

R E P O R T

AN INVESTIGATION OF THE POTENTIALITIES FOR THE ABSORPTION OF LEAD
BY THE USERS OF A LEAD-CONTAINING HAIR DYE

An Experiment Wherein Human Subjects
Applied a Representative Formulation
in Simulation of the Scheduled Use of
Presently Available Products, While Under
Periodic Surveillance of Their Absorption of Lead

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INTRODUCTION

A group of manufacturers and distributors of certain lead-containing hair dyes has found it necessary to seek an answer to the question of the extent, if any, of the hazard of the absorption of lead by the users of these hair dyes.

The preparations distributed by the members of this group of manufacturers vary from each other in certain ancillary components which have little relevance to the principle of their action. The latter is based on a slow interaction, upon or within the hair, of lead acetate with flowers of sulphur, whereby there is a darkening of the hair.

We have undertaken, by experimental means, to answer the question at issue, through the use of human subjects who have volunteered to apply a formulation chosen to be typical of those being distributed commercially by this group of manufacturers, on a schedule designed to duplicate, for a time, the procedure employed by regular users of these hair dyes. Before, during, and after the termination of the applications of the formulation upon the hair and scalp, specimens of the urine and blood of each of the subjects were obtained for determinations of their lead content, in search of evidence of the extent, if any, of the absorption of lead by the subject, under the experimental conditions.

Preparations of this type have been marketed for many years, and have been used by considerable numbers of people throughout this country, without, so far as is known, illnesses or credible claims of illness due to the use of these hair dyes. It is conceivable, however, that lead may be absorbed by the user into the hair, skin (scalp, and hands used to apply the hair dye) and its glandular structures,

including the regional lymph and blood stream, thence into the general circulation and into the other tissues of the body in accordance with the typical physiological pattern, and, further, into the excreta (of which the urine is the most sensitive indicator of absorption, in view of the appreciable and variable quantities of lead which are always to be found in transit in the alimentary tract). In addition, the contamination of the hands with lead, not only in the course of applying the formulation upon the hair, but also in touching the hair with the hands from time to time after the applications have been made, whether or not the hands have been washed entirely free of the lead compound, will have provided some opportunity for ingesting (swallowing) some quantity of lead supplied by the formulation, and for its partial absorption from the alimentary tract. Only through meticulous care on the part of the user, whether experimentally or for his own purposes, could he avoid entirely the absorption of lead from this source, and while it might well be supposed that our subjects, urged to exercise care to limit the absorption, if any, to the percutaneous route, would be more cautious in this respect than would the ordinary users of these products, one would not be surprised if some absorption were to occur from time to time or in specific instances involving some degree of hasty technique or some difficulty in following the prescribed precautions. The only likely evidence of an occurrence of this type, under the conditions of our experiments, would be some degree of elevation of the urinary excretion of lead (perhaps the concentration of lead in the blood as well), and this, if slight and of uniform occurrence among the subjects, could not be differentiated from the correspondingly slight elevation that might follow a minimum of absorption from the scalp. As a further point of differentiation, occasional sharp increases in the output of lead in the feces might be discovered by following the output of lead in the feces analytically during the period of use of the hair dye, but such increases could be identified as due in all likelihood to the ingestion

of the hair dye, only if they were of highly unusual magnitude, or if duplicates of all food and beverages consumed during the experiment were analysed in toto and found to be disproportionately low in their lead content. These procedures were considered to be unduly elaborate for the purposes of this experiment.

Our objective was to determine whether or not a physiologically significant (i.e., beyond the limits of currently normal variability) degree of absorption of lead would occur among the group of generally healthy male and female subjects, kept under a scheduled regimen of analytical surveillance of their urine and blood, while they followed a procedure simulating that employed by the ordinary users of this type of hair dye over such a period of time as was considered to be adequate for the portrayal of the outcome.

EXPERIMENTAL PROCEDURES

The Formulation.

The formulae of the hair dyes distributed by the individual manufacturers in the group were supplied to one of us (RAK), in strict confidentiality, and were assembled for comparison. These formulae differed from each other in minor matters such as the proportions of water and organic solvents employed, types and concentrations of surface-active agents, types and concentrations of perfumes, and the proportionate relationship of lead acetate and flowers of sulphur.

A single formula was devised in which the principal ingredients of the formulae submitted were represented in their maximum concentration. This formulation is given below, together with the manner of its preparation for experimental use.

DE

Lead Acetate (Reagent Quality)	20.00 grams
Sulphur (Sublimed)	10.00 grams
Glycerol (USP)	100.00 grams
Ethyl Alcohol (USP, 95 percent)	60.00 ml.
Tween 20	0.25 ml.
Distilled Water q.s.	1000.00 ml.

The lead acetate was dissolved in a sufficient amount of distilled water, to which, then, the glycerol, alcohol and Tween 20 were added. This liquid was added to the dry sulphur, with stirring, in sufficient quantity to make a homogeneous paste, after which the remainder of the liquid was added in divided portions, with stirring each time. The formulation was then brought to the desired volume by the addition of distilled water. [In order to distribute the undissolved sulphur (which will separate out from the supernatant liquid in any container) throughout the mixture of other ingredients, the container and its contents must always be shaken thoroughly before the discharge of any of the contents.]

The Subjects and Their Briefing.

Eight men and two women, who volunteered their services as the subjects of this experiment, were engaged to make use of the formulation described above, in accordance with verbal and written instructions. Nine were members of the technical and secretarial staff of the Kettering Laboratory in the Department of Environmental Health of the College of Medicine, while the one other was a physician in the Department of Dermatology of the College.

All of the members of the Kettering staff had been examined prior to their employment in the Laboratory and also periodically thereafter, by physicians in the Clinical Division of the Laboratory, and were known, through our informal access to the advice of such physicians, to be in good health. They were aware, generally, of the policies, procedures and precautionary measures employed in the

Kettering Laboratory in the conduct of experiments involving the use of human subjects (All of those in the Kettering Laboratory knew about the experiences of a series of human subjects who had been engaged for many years in experiments in the Laboratory involving the absorption and excretion of lead under a variety of conditions of experimental exposure to lead compounds. Two of the subjects in the present group had been subjects in such experiments.) All of them were advised in detail of the purposes of the present experiment, of the opportunities for the absorption of lead afforded thereby, and of the potential hazard so involved. They were warned that any abrasions of their scalp, which might occur in any manner, should be brought promptly to the attention of the physician (and of the nurse on duty daily in the Laboratory) in charge of the experimental procedures, whose responsibility it was to decide whether the experiment, or the involvement of any subject therein, should be terminated. (Our experience in such matters had established the fact that no risk to a subject or to the validity of this experiment would be imposed by the presence of a sharply localized lesion of his scalp, such as a small furuncle. Nevertheless, in the desire to avoid any suggestion of a cutaneous artifact, it was agreed that the occurrence of even such a lesion should be the signal for the discontinuance of the applications for so long as was required for subsidence of the lesion.)

The printed instructions issued to the subjects were as follows:

Shampoo the hair in the usual, desired manner. When the hair is dry, apply the hair dye by shaking it from a shaker type of bottle. Use only enough of the hair dye to dampen the hair evenly. This will probably not exceed 5 ml. per application, and may be less for short hair. Distribute it through the hair by rubbing with the fingers for a time, and then groom the hair with comb or brush. Always wash the hands thoroughly immediately after applying. Apply daily for two weeks and then twice-weekly thereafter. During the first two weeks, do not shampoo more than once each week. Thereafter shampoo as often as required. After shampooing, reapply only after the hair has dried.

Warning: Do not apply the hair dye if the skin is abraded or irritated.

These instructions were augmented, in the case of the female subjects (the normal users of these products are predominantly male), by advice to use such

olume of the hair dye as might be required to moisten their hair in a uniform manner. Further advice was given by the nurse (as authorized), in response to the questions posed by certain male subjects from time to time, especially as to the frequency of showering, with some attendant wetting of the hair.

The subjects were instructed further to appear between 8:00 and 9:00 a.m. at the clinic in the Kettering Laboratory on Wednesday's, according to the following schedule, and to carry out the applications of the hair dye in accordance therewith.

Schedule and Procedures
of Visits of Subjects to Clinic

Visit	Date	Purpose	Procedures
1	May 15	Preliminary briefing	First sampling of urine and blood
2	May 22	Second interview	Second sampling of urine and blood
3	May 29	Observation and inquiry after 1st week of daily applications	Third sampling of urine and blood
4	June 5	Observation and inquiry after 2nd week of daily applications	Fourth sampling of urine and blood
5	June 12	Observation and inquiry after 1st week of twice-weekly applications	Fifth sampling of urine and blood
6	June 19	Observation and inquiry after 2nd week of twice-weekly applications	Sixth sampling of urine and blood
7	July 3	Observation and inquiry after 3rd and 5th (final) week of twice-weekly applications	Seventh sampling of urine and blood
8	July 17	Observation and inquiry after 2 weeks of freedom from applications	Eighth sampling of urine and blood

The specimens of urine were obtained in the clinic of the Kettering Laboratory, each being of the volume voided at one time; those of the blood were obtained by one of us (EAK), by venipuncture into chemically clean evacuated glass tubes, the individual volume approximating 20 milliliters. All of the manipulations of these specimens, thereafter, were carried out in the analytical facilities of the Kettering Laboratory by methods that have been in use for many years.

PROCEDURES

Performance of Subjects.

One subject withdrew from the experiment during the preliminary observations because of his dislike of the venipunctures.

The quantity of the experimental hair dye used by the subjects varied considerably among the men, to some extent according to the characteristics of their hair, but also according to their manner of following instructions.

Subject 8-Su, whose hair was white, undertook to obtain a uniform darkening of his hair, and so applied the dye generously on each occasion according to the schedule, using approximately 150 milliliters in the course of the experiment. He achieved a satisfactory degree of darkening of his hair, and he proposes to continue the applications on a reduced, convenient schedule. (Periodic analytical observations will be continued, as a matter of mutual interest.)

Another Subject, 3-L, possessed of an oily scalp, had been in the habit of washing his hair daily while bathing (shower). During the period of the daily applications, he limited the washing of his hair to once per week, but he returned to daily washing of the hair (finding the lapse unsatisfactory) during the remaining period of the experiment. In order to counteract the effects of the daily washing, he applied the hair dye daily, after allowing his washed hair to dry thoroughly.

A small furuncle (unrelated to the applications) was found on the scalp of Subject 9-Sv early in the period during which applications were being made daily. The applications were discontinued for two days. The lesion subsided promptly, after which the applications were resumed.

The two female subjects whose hair was short, by one-time standards, (that of 5-P was deep brown, fine and moderately short, while that of 7-St

THE CONCENTRATION OF LEAD IN THE URINE OF EXPERIMENTAL SUBJECTS UNDER INVESTIGATION

Concentration of Lead in Twenty-four Hour Specimens

IDENTIFICATION OF SUBJECT	APRIL					MAY					
	2	9	16	23	30	7	14	21	28	4	11
I.F.	-	-	0.050	0.055	0.035	0.060	0.065	0.045	0.040	0.030	0.030
I.F.	0.020	0.020	0.020	0.025	0.030	0.030	0.020	0.020	0.020	0.025	0.025
S.W.	0.020	0.020	0.030	0.010	0.035	0.025	0.025	0.020	0.020	0.025	0.025
M.B.	-	-	-	0.040	0.025	0.030	0.030	0.020	0.025	0.045	0.045
F.C.	-	-	-	-	-	0.020	0.015	0.015	0.015	0.020	0.020
Ju.S.	-	-	-	-	-	-	-	0.015	0.020	0.030	0.030
S.B.	0.020	0.030	0.030	0.030	0.030	0.040	0.040	0.040	0.030	0.030	0.030
L.D.	0.025	0.025	0.025	0.030	0.020	0.020	0.025	0.025	0.030	0.025	0.025
Jo.S.	0.020	0.025	0.025	0.010	0.020	0.020	0.020	0.015	0.025	0.015	0.015
N.K.	0.030	0.020	0.015	0.020	0.015	0.025	0.025	0.020	0.025	0.025	0.025
N.S.	0.020	0.020	0.025	0.025	0.015	0.015	0.020	0.020	0.015	0.025	0.025
H.R.	0.025	0.030	0.040	0.040	0.025	0.045	0.030	0.025	0.020	0.030	0.030

OUTPUT OF LEAD UNDER PREVALENT ENVIRONMENTAL CONDITIONS

on the Dates Indicated Below

JULY					AUGUST		
2	9	16	23	30	6	13	20
0.055	0.020	0.020	0.030	0.040	0.025	0.050	0.020
0.020	0.010	-	-	-	-	-	-
0.020	0.025	-	-	0.035	0.025	0.040	0.030
0.035	0.040	0.035	0.030	0.035	0.035	0.030	0.035
0.025	0.030	0.035	0.025	0.035	0.035	0.040	0.055
0.010	0.020	0.015	0.020	0.020	0.020	0.030	0.015
0.030	0.030	0.030	0.030	0.030	0.030	0.030	0.040
0.030	0.025	0.015	0.025	0.020	0.015	0.020	0.025
0.030	0.025	0.020	0.020	0.020	0.020	-	-
0.030	0.020	0.020	0.025	0.025	0.035	0.030	0.015
0.015	0.030	0.020	0.020	0.020	0.020	0.025	0.025
0.020	0.030	0.025	0.025	0.040	0.025	0.015	-

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0.030

0.050

0.040

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0.035

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was medium dark brown, heavy, somewhat coarse, and somewhat longer), found it necessary to use much larger volumes of the hair dye than did the males. Subject 5-P used about 550 milliliters, while Subject 7-St used approximately 525 milliliters. The former washed her hair once per week during the first and second weeks of the use of the hair dye (daily applications), but in the third week of use (twice weekly applications), finding her hair increasingly "sticky" washed it twenty-four hours after each of the two applications. When advised that this was not the prescribed procedure, she discontinued this practice and, for the remaining three weeks, washed her hair once per week.

It may be supposed that similar variations in the manner of using the commercial formulations occur among the regular users of such hair dyes, and that the performance of these experimental subjects represented some portion of the spectrum of the variability in the intensity of the exposure to lead on the part of the users. Certainly, in any quantitative consideration of this matter, the female subjects would be credited with having had considerably greater potential for the absorption of lead in the course of this experiment, than would the male subjects.

RESULTS

The experiment was carried out according to plan with two slight exceptions. When one of the Subjects, 6-R, withdrew from the experiment, after providing the first two sets of samples (controls), he was replaced by another member of the technical staff of the Kettering Laboratory, who had indicated his availability. This meant that the entire sequence of the experimental procedures was delayed, in the case of the substituted subject, to the extent of his delay in starting.

In Table 1, the results of the analysis of the specimens of urine

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are listed, according to the subjects who provided them, the dates on which they were obtained, and their temporal relationship to experimental conditions. The dates on which the samples were collected from Subject 11-U are indicated just above the relevant analytical findings, and are averaged in with the others obtained at the corresponding stage of the experiment.

Examination of the foregoing data reveals a considerable degree of irregular variability from subject to subject and from time to time in the same subject. This occurs within the relatively narrow range of the findings obtained commonly by analysis of singly voided specimens of the urine of persons who have not been subjected to any discoverable exposure to lead beyond that provided by their general environment. When the results of the analysis of the specimens of the urine of the subjects, as grouped under each date in Table 1, have been averaged, some tendency toward an increase in the concentration of lead during the period of the experimental use of the hair dye may be noted, but the magnitude of the trend is small, and its meaning is uncertain. Its significance is rendered even more dubious, when the average values for the preliminary period and the period of use of the hair dye are represented, as in Table 2, for each individual subject. Here it may be seen that four of the ten subjects show no increase during the period of testing, while two others exhibit increases so slight as hardly to be significant. The concentration in the urine of only four of the subjects appears to have sustained a significant average increase during the period of use of the hair dye.

Balance experiments conducted over prolonged periods of time (one or more years) have demonstrated the fact that the output of lead from the body, (in relation to the intake, as well as to prior and subsequent periods of time), undergoes an increase during the warm months of the year (in the temperate zone as represented by Cincinnati).¹ It is likely that the results of the present experiment, conducted during the spring and early summer, were influenced, in some degree, by this seasonal factor. To a large extent, however, the unpredictable and incompletely

understood variability in the rate of excretion of lead in the urine, from hour to hour and day to day, would seem to have been responsible for the apparent but somewhat inconsistent temporal trends in the data. An opportunity was afforded for the examination of such variability in the excretion of lead during the year, and specifically during this period of the year, through the availability of data which have not yet been fully assembled, explored and published. During the past thirty years, seventeen young to early middle-aged men have been engaged as the subjects of experiments involving the ingestion or inhalation of certain inorganic compounds of lead in variable dosages, while the total intake of lead in food and beverages and the total output in the feces and urine have been followed analytically day by day over prolonged periods of time. Each subject has been followed similarly, during short or long periods of time, prior to the ingestion or inhalation of lead experimentally, so as to obtain the pattern of the normal (usual) metabolic processes with respect to the intake and output of lead, as indicated above, under ordinary environmental conditions. Many other data of medical and physiological interest have been gathered during and as part of the experimental regimen, but for present purposes, the levels of the concentration of lead in the urine voided day by day by individual subjects, during the preliminary period of the observations, are pertinent. These data were assembled throughout these periods, and from them were extracted those concerned with the concentration of lead in the specimens gathered on the same day of each week, i.e., the specimens obtained at intervals of one week, in order to bring them into correspondence with the data of the experiment being reported herein. It should be noted that the results of the latter experiment were obtained by the analysis of single voidings (spot samples) of urine, while those relating to the subjects of balance experiments were derived from the analysis of the total volume of urine voided during each twenty-four-hour day. It has been shown that specimens of urine of small volume

voided at intervals through the day vary more widely in their lead content, both as to their upper and lower limits, than do the corresponding twenty-four-hour specimens.² The two sets of data are not, therefore, strictly identical in the quantitative sense, but are comparable otherwise.⁷

The data described above have been assembled in Table 3, to the extent that they fall consecutively within the spring and summer months of the years during which such observations were made. Eleven of the subjects fitted into this requirement, (one, Subject I.F., having done so in two successive years). Simple inspection of these data reveals two pertinent facts, among others of interest. It may readily be seen that the variability in the concentration of lead in the urine voided day by day is of the same type and, at the least, in the same degree as those of Table 1. Furthermore, there is a somewhat irregular trend, in several instances, toward an increase in the concentration of lead in the urine beginning at some point within the springtime and extending into the summer months. This trend is neither pronounced nor sustained in any instance, but is evident in the data obtained from four of the eleven subjects, and suggestive but slight in two others. In only three of the twelve years (11 subjects) is there a trend, actual or suggestive, toward a decrease in the concentration of lead in the urine, beginning in the spring and extending into the summer months. One of the subjects (I.F.) exhibited a slight and uneven trend toward an increase, in one of the years, and a correspondingly irregular trend toward a decrease in the other.⁷

In Table 4, the results obtained by the analysis of the specimens of the blood of the subjects (analogous to those of the urine in Table 1) have been assembled. It is apparent, on both cursory and detailed examination of these data, that none of the subjects incurred any significant change in the concentration of lead in his blood in consequence of any experimental attempt to alter his absorption of lead.

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DISCUSSION

The most striking characteristic of the results of this experiment is the lack of satisfactory evidence of experimentally induced absorption of lead by the subjects. Thus, it is impossible to estimate the extent of such absorption, or even to ascertain whether it actually occurred. This fact arises through diurnal, seasonal and other factors which bring about variability in the rate of the excretion of lead in the urine. In consequence, the rate of any environmentally or experimentally induced absorption of lead must reach a certain magnitude to be recognized as such within a short period of time, while a considerably lower rate which persists can be detected by the occurrence of a gradual elevation in the average or mean concentration of lead in both urine and blood. In this instance, as in certain other situations involving dubious or seemingly insignificant degrees of absorption of lead, the concentration of lead in the blood remained unchanged.

Certain factors, as indicated above, are known to exert their influence on the rate of the urinary excretion of lead under ordinary and fairly uniform conditions of lead intake. Under ordinary environmental and physiological conditions, an increase in the flow of water through the body will increase the output of lead therefrom via the kidneys.^{2,3} Fairly wide daily variations in the ambient temperature, such as those which occur at certain seasons in temperate climates, and elsewhere in all probability, induce appreciable changes in the renal throughput of water, with significant alterations in the concentration of lead therein.³ There are also seasonal variations in the metabolism of lead in the body, independent of the rate of absorption of lead, and also independent of the renal throughput of water. In balance experiments on human subjects in Cincinnati, for example, we have found clear evidence of a considerable augmentation of the loss of lead from the body (without corresponding increase in the absorption of lead) during the summer months. Under the conditions of balance experiments, it seems reasonably certain that this

increased loss results either in a modest depletion of the skeletal tissues, or in the shift of somewhat larger quantities of lead, during the course of absorption, into the soft tissues. How this comes about is entirely speculative. However, this fact makes for a degree of temporal variability in the results of moderately prolonged experiments, with respect to the relationship between the rates of the absorption and those of excretion at any one time.

An additional difficulty in the interpretation of the results of an experiment of the type performed and described herein, lies in the fact that, while the intake of lead from the general or ambient atmosphere is relatively small and fairly constant from day to day, as is also the absorption from this source, the intake in food and beverages (and from other articles introduced into the mouth) is relatively large and fairly variable from day to day, and is absorbed in variable daily quantities from the alimentary tract.⁴ This results in variability in the excretion of lead in the urine, which can be assessed only by the collection and analysis of precise duplicates of everything eaten and drunk and of every other article (medication, etc.) swallowed during every day that is involved in the investigation.⁵

For such reasons as the foregoing, it is not possible to interpret, with certainty, the significance of the minor variations in the rate of the excretion of lead in the urine of the subjects. The most that can be said of the composite results of this experiment, is that there may have been some degree of absorption of lead by the subjects during the period of their use of the experimental formulation of the hair dye, either through their skin, or from their alimentary tracts, or from both. If such was the case, the quantity absorbed by certain subjects was too small to offset the variability of their absorption of lead from extra-experimental sources. In other cases, it was of sufficient magnitude to make itself manifest or, at least, apparent, under the metabolic conditions with

respect to the extra-experimental absorption and excretion of lead. But it is doubtful that there were differences of sufficient magnitude in the quantities of lead absorbed by individual subjects through their use of the hair dye to account for the differences in their urinary excretion of lead. It was noted previously that both of the female subjects applied much greater volumes of the preparation to their hair than did the men, but only one of the two appeared to have sustained greater increase in the level of the concentration of lead in her urine, during the period of use of the hair dye, than did any of the men, and then only because of an unusually low concentration of lead in the specimens procured during the preliminary period, i.e., not because the peak concentration during the period of experimental exposure was beyond the generally "normal" range of such values. In a further comparison, male Subject 8-Su, who used more of the hair dye than did any of the other men, ranked third among the men by the same standard of performance. No plausible explanation, in terms of the experimental conditions, can be given for this variability among the subjects, and it seems more likely that it derived from dietary variations than from any other source.

When full allowance is made for the possibility that certain of the subjects absorbed small quantities of lead in consequence of their experimental use of the hair dye, the question arises as to the degree of hazard which might be associated with the use of hair dyes of this type for many years (such as would be the case of an individual whose hair had become gray in his late twenties or early thirties). The evidence on this score derives from balance experiments on human subjects which have made it possible to visualize the role of intermittency in the absorption of lead, as differentiated from that of continuous absorption, in relation to the accumulation of lead in the body. On the one hand, continuous absorption of lead, above a certain rate, results in accumulation, indefinitely, at an approximately constant rate which is dependent upon the rate of absorption.

On the other hand, intermittent absorption, as seen in our experiments and best observed over many years under fairly uniform conditions such as those provided by well-controlled environmental conditions in certain industries, results in the development of an equilibrium between the periods of exposure (when absorption is of greater magnitude than excretion) and the periods of non-exposure (when the reverse is true), whereby the rate of excretion is elevated to an average steady state so long as the environmental conditions are maintained at a substantially constant level.⁶ Under the conditions of the use of hair dyes of the type here under examination, the exposure to and absorption of lead following the achievement of the desired coloration of the hair, would certainly be slight, if not negligible. Further, over the prolonged period of intermittent use required to maintain the color, the intervals between applications would insure against a significant rate of accumulation of lead in the tissues of the users. That is to say, that even after many years of use, any increase in the rate of the urinary excretion of lead, as well as the rate of increase in the concentration of lead in the blood, may confidently be expected to be negligible.

CONCLUSIONS

1. The ten persons, eight males and two females, who were subjected to the use of a preparation of a lead-containing hair dye over the period of ten weeks, under experimental conditions simulating those of the normal use of hair dyes of that type, failed to absorb lead therefrom with such uniformity or in such quantities, as indicated by the rate of their excretion of lead in their urine, as clearly to demonstrate that such absorption had actually occurred. (None of them gave evidence of any elevation of the lead content of his or her blood during the course of the experiment.) That some lead may have been absorbed by some or, perhaps, by all of the subjects, as the result of the experimental exposure to lead cannot be disproved, but neither can it be affirmed, since four of them failed to

go any increase in the rate of their urinary excretion of lead during the treatment, while the elevation of the lead content of the urine of the other subjects, during the period of experimental exposure, was so irregular or so great as to be within the limits of variability displayed by specimens of urine of similar volume obtained from persons whose exposure to lead is limited to that of the prevalent human environment (as the result of their ingestion of food and beverages and their inhalation of the ambient air).

2. Considering the slightness of the evidence for the absorption of lead induced by the experimental regimen, and in view of the intermittent character of the exposure to lead involved in the prolonged use of the hair dye, the likelihood of the progressive accumulation of lead in the bodies of the regular users of hair dyes of this type is regarded as remote.

3. The hazard of lead absorption among the users of these products appears to be negligible.

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TABLE 1

THE CONCENTRATION OF LEAD IN MILLIGRAM PER LITER IN THE URINE OF THE SUBJECTS ON THE
 ACCORDING TO DATES OF SAMPLING, SEQUENCE OF EXPERIMENTAL PERIODS AND ASSOCIATED EXPERIMENTAL

IDENTIFICATION		CONTROL PERIOD		PERIOD OF APPLICATIONS			
OF	SUBJECT	FIRST	SECOND	FIRST	SECOND	THIRD	FOURTH
		After 1st Week of Experiment	After 2nd Week of Experiment	After 1st Week of Daily Applications	After 2nd Week of Daily Applications	After 1st Week of Twice-Weekly Applications	After 2nd Week of Twice-Weekly Applications
		May 15	May 22	May 29	June 5	June 12	June 19
1	B	0.039	0.033	0.039	0.047	0.033	0.027
2	I	0.018	0.015	0.016	0.032	0.028	0.033
3	L	0.015	0.020	0.010	0.024	0.037	0.036
4	M	0.013	0.018	0.022	0.018	0.016	0.023
5	P	0.015	0.009	0.029	0.030	0.028	0.023
6	R	0.008*	0.014*	-	-	-	-
7	St	0.015	0.012	0.013	0.016	0.028	0.028
8	Su	0.023	0.018	0.024	0.022	0.036	0.028
9	Sv	0.035	0.026	0.028	0.028	0.036	0.028
10	T	0.033	0.024	0.025	0.027	0.027	0.030
		May 29	June 5	June 12	June 19	June 26	July 3
11	U	0.028	0.028	0.033	0.026	0.024	0.025
Averages:		0.023	0.020	0.024	0.027	0.029	0.028

* Excluded from average

TABLE 2

COMPARISON OF THE EXCRETORY RESPONSES OF THE SUBJECTS TO THE EXPERIMENTAL REGIMEN.
The Average Quantities of Lead in Specimens of the Urine of Individual
Subjects Obtained During the Preliminary (Control) and Experimental (Test) Periods

(Values Expressed as Milligram of Lead Per Liter of Urine)

Subject:	1-B	2-I	3-L	4-M	5-P	7-St	8-Su	9-Sv	10-T	11-U
Control:	.036	.017	.018	.016	.012	.014	.021	.031	.029	.028
Test:	.036	.029	.028	.022	.029	.021	.029	.030	.028	.028

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THE CONCENTRATION OF LEAD IN MILLIGRAM PER 100 GRAMS IN THE BLOOD OF THE SUBJECTS ON THE OCCASIONS INDICATED
ACCORDING TO DATES OF SAMPLING, SEQUENCE OF EXPERIMENTAL PERIODS AND ASSOCIATED EXPERIMENTAL CONDITIONS

IDENTIFICATION OF SUBJECT	CONTROL PERIOD		PERIOD OF APPLICATIONS							AVERAGES								
	AFTER FIRST WEEK OF EXPERIMENT		AFTER SECOND WEEK OF EXPERIMENT		AFTER 1ST WEEK OF DAILY APPLICATIONS		AFTER 2ND WEEK OF DAILY APPLICATIONS		AFTER 1ST WEEK OF TWICE-WEEKLY APPLICATIONS		AFTER 2ND WEEK OF TWICE-WEEKLY APPLICATIONS		AFTER 4TH WEEK OF TWICE WEEKLY APPLICATIONS		PERIOD AFTER TERMINATION OF APPLICATIONS		AVERAGES CONTROL PERIOD	
B	0.030	0.024	0.027	0.028	0.023	0.026	0.020	0.027	0	0	0	0	0	0	0	0	0	0
I	0.020	0.015	0.026	0.018	0.022	0.021	0.023	0.018	0	0	0	0	0	0	0	0	0	0
L	0.020	0.028	0.026	0.026	0.021	0.024	0.031	0.024	0	0	0	0	0	0	0	0	0	0
M	0.030	0.027	0.027	0.025	0.026	0.025	0.024	0.026	0	0	0	0	0	0	0	0	0	0
P	0.020	0.016	0.020	0.024	0.022	0.017	0.022	0.021	0	0	0	0	0	0	0	0	0	0
R	0.030	0.021	-	-	-	-	-	-	0.026	0.018	0.026	0.018	0	0	0	0	0	0
S	0.020	0.019	0.036	0.024	0.021	0.029	0.021	0.017	0.020	0.020	0.020	0.020	0	0	0	0	0	0
Su	0.020	0.032	0.027	0.028	0.029	0.027	0.026	0.022	0.026	0.026	0.026	0.026	0	0	0	0	0	0
Sv	0.030	0.028	0.024	0.029	0.022	0.025	0.021	0.024	0.020	0.020	0.020	0.020	0	0	0	0	0	0
T	0.025	0.024	0.020	0.028	0.030	0.033	0.032	0.021	0.025	0.025	0.025	0.025	0	0	0	0	0	0
U	0.024	0.027	0.024	0.020	0.025	0.026	0.026	0.023	0.026	0.026	0.026	0.026	0	0	0	0	0	0
Averages:	0.024	0.024	0.026	0.025	0.025	0.025	0.024	0.022	0.022	0.022	0.022	0.022	0.024	0	0	0	0	0

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