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252 MADISON AVENUE, NEW YORK, N.Y. 10017
TELEPHONE 679-0050 AREA CODE 212
CABLE ADDRESS: NYNEXO NEW YORK

Miss O'Hara

To: All Members of the Lead Environmental Health Committee

Re: LH-114 Proposal from Foster D. Soell

Attached for your consideration is a complete copy of the proposal as received from Foster D. Snell. This is the first proposal that we have received that comes close to what we have in mind for this study. Since this is the fourth or fifth proposal to arrive in our office, I must conclude that in general American toxicologists are not very well informed about conditioned reflex type investigations. However, I hope to have others for your consideration in the near future.

If you have any suggestions or comments about this project and this proposal, I shall be very pleased to receive them at any time. Kindly keep this document in your files for future reference, as we will be considering awarding a contract in the near future, I hope.

Very truly yours,

Don 27 October

Don G. Fowler
Project Manager

DDF:sv
enclosure

Mr. Donald Fowler
January 23, 1967
Page Two

OUR UNDERSTANDING OF THE PROBLEM

A review of the literature on "Air Pollution and Related Diseases" shows that the Russians have adopted a much lower maximum "safe-level" for lead than is considered applicable in the United States. Changes in behavior, learning, and/or memory have been reported in the USSR following relatively low levels of inorganic lead ingestion^{2,3}. Recently, in reporting functional shifts in higher nervous activity, it has been suggested that the people of the United States are being subjected to chronic lead insult from industrial sources⁴. However, in direct contradiction, Dr. Thomas J. Haley of Los Angeles, after reviewing the subject, came to the conclusion that "the supposed chronic lead intoxication from environmental contamination is a myth, not a fact". These conflicting reports have raised doubt in the minds of public health officials as to the safety of these levels of lead ingestion.

It has thus become necessary to carry out studies to demonstrate the safety of continued ingestion of lead at present levels. Since many studies have shown that no overt, clinically-evident shifts in biochemical, metabolic or physiologic functions occur, the new experiments must be directed toward evaluation of minimal shifts in activity or performance—the so-called sub-clinical effects.

OUR APPROACH TO THE PROBLEM

Our program is intended to define performance decrements following ingestion of graded levels of lead intakes. The doses selected should be equal to or part of the usual human intake to be valid as indicators of effects of present exposure levels. No significant biochemical or metabolic studies are contemplated nor will there be any overt effort intended to show alterations in tissue or cellular architecture. Rather, alterations in behavior, mental performance and muscular work output are sought. The experimental design has been selected so that the probability of finding as little as a 10 per cent shift in total performance will be high, i.e., greater than 95 per cent.

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2. Shalaubonds, Y.P., *Ogizhenia i sanitariya* 3, 751 (1959)
 3. Glavnyy, T., *Geofizicheskoy Observatorii Imeni A.I. Voznyakov*, 130 (1963)
 4. Patterson, C.C., *Arch. Environ. Health* 11, 344 (1965)

Mr. Donald Fowler
January 23, 1967
Page Three

The task assigned to test animals will include:

- . Decision making
- . Learning capacity
- . Visual recognition
- . Muscular work output
- . Muscular coordination

The single parameter to be followed is time for completion of five runs through a maze, thus reflecting summation of all of these psychomotor functions. As a simple, direct evaluation of total capability, it is intended to show any subclinical effect of lead.

Although no effort is to be made to delineate the site of activity, if any, of the lead salts, this 6-month long study may permit some evaluation of the effects of lead on other normal physiological developments such as growth, food intake, feces formation, etc. Some preliminary indication about carcinogenic effects of chronic feeding of accepted levels of inorganic lead salts may also be forthcoming.

The highest level to be administered to the test animals is to be the equivalent of the daily intake of 0.33 mg lead per human³. Assuming that the average man weighs approximately 100 pounds, this is an ingestion of 0.075 mg per kg per day, the highest dose to be used in these studies. Two lower doses, 0.034 and 0.0075 mg per kg per day, are included so that the three doses are in geometric progression using the square root of 10 as the factor. A dose response curve can then be plotted logarithmically permitting comparison among treated groups and between each group and the fourth group, the controls, receiving no dose.

3. Kehoe, R.A., Arch. Environ. Health, 8, 232 (1964)

Mr. Donald Fowler
January 23, 1967
Page Four

The test material is to be administered in the diet since over 90 per cent of the daily lead intake in man occurs per os⁶. The concentration in the food will be selected to give the desired level of lead ingestion. We feel that you should suggest the lead salt to be used from your knowledge of the availability and utility of the various compounds.

The weight, food consumption and efficiency of food utilization will be followed closely since these have been reported to be the most useful parameters in showing toxicity.

Performance is to be followed in 6 animals per group per week using a water maze (Figure I). The total elapsed time for five runs from introduction (A) to arrival at food cup (F) will be determined for each rat. Muscular activity will affect completion of the water course (B). Decision making occurs at two points (C₁ and C₂) and muscular coordination will be reflected in elapsed time for movement up the inclined plane (D). At C₂ the rat is presented with a visual problem seeing the empty food cup (E) and the desired cup (F), which has a familiar marking. At all feeding periods each rat is to be presented with two cups, one full and bearing specific markings and one empty and unmarked. If there is no difference in learning potential between treated and control rats then we anticipate that all rat groups will make the equivalent number of correct decisions at point C₂.

Using 36 rats per group in a 24-week feeding study we will have put all the rats through the test period in the initial 6-week period. These will then be randomized and used again during the second 6-week (7 through 12), the third 6-week (13 through 18) and the final (19 through 24) week periods. During these subsequent trials, memory and data retention will also be factors.

At that time the data will be reviewed and the findings presented to the International Lead-Zinc Research Organization for decisions as to further studies. Our program does not include provisions for sacrifice, or for biochemical or histological studies, although blood and urine samples are to be collected periodically.

6. Kehoe, R.A., J. Roy Inst. Pub. Health Hyg., 24, 81 (1961)

Mr. Donald Fowler
January 23, 1967
Page Five

This program is directed toward elucidation of any changes in performance based on psychomotor activity. The total task involving, as it does, exertion, coordination and psychic activity should show any subclinical effects that arise.

THE PROGRAM

A total of 144 male, weanling rats will be used in this study, divided into four groups of 36 each as follows:

Group I	-	CONTROLS	-	receiving a ground basal (commercial) ration
Group II	-	LOW	-	receiving a ground basal ration containing sufficient lead salts to yield a daily intake of lead of 0.0075 mg per kg.
Group III	-	MID	-	receiving a ground basal ration containing sufficient lead salts to yield a daily intake of 0.035 mg per kg.
Group IV	-	HIGH	-	receiving a ground basal ration containing sufficient lead salts to yield a daily intake of 0.075 mg per kg.

Food will be available one hour per day, 6 days per week. The food cups will be plainly marked. At the time of presentation of the food a second, empty, unmarked, cup will be placed in the cage. Ample supplies of fresh tap water will be available at all times.

The rats will be housed individually in raised-floor, wire-mesh cages. Observations will be made daily for survival, behavior, appearance of the excreta, and general physical condition. Food intake and body weights for 10 rats per group will be recorded weekly and efficiency of food utilization (g gained per 100 g food eaten) calculated after 12 weeks.

Mr. Donald Fowler
January 23, 1967
Page Six

On the fifth day of the study and weekly thereafter (after a 48-hour fast), 6 rats of each group will be introduced to the maze and total time for 5 trials for each rat determined. Comparisons will be made among groups to determine performance differences. Each rat will thus be tested 4 times, once during each of the four 6-week periods of the study. In addition to the objective criterion of elapsed time, subjective evaluation of rat performance will also be made.

The data reported will reflect effects of the various doses on growth and mental and muscular performance. At the end of each 6-week period these samples will be collected from 10 rats per group other than those used for food consumption and weight measurements, and these will be stored at -20°C should analysis become desirable at some future time. The animals will be sacrificed only as directed by the International Lead-Zinc Research Organization.

ORGANIZATION OF THE WORK

The project will be carried out under my supervision. A short resume summarizing my capabilities and background and a bibliography describing my past efforts have been included for your review. The specific animal work will be carried out by Mr. Juan Dorado, Senior Technician with assistance from Dr. Adolfo Hernandez, Research Veterinarian. Specific assistance in the psychological studies will be given by Mr. Harold Wallach who has been engaged in behavioral research for 8 years. Animals handlers will be assigned as needed.

The data will be reviewed mathematically by Dr. Theodore Horner, Senior Statistician of Booz, Allen Applied Research, Inc.

As Senior Investigator, I will submit a letter report to the Organization approximately once per month during the course of the studies. A quarterly report reflecting the findings during the first and second 6-week periods will be issued after the first 12 weeks of the study and a formal final report will be issued after 24 weeks during which time the animals will still be alive. This is expected to permit the Organization to decide the future of these test animals.

Mr. Donald Fowler
January 23, 1967
Page Seven

OUR QUALIFICATIONS

The specific personnel to be assigned to the project are described above.

The combined experience of our personnel in toxicological and behavioral studies will be brought to bear in the solution of this problem.

Studies involving administration of drugs, foods, cosmetics, and chemicals by oral, ocular, topical, parenteral, and inhalation routes have been carried out in this facility within the past year. Clinical and biochemical laboratories are located on the premises, and a complete histological service is maintained. In addition to investigations performed with animals, the following studies are frequently performed with human subjects: dermal irritation, sensitization, blood-level determinations, and metabolism.

We provide facilities and manpower for housing, treating, and observing animals during tests in our fully-staffed, air-conditioned, well-equipped laboratories.

As an independent laboratory with nearly half a century of service to industry and government, Foster D. Snell, Inc. clearly recognizes the need for confidentiality in all client matters. We are, therefore, prepared to enter into agreements providing for control of confidential information.

As part of the Boos, Allen family of professional service organizations, we offer not only the biological services in which we specialize, but also the broad chemical experience of the remainder of the Snell organization and the extensive engineering, statistical, and management capabilities of the other members of the Boos, Allen group.

ESTIMATED TIME AND COST

The studies can be initiated within 30 days of receipt of your authorization to proceed. In the present outline, with no post-mortem or terminal biochemical studies contemplated, the studies would be completed within 7 months of receipt of your authorization and the report delivered to you within one month thereafter. This is required to permit

Mr. Donald Fowler
January 23, 1967
Page Eight

complete and comprehensive analysis of the data. However, we realize that our projected ORGANIZATION OF THE WORK, including monthly reports, permits application of principles of continuous review so that you retain control of the study. Changes in procedures, protocols, etc. may be affected at any time, shortening or increasing the duration of the study, adding a recovery phase, etc.

Costs presented are our best estimates for performance of the entire study with maintenance of treated animals during the time required for report preparation. These are based on the time required for the professional and technical staff in planning, performing, analyzing and reporting the experiments and include the costs of animals and materials.

We also ask reimbursement for costs of travel, communication and other expenses incurred directly in support of this project which are not included in THE PROGRAM presented above.

Based on the work outlined in this letter and experience with similar projects we estimate that the charges for study of sub-clinical effects of inorganic lead salt ingestion will range from \$11,000 to \$13,500.

This estimate will remain unchanged for sixty days, after which time business conditions may require changing our estimate of charges.

It is our practice to operate strictly on a professional service basis with the work standing on its own merits. Any assignment is cancellable at any time at your discretion without any liability beyond the charges incurred to the point.

The great importance of the study to be carried out is evident. We are keenly interested in the opportunity to work with you to resolve the open question of potential subclinical effects of lead ingestion at levels of 0.33 mg per human. Application of our substantial capability and experience should yield the solution. We are looking forward to receipt of your authorization to proceed.

Mr. Donald Fowler
January 23, 1967
Page Nine

If there are any questions please do not hesitate to call on us and discuss these.

Sincerely yours,

BIOLOGICAL SCIENCE LABORATORIES

Myron S. Weinberg, Ph.D.
Director

R.J. Bouthilet, Ph.D.,
President

FIGURE I
SCHEMATIC DIAGRAM OF WATER MAZE

