workmen, sailors, chauffeurs, and clerks. Most of them were in youthful or middle life.

2. Type of Data Obtained.

All subjects were questioned carefully as to their occupation over a period of not less than five years previously, with special reference to exposure to any type of lead. Exposed men were asked as to the details of their daily work during the time of their exposure. Control subjects were asked leading questions as to their having been employed in industries known to involve contact with lead. A list of industries known to use lead to a greater or less extent was prepared and used in such questioning. When the appended data show that a given subject has no history of exposure to lead, it is to be remembered that every effort was made to discover some possible source of lead exposure in this case.

A history of previous illnesses was recorded for every subject, in order to note any relationships which might exist between such illness and present health. At the same time careful questions sought out the presence of any present symptoms of illness.

A physical examination was made by an examiner (Dr. Kehoe examined all

subjects) who had no knowledge of the contents of the history. In every case an analysis of the urine, the determination of the haemoglobin of the blood, and a microscopic examination of a stained blood smear were made, in order to determine the presence of abnormalities.

Appended hereto are typical histories and reports of examination of one control and one exposed subject, upon the blanks used in the investigation.

Each subject was furnished with a pint size Mason fruit jar, and a gallon size glass bottle such as is used for vinegar, molasses, etc. The containers were chemically clean and were kept tightly closed. The subject was carefully instructed to put the excreta directly into the container, and under no conditions to use any intermediate container. The specimens of the exposed men were obtained under the supervision of the foreman. Those at Columbus were obtained in a room rented for the purpose, during the course of two or three eight hour days. The containers were not permitted to leave the room, but they and the subjects were kept under observation during the periods of collection. Close observation and rigid discipline were necessary in this group to prevent deception, errors, and practical jokes, which might result in loss or mixture of specimens. One or two incidents occurred which necessitated the omission of the specimen obtained, from examination. (One man was found to be adding water from a bottle which he carried with him.) In the case of each subject a fair sized specimen of faeces, and one to three liters of urine ware obtained. Diuretics and cathartics were not used.

The containers, which had been labeled previous to their use, with the name of the subject, and a number assigned to him by the examiner, were turned over to the analytical laboratory. The subjects were all unknown to the analysts, whose only information consisted of a name and a number on the container.

III Data.

The mass of data obtained in the histories and in the examination of the two groups of men is too unwieldy for full presentation. The tables below indicate the frequency of occurrence of certain variations from the normal in the two groups, from which comparison may be made of the two groups. The analytical findings are tabulated opposite the history of employment and exposure, where their significance may be adjudged to the extent to which that is possible. In Tables II, III, and IV, the expression"Questionable lead line" is apparently not a happily chosen one. It refers, however, to a bluish color of the gums, adjoining the teeth, which is seen frequently in pyorrhoea, but which, when there is a history of lead exposure, may sometimes be thought to be significant. It is sometimes, quite difficult to determine the presence or absence of the bluish or black precipitate which is formed in the gum margin when lead or any other metal which gives a black sulphide is being secreted. For that reason, the examiner made note of every bluish discoloration of the gums, even where he was certain that it was the result of circulatory abnormalities in the gum margin, and labeled it as questionable, or as simulating to some degree a "lead line".

It is hardly proper to make comparison of the analytical data until it is reduced to some standard. The specimens of faeces and urine are not only different in volume and weight, but they represent varying periods of time of secretion. It is not possible to collect specimens without these variations. It has been considered advisable on this account to make calculations of the lead on the basis of unit weights of faeces, and unit volumes of urine. Table V and VI show the results of such calculations for both groups of subjects.

HISTORY

No. 14 Name [redacted] Date May 8th Age 22

Employment for past 5 years:

Dayton Power and Light Co. entire time
Filling cars with gas and oil up to Jan., 1925. Rough repair work since, fender work, steering gear repair. Has torn down motors.

Period of handling Ethyl fluid: Exposure at intervals for more than year.
Period of handling Ethylized Gasoline: Exposure at times for year or more.
Period of handling both:
Nature of exposure: Spilled fluid and gasoline on hands at times
(a) Exhaust gas: When testing motors.
(b) Gasoline vapor: When testing cars, and checking up on gas.
(c) Spillage of Ethyl Fluid: Several times.
(d) Spillage of gasoline: Spilled in garage at times.
Previous Illness: Tonsilitis and common colds. Nothing more.
(a) Illness and disease in words of subject:

(b) Symptoms and duration: Common colds of short duration.

(c) Presumable diagnosis:

Symptoms of any type now present.

Eg. Periodic headache: None
Susceptibility of colds their duration: No.
Digestive disturbances: Never
Habits as to sleep: Sleeps very well
Dreaming: Has dreamed at times for years, with nightmare
Appetite: Good always
Polyuria: No
Nocturia: No
Itching or burning of skin: No
Changes of weight: Has gained since 1923. 6 lbs.
Cramps: Never
Weakness: No
Nervousness: No

Miscellaneous

No. 14 Name [redacted] Date May 8th

General Appearance Healthy and robust Temperature 98.8
Nourishment Well nourished Pulse 72
Blood pressure 120-70

Eyes - Reaction to Light Normal Accommodation Normal
Extrinsic Muscles O.K.
Vision Good
Pathological Conditions None

Nose - Abnormalities Obstruction on left
Inflamations Acute rhinitis. Common cold.

Mouth & Throat Irritated and red.
Teeth Fairly good
Phorrhoea Present Caries None Lead-Line None
Tonsils and Pharynx Red especially around pillars.

Ears - Audition Good
Abnormalities None
Adenopathy None
Skin Normal

Chest (positive findings only) None

Abdomen Normal

Pelvis Normal

Extremities Normal
Central Nervous System (Reflexes Normal
(Tremor None
(Abnormalities None

Urinalysis - Reaction to M.R. Alk.
Albumin Neg.
Sugar Neg.
Acetone Neg.

Blood Hb. 95 Dare Smears Normal

REMARKS: No suggestion of any lead poisoning.

HISTORY

No. 23 Name [redacted] Date May 7th Age 28

Employment for past 5 years:

Teamster 2 years 1920-22

Truck driver 3 years, 1922-1925

Odd jobs at times, as carpenter's helper, digging for plumbing jobs.

No actual plumbing.

Period of handling Ethyl Fluid None
Period of handling Ethylized Gasoline None
Period of handling both None
Nature of exposure None
(a) Exhaust gas
(b) Gasoline vapor
(c) Spillage of Ethyl Fluid
(d) Spillage of gasoline

Previous illness Measles as child. Influenza 1922
(a) Illness and disease in words of subject
Pleurisy after "flu" for about one week. Pains around heart at times for years. Catarrh since flu.
(b) Symptoms and duration
Pains were sharpest in breathing.
(c) Presumable diagnosis Post influenzal pleurisy.
Probably adhesions remaining.

Symptoms of any type now present

Eg. Periodic headache No
Susceptibility of colds their duration Frequent colds
Digestive disturbances Cramps in stomach several weeks 1923
Habits as to sleep Restless every night for 3-4 years
Dreaming Scary draams 2 X weekly
Appetite Good
Polyuria No
Nocturia At times. Not frequent
Itching or burning of skin None
Changes of weight Very slight for years
Cramps In stomach after meals at times
Weakness Tired most of time
Nervousness At nights so as not to sleep well.

Miscellaneous

No. 23 Name [redacted] Date May 7th

General Appearance Healthy dark eyes and hair Temperature 97.8
Nourishment Medium Pulse 72
Blood Pressure 122-70

Eyes - Reaction to Light Normal Accommodation Normal
Extrinsic Muscles O.K.
Vision Good
Pathological Conditions None

Nose - Abnormalities Slight obstruction due to deflected septum.
Inflamations None

Mouth & Throat Mucous membranes pale
Teeth Fairly good
Phorrhoea Present - slight Caries None Lead-line Typical on incisors above
Tonsils and Pharynx Red. Buried. Cryptic.

Ears - Audition Good
Abnormalities None

Adenopathy None
Skin Scattered acne lesions on back and face.

Chest (positive findings only) None

Abdomen None

Pelvis None

Extremities O.K.
Central Nervous System (Reflexes Normal
(Tremor None
(Abnormalities None

Urinalysis - Reaction to M.R. Alk.
Albumin Neg.
Sugar Neg.
Acetone Net.

Blood Hb. 80 Smears

REMARKS: Blood smears show considerable numbers of polychromatophilic Erythrocytes but no stippling.

TABLE I

SYMPTONS OF ILLNESS PRESENT IN EXPOSED AND UNEXPOSED MEN

SYMPTON	Frequency of Occurrence		Percentage Occurrence	
	Exposed 22 subjects	Unexposed 64 subjects	Exposed	Unexposed
Periodic Headache	1	18	4.5	28
Susceptibility to colds	1	16	4.5	25
Chronic Constipation	2	10	9	15.6
Gastric Cramps	1[3]	11	4.5	17.2
Insomnia	0	5	0	7.8
Disturbing Dreams	1[1]	5	4.5	7.8
Poor Appetite	0	1	0	1.6
Loss of Weight	0	5	0	7.8
Polyuria	0	3	0	4.7
Nocturia	0	4	0	6.2
Fatigue	0	5	0	7.8
Nervous Irritability	1[2]	1	4.5	1.6

[1] This man has had occasional nightmare for years.

[2] This man was a victim of shell-shock in the war.

[3] This man has had "dyspepsia" for many years.

TABLE II

PHYSICAL VARIATIONS FROM THE NORMAL IN EXPOSED AND UNEXPOSED MEN

ABNORMALITY	Frequency of Occurrence		Percentage Occurrence	
	Exposed 22 Subjects	Unexposed 64 subjects	Exposed	Unexposed
Subnormal Temperature	3	29	13.6	45.3
Elevated Temperature	4	5	18	7.8
Subnormal Bl. Pressure	6	19	27	30
Elevated Bl. Pressure	2	12	9	18.6
Pyorrhoea Alveolaris	13	47	60	73
Severe Gingivitis	6	43	27	67
Albuminuria	1	5	4.5	7.8
Glycosuria	0	1	0	1.6
Questionable Lead Line	3	15	29	23.4
Definite Lead Line	0	2	0	3.2
Polychrom-atolphilia	1	3	4.5	4.7
Stippling	0	4	0	6.2

Under subnormal temperature everything under 98 degrees is noted.
Under Elevated temperature everything above 98.8.
Subnormal blood pressure refers to everything below 115 Systolic.
Elevated blood pressure refers to everything above 140 Systolic.
Under "Questionable lead line" is listed every bluish discoloration of the gums which simulates in any way a lead line. Such discolorations are common in mouths which show considerable amounts of gum inflammation. A definitely recognizable lead line is something about which there is no question. The questionable ones are noted because of the desire to omit no lesion about which there could be any question.
Polychromatophilia refers to a slight abnormality in the staining of red blood corpuscles, the significance of which is unknown.
Stippling is noted in any case in which a single stippled red blood corpuscle may be found in an entire smear, after staining by a standard, well controlled method. In one case four stippled cells could be found in 100 fields. In no others was more than one found.

TABLE III

HISTORY OF EXPOSURE, FINDINGS AND ANALYTICAL DATA
DAYTON POWER AND LIGHT SUBJECTS

Number of Subject	Period of Exposure	Occupation in Garage	Nature of Exposure	Findings	Lead Faec
1	2 years	Blender Filler	Ethyl Fluid Gasoline Exhaust	97.6 Temperature 114-70 Bl. Pressure Nothing else.	0
2	2 years	Driver Part time filler	Ethyl Fluid Gasoline Exhaust	None	0
3	2 years	Driver Part time filler	Gasoline Exhaust	97.6 Temp. Nothing questionable -(else lead line	0
4	2 years	Driver Part time filler	Gasoline Exhaust	None	0
5	18 mo.	Driver	Exhaust	97.6 Temperature None other	0
6	2 years	Driver	Exhaust	Questionable Lead line	0
7	2 years	Driver	Exhaust	112-60 Bl. Pressure	0
8	2 years	Driver	Exhaust	110-74 Bl. Pressure	0
9	3 mos.	Driver (in school previously)	Exhaust	112-74 Bl. Pressure	0
10	2 years	Driver	Exhaust	None	0

5	18 mo.	Driver	Exhaust	97.6 Temperature None other	0
6	2 years	Driver	Exhaust	Questionable Lead line	0
7	2 years	Driver	Exhaust	112-60 Bl. Pressure	0
8	2 years	Driver	Exhaust	110-74 Bl. Pressure	0
9	3 mos.	Driver (in school previously)	Exhaust	112-74 Bl. Pressure	0
10	2 years	Driver	Exhaust	None	0
11	2 years	Driver	Exhaust	Questionable Lead line	0
12	2 years	Driver	Exhaust	None	0
13	2 years	Mechanic Blender	Ethyl Fluid Gasoline Exhaust	None	0
14	2 years	Mechanic Blender Filler	Ethyl Fluid Gasoline Exhaust	None	1
15	2 years	Mechanic Filler	Ethyl Fluid Gasoline Exhaust	None	0
16	2 years	Mechanic	Ethyl Fluid Gasoline Exhaust	Polychromatophilia	0
17	2 years	Foreman Blender	Ethyl Fluid Gasoline Exhaust	None	0
18	2 years	Driver	Exhaust	106-66 Bl. Pressure Healed Tubc.	
85	2 years	Driver	Exhaust	97.2 Temperature 104-64 Bl. Pressure	0
86	2 years	Driver	Exhaust	None	0
87	1 year	Driver	Exhaust	None	0

TABLE IV

HISTORY OF EXPOSURE, FINDINGS, AND ANALYTICAL DATA OF CONTROLS

Number of Subject	History of Lead Exposure	Findings
19	No history of exposure	None
20	Occasional odd jobs of painting	Questionable lead line
21	1-1/2 years pipe joining with lead	None
22	Enamel mfg. 1 yr. Occasional paint.	None
23	No history of exposure	Typical lead line 97.8 Temperature
24	Occasional painting at home	104-64 Bl. Pr. 97.8 Temp.
25	Trucking metal. lead. Steam fitting.	97.8 Temperature
26	Occasional odd job of painting	None
27	Coal miner. No other history.	None
28	No history of exposure	112-80 Bl. pressure
29	Marble worker. Occasional painting	None
30	No history of exposure	112-70 Bl. Pr.
31	Odd jobs painting and plumbing occasionally	Questionable lead line Definite stippling 1-100
32	Occasional painting.	110-80 Bl. Pressure
33	Painting 5 mo. recently	114-72 Bl. Pressure
34	Plumber's helper occasionally	110-76 Bl. Pr. 97.2 Temp.
35	No history obtained.	No examination made
36	No history of exposure	97.4 Temp.
37	Plumber's helper 1923	Stippling 1-100
38	Painter 3 years	102-60 Bl. Pr.
39	Painting 3 mo. 1925	Questionable lead line. 97.2 Temp.
40	Farmer. No hist. of exposure	97.2 Temp, 100-60 Bl. Pr.
41	Mixed paint 3 mo. 1924	96.6 Temp.
42	No history of exposure	104-60 Bl. pressure Questionable lead line
43	Engine Painter 3 years	97.4 Temperature
44	Vulcanized tires 1923-1924	110-70 Bl. pressure
45	Painting 6 mo. 1924	96.4 Temp. 112-70 Bl. Pressure
46	Plumber and pipe fitter	96.0 Temperature
47	Occasional job painting	97.6 Temp. 114-74 Bl. Pressure Questionable lead line
48	Plumber	Questionable lead line
49	Released because of deception before being examined.	Samples discarded.
50	No history of exposure	97.0 Temp. 102-60 Bl. Pr.
51	No history of exposure	97.6 Temp. 112-60 Bl. Pr. Questionable lead line
52	Occasional odd job painting	110-70 Bl. Pressure
53	No history of exposure	Questionable lead line

47	Occasional job painting	97.6 Temp. 114-74 Bl. Pressure Questionable lead line	C
48	Plumber	Questionable lead line	C
49	Released because of deception before being examined.	Samples discarded.	
50	No history of exposure	97.0 Temp. 102-60 Bl. Pr.	C
51	No history of exposure	97.6 Temp. 112-60 Bl. Pr. Questionable lead line	C
52	Occasional odd job painting	110-70 Bl. Pressure	C
53	No history of exposure	Questionable lead line	C
54	No history of exposure	None	C
55	Plumber 2 years	None	C
56	Painting 2 wks. recently	97.8 Temp.	C
57	No history of exposure	None	C
58	Occasional odd jobs painting Short periods as plumber's helper	Questionable lead line	C
59	Painter	None	No
60	No history of exposure	Questionable lead line Stippling 1-100	C
61	Tinner and roofer's helper 1923	97.8 Temperature	C
62	No history of exposure	97.0 Temperature	C
63	No history of exposure	100-80 Bl. Pressure	C
64	Painter for 3 years before 1920	97.6 Temperature	C
65	Plumber's helper 4 mo. 1923 Storage battery mfg. 4 mo. 1924	97.8 Temperature	C
66	No history of exposure	97.4 Temperature	Sa
67	No history of exposure	97.4 Temperature, Questionable lead li	
68	No history of exposure	None	C
69	No history of exposure	97.4 Temperature Questionable lead line	C
70	Short period painting 1923 In tire factory 1921	None	C
71	Occasional odd job painting	97.0 Temperature 112-72 Bl pressure	C
72	No history of exposure	97.6 Temperature	C
73	No history of exposure	97.8 Temperature	C
74	Plumber's helper and painter	None	C
75	Street Cleaner	96 Temperature	C
76	No history of exposure	114-78 Bl. Pressure Questionable lead line	C
77	No history of exposure	97.8 Temp. 100-60 Bl. pressure Questionable lead line	C
78	No history of exposure	None	C
79	No history of exposure	Questionable lead line	C
80	Plumber's helper 3 mo.	None	C
81	No history of exposure	97 Temperature	C
82	Tire mfg. 6 mo. 1924	97.4 Temp. Slight anemia. Tbc. stippling 4-100	C
83	Plumber's helper 3 mo. 1925	97 Temp. Stippling 2-100	C

METHOD OF ANALYSIS

Treatment of Samples

(1) The samples of urine were received by the laboratory in gallon jugs of the type used for water, fruit juices, etc. The volume was measured and the samples made ammoniacal soon after they were received. The details of analytical procedure are given below.

(2) The samples of faeces were received in pint fruit jars. They were transferred to silica dishes and dried on a steam plate to "apparent dryness". No attempt was made to bring them exactly to constant weight.

Urine: Preparation of Solution for Analysis

(3) The ammoniacal urine, after standing for at least 12 hours, is filtered on 18 cm. folder filter paper, care being taken that all of the precipitate is transferred to the filter. Without being washed, the filter paper is transferred to a silica or porcelain dish, and after being dried on a steam plate, is ignited in an electric muffle furnace at a temperature not exceeding 600 degrees C. When the organic matter has been completely destroyed, the residue is treated as follows: The ash is moistened carefully with distilled water, and 5 cc of conc. HCl is added, together with enough water to make the volume about 25 cc. The mixture is digested on a steam plate, and is filtered into a 400 cc beaker, any residue being washed six times alternately with hot 10% HCl, and hot water. The filtrate in the beaker should contain all the lead. The residue is usually insignificant, and in these experiments have been disregarded.

Analysis of Solution

(4) The solution from (3) is neutralized by adding 25% NaOH solution until a faint turbidity persists. If too much sodium hydroxide is added, the solution should be treated with hydrochloric acid again until it is perfectly clear and sodium hydroxide again added until a faint turbidity appears. The solution is diluted

to 300 cc and after cooling, if necessary, is treated with hydrogen sulfied gas for one hour, and allowed to stand over night. The mixture is filtered on a 12.5 cm. Whatman No. 40 filter paper, any precipitate being transferred to the paper by means of freshly prepared hydrogen sulfied water acidified with 1 cc. of conc. HCl per liter. The paper and its contents are transferred to the beaker in which the sulfied precipitation was made. The sides of the beaker are washed down with 25 cc of warm (1-1) hydrochloric acid containing 10 drops of conc. HNO_3. The mixture is digested with the acid until all sulfides have been dissolved and the paper is thoroughly white. The solution is diluted to 50 cc with hot water, and is filtered, the residue being washed 15 times with hot water. To the filtrate, which should be about 200 cc add 4 drops of a solution of methyl red, make just alkaline with 25% NaOH, then just acid with (1:2) hydrochloric acid, and add 1 cc of (1:2) hydrochloric acid in excess. Dilute to 300 cc and after cooling treat with hydrogen sulfied gas for one hour and allow to stand over night. The mixture is filtered on a 12.5 cen. Whatman No. 40 filter paper, the precipitate beingtransferred to the paper by means of freshly prepared hydrogen sulfied water containing 1 cc of conc. HCl per liter. The beaker is washed carefully with this wash water and the filter is washed ten times.

The sulfides are dissolved from the paper with 10-20 cc of hot (1:1) nitric acid, the solution being caught in the beaker in which the precipitation was made. The paper is washed 15 times with hot water. The sides of the beaker, and the inside and outside of the tube from the hydrogen sulfied generator are washed with hot (1:1) nitric acid. The solution is evaporated to 5 cc diluted to 25 cc with hot water, and filtered through a 7 cm filter paper into a 150 cc beaker. The beaker is carefully washed with hot water and the filter paper is washed 15 times with hot water. The filtrate and washings are evaporated to 25 cc.

and neutralized with 25% NaOH, free from iron and aluminum, a slightly alkaline water solution of phenolphthalain being used as indicator. The solution is made faintly pink with alkali, the color is just discharged with 5% acetic acid, and 2 cc of 5% acetic acid is added in excess. The solution is brought to boiling and 1 cc of 1% potassium chromate solution is added. The mixture is placed on the steam bath for one hour, and is allowed to stand in a warm place over night. The mixture is then brought to boiling, filtered on a 7 cm. ashless filter paper, the beaker being washed carefully with hot water, and the filter paper being washed 15 times with hot water. Any residue is dissolved in 5 to 15 cc of cold (1:2) hydrochloric acid, the paper being washed at once with cold water, the solution and washings being caught in the beaker in which the chromate precipitation was made. The sides of the beaker and the stirring rod are washed with cold (1:2) hydrochloric acid.

Colorimetric Estimation of Lead

(5) A standard solution of lead (1 cc = 0.10 mg. lead) is made by dissolving 0.1560 grams of pure lead chromate in 200 cc of 10% HCl and diluting to one liter. This solution may be checked colorimetrically against standard potassium chromate solution. A one per cent solution of C.P. s-diphenyl carbazide in glacial acetic acid is used as reagent.

For the analysis an aliquot portion of the solution of lead chromate obtained from (4) is taken, which should contain not more than 0.40 to 0.50 mg. of lead, the best results being obtained with about 0.20 mg.

The sample is diluted to about 100 cc in a Nessler tube, and an equal volume of water put in another Nessler tube. 2 cc of diphenylcarbazide reagent is added to each tube. From the color which develops in the first tube a rough visual estimate is made of the quantity of lead present and an amount of standard lead

chromate slightly smaller than this is added to the blank tube. The colors of the two solutions are compared, and standard lead chromate is added drop by drop to the standard tube until an exact match is obtained.

Feces

(6) The sample of dried feces is ashed in a silica dish in an electric muffle at 500°C-600°C. When the organic matter is apparently destroyed the residue is moistened with water, acidified with HCl, and digested on the steam plate for some time. The mixture is then filtered, the residue washed with hot dilute HCl and hot water, and the filter paper and its contents returned to the silica dish in which the original ashing was done. After being dried on the steam plate the dish and its contents are returned to the furnace and ashed again at 500°C-600°C. The residue is treated with water and HCl as before, and after being digested on the steam plate, is filtered, the filtrate being added to the first filtrate. The residue may contain traces of lead, and may be reashed and brought into solution with HF, and added to the main solution. The lead in the residue is, however, always in our experience a very small fraction of the total lead present, and it seemed justifiable to neglect it in this particular case.

(7) The solution obtained from (6) is diluted and neutralized with 25% NaOh, exactly as described in (4) above. The remainder of the analysis is exactly like that described under (4) and (5), except that the second hydrogen sulfide precipitation is repeated, the third precipitation being made exactly as the second is made. The precipitation as chromate and the colorimetric estimation are made just as in (4) and (5).

Notes

(a) Reagents and Apparatus

Lead free reagents were employed throughout. It is possible to purchase them in the market, but they must be carefully tested for lead.

(b) Pyrex glass beakers, flasks, wash bottles, etc. were employed throughout. Soda glass funnels were employed. The dishes used for ashing the feces were 400 cc opaque silica; those used for ashing the urines were of opaque silica or porcelain.

(c) General

The ordinary technique of good analytical work must be observed most scrupulously if satisfactory results are to be obtained in analyzing organic material for small quantities of lead. Precautions as to the cleanness of the laboratory and of the apparatus are essential. In the present case no other work than the analyses in question was carried out in the laboratory employed for all work but the preparation of the samples.

(d) (1) It has apparently been shown that ammonia precipitates practically all of the lead from urine as lead phosphate, which is carried down with the other alkaline earth phosphates. The procedure is so much more convenient than that involving the evaporation of the urine, that it was employed throughout. If it is in error at all it is in the direction of giving slightly low results.

Ref. Dr. L.T. Fairhall, J. Biol. Chem. (60) 485-8 (1924). As little as .05 mg. of lead in 3500 cc of urine can be detected.

(e) (4) The exact details of the analysis are those decided upon after a careful study in this laboratory, and after a careful investigation of the literature. The essential features (with the exception of the colorimetric method) are those of Dr. L.T. Fairhall (J. Ind. Hyg. (4) 9-20 (1922), but the exact conditions have been modified in several respects which have given more satisfactory results than the original Fairhall method. Numerous tests of the method have been carried by working with lead free material and material to which known amounts of lead had been added. The following data are more or less characteristic:

To test the accuracy of the method for urine two composite samples of urine were collected from workers at the laboratory. One of these was divided into six portions of 1 liter each and varying quantities of lead were added to them by members of the research staff outside the analytical laboratory. The samples were then analyzed by the usual procedure. Sample No. 2 was divided into five portions and was analyzed after the addition of much smaller amounts of lead. The results follow:

	Lead Added	Lead Found
Sample No. 1	1.10 mg.	1.13 mg.
	7.00	7.47
	7.00	7.00
	11.00	11.00
	7.00	6.75
	11.00	11.00
Sample No. 2	None	0.10
	None	0.10
	0.01	0.08
	0.10	0.20
	0.10	0.20

To test the accuracy of the analytical method for feces and the possible influence of other metals, four samples were prepared as follows: 4 lbs. of beef was obtained bone and fat separated and the remainder divided into four 400 g. portions. All of these were ashed in silica dishes with or without the addition of known quantities of lead and other metals, the procedure being that given above for the analysis of feces. Several filter papers were also added before ashing. The results follow:

Sample	Lead Added	Lead Found
No. 1	None	.07 mg.
No. 2	1 mg.	0.73 mg.
No. 3	None	0.08 mg.
No. 4	1 mg.	0.73 mg.

Samples Nos. 3 and 4 had 1 mg. each of cadmium, bismuth, mercury, copper, arsenic, antimony, and tin added before ignition.

The actual quantity of material in these samples is much larger than the samples of feces and required considerably larger amounts of reagents throughout.

As a check on the accuracy of the colorimetric method, samples containing various quantities of lead chromate were analyzed by the colorimetric procedure.

Lead Added	Lead Found
.38	.38
.63	.60
.03	.03
.02	.02
.95	.93
.02	.02
.07	.06
.30	.30
.56	.56

Note the following: All indications are that the experimental method for lead gives results which are, if anything, slightly too low. Considerable study has been made of the losses of lead which may occur at different steps. Thus the neglect of the insoluble residue may introduce slight losses, which are apt to be greater the greater the amount of insoluble residue. Furthermore the study of the precipitation of lead as chromate under the conditions followed showed that a slight loss amounting to perhaps a few hundredths of a milligram of lead is apt to occur, although the loss is considerably less than that observed when following the procedure of Fairhall (Loc. Cit.).

The data on the samples of urine and feces from Dayton and Columbus follow. In the attached blue print these data have been plotted to show the general trend of the results. The interpretation of these data is discussed below. Since the samples of feces could not be regarded as definite 24 hour samples it has seemed preferable to plot the lead per 100 grams of dried feces rather than the lead in the individual sample. The urine probably represents a more nearly true 24 hour sample but for the sake of uniformity the results have been plotted in milligrams of lead per liter or urine.

Sample Number	Origin of Sample	Analysis Number	Lead mgs.	Lead mgs. per 100 gms. dry feces	Dry weight gms.	Analysis Number	Lead mg.	Lead mgs per liter	Volume in cc.	
1	[redacted]	127	0.50	1.83	27.4	113	0.14	0.20	700	
2	[redacted]	138	0.30	0.97	31.1	123	0.12	0.08	1450	
3	[redacted]	128	0.32	1.52	21.1	114	0.17	0.14	1200	
4	[redacted]	129	0.11	0.99	11.1	115	0.22	0.07	3000	
5	[redacted]	188	0.45	1.49	30.3	234	0.08	0.10	820	
6	[redacted]	130	0.14	3.11	4.5	116	0.12	0.25	470	
7	[redacted]	139	0.33	1.05	31.5	125	0.15	0.12	1270	
8	[redacted]	131	0.65	1.62	40.2	117	0.17	0.14	1200	
9	[redacted]	189	0.27	1.62	16.7	235	0.12	0.05	2450	
10	[redacted]	140	0.42	1.73	25.4	126	0.09	0.06	1500	
11	[redacted]	132	0.32	1.08	29.8	118	0.08	0.05	1500	
12	[redacted]	141	0.27	3.70	7.3	124	Nil	Nil	750	
13	[redacted]	133	0.30	3.85	7.8	119	0.10	0.07	1400	
14	[redacted]	134	1.91	1.63	117.3	120	0.14	0.05	2900	
15	[redacted]	135	0.29	0.88	33.0	112	0.56	0.40	1350	
16	[redacted]	136	0.51	1.44	35.5	121	0.15	0.11	1350	
17	[redacted]	137	0.65	1.70	38.3	122	0.12	0.08	1530	
85	[redacted]	284	0.73	1.04	69.9	237	0.25	0.11	2220	
86	[redacted]	283	0.25	1.95	12.8	241	0.25	0.12	2100	
87	[redacted]	285	0.46	0.87	52.8	242	0.23	0.11	2100	
88	[redacted]	282	0.27	1.63	15.3	236	0.12	0.04	2920	

SAMPLES FROM COLUMBUS, OHIO

Sample Number	Origin of Sample	FECES				URINE			
		Analysis Number	Lead mgs.	Lead mgs. per 100 gms. dry feces	Dry weight gms.	Analysis Number	Lead mgs.	Lead mgs. per liter	Volume in cc.
19	[redacted]	267	0.16	2.26	7.1	220	0.17	0.09	1820
20	[redacted]	198	0.53	2.22	23.9	231	0.24	0.16	1460
21	[redacted]	258	0.35	0.92	38.0	227	0.06	0.04	1460
22	[redacted]	269	0.27	2.88	9.4	208	0.16	0.10	1640
23	[redacted]	275	0.37	1.23	30.0	207	0.10	0.05	2160
24	[redacted]	279	0.15	1.25	12.0	214	0.21	0.08	2580
25	[redacted]	286	0.45	0.93	48.5	180	0.13	0.22	600
26	[redacted]	287	0.17	1.95	8.7	202	0.12	0.08	1520
27	[redacted]	182	0.35	6.73	5.2	161	Nil	Nil	2000
28	[redacted]	265	0.33	1.35	24.5	156	0.02	0.01	2620
29	[redacted]	185	0.74	3.27	22.7	155	0.14	0.06	2200
30	[redacted]	281	0.23	1.17	19.7	152	0.12	0.07	1760
31	[redacted]	266	0.68	3.13	21.7	230	0.16	0.13	1200
32	[redacted]	193	0.62	0.75	82.8	229	0.14	0.10	1460
33	[redacted]	280	0.05	0.43	11.6	225	0.09	0.05	1940
34	[redacted]	277	0.32	1.05	30.5	233	0.07	0.03	2220
35	[redacted]		No sample			239	0.08	0.17	470
36	[redacted]	191	0.14	0.35	40.6	232	0.10	0.07	1460
37	[redacted]	181	0.06	1.58	3.8	178	0.13	0.07	1880
38	[redacted]	278	0.38	2.30	16.5	206	0.03	0.02	1760
39	[redacted]	194	0.24	0.39	61.5	153	0.04	0.02	1830
40	[redacted]	268	0.24	1.07	22.5	203	0.15	0.11	1360

SAMPLES FROM COLUMBUS, Cont'd.

Sample Number	Origin of Sample	FECES				URINE			
		Analysis Number	Lead mgs.	Lead mgs. per 100 gms. dry feces	Dry weight gms.	Analysis Number	Lead mgs.	Lead mgs. per liter	Volume in cc.
41	[redacted]	190	0.30	1.68	17.9	150	Nil	Nil	1220
42	[redacted]	276	0.28	1.35	20.7	159	0.04	0.02	1830
43	[redacted]	195	0.30	1.42	21.2	154	0.05	0.02	2230
44	[redacted]	251	0.30	3.30	9.1	170	0.07	0.06	1180
45	[redacted]	184	0.41	1.59	25.8	209	0.16	0.10	1520
46	[redacted]	No Sample				179	0.17	0.11	1520
47	[redacted]	199	0.18	0.39	46.5	238 212	0.20	0.07	2680
48	[redacted] J.	263	0.55	1.87	29.4	205	0.13	0.09	1400
50	[redacted]	255	0.38	0.88	43.0	169	0.13	0.09	1460
51	[redacted]	262	0.37	1.49	24.9	223	0.20	0.14	1460
52	[redacted]	270	0.55	1.35	40.6	228	0.06	0.04	1520
53	[redacted]	260	0.33	0.73	45.1	221	0.18	0.12	1520
54	[redacted]	197	0.42	0.45	92.9	226	0.13	0.09	1400
55	[redacted]	254	0.42	1.99	21.1	222	0.18	0.12	1520
56	[redacted]	257	0.53	2.69	19.7	224	0.08	0.05	1520
57	[redacted]	259	0.31	0.92	33.8	216	0.18	0.10	1760
58	[redacted]	264	0.34	1.89	18.0	219	0.14	0.16	870
59	[redacted]		No Sample			215	0.20	0.10	2140
60	[redacted]	261	0.21	1.54	13.6	213	0.13	0.06	2200
61	[redacted]	290	0.40	1.48	27.0	218	0.18	0.10	1760
62	[redacted]	288	0.33	0.72	45.9	217	0.15	0.09	1640
63	[redacted]	192	0.20	0.73	27.5	164	0.11	0.06	1750
64	[redacted]	256	0.37	2.03	18.2	165	0.04	0.02	1690

SAMPLES FROM COLUMBUS, Con'd.

Sample Number	Origin of Sample	FECES				URINE			
		Analysis Number	Lead mgs.	Lead mgs. per 100 gms. dry feces	Dry weight gms	Analysis Number	Lead mgs.	Lead mgs. per liter	Volume in cc
65	[redacted]	253	0.14	0.61	23.0	168	Nil	Nil	1640
66	[redacted]	272	Sample Lost		19.2	211	0.12	0.08	1760
67	[redacted]	289	0.24	0.81	29.6	162	Nil	Nil	2160
68	[redacted]	144	0.32	0.90	35.6	163	0.11	0.07	1650
69	[redacted]	252	0.60	0.80	74.7	167	0.05	0.03	1600
70	[redacted]	187	0.20	2.28	8.8	210	0.17	0.10	1640
71	[redacted]	273	0.24	1.97	12.2	166	0.11	0.06	1880
72	[redacted]	200	0.60	2.42	24.8	201	0.20	0.08	2530
73	[redacted]	146	0.43	0.42	103.4	175	0.30	0.23	1300
74	[redacted]	145	0.24	0.53	45.4	173	0.15	0.11	1300
75	[redacted]	196	0.23	0.33	70.2	151	0.12	0.09	1300
76	[redacted]	271	0.37	1.91	19.4	158	0.04	0.06	700
77	[redacted]	142	0.72	1.44	50.0	160	0.07	0.06	1180
78	[redacted]	186	0.16	1.36	11.8	157	0.15	0.08	1850
79	[redacted]	274	0.11	1.64	6.7	204	0.15	0.09	1760
80	[redacted]	143	0.22	1.20	18.3	172	0.10	0.08	1320
81	[redacted]	147	0.14	0.50	28.4	177	0.07	0.05	1400
82	[redacted]	183	0.18	2.31	7.8	171	0.05	0.03	1950
83	[redacted]	148	0.50	1.52	32.8	174	0.07	0.10	710
84	[redacted]	149	0.54	2.31	23.4	176	Nil	Nil	1170

We failed to find lead in analyses of Dayton and Columbus city waters.

VI CONCLUSIONS

A consideration of the data and the calculations, together with the study of the grouping of symptoms and clinical signs in individual subjects justifies and compels the following conclusions:

1. No individual in the two groups is suffering from lead poisoning.
2. All the individuals in the two groups have absorbed lead into their bodies from some source.
3. A group of twenty-two workmen who have been exposed to, apparently, the worst hazards associated with the sale, distribution and use of Ethyl gasoline, fails to show any indication of having been poisoned by lead. A comparison of their ailments of an indefinite type, which might conceivably be due to a greater opportunity for lead absorption, that is, normally, shows they are in better physical condition and that the occurrence of abnormalities among them is lower than among a controlled group of workmen taken at random through an employment agency at Columbus, Ohio.
4. It is perhaps to be expected that the group of twenty-two workmen regularly employed by the Dayton Power and Light Company should be normally in somewhat better physical condition than such a group as that obtained from the Columbus employment agency, but in any case, the data gives no indication whatever that the employees of the Dayton Power and Light Company have been in any way injured by their contact with tetra ethyl lead.
5. From the evidence presented herein it is not possible to state that these men so exposed to the hazards which some have claimed exist in connection with Ethyl gasoline have received any damage or have absorbed any lead as a result of their exposure to Ethyl gasoline. In fact, it seems quite probable, in view of the evidence, that the lead which they have absorbed has come from other sources.