BEHAVIORAL TOXICOLOGY

EARLY DETECTION

OF

OCCUPATIONAL HAZARDS

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"To assure safe and healthful working conditions for working men and women"

The Occupational Safety and Health Act of 1970 seeks to provide American workers with protection against personal injury and illness resulting from hazardous working conditions. The National Institute for Occupational Safety and Health (NIOSH), Department of Health, Education and Welfare is responsible for conducting and supporting research on which new and improved standards can be based. The responsibility for promulgating and enforcing occupational safety and health standards rests with the Department of Labor.

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FOREWORD

The Behavioral Toxicology Workshop for Early Detection of Occupational Hazards was held in Cincinnati, Ohio, June 24-29, 1973. It was sponsored by the National Institute for Occupational Safety and Health (NIOSH) and the University of Cincinnati, College of Community Services, with cooperation from the National Academy of Sciences, National Research Council, the International Brain Research Organization, and the International Association for Occupational Health. The workshop was supported by Grant No. OH-510 to the University of Cincinnati.

This publication is responsive to the NIOSH mission as identified in the Occupational Safety and Health Act of 1970. Sections 20(a)(1) and 20(a)(4) of the Act emphasize the need to include behavioral factors in examining problems of occupational safety and health and the need to develop new methods, techniques and approaches in handling problems of worker safety and health.

The workshop was conducted to provide an opportunity for the exchange of current information and test methods among leaders in behavioral toxicology research who are concerned with (1) methods for assessing the adequacy of occupational health standards and for quantifying and evaluating functional impairments in the worker based on behavioral performance measures and neurophysiological changes, (2) the application of behavioral/neurophysiological indicators for the monitoring and early detection of potential occupational health and safety problems, and (3) the ultimate establishment of occupational health and safety standards based on the preservation of behavioral functions and worker capabilities.

It is hoped that NIOSH support of behavioral toxicology research will result in information useful in detecting early reversible functional changes in workers experiencing long-term exposure to industrial chemicals. This information may be significant in terms of human capability to work safely and may be used in developing early indicators of clinical disease. Research findings will be useful in evaluating the adequacy of recommended safe exposure levels and for rating the risk of safety and health problems on the job.

Early awareness and rapid communication of findings in worker safety and health research are essential for the early detection and control of occupational hazards. NIOSH will make every effort to be responsive to this need.

Marcus M. Key, M.D.
Director, National Institute
for Occupational Safety and Health

ACKNOWLEDGMENT

To plan and conduct the Behavioral Toxicology Workshop for Early Detection of Occupational Hazards required the labor of many individuals. We especially thank Bruce Gutnik, M.D., Robert Baloh, M.D., and John Bryant who, as members of the Scientific Planning Committee, contributed so significantly to the success of the workshop and Sherry Selevan, Executive Secretary of the workshop for her tireless and courageous effort in resolving problems encountered by the participants. Staff of NIOSH's Behavioral Studies Laboratory, Dr. H. Harvey Cohen, W. Kent Anger, Robert Struble and James Setzer are to be commended for their untiring effort in the planning and conduct of the workshop. Nadine Dickerson deserves special credit for her secretarial help during all phases of the workshop and for her assistance in transcribing and typing workshop presentations.

Dr. Edward J. Fairchild, II, Dr. Elliott Harris, John Bryant, Dr. Alexander Cohen, and Dr. Alan Stevens of NIOSH have given continued encouragement and support to behavioral toxicology research and deserve special recognition. Early publication of the proceedings of the workshop is the result of concern by NIOSH's Printing Officer, Russ Hinton.

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University of Michigan

WELCOMING REMARKS FROM NIOSH

E. J. Fairchild, II, Ph.D.

Mr. Chairman, Honorable Senator Taft, participants and guests. It is with great pleasure that I stand before you this morning to extend a warm welcome to all of you as distinguished guests and participants in this workshop. I speak not only for myself but also for Dr. Marcus Key, Director of the National Institute for Occupational Safety and Health, who extends his regrets for not being able to be here today.

I speak with genuine sincerity when I say that we, as well as all of NIOSH, are proud to have the opportunity to sponsor and co-host such a workshop. We envision it as a forerunner of the Institute's intent to lead the way in the delineation, development and interpretation of criteria whereby men and women are afforded a safe and healthful work environment.

Other meetings and conferences somewhat analogous to this workshop have been sponsored by NIOSH in its brief existence as an Institute. However, special note should be made that the workshop beginning here today is, in my own opinion, unique. Previously sponsored conferences have dealt with more clearly defined areas whereas behavioral toxicology today encompasses broader areas with many controversial ramifications as most of you are aware.

I believe that we are all in agreement that new sciences in general offer controversies which require time and dedication to resolve. No less is true for the behavioral sciences and it is precisely for this reason that many of the experts are gathered here this week. Therefore, it is my belief that the uniqueness of NIOSH's effort lies in the challenge to behavioral scientists of today and specifically those in attendance at this workshop.

NIOSH is mandated by the Occupational Safety and Health Act of 1970 to "Conduct research into the motivational and behavioral factors relating to the field of occupational safety and health," and this of course goes hand in hand with other requirements of the Act which recognize needs for new and innovative research. The OSHA of 1970 also requires that NIOSH "develop criteria dealing with toxic materials and harmful physical agents and substances which will describe exposure levels which are safe for various periods of employment, including but not limited to the exposure levels at which no employee will suffer impaired health or functional capacities or diminished life expectancy as a result of his work experience."

Dr. Edward J. Fairchild, II is Associate Director for Cincinnati Operations, National Institute for Occupational Safety and Health.

It is very clear that the charges in the OSHA of 1970 relate to the kinds of things that are going to be discussed in this workshop. This is indeed a key challenge. It is obvious that studies of functional capacity put claim on many disciplines but the most serious concern is for behavioral science research to provide answers that will disquiet the mounting concern of many. As an example, there are those who ask whether the more stringent standards of the Soviets reflect a philosophy and long term understanding that will ultimately yield more efficient preservation of human resources. Putting this differently, do they care more about people than do we? I don't believe that such is the case, but are we doing enough to demonstrate it?

I am fully aware of the arguments that indicate the Soviet Union cannot fully comply with their avowed standards, but I am now speaking of the philosophies that are involved. The behavioral toxicologists are going to have to come up with the information which gives credence to the proper balance between realism and ideology. Accordingly, you behavioral scientists have a great challenge. You have your work cut out for you.

I wish you good luck in your endeavor on behalf of NIOSH. I particularly wish to thank the University of Cincinnati for their splendid cooperation and the participants in making this a successful meeting.

WELCOMING REMARKS FROM THE UNIVERSITY OF CINCINNATI

Robert Taft, III

Representing the Vice President for Metropolitan Affairs of the University of Cincinnati, Charles Johnson, who was not able to be here this morning, it is my pleasure and privilege to welcome you to the City of Cincinnati and the University of Cincinnati. This is a beautiful city with many recreational, cultural and culinary attractions and my only hope is that the rigor of your conference schedule will not prevent you from taking advantage of these.

In an important respect the City and the University are indivisible. As you may know the University of Cincinnati is the second oldest and second largest municipal university in the United States. It is within a long tradition of community and national service that we are enthusiastic in co-hosting this workshop as a joint endeavor with the National Institute for Occupational Safety and Health. The recent designation of Cincinnati as the permanent research headquarters for the Institute make it particularly appropriate that Cincinnati should be the site for this workshop.

On the part of the University, we are hopeful that this will be the first in an expanding step of interaction between the National Institute and the faculty and students of the University. We are proud of the resources in the College of Community Services, College of Medicine and other colleges and departments at the university, which bear on the problems on the detection and treatment of occupational hazards. We are hopeful that through this conference these resources will be further stimulated to focus on the research and problems of preserving occupational health in this country.

In the interest of establishing, or setting up a rather unique situation for you this morning and perhaps in order to wake you up because of the starting time, I have been requested to do double duty this morning and introduce the keynote speaker. It is very difficult to determine where to begin in introducing one's father. In deciding to return to Cincinnati recently I knew that living with the legacy of successive generations of my family would be a serious occupational hazard I would have to face. I did not know that it would confront me so soon.

Let me say frankly though that if I were so foolhardy to embark on a political career myself my father is the kind of legislator that I would like to be. He works hard, does his homework and spends

Mr. Robert Taft, III is affiliated with the Office of Metropolitan Affairs, University of Cincinnati.

none of his time running for president. His political experience and his legislative interest qualify him well to address this conference.

He worked particularly hard in the Ohio General Assembly where he was prominent in establishing the Ohio Bureau of Workmen's Compensation and in producing the first rewrite of the Ohio Workmen's Compensation Law since 1913. In 1969, as a member of the United States House of Representatives, he supported and worked for the passage of the comprehensive Coal Mine and Safety Act. Upon election to the Senate, he was appointed to the Senate Labor and Public Welfare Committee where he sits on both the Labor and Health Sub-committees. In 1970 he supported and worked for the passage of the Occupational Safety and Health Act of that year. Most recently and most important in February 1973 he introduced the Respiratory Disease and Benefits Act which is designed to provide workers who contract occupationally related respiratory diseases the same benefits that coal miners receive under the Black Lung Benefits Act.

So as not to steal anymore of his thunder, particularly in view of the possible consequences to myself, let me introduce my father, Senator Robert Taft, Jr.

FEDERAL COMMITMENT TO OCCUPATIONAL SAFETY AND HEALTH

Robert Taft, Jr.

Thank you very much Bob Taft, distinguished panelists, ladies and gentlemen. Bob mentioned the hazard that he suffered and being introduced by your son is one that I hadn't experienced myself but I am indeed pleased and honored to have him here with the University of Cincinnati and in a position where it is appropriate that he extend greetings to this group. Coming to talk to you at this early hour of the morning, I am sure, is not an easy task.

I am happy to be here to talk to the participants of the Behavioral Toxicology Workshop. However, I might say, with all of the background and expertise my son attempted to lay at my door when I was asked to come to a behavioral toxicology workshop and speak on this subject, I recalled my uncle who was a school master and the story that he frequently told of the english exam. One of the questions on the exam that was put to one of the less expert students was write all you know about Keats. The student thought for awhile and his answer was I don't even know what Keats are. This is a little the way I felt when asked to speak at this "behavioral toxicology" workshop. But, I am delighted to be here particularly with the association between the National Institute for Occupational Safety and Health and the University of Cincinnati. Along with Bill Keating and Don Clancy, our Congressmen from this area, we worked long and hard to keep the Institute here and indeed I am delighted that even though it is having some problems it is here and is sponsoring this particular workshop.

I would like to talk to you this morning about occupational safety and health and about some of the prospects and problems as I see them on the national scene. I think that it is clear in the minds of the Congress generally that there is a tremendous need in providing more and better research in the field of occupational safety and health. We can't overstress this area. Behavioral Toxicology is, of course, a part of it.

Frequency rate of disabling injuries and diseases per million employee work hours has rapidly increased as productivity has increased. The Bureau of Labor Statistics has estimated that the frequency rate of injuries has risen from 11.8 percent in 1960 to 15.2 percent in 1970. These percentage figures translated to numerical data represented in 1971 more than 14,000 employees killed and an estimate of 2.2 million employees who suffered from disabling injuries or disease as a result of occupational hazards.

Mr. Robert Taft, Jr. is the Honorable U.S. Senator from Ohio.

The Social Security Administration estimates that the total cost of Workmen's Compensation to employers in 1970 was a staggering figure of 4.82 billion dollars. Now as shocking as these figures are, they do not represent the great number of minor and non-disabling injuries and more importantly the number of workers who become ill after being exposed to hazardous working conditions.

According to data being released in 1972 by the Department of HEW there are more than 13,000 toxic substances which could cause harm to workers. The exact effect and causal relationship, however, of many of these substances still is in a very preliminary stage of research in most instances. The total human and economic consequences of occupational diseases, injuries and illnesses, of course, can't ever really be measured. An individual who is injured on the job may be forced not only to file for workmen's compensation payment but also for other types of assistance including welfare and this is especially true if the individual must support a family. Such a person may not ever be able to obtain employment due to a physical impairment, furthermore, in many cases he may suffer from employment discrimination if he is classified as a safety risk.

The major legislative breakthrough with regard to occupationally related injuries was the Williams-Steiger Bill of 1970 of which I am sure that you are familiar. This legislation, the Occupational Safety and Health Act of 1970, was signed into law by President Nixon on December 29, 1970 and covers about three quarters of the civilian labor force. The Congressional intent of the Act was stated as follows: "To assure, so far as possible, every working man and woman in the nation safe and healthful working conditions." Every effort should be made to attain this goal as rapidly as possible, but as many of you know some problems have arisen with regard to enforcement of the Act. These problems are basically practical ones and can be corrected without impairing the primary intent of the Act. I have introduced, along with others, legislation in this Congress to alleviate some of these problems. My proposal would not change the coverage of the Act but would permit consultative visits at the worksite by OSHA inspectors. Hopefully, action will be taken by the Congress in this session to correct some of the more practical enforcement problems. I am certain, however, that congress will continue to express strong support for the Act.

As Bob mentioned, I introduced legislation to further our national commitment to occupational safety and health. The bill, The Respiratory Disease Benefits Act of 1973, is patterned after the Federal Coal Mine Health and Safety Act and Black Lung or Pneumoconiosis Benefits. It is the first proposal advanced in con-

gress to recognize the concern for occupationally related respiratory diseases other than pneumoconiosis. The compensation levels established by the Black Lung Act are extended under this proposal to all workers suffering from occupationally related diseases with the Federal Government assuming such liability for the first two years of the program. I am quite hopeful that legislation of this type can be enacted by the congress. It seems to have broad support and interest. We worked very closely with national labor groups such as the AFL-CIO, in the drafting of it, as well as with other industrial groups. It would cover such diseases as silicosis, asbestosis, berylliosis, and byssinosis that are certainly as serious as black lung disease although they are not, of course, as prevalent. Under most state workmen's compensation programs, coverage of occupationally related diseases has lagged behind coverage of accidental injuries. A hearing is yet to be scheduled on the proposal. I am hoping, however, that we will get some action by the Senate Labor and Public Welfare Committee this fall in at least considering the proposals.

One of the practical problems that you may be interested in is the bill presents a rather difficult choice for some members of congress who are concerned with this area but whose approach to it has been that we ought to federalize workmen's compensation or establish some federal standards under workmen's compensation. You may feel that putting emphasis in the respiratory disease act, particularly on the diseases I noted earlier might perhaps slow down activity in this direction. The representatives of organized labor with whom we have been working are of a contrary opinion and realize the practicality of the situation. It seems pretty clear that the likelihood of moving on federalization or even minimum standards legislation for workmen's compensation at the federal level is not very good and the respiratory disease problem is here with us now. I think we ought to step forward and at least make an attempt to move into this area whatever the outcome might be ultimately on the question of federalization or federal standards as far as workmen's compensation is concerned. It is distressing that we are having such a hard time in getting it moving and getting it to a hearing. Any help we can get in this regard I would certainly welcome. So much for where that particular legislation stands.

It would be amiss, I think, if I didn't mention the problems that we seem to be having on the national scene in so far as financing activities in this field is concerned. I am upset to learn of the reduction in force and grade level in the National Institute for Occupational Safety and Health. It is particularly distressing that highly qualified personnel have sometimes been shifted from

their areas of expertise in some cases to areas actually out of the field entirely. I and other members of the Senate Labor and Welfare Committee intend to have Secretary Weinberger before us to discuss this problem sometime in the near future. I have already discussed it with the Chairman, Senator Williams of New Jersey. As the situation currently exists clearly something must be done.

The President in his 1972 report to the congress on occupational safety and health stated "Like many problems we face today the improvement of job safety and health cannot be accomplished simply by pressing a button. If we are to reduce injuries, the illnesses and the deaths that are connected with working conditions we must take determined actions: we must increase the number of people who are trained in health and safety techniques; knowledge of the causes of accidents and illnesses must be developed; this knowledge must be translated into effective standards; employers and employees require adequate instructions; and standards must be enforced through energetic and rigorous inspection programs." I believe that there is no reason to retreat from such an approach.

NIOSH, and other related occupational safety and health programs, must be funded and staffed at an adequate level. The Federal government must not abandon its commitment to America's working men and women in the occupational safety and health field. I have tried to highlight in my remarks the feeling congress has for your field of interest. It is true that there are current problems but this by no means indicates a lack of attention or turning of attention away from this field and I feel that it is going to continue to grow and to continue to face up to and solve the problems.

I hope that this will be a most successful workshop and that you will contribute materially in providing safe and healthful work conditions for every working man and woman in the Nation.

DISCUSSION

Dr. B. Dinman: Mr. Senator, those of us who have worked in this field of occupational safety and health for many years can't help recalling at moments like this the shape of the scene as it was just three or four years ago. To hear any expressed concern on the part of the national leadership for occupational safety and health both in the legislature and executive branch was an unusual situation. Now I think we have turned a tremendous corner in the last four years, and it certainly makes us feel as if people are now listening to what many of us have felt for years. Now we can get action in promoting occupational safety and health and in promoting the safety and health of probably our most precious

asset, the American worker. We also feel somewhat more reassured in hearing your words of support for NIOSH and for the Occupational Safety and Health Act, that I understand is under some attack at this moment. We will look to your continued support of this very important area.

INTERNATIONAL BRAIN RESEARCH ORGANIZATION

Peter Dews, M.B.

Mr. Chairman, Senator Taft and colleagues. The impressive collection of organizations with whose blessing we meet here today, acronyms, IBRO, IAOH, NIOSH, NAS, sound like the names of a bunch of reindeer. Of them, IBRO, the International Brain Research Organization whose greetings I bring is probably the least familiar to you.

IBRO was founded about a dozen years ago under the auspices of UNESCO for the purpose of fostering research in education, in the study of brain sciences and of behavior by international cooperation throughout the world. It has since developed strong ties with the World Health Organization. It has fostered fellowship programs and has sponsored teaching workshops and symposia. The major activity has been facilitating exchange with eastern European countries. We have held workshops in Poland, Hungary, USSR, Rumania and Yugoslavia.

Our job in this regard has become progressively easier and we've been joined in this purpose in late years by quite influential people. It was heartening to see in Washington last week the lamp post adorned by both the Stars and Stripes and the Hammer and Sickle. One of our prominent members is currently in China and although it is not his main purpose we are hoping he will be able to hasten major interchanges with China, for IBRO.

Another main thrust of IBRO, as might be anticipated from our association with UNESCO and WHO, has been to disseminate information and techniques to developing countries. We have held workshops in India, Pakistan, Iran and Nigeria and others are in the state of planning.

Behavioral Toxicology is obviously a field of central interest to IBRO and we were glad for the opportunity to cooperate with Dr. Xintaras and his planning group and give moral support to this workshop. IBRO is not an affluent organization so our help has indeed been in moral support but nevertheless sincere. I will make only two points about behavioral toxicology in the relatively undeveloped parts of the world.

First, it is a mistake to think of pollution and occupational hazards as only a problem of places with vast chemical and metal industries and one car per 1.2 inhabitants. Most all the hazardous substances permeate their way throughout the world, in the villages

Dr. Peter Dews is Executive Secretary of the International Brain Research Organization and Director, Laboratory of Psychobiology, Harvard Medical School, Boston, Massachusetts.

and towns of Africa and Asia and the lesser absolute amounts of hazardous chemicals are largely offset by the greater intimacy with which the poor people live with their environment. We've heard in our own country how the children at greatest hazard from lead are not the children of the rich whose cars burn most leaded gas but the children of the poor. Poor have the most contact with the dirt of the streets where most of the lead ends up. A similar situation obtains with metals and insecticides and other chemicals in the crowded villages and towns of undeveloped countries.

The second point is that behavioral toxicology is a subject which should rapidly become excellently suited for field studies anywhere in the world. Sophisticated behavioral tests to assess minimal damage require sophisticated equipment as you will see later this week. But in contrast to chemical and physiological assessment the apparatus for behavioral tests can in principle be made rugged and reliable for use anywhere in the world. The development of integrated circuitry makes it possible to carry a computer in a suitcase, a computer which can run indefinitely off an inexpensive battery.

The main problem in behavioral toxicology research is to devise the right test, discriminating and informative and that is the main purpose of Dr. Xintaras and his group with NIOSH and of this workshop. You are furthering a program that will benefit people of all the world with detriment to almost none and it is not many enterprises in which that could be truly said.

On behalf of IBRO we wish you well in this workshop this week.

INTERNATIONAL ASSOCIATION FOR OCCUPATIONAL HEALTH

B. Dinman, M.D.

Unfortunately, Professor Milan Horvath of Czechoslovakia was unable to be here this morning to extend greetings from IAOH but, as a member of the American delegation of the Permanent Commission and International Association for Occupational Health, I will extend on behalf of the organization at this moment its greetings and its support for the worthwhile efforts which you will be engaged in here this week.

The Permanent Commission and International Association has over the years provided a forum, a meeting place for scientists and health workers around the world who were concerned with worker safety and health. This forum provided us a place where we might be able to carry out the dialogue for workers in this area who at many points because of differing biophilosophic points of view had differing opinions. Nevertheless, under such aegis it was possible that we might at least aid in some convergence of understanding between the various groups in various parts of the world.

First, one of the most noteworthy of the areas of disagreement has been in the area of behavioral toxicology. The point of view of our eastern European colleagues was quite diametrically opposed it seemed in substance but perhaps more apparent than real from the point of view that was more current in the west as regards the utilization of techniques for the protection, understanding for the protection, of worker health. With, of course, the rather rapid development of the national concern with occupational safety and health perhaps our horizons were broadened somewhat in the United States when we began to appreciate more completely the concern of workers not just for the preservation of health in its very narrowest definition but also for the augmentation and promotion of health not only as the absence of pathology but health as a state of well being, as a state or expression of quality of life.

It was in this particular area of some objective, perhaps, hopefully objective approach to assessment of the quality of life as it relates to the broad definition of health that this area of behavioral toxicology possibly offered a way for us in the west to approach such an elucidation, such an objective definition of well being in addition to just the absence of pathology. It is fortunate that the International Association has provided over the years a forum for discussion between the various points of view, east and west,

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in this regard and so we could turn back to our colleagues in the International Association to augment our interest or at least to give us pause for reconsideration of our previous points of view.

In the promotion of this interchange between differing points of view the International Association established a Subcommittee on Higher Nervous Functions. This subcommittee is composed of scientists from both east and west and this hopefully provides an additional forum at which we can come down to the very hard questions of disagreement and at least approach the solution of these questions through mutual discussions. Anything which promotes an understanding of these problems of behavior as it applies to behavioral toxicology particularly in the west is a function which the International Association has considerable pleasure and certainly interest in promoting.

Accordingly, the International Association for Occupational Health brings greetings and we hope, of course, that you will have a successful and useful workshop this week.

NATIONAL RESEARCH COUNCIL, NATIONAL ACADEMY OF SCIENCES

Ralph Wands and Louise H. Marshall, Ph.D.

Thank you Dr. Dinman, morning greetings to our panel members. I bring participants in the workshop greetings from the National Academy of Sciences and also the regrets from my compatriot, Dr. Louise Marshall, who also participated in the planning of this conference with us but was unable at the last moment to be here this morning.

In the very early planning of the conference, Dr. Xintaras discussed the concept with Dr. Charles Dunham who then was chairman of the Division of Medical Sciences of the National Academy of Sciences, National Research Council. Committee members and staff members of the Academy's committees on brain sciences, problems of drug dependence, biological effects of atmospheric pollutants, and advisory center on toxicology joined with Dr. Xintaras and several other speakers at this table in discussing the need for this workshop which would stress behavioral methods for toxicology and also in considering possible sources of funding. We are pleased to have had the opportunity to cooperate at an early stage in planning for this very important event.

We are impressed with the broader applications of behavioral toxicology beyond the early detection of occupational injury. Important as this may be, we believe that the fruits of this conference are going to have much greater applications than just simply to the occupational members of our total population. Let me give you two examples. One of these is the detection in children of adverse effects from exposures to low doses over long periods of time. The behavioral methods for adults may not necessarily be applicable to children for very many obvious reasons. Yet, as in the case of lead, children may constitute a high risk population. Another application to the general public is coming to the foreground from our space travel. Very little is known about the amount of high-energy heavy ions that can be received by the central nervous system without causing behavioral effects. I am sure that all participants can add examples of the broader aspects of behavioral toxicology to their own thinking.

We extend our greetings to the participants, wish them a totally successful workshop, and urge that they all keep in mind the widest possible applications of the methodology which you will be examining.

Mr. Ralph Wands is Director, Advisory Center for Toxicology, National Academy of Sciences, National Research Council, Washington, D.C.

Dr. Louise Marshall is a Professional Associate, National Academy of Sciences, National Research Council, Washington, D.C.

BEHAVIORAL TOXICOLOGY IN THE DEVELOPMENT OF THRESHOLD LIMIT VALUES

H. E. Stokinger, Ph.D.

The setting of standards for substances in the environment of man is one of the major endeavors of contemporary toxicology. As Dr. Xintaras has so ably envisioned it in his background statements for this Behavioral Toxicology Workshop, this week is to be devoted to the very practical endeavor of directing attention of workers in the behavioral sciences and of exploring ways to detect, at the earliest possible moment in biologic time, changes that are occcurring in the animal organism from exposure to industrial substances in the workroom air, that may be affecting workers, be they of systemic or of nuisance nature.

We already have a considerable number of industrial air standards, about 550 of them, listed in the TLV Booklet for 1973 complete with Documentation of these TLVs. But we note that only for 3 of them, Carbon Monoxide, and 2 Freons, has there been any contribution to their TLVs by behavioral toxicology. On the contrary, in the USSR, behavioral toxicology has contributed much to the industrial air standards. Behavioral toxicology there has been credited, at least by Soviet admission, for their lower, and in their eyes, more appropriate standards.

NATURE OF INDUSTRIAL AIR STANDARDS — U.S.A. THRESHOLD LIMIT VALUES (TLVs)

For the interests of this group, and to see clearly what the objectives of this workshop are, and what is involved for behavioral toxicologists, the TLVs, Threshold Limit Values, are numbers representing safe limits of exposure for industrial workers for a 7 to 8-hour workday, 5 days a week, for a working lifetime and even thereafter; for we do not intend that a worker retire from employment only to come down with a job-related disease.

From the standpoint of developing useful behavioral criteria for basing TLVs, it must be appreciated that TLVs concern two broad types of reacting substances. One is the short, fast-acting type, typified by the irritants, the narcosis-producing substances and the lung sensitizers, which after a relatively brief exposure, may, after a suitable latent period, result in sensitization from a single brief exposure.

The second includes those substances whose action requires long-repeated exposures for manifestation of disease, substances

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such as carbon disulfide, benzene, lead, silica and fluoride. Of course, there are some substances with both actions, irritation and/or narcosis followed by systemic effects. To repeat: Realizing these possible actions, it is the obligation of the behavioral toxicologist to so design his experiments that the period of exposure conforms to the toxicologic actions of the substance for which the early behavioral indicators are being sought.

Objectives of Behavioral Toxicology in Standard-Setting

There are two broad objectives for research in behavioral toxicology that are directed toward finding the most sensitive indicators of response from inhaling industrial chemicals.

(1) The development of criteria on which to base *new* TLVs, and

(2) The development of criteria for *refining* or *validating* already adopted or accepted industrial air standards.

Nature of Behavioral Studies for TLV Criteria

It is obvious that any behavioral data for industrial TLVs would be of greatest use if derived from man; data on human behavioral response obviously take precedence over those derived from any other species (it is not our present purpose to define an environment safe for rats and mice).

I see that the great majority of the people here are working on human material. That is as it should be. But at the same time, you must realize that it cannot be forever this way. Human studies are performed for the most part on substances for which a TLV already has been set, and consequently behavioral work merely provides criteria in one area, in refining or validating TLVs. Sooner or later this area of endeavor will become exhausted. The development of criteria for new TLVs must perforce be done in animals, the ethic of our Society being what it is.

Correlation of Behavioral Response in Man and Animal*— A Needed Area of Research

Irrespective of whether one prefers man or animal as test object, there is one virtually untouched area of investigation that requires both, namely, the correlation of human and animal response to various type substances, at equivalent dosages. Such correlation would provide a guide to the relative sensitivity of animal species to man, thus supplying badly needed information.

Animal Behavioral Toxicology — Limitations and Concerns

Resort to animal experimentation instead of man, eliminates one whole area of response, namely, the organoleptic or sensory

*I am indebted to Ralph Wands for this contribution.

response, the entire area of subjective effects of irritation, headache, nausea, dizziness and the like; animals simply can't communicate these finer sensibilities. Because of this, animal species, including the monkey, tend to respond less sensitively than man to the fast-acting substances, the irritants and the narcotics. It should be appreciated also that animals commonly respond differently than man to substances giving the following types of responses: metal fume fever, pulmonary sensitization, irritation to the eye, skin and mucous membranes. It is apparent, therefore, that considerable toxicologic judgment must go into translating the results of animal behavioral work to man, even with the use of nonhuman primates.

Protocols and Problems

As chairman of the TLV Committee, I feel constrained to mention 4 very practical items of research protocol that seem from my vantage point either not to be appreciated, or if appreciated, have not been given the serious attention or corrective effort needed, if behavioral work is to be useful for developing standards for new substances or refining adopted standards.

(1) *Number of Subjects*
The number of experimental subjects must be increased. This is an absolute "must," if data are to be used for TLVs. No one responsible for setting industrial air standards is going to pay attention to results on ½ dozen individuals or on 2 monkeys, or 6 rats, when the health of 80 million workers, or the huge costs of over-engineering are at stake. But there are other reasons. Nonuniformity of response alone, particularly marked in monkeys but seen also in inbred rats, dictates larger numbers than are now commonly used. Moreover, Soviet Pavlovian behavioral scientists have long recognized 4 groups of central nervous system reactors, ranging in response from the plethoric to the highstrung, with correspondingly reflected toxicity responses.

(2) *Variation in Response*
Because of the unacceptable variation in response seen in randomly selected human populations or noted in monkeys obtained in the wild, I see no suitable alternative than to select subjects from definable cohorts or to *obtain monkeys from colonies where heritage and environment are known,* and hence variation in response is greatly reduced and at least, better understood.

(3) *Determination of Physiologic Significance*
In order for the highly sensitive indicators of behavioral

toxicology to serve as valid criteria for TLVs, a second investigative step is necessary. It is not enough merely to define the dose that will produce a borderline response. The next important step is to determine whether the response at the defined dosage has any over-all *physiologic significance to the industrial worker*, who is on the job day after day, and may become inured through homeostatic mechanisms. Note also, we are not especially concerned with individuals in highly sensitive activities such as astronauts, jet pilots or atomic submarine crews. What standard-setting bodies are concerned about is that industrial air standards are not based on physiologically meaningless changes that are within normal homeostatic control. Changes in the visually evoked response (VER) are presently not sufficiently well understood in over-all physiologic terms to serve as basic criteria for air standards. Not to make the determination of physiologic significance can result in undue alarm for health and safety of the worker and, more importantly, can result in costly over-engineering — costs that are ultimately passed on to you and me, the consumer.

(4) *Motivation and Performance*

And now we come to the fourth and last and probably the most difficult problem to resolve in all of behavioral toxicology: How much of a role does motivation play in the responses to toxic stimuli? It has been noted, for example, that responses to the same exposure conditions differ according to whether women are tested with men or tested separately. Certainly motivation must play a tremendous role when self-preservation is at stake, — the pilot who smells smoke in landing his plane while flying blind is considerably better motivated for better performance than is the pilot trainee who is flying blind in a simulated situation. Accordingly, the question that might be given some airing at this workshop is, how to relate animal performance when their motivation for response is food or shock avoidance when human concern is for health and safety?

Interpretation of Results

Finally, in view of the subtleties of behavioral toxicology, I cannot urge too strongly that all data that are developed for the scrutiny of standard-setting bodies contain thorough discussions and interpretations in terms that can serve as relevant criteria for TLVs; poorly controlled, equivocal, or scanty data will not be found acceptable.

NIOSH BEHAVIORAL RESEARCH PROGRAMS

Alexander Cohen, Ph.D.

Aside from other cogent considerations, behavioral research within the National Institute for Occupational Safety and Health or NIOSH has a statutory reason for being. Indeed, the Occupational Safety and Health Act of 1970[1] gives NIOSH the following research directives:

1. To include psychological, behavioral and motivational factors in researching problems of worker safety and health and to consider these factors in developing new methods, techniques or approaches in handling such problems (in Sections 20 (a) (1), 20 (a) (4)).
2. To determine exposure limits and the physical conditions at the workplace which are not only safe for health, but which pose no threat to one's functional capacity (in Section 20 (a) (3)).
3. To evaluate the effects of job stresses on the potential for illness, disease or loss of functional capacity in aging adults (in Section 20 (a) (7)).

NIOSH activities concerned with behavioral, psychological and motivational factors fall into three program areas that parallel the three directives just stated.

HUMAN FACTORS IN ACCIDENT POTENTIAL AND STRATEGIES FOR IMPROVED WORKER SAFETY

One such program deals with human factors bearing on accident potential, especially in high risk jobs, and the effectiveness of behavioral science approaches in accident control. Work here will include the design of a technique for probing human factor causes of accidents or "near misses" at the workplace, and the prospective use of this scheme for improved accident reporting in industry. While the human element is believed to be the single most important cause of industrial accidents, current investigatory procedures yield little more than a notation of the agent that actually injured the person and the extent of the injury. Such data provide no real insights into accident causation due to human or other factors.

Another effort involves contrasting attitudes towards safety rules, perceptions of job risk, emotional states, behavioral performance capacities, and work habits of workers having few versus many accidents in hazardous occupations. Those currently being

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studied are roofing workers and coal miners. These occupations rank among the highest in accident rates and disabling injuries. Also being compared are company practices and management attitudes towards safety in work establishments with histories of good and poor safety performance. This research is intended to dramatize the importance of certain human factor variables in worker accidents and management practices that may influence or shape these factors.

Still another project within this program area is designed to show the utility of behavioral science principles in reducing the possibility of worker accidents. One contract study, almost complete, will provide for an inventory of contingency plans for accident control which are based upon psychological principles as found in the literature on learning and motivation, organizational behavior, behavior modification, and human factors engineering. A follow-on effort here will include the development of a diagnostic technique which a foreman can use in analyzing accidents or unsafe acts connected with a specific job. This technique would allow the foreman to select an appropriate behavioral strategy for control from the inventory just noted. Plans call for field testing of this diagnostic technique and the actual implementation of the prescribed control strategies in four job settings where there is evidence of frequent worker accidents or "near misses."

The activities in this program area are expected to offer the following outputs:

1. A better understanding of the human factor in accident occurrences and manuals of good work practices in dealing with this element in select high risk occupations.
2. The description of model safety programs referencing optimal management practices, aspects of hazard control, safety promotions, effective training, and sensitive record-keeping procedures.
3. Criteria for selecting workers for risky jobs that may not be be feasibly controlled through engineering means.

BEHAVIORAL AND NEURAL FUNCTION CHANGES DUE TO CHEMICAL AND PHYSICAL AGENTS

The second program area concerns the identification of adverse behavioral and neural function changes due to chemical and physical agents. NIOSH has given the highest priority to developing exposure limits for potentially harmful chemicals and physical agents found at the workplace. The schedule of criteria development for recommended standards includes chemicals having neurotoxic properties (e.g., pesticides (parathion), solvents (carbon disulfide, perchloroethylene,) heavy metals (lead, mercury),

exhaust gases (CO), irritant vapors (formaldehyde). Physical agents such as noise, vibration, and heat are also listed which can be injurious to sensory organs or physiologic functions. The protection goal in setting limits for these chemical and physical hazards has been to safeguard workers against those pathologic changes or disabilities which are specific to the different agents. Exposures at or even below these limits, however, may evoke behavioral and nervous system changes whose implications for safety and health are in need of more study. This workshop will address itself to the question of whether these preclinical behavioral/neurological changes may be an early indicator of a potential disease process. Also, whether the nature of the behavioral changes induced may increase accident risk on or off-the-job. A slowed reaction time, erratic eye-hand coordination, diminished perceptual capacity are some of the behavioral losses seen in low level exposures to chemical agents which could be predisposing factors in accidents[2, 3, 4]. With regard to one physical agent, noise, current limits for safeguarding hearing can permit high enough levels for short exposure durations to trigger a number of non-auditory effects which again could increase accident potential[5, 6].

NIOSH supported studies already completed or in progress in this program are intended to generate behavioral/neurological response data associated with exposures to mercury, lead, CO and noise at or below suggested threshold limit values. Still another investigation is testing for the influence of trace levels of anesthetics found in operating rooms on the performance effectiveness of operating room personnel, notably, anesthesiologists. This latter work is part of a much larger project designed to justify the need for stricter ventilation requirements in surgery facilities.

Behavioral test batteries used in the above-mentioned studies consist of perceptual, psychomotor and cognitive-type tasks which will be demonstrated in the course of this workshop. These performance tasks, or variations of them, are also planned for use in future NIOSH behavioral investigations of workers exposed to solvents and pesticides.

The information generated within this program area is to be incorporated into the data base for setting exposure limits for chemical agents so as to duly account for added health and safety considerations. In addition, this research will also yield insights into the benefits of behavioral and neurophysiological monitoring techniques for providing early detection of potential hazards due to these agents.

PSYCHOLOGICAL JOB STRESS AND WORKER HEALTH

The third and last program area deals with the impact of

psychological job stresses on the worker's physical and mental health. It is important to realize that a segment of the workforce may experience stress factors at work other than or in addition to those posed by chemical or physical agents. These types of stressors defy measurement by impingers, sound-level meters, gas detectors and other instruments used in characterizing the physical environment. These stressors are psychological in nature, stemming from the demands of the job or other aspects of one's work situation. Information overload, role conflicts, decision-making affecting the safety or well-being of others are examples of psychological job stressors, and there is growing evidence linking these kinds of stress factors with increased risk of health ailments including coronary heart disease, ulcers, and nervous disorders[7]. Perhaps the classic example of a work group subjected to psychological job stress with consequent health problems is the air traffc controllers. A sample of workers in this occupation and others are presently being studied by NIOSH in an effort to define the degree of relationship between elements of stress in twenty different job types, actual perception of stress by the jobholders, and evidence of health problems, either physical or mental, among these workers. The latter data are taken from psychological interviews, adjustment inventories, medical histories, and biochemical determinations on the subject workers.

These studies should yield more systematic evidence acknowledging psychological job stress as a health problem and identify the more potent sources of this stress. The latter in turn, are to provide a basis for developing stress profiles for a variety of occupations making up the technical/professional, management/supervisory or typical white collar worker groups. There are 31 million workers who currently fall into this occupational category. Also envisioned in this area of job stress and health are investigations of the stress impact of certain job features or work regimens which apply to a cross-section of the workforce, both white and blue collar. One such variable is work hour regimens. Still another effort is going to concentrate on the role of psychological stress factors at work as a primary or contributing factor in coronary heart disease.

To ascertain the status of knowledge and to advance research interest in job stress, NIOSH sponsored last year the Cornell Conference on Occupational Stress[8]. That meeting, as this one, brought together professionals from diverse disciplines who interacted on issues of problem definition, methods for measurement, health impact, remedial strategies and the like. The meeting was a successful one in providing multi-disciplinary views of job stress and defining the areas where research attention needs to be

focused. I have every confidence that this conference will also satisfy its aims.

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BEHAVIORAL SCIENCE AND OCCUPATIONAL HEALTH

Sheldon W. Samuels

I can add only one remark to the chairman's introduction and it is that in this city, Cincinnati, our oldest union was founded after the Civil War: the Moulders Union. To illustrate that worker health is not a "new" concern, the Moulders Union was founded in part for the purpose of protecting workers' health. I think all 85,000 members would like this recognition.

About a year ago, when this conference was still recorded only as a wild rhythm in Dr. Xintaras' encephalogram, Charlie and I met for a full day in his laboratory here in Cincinnati. I wanted to spend time with him because of the critical importance of his research to the labor movement. He seemed pleased but surprised that we rate this field as the nation's first priority in health research. As a means of fulfilling my obligation here, let me reiterate to you the rationale I provided him for this bias.

The labor movement, abroad as well as in this country, has been the subject of intense research by the behavioral scientists since the beginnings of the industrial revolution. For what purposes?

(1) Incentives research to increase "productivity,"
(2) Identification of psychological stresses that decrease "productivity,"
(3) Delineation of sociological or cultural patterns that interfere with or enhance "productivity," and
(4) Performance research geared to production task training for "productivity."

These have been the major areas of concern. But they have little to do with concern for the worker, rather they focus on increasing the efficiency with which the worker is used.

There is one notable but minor addition to this list: the study of the causation of the so-called "unsafe act" as a basis for "safety" training. What makes this line of endeavor notable but minor is that it is perverted by an assumption that has little relation to reality. Even if we assume that occupational injuries are caused by either unsafe acts or unsafe conditions, the unsafe act explains but a small portion of all injuries and injuries are a fraction of the total risk in the workplace. We estimate that of the 10,000 deaths caused both by disease and injury in our workplaces each month, 1,000 or 1/10 is due to injury. On the basis of fantasy and guesses, Heinrich has alleged that about 90 percent of these 1000 are due to "unsafe acts." But the only objective

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study of which we are aware, currently in process at the University of Wisconsin, without Federal support, indicates just the opposite — 90 percent of the injury rate is not accidental at all but due to working conditions.

Thus, unfortunately, because of inadequate studies, behavioral research in this area is focused on the cause and prevention of 100 deaths per month and virtually no attention is being given to the cause and prevention of the 900 other deaths. The malfocus has little chance to result in saving the lives of even the 100 since its aim is to provide a basis for a regime of safety training, which means training workers to adapt to environmental, physical and psychological hazards and stresses. This approach can, without doubt, reduce injury rates among young, inexperienced workers. But as the worker gains in age, the effects of aging plus lead or carbon monoxide or other substances or agents which impact on the nervous system are less easily overcome and — as might be predicted the ability to continue the learned adaptation decreases and injury rates rapidly rise.

Unfortunately for the older worker this pattern coincides with decreased productivity due to age and an increase in employer shared medical expenses. Thus it is cheaper to replace dead workers over 50 or to lever older workers into less remunerative but "safer" positions or out of the plant altogether, than to eliminate unsafe conditions!

Obviously the labor movement will have little to do with a line of research that reinforces this pattern. Rather, it is obviously in our interest that the focus be on unsafe conditions rather than the largely mythical unsafe act. Yet large-scale studies of relationships between the immediate cause of injury and environmental or psychological stress have just begun in this country. We are at least a decade behind our European brothers. We need studies such as Jansen's demonstration of the link between noise and equilibrium and Broadbent's study relating noise and peripheral vasoconstriction (with its obvious implications for impaired finger movement).

Labor's primary contribution has been and will be the creation of a climate in which these studies can be done. But studies relative to injury will not fulfill the potential of behavioral science. Rapid expansion of our knowledge of the effects of toxic substances on performance, for example, will unquestionably result in lower injury rates. But we are convinced that they will result also in fast, sensitive methods for detecting exposures deleterious to health itself; that is — they will result in a reduction of the estimated 9000 deaths per month due to occupational disease. In our increasingly synthetic universe, no research has more critical biological, economic and technical impact than research that will

provide the earliest warnings on the basis of which life can be protected. Let there be no misunderstanding of what I mean. Life, not life style, is paramount.

The direction and even the continued development of our technology will depend upon the best estimate of whether a technology or technological artifact contributes to or inhibits existence. At the present time the greatest potential for such prediction lies in the behavioral assay of the synthetic condition or substance. To the actualization of this potential, organized labor has already begun a program of systematic support.

WORKSHOP PERSPECTIVES

B. Dinman, M.D.

If I may summarize in a moment the purpose of this workshop. It should provide us this week an opportunity for interchange of current information and test methods among those with vital concern for how behavioral toxicology research methods are used in academia, industry, government or by labor and how the results can be applied in the establishment of protective standards designed to protect not only the health in the strictest sense of the term but to also protect and maintain the functional capacity of the working people in the United States.

To put the workshop in perspective for the rest of the week, we should note that we are concerned not only with the demonstration and elucidation of indicators of change in itself but should keep in mind the implications of these changes for health and well-being for both the immediate and long term - life time if you will - health of the individual.

It is not enough to discuss and consider during this week that changes do occur. This is not sufficient by itself. For instance, one recalls the work of a scientist in 1948 who demonstrated that the passage of an air current across the cheek acting on the skin of the cheek enhanced the sensitivity of peripheral vision. In the Soviet view this represented stimulation of trigeminal nerve acting on the cortical sensory analyzer. Such stimulation of the sensory center, by radiation also produced stimulation of the optic center which resulted in a decreased visual threshold. Now this describes a fairly benign process in our point of view which to us would be an essentially secondary manifestation of environmental awareness. That this represents a change, there is no argument, but that we demonstrated such a change does not necessarily indicate that a change in and of itself, has a deleterious implication. Yet, in the late 1950's Professor Bushtueva was able to demonstrate that 0.7 and 0.9 miligram per cubic meter of H_2SO_4 mist produced a similar change, that is a decreased visual threshold, increased ability to perceive light changes. Now in her report she essentially stated that this represented a response to a noxious stimulus and that since this change was associated with a noxious stimulus it was deleterious.

It seems strange in our point of view that for one individual a passage of air across the cheek, certainly having no deleterious implications, produced a change in responsiveness and to another just

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because this change was induced by H_2SO_4 mist, that this necessarily demonstrated a deleterious change. We believe that herein lies the crux of the problems for western industrial toxicologists. To the Soviet toxicologist such changes in cortical centers or analyzers as they refer to them are early manifestations of toxicity, change per se almost as an early manifestation of toxicity and associated with instability of nervous regulation arising against the background of a changed functional state of the cortex and subcortical region. These are believed in turn to lead to altered reactivity of the vascular and endocrine system and instability of neurocirculatory processes. The manifestations of such changes in the affected individual are the general nonspecific symptoms of toxicity arising from such autonomic disequilibrium. Now herein lies an indisputable proposition. That is, autonomic, or in the verbiage of the east, neurovegetative changes, may well be at the root of nonspecific alterations seen in early toxicity.

Indeed, the considerable attention paid to such alterations by Soviet toxicologists should give their Western counterparts pause. Undoubtedly, except in a few U.S. studies, little attention is paid to such subjective changes which are so difficult to evaluate objectively. Conversely, more statistically valid characterizations of changes in exposed populations are needed to confirm the clinical and neurophysiological evaluations noted in the Soviet literature. For these reasons we believe that considerably more attention should be devoted to objective delineation of what we in the west would call nonspecific change.

Through the techniques of neurophysiologic/behavioral testing such insults of a nonspecific nature might be dealt with objectively. It is not sufficient to be protected against toxicity per se. We have no justification for requiring American workers indeed to live with such nonspecific changes that while not leading necessarily to toxicity, nevertheless do lead to impaired functional capacity, or if you will even impaired quality of life. But before saying we see no reason for a worker to have to put up with the hardening process encountered let's say in exposure to phenolformaldehyde polymers we should be prepared to provide objective measurements that define such nonspecific changes so that the most judicious scientist can be intellectually satisfied.

It would seem that through this route of behavioral toxicology, if we read our Russian colleagues right they are saying in effect that animal testing and human testing using neurophysiologic/neurotoxicologic principles can aid in arriving at some objective delineation of these nonspecific alterations that we in the west have tended to dismiss. As individuals we all know that as a precursor, as a preliminary, to clinical disease this is a nasty, difficult,

unhappy way to live. So, I would like to harken back once more, even though we will be talking this week of the methodologies or demonstration of neurophysiological change, we should keep this within the context of understanding that we are not talking about or concerned with change for changes sake in itself. We should keep in mind that whatever methodologies, whatever techniques we apply to this area that we consider these changes, so delineated, for what their long term implications are for occupational safety and health, both for the workers as a group and for the individual workers.

WORKER EXPOSURE TO SOLVENTS

SESSION II

Chairman and Keynote Speaker

RICHARD D. STEWART, M.D.
Medical College of Wisconsin

SOLVENT SEMINAR KEYNOTE ADDRESS

Richard D. Stewart, M.D.

Twentieth-century America is becoming increasingly aware of her responsibility to the workman. The work environment must be safe, and the impact of the exposure to chemicals, heat, radiation, and noise such that even transient physiological or psychological dysfunction does not occur.

In the 1940's, industrial exposures were controlled so as to prevent overt and permanent organic injury. In my home town, the citadel of a major chemical company, I can recall my father coming home to tell of an aspirin process in which carbon tetrachloride was used as the solvent. He extolled the virtues of this beautiful process because the workmen could wash their soiled caps in the open vat of carbon tetrachloride, flick off the excess solvent, don the damp caps, and whistle as they went on with their work. During the lunch hour it was common practice to dry clean coveralls in open buckets of carbon tetrachloride. There were no measurements of the breathing-zone concentration of the solvent in the work area. Untoward subjective responses were completely ignored. "Behavioral toxicology" was a term yet to be coined.

Workmen who stopped for a beer on the way home would experience nausea later in the evening. A few became jaundiced. The ill complained to the Medical Department, and because they were looked upon as somewhat less than stoic, they were transferred to other departments. The non-drinkers were able to tolerate the daily exposures and continued working.

When I returned to my home town in the mid-1950's as an industrial physician, this same chemical company had instituted a medical surveillance program in its effort to define the effect of chemical exposures upon the health of the workmen, and I had the opportunity to palpate the damaged livers of the aspirin workers. To prevent further injury, the process was changed and water was substituted for carbon tetrachloride. However, in the manufacture of chlorine in this same chemical company, transient injury was still permitted. When a workman was accidentally gassed with chlorine he would agonize for hours, coughing, but so long as no permanent damage occurred, the situation was tolerated.

In the next five years this philosophy was modified further to exclude exposure capable of producing untoward subjective responses. But the problem was that the few American industries

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which were developing a genuine concern for their workmen found that a preventive health program was costly. To have a corporate "health" conscience placed one at an economic disadvantage. Industries with underdeveloped, Neanderthal-type consciences profited in the marketplace.

Then in 1970, the Occupational Safety and Health Act legislated a conscience, if you will, into all American industry. Industrial work and safety standards were established. Surveillance programs to identify problems were commenced. Research to establish dose-effect relationships and to enable the valid recommendation of standards was begun. This endeavor was a titanic task because in our incredibly complex industrial array were 60 to 80 million workers distributed over 5 million workplaces engaged in thousands of activities where exposure to between 500,000 and 600,000 chemical substances occurred.

Our country is now endeavoring to place the entire workforce under medical surveillance through the use of periodical physical examinations to define health status. Such a program when complemented by the simultaneous monitoring of the work environment will permit the critical prospective studies which will allow us to define the true effect of the various environmental factors upon man.

This seminar is concerned with the health effects of industrial solvents and is placing special emphasis on behavioral toxicology. To monitor the effects of solvent exposure requires that the work force be placed under good medical surveillance. In the majority of large industries this has already been done, and has been done well for more than a decade. To define the behavioral effects of solvents and to supply the toxicological information we lack requires the use of specialized skills. This we must now commence.

Measuring the magnitude of the solvent exposure in the workplace presents a major problem. It requires well-trained personnel and expensive equipment to adequately define the environmental exposure. We lack sufficient personnel to do the job and we have not developed a training program adequate to supply our current or future needs. Hopefully, Biological Threshold Limit values will be developed so that man can serve as his own biological monitor in whatever workplace he finds himself. This would reduce the number of industrial hygienists required by reducing the number of industrial hygiene surveys. Special surveys would need be done only when the Biologic Threshold Limit values indicated that a problem existed. This is where we at this conference come in. Before reliable Biologic Threshold Limit values can be established for an industrial solvent, the precise effect of that solvent upon the physiological and the behavioral function of man must be

known. To date the necessary toxicological facts to do this have not been generated for a single solvent.

Behavioral toxicologists need to address the following questions: What is the solvent concentration and exposure time capable of altering physiological responses, producing decrements in cognitive task performance, or causing changes in behavioral responses? First, this must be defined for healthy adults, and then for the more sensitive segment of the population — the workman whose job performance is already compromised by the presence of a disease state. Before scientists can logically proceed to collect these data, they should have information regarding the solvent's metabolism, its drug interactions, and the modifying effect of variables such as temperature, altitude and pressure upon the solvent's toxicity. What happens to a workman when he is exposed to a solvent and drinks alcohol or takes a medicine which could interfere with the metabolic fate of the solvent? Is there a potentially hazardous interaction? There is a vast body of knowledge we must now acquire if we are to make the workplace safe for all.

Let us consider this situation: there was a workman who had significant coronary heart disease. He was asked to do a simple paint- stripping operation. This was light work thought not to increase his cardiac output by more than a few percent. He began the paint stripping and after four hours developed severe, crushing, substernal pain. He was taken to the hospital with what proved to be a fatal myocardial infarction. The reason for the heart attack at that specific time escaped everyone until recently when it was discovered that the methylene chloride in the paint stripper is metabolized to carbon monoxide[1]. Exposure to 1000 ppm for two hours can produce a carboxyhemoglobin level of 15% saturation, a concentration well above that known to be detrimental to patients with heart disease.

Let us consider a second situation: the case of an individual who worked with trichloroethylene during the day and then drank beer in the evening. After he had consumed one quart of beer his face became covered with fiery red blotches, making him appear like the circus clown. The laughter this invoked forced him to give up drinking for he refused to drink alone. This interesting phenomenon is known as the "degreasers' flush." A few physicians had seen the phenomenon, but no one had photographed it or described it in the world literature. It was not known whether this occurrence was universal. Would it happen to everyone exposed to trichloroethylene and who then drank ethanol?

To investigate this "degreasers' flush" we have available an investigative tool which should prove of great value to behavioral toxicologists - the controlled-environment chamber, a laboratory

room into which can be introduced the exact amount of the vapors of an organic solvent so that its effect on healthy human volunteers can be investigated. In this setting behavioral toxicological data can be meticulously collected and correlated to the blood stream concentration of the solvent. In such a setting these photographs of the "degreasers' flush" were obtained as the mechanism of this interesting dermatologic response was investigated recently[2].

FIGURE 1. Appearance of normal facial skin after 5½ hours exposure to trichloroethylene vapor, 200 ppm, 8 minutes after drinking one glass of beer. This volunteer subject had been exposed to trichloroethylene vapor concentrations ranging from 50 to 100 ppm over the three-week period immediately preceding this exposure.

FIGURE 2. The subject has finished drinking one quart of beer and the "degreaser's flush" is evident.

FIGURE 3. The facial flush has reached its peak intensity. Note the distinctive areas of sparing of the skin about the eyes, eyebrows, and neck.

FIGURE 4. Close inspection of the skin reveals that the flush is due primarily to an intense vasodilatation of the superficial skin vessels.

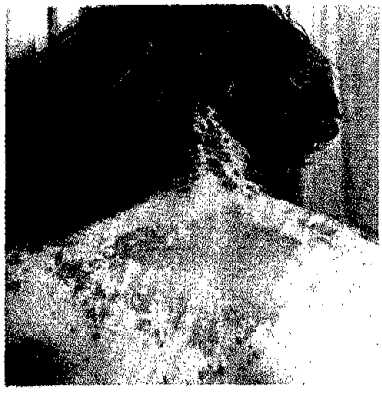

FIGURE 5. Dermal lesions on the shoulders and between the scapulae accompany the facial flush.

Finally - and this is most important, those "truths" discovered in the laboratory must be proved to be truths in the workplace. The individual may possess the ability to adapt to a chemical exposure which initially produced an untoward physiologic or behavioral response. For example, a recently completed national survey of blood donors has very nicely demonstrated that nonsmokers living in Los Angeles compensate for their higher exposure to carbon monoxide by increasing their red blood cell mass[3]. Man often has the ability to adapt to his environmental stresses, and the extent to which this is possible must be determined for each industrial stress.

In conclusion, it is apparent that a new era of safety and health is being ushered into the workplace. The new work environment will be one in which each workman will enjoy optimum well-being, not just the absence of overt disease. It will be an exciting time for behavioral toxicologists, industrial hygienists, and occupational physicians. It will be a challenging time for the people of NIOSH, as they attempt to coordinate the efforts of industry and the scientist to achieve the greatest good for the greatest number in a limited span of time.

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TRACE ANESTHETIC EFFECT ON VIGILANCE

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The anesthesiologist, in addition to maintaining a state of sleep in his patient, has the responsibility for monitoring pulse, blood pressure, skin color and temperature, depth and frequency of respiration, electrocardiographic display, type and rate of intravenous infusion and degree of muscle relaxation and color of blood in the surgical field. It is therefore fitting that the motto of the American Society of Anesthesiologists is "Vigilance." Anything which might impair the perceptual, cognitive or motor skills of the anesthesiologist could constitute a threat not only to his well-being but also to that of the patient for whom he provides care.

Current practice of anesthetic administration follows a form wherein sleep is induced by the injection of an ultra-short acting barbiturate, causing loss of consciousness, then this state maintained by the administration of inhalation agents. Commonly, the patient breathes the vapors of a halogenated hydrocarbon, halothane (2-bromo-2-chloro-1,1,1 trifluoroethane) carried in a mixture of 50% nitrous oxide, 50% oxygen. Due to the intermittent pattern of respiratory requirements, the design of anesthesia machines incorporates the feature of a reservoir bag which, in turn, necessitates inclusion of a pressure relief valve in the system. From this valve, spillage of overflow gases and vapors into the ambient air of the operating room occurs. Due to the proximity of the overflow valve to the anesthesiologist, he is exposed to these traces of anesthetic agents more than are other personnel. In addition, he is exposed more hours daily, for he may administer anesthesia for several operations, performed by different surgical teams, in the course of a working day.

To what concentrations is he exposed? We reported measurements of ambient levels of nitrous oxide and halothane in the vicinity of the anesthetist to be about 130 and 10 ppm, respectively[1]. The halothane figure was confirmed in studies at Stanford[2] and Michigan[3]. Since our publication, we have re-investigated nitrous oxide and found a figure of 455 ppm[4]. Others have reported nitrous oxide values closer to 1,000 ppm[5, 6], so we regard 500 ppm as a realistic and, possibly, conservative figure for ambient levels of this agent. To mimic actual occupational ex-

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posure to halothane and nitrous oxide we chose the concentrations of 15 and 500 ppm, respectively, as the exposure levels.

MATERIALS AND METHODS

Twenty paid volunteers were selected among dental and medical students at Northwestern University. Only male subjects were chosen, to avoid any question of the effect of trace anesthetics on an early pregnancy in a female subject. Care was taken to accept only those who had not had any significant exposure to anesthetics either as a patient or during clinical training experiences. Further screening questions were asked to assure that these individuals were in good general health, taking no prescription drugs, understood the purpose of the study and agreed to it. Each subject signed an informed consent form previously approved by the Research Committee of Northwestern University. The subject was assigned a day in which he would be tested at a given hour. He was then scheduled to be tested on that day and at the same hour one week later. On one of these days, he was exposed to air, and on the other to the trace anesthetics in air. The order was counterbalanced so that half the subjects were tested with the air followed by the anesthetic, and the other half in the reverse order.

The subject was seated in a reclining chair, over which was suspended a standard hospital oxygen tent. The individual was not restricted in his movements within this tent, was free to read or do as he pleased, but was cautioned not to sleep in order that conditions might simulate those of the operating room. A 70 lpm flow rate of filtered, compressed air provided adequate ventilation as determined both by the comfort of the subject reported to us, and normal measurements of oxygen and CO_2 made on samples obtained from the tent during the exposure. During the four hours the subject was seated in this situation, samples were taken periodically from a catheter inserted through the top of the tent and suspended within a few inches of the breathing zone of the subject (Figure 1).

The anesthetics were administered by metering a small amount of 1% halothane in nitrogen from a tank via a line of Teflon tubing into a larger Teflon line carrying the 70 lpm flow of filtered air to the subject. A third line passed from an anesthesia machine delivering a low flow of nitrous oxide into the air line. The resultant mixture passed over a screen which prevented the subject from seeing the equipment, and connected to the top of the tent. Air samples were subjected to gas chromatographic analyses for content of CO_2 and anesthetics. The flows of anesthetics were adjusted when necessary to maintain concentrations within 10% of the prescribed levels of 15 and 500 ppm. The gas exit was by simple

FIGURE 1. Volunteer in exposure tent described in text, through which 70 lpm air is flowing via tubing from above. Ambient air is being sampled from Teflon catheter suspended near subject's breathing zone.

leakage around the perimeter of the foot of the tent, below the breathing zone of the subject. At the end of four hours, the subject was taken immediately from the tent to a testing room across the hall and within five minutes, testing was begun in the sequence shown in Table I.

Table I. SEQUENCE OF TESTS

Audiovisual task	WAIS
Wechsler memory scale	Similarities
Mental control	Digit span
Memory passages	Digit symbol
Visual reproductions	Picture completion
Paired associates	Block design
Tachistoscopic task	Picture arrangement

The audiovisual task involved perception of, and appropriate reaction to independent changes in auditory and visual signals. The experimental equipment consisted of a two channel oscilloscope

(Hewlett-Packard), a four channel FM instrumentation tape recorder (Hewlett-Packard), earphones to perceive the auditory output from the recorder and a response panel which was custom made for this equipment. On the first two channels of the magnetic tape were recorded a pattern of ventricular fibrillation from a commercial electrocardiographic simulator; on the fourth channel was recorded the clicking of a metronome at either 100 or 200 clicks per minute; on the third channel the responses of each subject were recorded as he pushed the appropriate button on the response panel. Figure 2 shows this equipment in use.

FIGURE 2. Volunteer seated at audiovisual test described in text. The tape recorder is sending auditory signals to the earphones and visual signals to the two channel oscilloscope. Changes in either modality are noted by the subject depressing the appropriate button on the response panel.

The visual pattern was programmed to appear either on the bottom or top channel of the oscilloscope. Thus, the two by two combination of conditions was presented to the subject as follows: visual high, sound slow; visual high, sound fast; visual low, sound slow; visual low, sound fast. On the response panel at the fingertips of the subject was one button for each of these four possibilities. As soon as he detected a change in either visual or auditory conditions, he depressed the button corresponding to the new set of conditions. There were 100 changes programmed into the tape (25 changes to each of the four conditions) and these occurred

over a period of seven minutes, 20 seconds. By recording the response of the subject on the empty channel of the tape, the test could be conducted quickly and the responses saved for grading after the subject had completed the other tests. At that time, the tape was played through a Grass polygraph recorder, which simultaneously printed out the signals for visual and auditory patterns, plus the response of the subject. From this written trace, in which the changes and signals were clearly identifiable, the time taken for the response of the subject could be measured. Mean reaction time could thus be calculated from the time of stimulus change to the time of occurrence of the correct response. Pilot work had indicated that subjects averaged about 97% correct on this task. A high level of correct performance is desirable for any reaction time measure.

Following this task, the subject was given the next item in the array of tests shown in Table I. The next four were standard psychological tests selected from Wechsler Memory Scale Forms I and II. Form I tests were always given during the first session. Mental control involved counting backward, saying the alphabet rapidly, and tasks of that sort. Memory passages refers to a test in which two paragraphs were read to the subject, his task being to remember as many "facts" as possible and to repeat them. Visual reproduction involved the subject drawing a picture that he had seen for ten seconds, following its removal. Paired associates was a test in which the subject was to say the second member of a pair of words when the first one was given.

Next, the subject was seated in front of a tachistoscope. A slide consisting of a square divided into nine small squares was displayed for 1/20th of a second. The small squares contained black circles which each time were varied in number (4, 5 or 6) and position. Fifteen such slides were seen, twice each, for a total of 30 presentations. The subject's task was to indicate on a grid comparable to the slide background the location of the circles in each case.

Following this, selected parts of the Wechsler Adult Intelligence Scale (WAIS) were given. Similarities denotes a test in which the subject was asked, "What makes these two words similar?" Digit Span was a task in which a series of numbers were recalled, both forward and backward. The Digit Symbol test involved giving the subject a written symbol for each of the digits, 1 through 9. His task was then to reproduce the correct symbol for each of a random array of these digits, scoring the maximum score achieved in 90 seconds. Picture Completion was a test asking the subject to tell what was missing from a picture, e.g. a United States flag with only 35 stars. Block Design involved reproducing

pictures using blocks with three kinds of faces, some tasks using four blocks for 2x2 designs and some using nine blocks for a 3x3 design. Finally, Picture Arrangement involved arranging sets of pictures to make a logical story. The repeat tests, following the second exposure, used the identical tests for Similarities and Digit Symbol. For the other sections chosen from the WAIS, half of the items were utilized for each testing session. The entire test session took approximately 40 minutes to complete. Following this, the subject was allowed to leave the laboratory, and the audiovisual task was recorded on paper by playing the tape through the polygraph as described. Results were tabulated at a later time and the data subjected to statistical analysis, using analysis of variance to differentiate the statistical significance of anesthetic effect from the learning effect of improvement from the first to the second test session. Assessment of a possible interaction of these two factors was also available in this analysis.

RESULTS

The subjects were asked to state whether they believed they were being exposed to the anesthetic or air condition on each exposure. Uniformly, they were unable to detect any difference in the odor of the air they were breathing, and the randomly correct answers given to this question were, by the admission of the subject himself, strictly a guess. It was quite clear to the experimenters that the subjects were uniformly unable to tell the order of exposure. Of interest is the fact that six subjects fell asleep spontaneously some time during the four hour exposure and in each of these cases the subject was in the trace anesthetic exposure condition. Even in these cases, the subject was unable to identify correctly his condition. None of these periods of sleep lasted more than 10 minutes, and each of the six subjects was awake and alert at the time of testing following exposure.

Table II. TESTS SHOWING A STATISTICALLY SIGNIFICANT EFFECT OF TRACE ANESTHETICS

Task	Level of significance
Audiovisual task	$p<0.001$
Tachistoscopic task	$p<0.025$
Memory passages	$p<0.05$
Digit span	$p<0.05$
Paired associates	$.05<p<0.10$

Those tests reaching statistical significance for anesthetic effect are listed in Table II. The most striking decrement in performance

Table III. MEAN REACTION TIMES FOR THE AUDIOVISUAL TASK

Order of exposure	Air	Air and trace anesthetics	
1st	1.48	1.81	
			Learning effect
2nd	1.28	1.67	
	Anesthetic effect		

produced by trace anesthetic exposure was on the audiovisual task. Table III summarizes the data from this task, giving the mean reaction time in the four conditions and indicating the effect of order of testing. Regardless of the order, the mean reaction time increased by almost ½ second in the face of the trace anesthetic. This is rather a large increase considering the short reaction times found in the control measurements from the same subject.

As may be seen in Table II, a statistically significant decrement in performance related to anesthetic exposure was also shown in the tachistoscopic task, memory passages and digit span. The paired associates task was of borderline significance. Most of the Wechsler tests, of the type used in determination of I.Q., showed no significant difference.

DISCUSSION

The most impressive results were from the audiovisual task. It might be argued that this test was the most sensitive since it was given soonest after discontinuance of anesthetic exposure, but the other tests showing significant changes were later in the sequence of those administered so this explanation is likely to be an over-simplification. We believe that the audiovisual task showed the greatest sensitivity for detection of subtle effects. The subjects in this study were told there would be changes in the pattern on this test, and prior to the first exposure had been given a three minute trial run to acquaint them with it and thereby minimize the learning effect from first to second testing. Thus, they were oriented toward the detection and rapid recognition of these changes in signal, yet they had approximately a 150% reaction time, compared to control conditions, following the trace anesthetic exposure. This differs from the clinically comparable situation where the anesthesiologist is occasionally looking at an oscilloscope displaying the electrocardiogram while listening with a stethoscope to the heart sounds. In that situation, he is oriented basically toward detection of a possible, rare change from normal — a vigilance task. His basic "set" is that everything is going along

normally, with no significant change in signal from minute to minute. It is quite possible that this basic orientation, if his perception and cognition were obtunded, might cause him to miss altogether a rare event such as a change in visual or auditory signal. However, we cannot extrapolate from our findings to the important question of the ability of the anesthesiologist to monitor adequately the patients under his care during surgery. We do feel that these highly significant changes in reaction time to the audiovisual task should be considered in any discussion of the anesthetist's occupational exposure to trace anesthetics.

The tachistoscopic task, which also showed a statistically significant decrement during anesthetic exposure was designed to resemble closely that reported by Salvini and his associates [7] during their studies of the effect of 110 ppm trichloroethylene on human subjects. Their study is of particular interest owing to the fact that trichloroethylene is used in combination with nitrous oxide and oxygen in a manner quite comparable to halothane. Due to its instability in the presence of the soda lime used in circle absorption systems to remove CO_2, trichloroethylene is not given in the same anesthesia system as are most other agents. Consequently, a high flow rate of anesthetic gases must be used with this agent in order that a turnover of gas sufficient to remove CO_2 is provided. In practical terms, this means a much higher flow rate and a consequent higher degree of escape of overflow gases into the operating room. We have made only a few measurements of trichloroethylene concentration in the vicinity of the anesthetist, since at our hospitals it is rarely if ever used except for teaching purposes, but we have found approximately 50 ppm during the measurements we did make. Thus, we would expect that the same sort of effects reported by them in an industrial setting might very well hold true during anesthetic exposure. This is the only case we know of where we may rely on the occupational toxicology literature for evidence corroborating the findings of our study. It does appear very likely that a similar effect was found in these two studies done from entirely different points of view.

These data demonstrate a definite decrement in performance on several tasks following an exposure to both concentrations of agents and duration of exposure which are closely comparable to the actual, clinical situation prompting the study. We have not done a dose-response curve, either in terms of time or concentration, to ascertain whether there is a threshold value, below which no such effects could be detected. This is of immediate, practical importance since there are now commercially available "scavenging" devices to lower the concentrations of anesthetics found in operating room air. We have reported recently[4] that we were able to

achieve a reduction of nitrous oxide from approximately 500 ppm to a figure of about 25 ppm by such means. It is of great interest to know whether such a reduction of approximately 95% in ambient air contamination would result in a corresponding reduction in effect on these tests. Although nitrous oxide disappears relatively rapidly from the body, due to its physical and chemical properties, highly fat soluble agents such as halothane and a host of other halogenated hydrocarbons persist for much longer periods of time. Corbett has recently reported findings of the disappearance curves of halothane and other agents in the breath of anesthetists [6] and finds that halothane may be detected in the end-expired air of the anesthetist for as long as 60 hours following his last administration of this anesthetic. Practically, this means that the anesthesiologist will, during the course of a week, build up a reservoir of this agent in his body, since on day 3, for example, he will not yet have totally eliminated that which he has accumulated in him during days 1 and 2. Thus, there is need for investigation of the duration of the effects as well as studies simply of a dose-response curve on an acute, one-exposure basis.

There are basically two implications of our findings. First, the anesthesiologist may, by occupational exposure to inhalation anesthetic agents in these trace amounts, not be as alert to departures from normal in the status of his patient as he would be were the atmosphere not so contaminated. This would have relevance to the quality of health care being provided for the millions of patients undergoing surgery under general anesthesia each year. The second area of interest relates to the personal health experience of the approximately 20,000 physicians and nurses administering anesthesia daily in operating rooms in the United States. Are these individuals, following a day's work, as alert as they should be when driving home? If they consume a modest amount of beverage alcohol, will it affect them in a manner similar to, greater than, or less than the response they have had when not previously exposed to anesthetics? Is their accident record, both in automobiles and in the home, different from workers in other fields? All of these questions are extremely interesting but have no answer at the present time.

SUMMARY

1. Twenty male, paid volunteer medical or dental students were exposed on two occasions to four hours of inhalation of either air or 500 ppm nitrous oxide and 15 ppm halothane in air.

2. A battery of tests of perceptual, cognitive and motor skills was administered to them immediately following each exposure period.

3. Compared to controls, there was a significant decrement in performance following anesthetic exposure in a test involving recognition of changes in auditory and visual signals, a tachistoscopic task, digit span and memory passages.

4. It is possible that these findings indicate a subtle but significant effect on the ability of anesthesiologists to be fully effective in providing vigilant care for their patients.

5. Investigation of possible long-term effects upon the health and accident record of the anesthetist was indicated.

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BEHAVIORAL EFFECTS OF OCCUPATIONAL CARBON DISULFIDE EXPOSURE

Ruth Lilis, M.D.

It is well known that carbon disulfide (CS_2) exposure may cause neurological and psychological changes. The neuro-psychiatric syndromes of CS_2 poisoning were first described more than 120 years ago, in workers of the rubber industry. The later development of the viscose rayon industry resulted in repeated outbreaks of CS_2 poisonings.

Acute toxic encephalopathy, manic-depressive psychosis and peripheral neuropathy were the main clinical syndromes described.

Gordy and Trumper (1938), Lewey (1941) and Alice Hamilton (1940) made major contributions to the recognition of this occupational hazard in the United States.

Mental symptoms, almost always present in CS_2 poisoning, may have varying severity. The more dramatic clinical aspects, as manic-depressive psychosis, were reported with decreasing frequency after efforts were made to lower CS_2 concentrations at the work place. Less attention has been given to the initial psychological changes which occur after exposure to *lower* CS_2 concentrations. Their importance cannot be overemphasized, since they may be related to the individual's working capacity, to the occurrence of accidents, at work or elsewhere, and even, as more recent work has shown, to a higher-than-expected suicide rate (Mancuso, 1972.)

METHODS AND PATIENTS

This report presents the results of two separate studies, conducted in two different viscose rayon plants, where CS_2 exposure levels, as well as the main characteristics of the working populations, were different.

Study A

One of the studies was concerned with a large number of young workers; most of them had been active in the spinning departments of a new rayon viscose factory; CS_2 concentrations almost continuously exceeded 60 ppm. Higher peak concentrations occurred, especially during technical difficulties and necessary repairs.

We report the findings in 84 cases of confirmed CS_2 poisoning. The group of patients was characterized by young age (one-third under 20 years of age, and 36% from 20 to 25 years.) The exposure time had been relatively short, not exceeding 6 years; more than two-thirds of the patients had been exposed for less than two years.

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Clinical Characteristics

The patients complained of "pseudo-neurasthenic" difficulties, often accompanied or followed by symptoms of toxic peripheral neuropathy. A marked decrease in libido and potentia was also often reported. Non specific digestive symptoms were present in half of the patients (Table I).

Table I. SYMPTOMS IN 84 PATIENTS WITH CHRONIC CS_2 POISONING

Symptom	Number of cases	%
"Pseudo neurasthenic syndrome"		
Headache	65	77
Weakness	61	72
Irritability	48	56
Sleepiness	47	56
Depression	30	35
Impairment of memory	30	35
Insomnia	29	34
Nightmares	22	26
Aggressiveness	6	7
Digestive symptoms	48	56
Weight loss	43	51
Symptoms of peripheral neuropathy		
Muscle pain	74	88
Fatigue while walking	74	88
Paresthesia	67	80
Diminished muscle strength	47	56
Disturbed gait	34	40
Hypesthesia (pain and touch) in the extremities	6	7

The pseudo-neurasthenic symptoms were usually noted first. They appeared soon after work with CS_2 started and had a progressive evolution, with their intensity and complexity increasing steadily over short periods of time (weeks or months.)

The patients complained of headache, fatigue, somnolence (frequently overwhelming), nightmares, increasing irritability which resulted in conflicts at work and at home, aggressiveness with outbursts of violence, decreasing memory and intellectual concentration capacity. In some cases a definite depressive change had taken place. Decrease in libido and potentia was also a frequent com-

plaint, and was thought to contribute to the anxiety and depression. The majority of the patients felt that they had undergone a marked change in behavior and character, and this was substantiated by close relatives and friends.

Although the pseudo-neurasthenic changes were rather impressive, they had been given little attention by the patients themselves or by supervisory personnel or by the medical service, until the symptoms of peripheral neuropathy developed. Only at this point was it usual for the workers to seek medical help and for the medical staff to consider the diagnosis of chronic CS_2 poisoning.

Although the exposure time has been rather short many cases of toxic peripheral neuropathy were diagnosed (Tables II and III).

Table II. RESULTS OF PHYSICAL EXAMINATIONS IN 84 PATIENTS WITH CHRONIC CS_2 POISONING

Signs	Number of cases	%
Distal sensory loss (light touch, pain and temperature sense)	56	66
Altered tendon reflexes		
hyperactive	25	29
diminished	9	11
absent, (mostly ankle jerk, sometimes knee jerk too)	17	20
Diminished muscle strength		
flexor group	46	54
extensor group	32	38
Muscle atrophy (slight to moderate)	11	13

Electrophysiologic Investigations

In order to ascertain the diagnosis of peripheral neuropathy, the response to electric stimuli (faradic and galvanic) as well as electromyographic patterns were studied. A reaction of nerve degeneration was found in 27 cases (total in two patients and partial in 25); in three cases a faradic hypoexcitability was detected. Electromyographic investigation (performed in the tibialis anterior and in the flexor digitorum communis) showed normal tracings in only 9 of 81 tested patients; in 72 persons abnormal results typical of lower motor neurone injury were found. In two cases, on the electromyographic tracings taken in the resting muscle, "fibrillation" potentials were observed, with an amplitude of 50-150 μV and a duration of 1-2 msec. These fibrillation potentials are characteristic for the denervation of motor units in the explored muscle, in

Table III. ELECTROMYOGRAPHIC PATTERNS IN 81 CASES OF CARBON DISULFIDE POISONING

Observed changes	Number of patients	%
"Fibrillation"	2	2
"Simple" pattern (lower and upper limbs)	6	7
"Simple" pattern in the lower limbs and "intermediary" pattern in the upper limbs	26	32
"Intermediary" pattern (lower and upper limbs)	29	36
"Intermediary" pattern in the lower limbs and normal "interference" pattern in the upper limbs	8	10
"Intermediary" pattern alternating with normal "interference" pattern in the lower limbs	1	1
Normal "interference" pattern (lower and upper limbs)	9	11

severe cases of peripheral neuropathy. In the other cases with abnormal electromyograms, on the tracings taken during maximum contraction of the muscle, a modified pattern, replacing the normal "interference" pattern, was found.

The tracings were characterized by an excessive amplitude or voltage of the potentials, and by an increased duration of the potentials (7-15 msec; normal range 3-5 msec).

In some cases with more pronounced changes the electromyographic pattern was "simple," pointing to a high-grade reduction of the number of active motor units, while in other cases the changes were less pronounced, the tracings being of an "intermediary" type.

In a few instances explored areas with "intermediary" electromyographic tracings alternated with areas of the same muscle where a normal "interference" pattern was obtained. Such results were considered to express a parcellar, low grade toxic lesion of the motor neurones (Table III).

In a significant number of cases the EMG changes found in the lower limbs (tibialis anterior) were more pronounced than were those observed in the upper limbs (flexor digitorum communis). As can be seen in Table III, in 26 (32%) of cases, a "simple" EMG pattern was found in the tibialis anterior, while in the upper limbs the changes were less pronounced, of the "inter-

mediary" pattern.

In the other 9 cases, electromyographic changes ("intermediary" pattern) were found only in the lower limbs.

Both electromyographic and electric excitability changes occurred with greater frequency and were more marked in workers of the staple fiber spinning department.

Follow up, including EMG examination, failed to demonstrate full recovery in any of these patients re-examined 3 to 14 months after the diagnosis was established and CS_2 exposure discontinued. Each had at least some residual abnormalities on EMG.

Medical supervision, including an effort to detect pseudo-neurasthenic troubles as early as possible, and the subsequent cessation of toxic exposure of the affected worker, might have avoided the development of toxic peripheral neuropathy in many cases. The potential benefits of such an approach for all those concerned are evident.

Study B

Besides the classical effects of carbon disulfide resulting from relatively high exposure levels, during recent decades a potentiating effect on the development of atherosclerosis has been described in workers with prolonged, relatively low levels of CS_2 exposure.

Gsell, Attinger, Vigliani and Cazullo, Uehlinger, Rechenberg and other investigators have reported a significant number of cases with marked cerebral vascular damage (confirmed by cerebral arteriography and post mortem examination). "Sulphocarbonic encephalovasculopathy" as described by the above mentioned authors, appeared as a pyramidal, extrapyramidal and/or pseudo-bulbar syndrome at a relatively early age (40-55 years) and was often associated with vascular renal sclerosis.

Tiller, Schilling and Morris (1968) reported a significant excess of deaths from coronary heart disease in men who had been exposed to CS_2 in a viscose rayon plant. Hernberg and coworkers (1970) also found a statistically significant excess of coronary deaths in viscose rayon workers, as well as an increased prevalence of angina.

Most of the cases of cerebral atherosclerosis in CS_2 exposed workers have been reported in disabled patients. We have studied 138 *active* workers employed in a viscose rayon plant in which CS_2 exposure levels had had a decreasing trend during the preceding 8 years, and had reached mean levels ranging from 7 to 15 ppm, with peak concentrations usually not exceeding 60 ppm. The mean length of exposure was 14.5 years (in 78% of cases the length of exposure was more than 10 years) and the mean age was 42.1 years (94% of workers below 50 years).

Complete neurological evaluation and optic fundi examination were part of the investigations performed in order to detect possible signs of atherosclerosis. Electrocardiographic (double Masters test) and oscillometric (before and after exercise) tests together with estimations of cholesterolemia, triglyceremia and beta/alpha lipoprotein ratios were also made.

Clinical Characteristics

Slight or moderate cerebral atherosclerosis was found to be present in 22 (17%) of the cases. Young age at onset of CS_2 exposure seemed to favour its development. All the workers in the age group 31-40 years in whom neurological signs of slight or moderate atherosclerosis were detected had been exposed for more than 15 years.

The optic fundi examination showed retinal vessel changes (classified as grade I or grade II) in 46 (33%) of the cases. These abnormalities were more frequent in those with longer CS_2 exposure (Table IV).

Table IV. RETINAL VESSEL CHANGES AS RELATED TO LENGTH OF EXPOSURE

Length of exposure (years)	Total number of workers	Number of workers with retinal vessel changes	%
less than 10	31	4	13
11-15	59	18	30
16-20	28	11	39
21 —>	20	13	65
Total	138	46	33

Since length of toxic exposure and age were correlated, and since age could affect the appearance of retinal vessel changes, the age group with the largest number of workers (41-50 years) was selected and the prevalence of retinal vessel changes as related to length of exposure was analyzed.

While in the workers of this age bracket with less than 10 years of CS_2 exposure retinal vessel changes were found in 20% of cases, in those with more than 20 years of exposure the prevalence of retinal vessel changes rose to 62% of cases. These results compare well with those of Goto, Hotta and Sugimoto (1971).

Atherosclerotic changes of the cerebral vessels may produce various neurologic and psychiatric syndromes.

Behavioral changes are likely to be the earliest manifestations.

Symptoms found in the 138 examined workers are shown in Table V.

Table V. SYMPTOMS IN 138 WORKERS WITH LONG EXTENDED CARBON DISULFIDE EXPOSURE

Symptom	Number of workers	%
Fatigue	64	46
Irritability	68	49
Sleep disturbance	57	37
Nightmares	33	24
Decreasing memory	47	34
Headache	31	23
Depression	11	8
Loss of libido and potentia	23	17

We were able to compare the prevalence of these symptoms in this group of CS_2 exposed workers with those found in three other groups: workers exposed to carbon monoxide, nitro and amino-aromatic compounds, and workers with no toxic exposure. The carbon disulfide exposed group had the highest prevalence of the above mentioned psychic disturbances.

DISCUSSION AND CONCLUSIONS

Psychic disturbances and behavioral changes are the earliest symptoms of carbon disulfide toxicity. They are very frequent in situations where relatively high exposure levels occur, even if only as peak concentrations. If these symptoms are not given the necessary attention, and toxic exposure is not discontinued, peripheral neuropathy may develop, and prove to be disabling for long periods of time. Behavioral changes are also present in workers with long-extended, low level exposure, and are probably mainly the manifestation of atherosclerotic cerebral vascular changes in these cases.

Decreased adreno-cortical function, secondary to the hypothalamo-pituitary impairment, and decreased testosterone and interstitial cell stimulating hormone secretion, recently detected in carbon disulfide exposed workers (Cavalleri, Lancranjan), may have a contributory role in the appearance of psychic disturbances and behavioral changes.

Such disturbances can have important effects on the general performance level, working capacity and productivity, inter-relationships at work, the frequency of accidents and also on the whole range of human social relationships.

Medical supervision of carbon disulfide exposed workers, to

be satisfactory, should aim at early detection of psychological and behavioral changes; cessation of toxic exposure could prevent more severe forms of chronic CS_2 poisoning, and also the above mentioned adverse effects on the workers' well-being.

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FIVE YEARS OF EXPERIENCE WITH CS_2

J. Lieben, M.D. and R. A. Williams

Before I discuss this subject I want to set the record straight. My experience with actual behavioral toxicology of CS_2 for the early detection of occupational hazards is limited to the literature.

At this point of my career I am not working at the plant level. I am employed as Group Medical Director of the largest rayon producer in the United States in a corporation office 150 miles away from the nearest rayon plant. Nonetheless, I see all, and sometimes initiate, correspondence with regard to toxicology of CS_2. I discuss problems with our plant physicians. I visit our three rayon plants frequently, talk to our workers, may examine them, and establish preventive measures in these plants. I also work closely with the Medical Director of another large rayon producer in this country. My duties are by no means limited to CS_2. My responsibilities include our Film & Packaging Division, our polyester operations, and several unrelated chemical plants.

While I have had only part-time experience with CS_2 over the last four years and nine months, it goes back much further.

Many of you probably do not know that the classic investigation of the viscose rayon industry by Alice Hamilton dealt with the Lewistown plant of American Viscose, a company which was merged with FMC Corporation, which is now my employer. In my previous position as Director of the Division of Occupational Health for the Commonwealth of Pennsylvania I have raised a few skeletons in the field of Occupational Illness. In this activity I also checked on the presence of skeletons from this Lewistown plant in the 1960s. Many of Alice Hamilton's chronic cases had ended up in the Harrisburg State Hospital, a mental hospital where all chronic psychiatric admissions from Lewistown would go. At that time I reviewed the question of continued admissions with the superintendent of the hospital, Dr. Eaton, and with one of the 1938 authors of the CS_2-H_2S Survey in the Viscose Rayon Industry, Dr. Braceland. I drew a complete blank—there had been no CS_2 intoxication admissions since the early 1940s.

Also, in the five years of my employment by the Fiber Division of FMC Corporation, no chronic case of CS_2 poisoning has ever come to my attention. As a matter of fact, during my early days of employment I searched for them extensively, but found none.

What we did have were several acute cases from one plant where we had many manufacturing problems. These cases occurred over a

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period of three years, and there have been no further cases for over a year since.

The early recognition of these cases was obscured by the fact that at the time we also had a problem with amphetamines among our shift workers. Most cases occurred in recently hired workers. The symptoms and signs in these men were all similar, and amounted to an acute attack of central nervous disease, such as ringing in the ears, irritability, confusion, excitability, feelings of angriness, depression, frequent crying, emotional instability and bizarre behavior —which were diagnosed by various psychiatrists not familiar with CS_2 poisoning as neurosis, depressive reactions, schizophrenic disorder, acute anxiety reaction, paranoid delusions, and organic brain syndrome.

All of the acute cases which we had occurred in our staple men who were exposed to several episodes of unusually high CS_2 levels.

Of the 12 cases which resulted in various absences from one week to several months, seven are now working in the same plant, no longer in areas with CS_2 exposure; one went to college; three are working elsewhere; and one we were unable to locate. One of the ones working is now also a Union shift steward; one is in Alaska; one in Ohio; and one works at a local power plant. The last case occurred in June, 1972. All cases occurred in one plant and no other cases have come to my attention, either from the Lewistown plant, which is now shut down as a result of flooding in last year's Hurricane Agnes, or from our other plants in Front Royal and Parkersburg.

At this point I think I should briefly explain the manufacture of rayon and some of the words which I have used which are not familiar to a large part of this audience.

The raw material for rayon is cellulose, which comes to us as pulp from the manufacturer in large white sheets, similar to thick blotting paper. This pulp is manufactured from trees and is nothing but chemically purified cellulose.

The pulp is steeped and shredded in caustic soda. It is then called crumbs. These crumbs are reacted with CS_2 in churns. This treatment produces a yellow compound which is further mixed with caustic soda and becomes a heavy viscous solvent similar to honey. Following filtration and aging, this viscose is pushed through a final filter and then through a jet, which is a sieve with many small holes, into a sulphuric acid bath, where it is coagulated into a fine thread. Several thousands of these threads make up the Tow or Staple, which contains residual spin bath along with liberated CS_2. In the cutter house this Tow is cut into desired lengths ranging from 1″ to 6″. Following the cutter house procedure, it is desulfurized, bleached, washed and dried—and from there it goes to the customer. It is in the cutter house operation where we had the problems.

In the old days there were problems in the churn rooms and the spinning rooms, but these we have eliminated many years ago. Hopefully, we can make the cutter house operation failsafe too.

Until about a year ago it was our general policy to take measurements of CS_2 at specified locations between the various machines at regular intervals. These were spot samples and not time-weighted exposures in the breathing zone of the operator. These samplings have given us over the years ballpark figures on what the operator might be exposed to.

Recognizing the inherent limitations of grab sample results and prompted by a NIOSH health hazard evaluation conducted at one of our major plants, we are currently using personal monitoring techniques to evaluate time weighted average exposure to CS_2. Full shift samples are now routinely collected on activated charcoal and these, as well as the grab samples, are analyzed photometrically following the copper diethylamine colorimetric method of Viles[1]. The charcoal tube sampling system that we are currently using is new, and is an adaptation of the NIOSH procedure which was field tested at our plant. The NIOSH method is apparently still under development and was reported in a paper presented at the recently concluded American Industrial Hygiene Conference last month in Boston. Our method differs somewhat from the NIOSH procedure in that we are utilizing a much larger sample collection tube, 5 grams vs. the NIOSH 150 milligrams tube, and feel that for our purposes the larger tube is more satisfactory, since it allows us to evaluate full shift breathing zone CS_2 concentrations in a single sample. Since our staple department personnel now routinely wear respiratory protection whenever they are required to perform tasks in potentially high exposure areas, we have designed our sample collection system in such a manner, so as to account for the protection afforded by the respirator. In this way, we are attempting to realistically represent actual breathing zone concentrations for full shift operations. We are relatively confident that with this system of measuring time weighted average concentrations and routine iodine-azide biological monitoring coupled with medical surveillance that the acute effects of CS_2 inhalation can be successfully predicted and prevented.

As a result of these cases and also influenced by the Health Hazard Evaluation by NIOSH, we have instituted routine iodine-azide testing of urines among cuttermen, where the cases had occurred. We had an extensive review of our ventilating system, have redesigned our cutter houses, which were the places where the potential for high short exposures was greatest, to a point where exposure levels have been reduced to less than 50%. One of the new cutter houses is in operation and several more have been ordered. Eventually all of them will be replaced. This change will cost over one-half

million dollars. We have completed negotiations with our Union to eliminate the job of a full-time cutterman and allow these men 50% of their working time in an area where CS_2 levels are generally much lower. This will further reduce the exposure.

Before I close my remarks I would like to say something about the future of the rayon industry in the United States.

In 1968 seven companies produced 1,104,000,000 lbs. of rayon. In 1972 there were four companies left which produced 964 million lbs. According to a forecast by our former Manager of Marketing Business Analysis, there will be a total rayon production of 1,310,-000,000 lbs. in 1982. In general, this is not a very bright forecast. Rayon tire cord and textile fibers are on the downtrend, but rayon staple will account for the increase in the projection.

This, of course, relieves us in no way of our responsibility to use all precautions and means to avoid any of our workers getting ill as a result of their occupation no matter what the cost.

We need tests which will tell us (1) who is sensitive to carbon disulfide, because I believe that there are some who are more sensitive to CS_2 than others. Unfortunately, the test described by Djuric and others in the June issue of the AMA Archives is not usable in the United States. (2) Nor are we able to utilize any of the tests described by Stokinger each year in the preamble to the ACGIH-TLV list. (3) The American worker just will not accept Antabuse as part of a preemployment evaluation, nor would he submit to it as a condition of his employment. Nor can we test him with exposures to CS_2 above the TLV prior to employment.

We also need simple objective tests which will predict that an exposed worker will develop carbon disulfide symptoms. I am rather doubtful that we shall have usable tests within the next five years. We can't wait that long, and for that reason the thrust in our company is toward eliminating the actual exposure to CS_2 through engineering and work changes, and I am confident that we shall accomplish this within the next two years.

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NEUROPHYSIOLOGICAL FINDINGS IN CARBON DISULFIDE EXPOSURE

Anna Maria Seppäläinen, M.D.

CHRONIC CS_2 POISONING

Our research group published last year a descriptive study on chronic CS_2 poisoning.[1] All the subjects had a long exposure to carbon disulfide. When dealing with long exposure, we are dealing with varying degrees of exposure. In a Finnish viscose rayon factory that was involved in this study the CS_2 concentrations were high in the 1940s, around 20-40 ppm in the 1950s and 10-30 ppm in the 1960s. The cases of poisoning that we see now originated probably from those earlier days. However, we still find, although this happens very seldom, new cases of CS_2 poisoning, of a slight degree, in workers who were first exposed at work in the 1960s. No doubt most of the subjects with poisoning had been exposed to very high concentrations—up to 1500-2000 ppm under exceptional conditions during equipment failure or the like.

CS_2 attacks the nervous system at several levels, namely the central and the peripheral nervous systems as well as the autonomic nervous system. The most common subjective symptoms in our study group were general fatigue, insomnia, paresthesias and headache (Table I). As one can see the symptoms are common and unspecific but they clearly occur much more frequently than in a control population of paper-mill workers.

Table I. CLINICAL SYMPTOMS

Symptom	Prevalence in CS_2 poisoning group, % (N=36)	Prevalence in control group, % (N=188)	P
General fatigue	69	25	0.001
Insomnia	74	12	0.001
Paresthesias	74	21	0.001
Headache	54	9	0.001

METHODS

The neurophysiological tests used were electroencephalography (EEG), electromyography (EMG) and conduction velocity studies of several peripheral nerves. The EEGs were routine recordings that were interpreted in the conventional way. In this paper I classify

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them as normal or abnormal. EMG was performed on several muscles of the upper and lower extremities; I used concentric needle electrodes and a Disa EMG. I measured the maximal motor conduction velocity (MCV) of the median, ulnar and deep peroneal nerves and also the conduction velocity of slower motor fibers (CVSF) of the ulnar nerve. This latter factor is a very useful and informative one. It is a quite sensitive measure of partial nerve damage, probably demyelination. On the other hand, a slowing of MCV needs a more substantial damage of peripheral nerve; the main part of the nerve fibers must be involved. The CVSF methodology was originally described by Hopf in 1962[2], but I have modified it and described this in 1971[3]. The method is based on giving paired electrical stimuli on two different sections of the same nerve and thereby studying at what interval of the paired stimuli the antidromic blocking begins. The patients were given a neurophysiological (NP) score based on EMG, MCV and CVSF findings (Tables II and III).

Table II. ELEMENTS OF THE EMG SCORE (0=NORMAL)

Pattern on maximal contraction	0-3
Changes in motor units	0-2
Fibrillations	0-2

Table III.
ELEMENTS OF CONDUCTION VELOCITY (CV) SCORE

MCV of the median nerve	<50 m/sec	1
MCV of the ulnar nerve	<50 m/sec	1
CVSF of the ulnar nerve	30-40 m/sec	1
CVSF of the ulnar nerve	<30 m/sec	2
MCV of the lateral popliteal nerve	<45 m/sec	1

RESULTS AND CONCLUSIONS

We divided the patients with chronic CS_2 poisoning into three groups based on the time passed since the diagnosis of poisoning. Group A represents a subacute stage, Group B an intermediate stage and C a very long-standing chronic poisoning (Table IV). In Group B we had a little lower NP score than in the other groups and, in particular, the slowing of conduction velocities was less frequent in this group. In Group A the slowing of CVs was most frequent.

I also performed a two minute tetanic stimulation test (20 supramaximal stimuli per second) on some of the patients who complained of easy and rapid muscular fatigue. Many of them showed a marked drop-off of the muscular response (Fig. 1). This symptom, as well

Table IV.
NEUROPHYSIOLOGICAL SCORES IN CHRONIC CS_2 POISONING

Poisoned for	Group A 0.5-2 years N=9	Group B 3-10 years N=17	Group C 10 years N=10
CV score $\bar{x}$	1.7	0.6	1.0
EMG score $\bar{x}$	1.9	2.4	2.6
Total NP score $\bar{x}$	3.6	3.0	3.6
NP score, range	1−7	0−8	0−6
Patients with normal neurophysiological findings	N=0	N=3	N=1

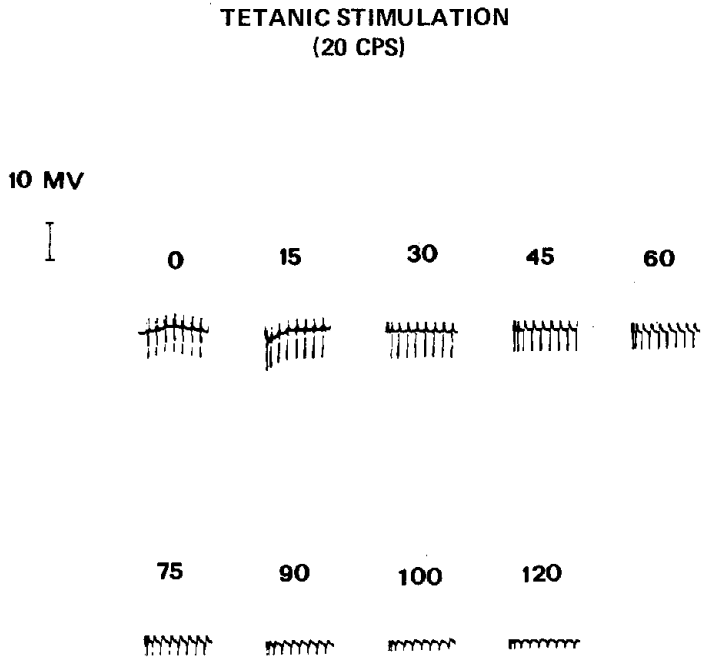

FIGURE 1. A tetanic stimulation test at 20 cps in a case of chronic CS_2 poisoning. Marked drop-off of the motor response in 120 sec. Numbers on top of the tracings indicate the seconds (0 = start to 120) from the beginning of the stimulation.

as abnormal findings in the test, was most frequent in Group A (Table V).

Table V.
FINDINGS IN TETANIC STIMULATION TEST
(20 cps for 2 minutes)

	Normal	Slightly abnormal	Markedly abnormal	Total
Group A 0.5-2 years	1	0	5	6
Group B 3-10 years	2	2	1	5
Group C 10 years	1	0	2	3

For further classification of neurophysiological findings in chronic CS_2 poisoning I contacted all the patients I had studied during the last four years and found 43 with this poisoning. The combinations of abnormal neurophysiological findings are shown in Figs. 2, 3 and 4. In Group A half of the cases had abnormal findings in EEG, EMG

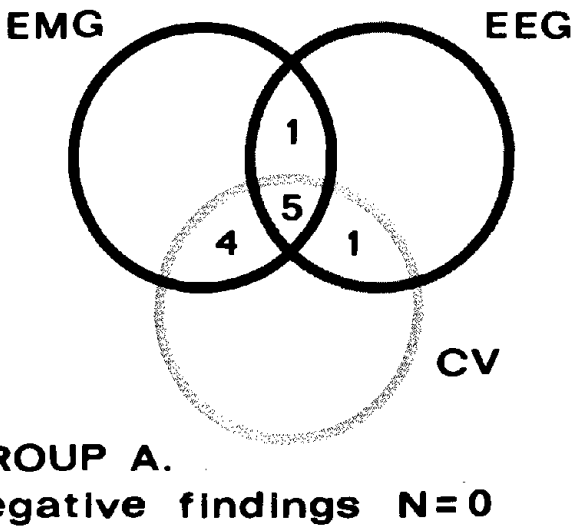

FIGURE 2. Combinations of abnormal neurophysiological findings in subacute stage of chronic CS_2 poisoning.

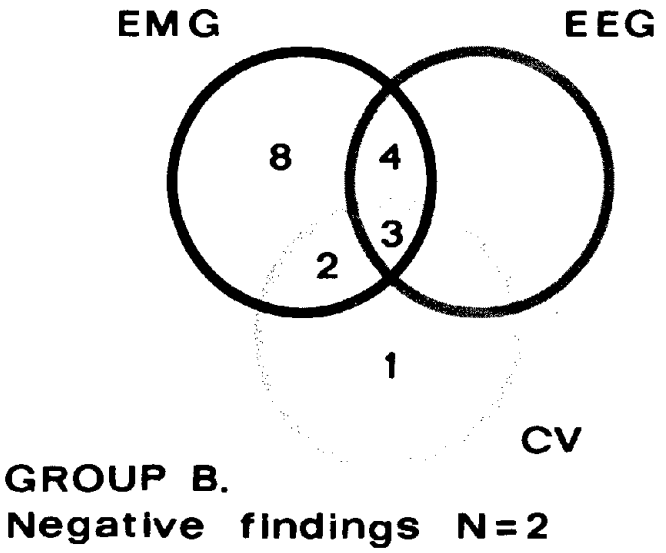

FIGURE 3. Combinations of abnormal neurophysiological findings in intermediate stage of chronic CS_2 poisoning.

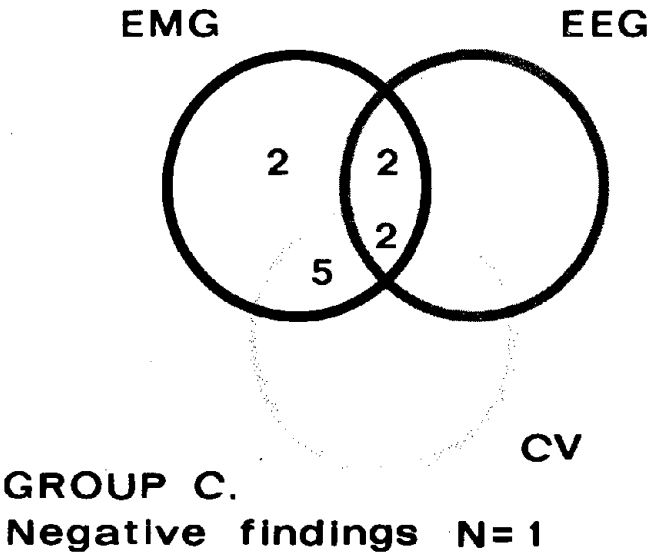

FIGURE 4. Combinations of abnormal neurophysiological findings in longstanding chronic CS_2 poisoning.

and conduction velocities and 9 out of 10 with abnormal EMG also had a slowing of CVs. In Group B (intermediate) the most frequent finding was abnormal EMG, which was the only abnormality in 8 out of 20. Altogether 17 cases had EMG abnormalities. In Group C abnormalities in multiple modalities were again more frequent. These differences are probably based on the following points.

1) Group C has a more severe poisoning—originating from the 1940s, when the concentrations in the workroom air were the highest.

2) In Group B the poisoning was perhaps originally less severe (the diagnosis had become possible at earlier stages because of new test methods) and with time, when exposure had been stopped, the abnormalities have lessened. There are experimental animal studies by Lukas[4] about the improvement of nerve conduction velocities when exposure was stopped and time was allowed for recovery.

3) In Group A in the subacute stage all the lesions are fresher, and no recovery has been possible.

From the EMG findings in this population of 43 patients, in which 38 had abnormal EMGs, I noted the following: All of them (Fig. 5) had a diminished number of motor units in maximal contraction but most of them had, in addition, either fibrillations or

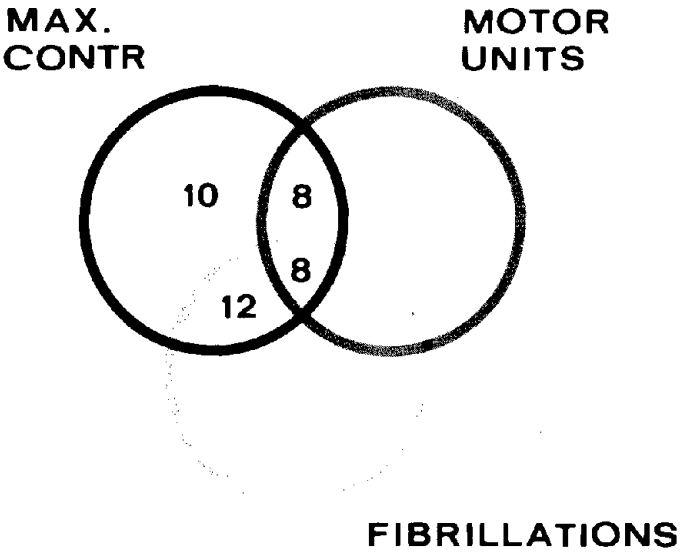

FIGURE 5. Electromyographic findings in 38 cases with chronic CS_2 poisoning. Max. contr. = diminished number of motor units in maximal contraction
Motor units = motor units of long duration and usually of high amplitude
Fibrillations - fibrillations found in some muscle(s)

neurogenic type motor units (of long duration and usually high amplitude) or both of these later abnormalities. The long duration, high amplitude motor units suggest a lesion at the spinal cord level —a common abnormality in long-standing CS_2 poisoning, especially in Group B.

I also looked for what type of EMG and CV abnormalities patients had to whom I had given a tetanic stimulation test (Fig. 6).

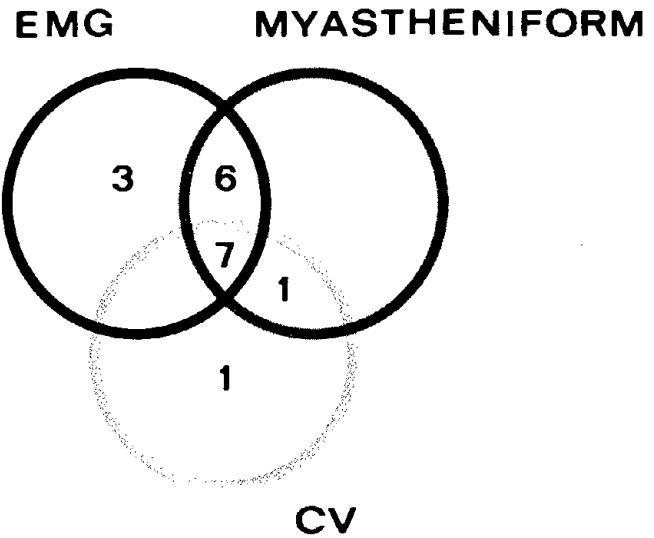

FIGURE 6. Electromyographic (EMG) and conduction velocity (CV) abnormalities and myastheniform reaction (Myastheniform) in tetanic stimulation test among cases with CS_2 poisoning.

All of them also had other abnormalities, mostly EMG abnormalities, but often in combination with a slowing of conduction velocities. This myastheniform reaction may depend on abnormality in the myoneural junction, although there are papers on myastheniform drop-off in tetanic stimulation in patients with peripheral neuropathy[5].

Fig. 7 shows the scattering of MCV and CVSF abnormalities and fibrillations as a denervation sign. Forty-three percent of patients with a slowing of conduction velocity also had fibrillations but there were still 10 patients with fibrillations and normal conduction velocities. This would suggest a spinal cord lesion in these people.

CHRONIC CS_2 EXPOSURE

Last year we ran a large series of studies on workers chronically

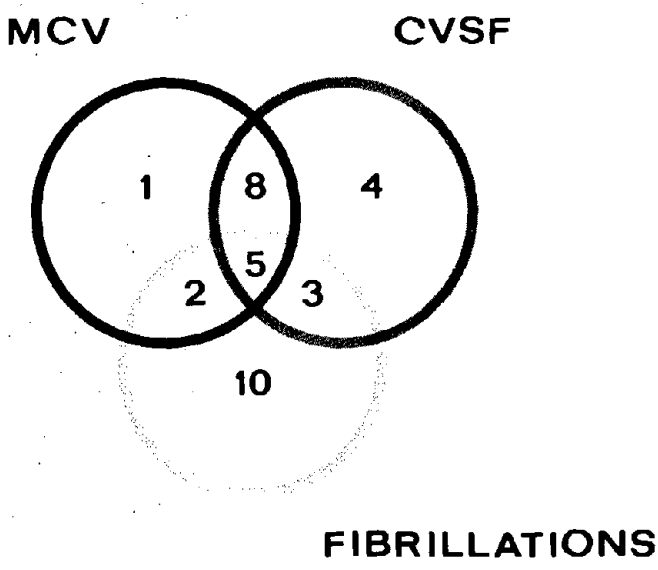

FIGURE 7. Slowing of maximal motor conduction velocity (MCV) and conduction velocity of slower motor fibers (CVSF) and fibrillations in the EMG of CS_2 - poisoning cases.

exposed to CS_2. This is part of a long prospective cohort study begun in 1967, when around 350 workers exposed for at least 5 years to CS_2 in a viscose rayon factory were identified. Each exposed worker was matched to a control worker from a paper-mill in the same town.

The first aim of the cohort study was to study coronary risk factors and coronary deaths in this population[6]. A cross-sectional study of these workers was carried out in 1972. Most of the exposed had by then worked for over 10 years and a large proportion for over 20 years, and were still working in the plant. Usually they work for six months in the exposure and for one month in a clean job in rotation. The exposed workers are employed as spinners, or in a xanthation room, at the sulfidation process or in the manufacture of CS_2.

The control population works in a paper-mill and has no exposure to CS_2. A random sample of 100 CS_2—exposed workers and 100 controls was sent for electroneuromyographic examination and to half of them an EEG examination was also given. No selection on the basis of possible neurological symptoms was considered in either of the populations so there may be neurological diseases or defects from other causes both in the CS_2-exposed and in the controls. We

have no reason to believe that there was a difference in alcohol consumption between the groups.

I carried out a battery of conduction velocity studies of nerves in the upper and lower extremities including maximal motor and sensory conduction velocities and also CVSF of certain nerves. To the CS_2-exposed workers I performed also a needle EMG, if conduction velocities were in an abnormal range.

In the EEG records of 48 CS_2-exposed subjects 18 were abnormal, whereas only six of 50 paper-mill workers had abnormal EEGs. This difference is statistically significant, Chi-square 7.29 and $p<0.01$.

All the conduction velocity measures indicated slower nerve conduction in the exposed workers. The difference between them and the controls was highly significant ($p<0.001$, t-test) concerning the CVSF of the ulnar and deep peroneal nerves and the motor distal latency of both median and ulnar nerves. The maximal motor conduction velocity of deep peroneal and posterior tibial nerves was significantly slower in the exposed than in the controls ($p<0.05$, t-test). This is in contrast to lead workers in whom I found significant slowing of MCV of arm nerves.

CONCLUSIONS

These studies have revealed nervous system lesions at the brain level, in peripheral nerves and suggest lesions at the spinal cord level in cases with chronic CS_2 poisoning. Among them there are possibly also defects in the myoneural junction. However, the nature of this defect needs further investigation.

Among people chronically exposed to CS_2, at times to high concentrations for short periods, a significant slowing of nerve conduction velocities was shown and also a rather high prevalence of abnormal EEGs. The EEG abnormalities may depend on the direct effect of CS_2 upon the brain or on circulatory changes as a result of atherosclerosis of the brain vessels caused by CS_2.

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BEHAVIORAL STUDY OF THE EFFECTS OF CARBON DISULFIDE

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In this paper we present the earlier unpublished results of the study on the behavioral effects of CS_2, as well as our most recent results. In addition, methodological problems and research strategies which can be used in these kinds of studies will be discussed.

Our investigations in behavioral toxicology started with practical aims in mind. Behavioral symptoms were observed during testing of patients affected by CS_2 or other solvents. It became evident that there was an increasing need for more systematic observations of a larger sample of exposed workers in order to control the diagnoses based on behavioral symptoms, and to find out whether workers who had been exposed but did not show manifest poisoning effects, had behavioral symptoms.

A clear theoretical framework, however, is still needed. It is known that CS_2 and other solvents affect the Central Nervous System, and that psychological processes depend on the CNS. Thus, one can assume that these compounds bring about behavioral changes. But the knowledge about *how* these solvents affect the CNS, and what regions of it are most affected, as well as knowledge about the effects of various CNS disturbances on human behavior, is still vague and fragmentary. For this reason we did not start with exact hypotheses about the effects. Instead, a large sample of behavioral observations—a large sample of test results—were collected, and the interrelations analyzed with multivariate techniques.

The approach adopted here is based on the assumption that the variables which we have selected for our study do measure different psychological reactions, traits or abilities, and that the differences in test results between two groups reflect differences in psychological reactions, traits or abilities. The degree to which a test variable measures the trait it is supposed to measure is the construct validity of the test variable. Using multivariate techniques we can determine the construct validity of the psychological methods, and if necessary revise the test battery or the interpretation of the results.

THE FACTOR—ANALYTICAL STUDY

Subjects and Statistical Methods

There were three groups of 50 subjects in the study on the behavioral effects of CS_2: unexposed workers, workers that had long been exposed but showed no manifest symptoms, and workers with

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clinical poisoning. For a more detailed description of the groups, see Hänninen[1].

Two multivariate techniques, discriminant analysis and factor analysis, were used. The results of the former have been published elsewhere[1]. Discriminant analysis proved valuable in validating diagnostic methods, but the interpretation of discriminant functions, in order to get a picture of the behavioral syndrome, is difficult. The psychological performance profile characteristic for each group was obtained better through factor analysis, the results of which are reported below.

Test Methods

The intelligence measure consisted of five subtests from the Wechsler Adult Intelligence Scale[2]: Digit Span, Similarities, Block Design, Picture Completion and Digit Symbol.

Other abilities were measured by the following tests:

(1) Bourdon-Wiersma vigilance test that measures sensorimotor speed and accuracy, i.e. speed, accuracy and fluctuation of speed;

(2) Santa Ana dexterity test[3]

(3) Symmetry drawing for detecting visual disturbances, and

(4) Benton test for measuring visual retention[4].

(5) The personality indicators consisted of five Rorschach test variables[5, 6] which were supposed to measure adaptability, emotionality, spontaneity, rational self control, and originality of perception.

(6) Psychomotor behavior and psychomotor ability were measured by the Mira test [7, 8]; eight variables were distinguished: three for pattern size, four indicating the qualitative aspects of drawings, and one for speed.

(7) In addition, a symptom inventory was administered to the subjects; four variables were taken into account: neurovegetative symptoms, sleep disturbances, symptoms of fatigue, and symptoms of neurotic behavior.

Table I presents the list of variables.

RESULTS AND DISCUSSION

Three analyses were made: the results of each group were subjected to a separate analysis. In each analysis six factors were extracted. Table II shows the factors obtained with Varimax rotation in each group.

Factors of the Unexposed Workers

There are two intelligence factors, one is *visual intelligence*, but involves also vigilance to some extent, and the other can be interpreted as a factor of *general intelligence*, and accounts also for general psychomotor ability. The third factor involves *motor speed and dexterity*. The fourth is a factor of symptoms revealed by in-

ventory, and is easily identified as a *neuroticism* factor, often found in factor analyses. The fifth is a factor of original and rich, but rationally controlled, ideation, revealed in the Rorschach test and combined with psychomotor uncertainty. This is more difficult to interpret, but might reflect an *introversive style of behavior.* The

Table I. LIST OF VARIABLES

Intelligence test
- Digit Span (D Sp)
- Similarities (Sim)
- Block Design (BD)
- Picture Completion (PC)
- Digit Symbol (D Sy)

Other abilities test
- Bourdon Wiersma speed (BW sp): number of rows
- Bourdon Wiersma errors (BW err): number of errors
- Bourdon Wiersma fluctuation (BW fl): time variation in completing a row
- Santa Ana (SA): right hand performance
- Symmetry drawing (Sym): number of reversions
- Benton (Ben): number of correct figures

Personality
- Rorschach: adaptability (Ro Ada)
- Rorschach, emotionality (Ro Emo)
- Rorschach, spontaneity (Ro Spon)
- Rorschach, rational self control (Ro Rat)
- Rorschach, original intellectual activity (Ro Orig)

Psychomotor behavior
- Mira I: length of lines (Mi I)
- Mira II: distance of lines (Mi II)
- Mira III: size of angles (Mi III)
- Mira IV: left-hand deviation (Mi IV)
- Mira V: right-hand deviation (Mi V)
- Mira VI: form level of straight lines (Mi VI)
- Mira VII: form level of broken lines (Mi VII)
- Mira VIII: performance time (Mi VIII)

Subjective symptoms
- Symptoms of neurovegetative lability (Labil)
- Sleep disturbances (Sleep)
- Symptoms of fatigue (Fatig)
- Symptoms of neurotic behavior (Neur)

For a more detailed description of the tests and variables, see Hänninen 1972.

Table II.

FACTOR STRUCTURES IN THE THREE GROUPS

	Unexposed workers	Exposed workers	CS_2-patients
Factor I	Visual intelligence PC 0.60 BD 0.53 Sym 0.58 Memory D Sp. 0.55 Vigilance B-W fl 0.48	Visual intelligence Sym. 0.72 BD 0.69 PC 0.56 Intelligence Sim 0.60 Mi VII 0.55 Psychomotor level Mi V 0.48 Sensorimotor accuracy B-W err −0.42 Memory Ben 0.61	Intelligence PC 0.77 BD 0.72 Sim 0.69 D Sy 0.74 Sym 0.52 Sensorimotor speed B-W 0.61 Memory D Sp 0.65 Ben 0.49 Vigilance B-W fl −0.59 Psychomotor level Mi VII 0.47
Factor II	Intelligence D Sy 0.64 Sim 0.54 Memory Ben 0.56 Psychomotor level Mi II −0.53 Mi VII 0.56	Motor speed SA 0.78 Sensorimotor speed B-W 0.71 Visuomotor speed D Sy 0.61 Psychomotor level Mi VII 0.45	Motor speed SA 0.81 Personality traits Ro, Ada 0.49 Ro, Orig 0.47
Factor III	Motor speed SA 0.75 Sensorimotor speed B-W 0.67 Psychomotor level Mi VII 0.48	Personality traits Ro, Spon 0.63 Psychomotor spontaneity Mi I −0.66 Mi II −0.62	Personality traits Ro, Emo −0.78 Ro, Rat 0.64 Psychomotor level Mi VII −0.42
Factor IV	Subjective symptoms Neur 0.68 Labil 0.57 Fatig 0.52 Sleep 0.47 Personality traits Ro, Spon 0.53 Ro, Rat 0.45	Subjective symptoms Labil 0.75 Neur 0.71 Fatig 0.54 Psychomotor uncertainty Mi IV 0.53	Subjective symptoms Sleep 0.63 Labil 0.51 Size of movements Mi III 0.50 Sensorimotor inaccuracy B-W err 0.47
Factor V	Personality traits Ro, Orig 0.50 Ro, Rat 0.40 Ro, Ada 0.42 Psychomotor uncertainty Mi V −0.56 Mi IV −0.43	Personality traits Ro, Spon 0.58 Ro, Orig 0.57 Ro, Emo 0.49 Psychomotor tempo Mi VIII 0.60 Size of movements Mi III 0.55	Subjective symptoms Neur 0.61 Fatig 0.42 Psychomotor uncertainty Mi IV −0.47 Mi VI −0.40
Factor VI	Psychomotor tempo Mi VIII 0.65 Subjective symptoms Sleep −0.41	Personality traits Ro, Rat −0.63 Memory D Sp 0.50	Personality traits Ro, Spon 0.65 Ro, Ada 0.51

last could be called a factor of *personal tempo* as it has its only high loading in the performance speed of the Mira test.

The results cannot be considered to be particularly elegant as the analysis did not yield a simple factor structure. This was expected, however, as the tests were not pure factor tests, measuring only a single trait, but tests intended to detect mental disturbances which are of a complex nature. Nonetheless, it was possible to interpret the factors as those generally found in a similar set of test variables obtained from (in the psychiatric sense) normal subjects.

Factors of the Exposed Workers

Three of the factors in this analysis can be identified as the same found in the unexposed group: *intelligence* (I), *dexterity and speed* (II) and *neuroticism* (IV). There are some differences, however:

In the exposed group the connection between visual and general intelligence is closer: only one intelligence factor has been extracted (I). Moreover, the test intended to measure visual disturbances (Symmetry drawing) has the highest loading here.

The dexterity and speed factor (II) corresponds closely to the dexterity factor of the control group except for the fact that the Digit Symbol test has a high loading and thus seems to measure speed rather than intelligence in this group.

The neuroticism factor (IV) shows that there is a closer connection between psychomotor disturbances and neurotic symptoms in this group than in the unexposed group.

In addition there are two factors which seem to reflect personal styles of behavior or affective states. One of them (III) reflects behavior from *spontaneity* to *inhibition* as it appears in the ideation process and in psychomotor behavior. The other (V) can be called a factor of *mood*. One end of the dimension is characterized by fast and large motor movement in the Mira test, and by mental flexibility in the Rorschach test. At the other end there are constricted reactions and a pedantic style of drawing which have here been interpreted to reflect depressive mood.

The sixth factor seems to show some connection between poor rational self control and good verbal memory, and is difficult to interpret.

What seems to be interesting — and this might be an important finding — is that some factors of the exposed group are easier to interpret as indicators of disturbance, rather than factors accounting for success in tests. This finding is in agreement with the fact that the test results of the exposed group were on a significantly lower level than those of the control group[1]. The exposed subjects were slower and had more psychomotor disturbances.

This kind of explanation of factors applies even better to the next group, where the general level of performance was lowest.

Factors of CS_2 Patients

The first factor in this analysis includes the speed factor and all but one of the variables of the two intelligence factors that were extracted in the group of unexposed workers. It can be supposed, that the deterioration and retardation to various degrees of the subjects' performances in this group[1] account for the decreased differentiation of the abilities. This we can call *a deterioration* factor.

A pure speed factor could not be found as the deterioration factor accounts for the most decline of speed, with the exception of speed in the Santa Ana dexterity test. Lowered speed in this test is connected with personality measures reflecting general rigidity of spontaneous mental processes. We can call this factor (II) a factor of rigidity, or a factor of depression.

The third factor has only two significant loadings, both in Rorschach variables. It is a bipolar factor and seems to indicate that the poisoned subjects show two types of emotional disturbances, either lack of emotional responses, or weakly controlled anxious reactions. It can be called a factor of *emotional disturbances.*

The subjective symptoms revealed by inventory are located here on two factors. One of these factors (IV) shows the connection between neurotic symptoms, fatigue and psychomotor disturbances among the poisoned. It seems to reflect a *neurasthenic syndrome. Sleep disturbances* and neurovegetative symptoms form another factor, and they are accompanied by clumsy drawing and inaccuracy in sensorimotor performance. These symptoms can be interpreted as consequences of lack of sleep.

The last factor again reflects spontaneous productivity in the Rorschach test and has no significant connection with the other tests.

The hypothesis that the factors in the patient group reflect various symptom types of CS_2 poisoning seems to give a meaningful interpretation to most of the factors here. The next step will be to investigate the relationship between behavioral symptoms and other data: the duration and intensity of exposure, neurological and neurophysiological findings, clinical observations, and so on.

SOME CORRELATIONS BETWEEN BEHAVIORAL AND NEUROPHYSIOLOGICAL VARIABLES

Up to now correlations between psychological tests and electroneurographic measures have been calculated in a sample of 93 CS_2 workers. (For a description of the sample and the electroneurographic measures see the paper by Seppäläinen[9] in this volume).

The correlations were calculated in order to test the hypothesis that disturbances in the peripheral nervous system account to some

extent for the retarded speed of the exposed workers. The psychological variables which were correlated with ENMG were all measures of performance speed.

Table III shows the correlations.

Table III. CORRELATIONS BETWEEN ENMG-VARIABLES AND SPEED OF PERFORMANCES

Behavioral variables	ENMG-variables 1	2	3	4	5	6	7	8
D S	.06	−.14	−.05	.16	.13	.20	.00	.05
SA, right	.11	−.03	−.03	.24*	.11	.10	−.11	.17
SA, left	.15	−.01	−.16	.21*	.16	.14	−.06	.22*
BW	.13	.02	−.15	.23*	.15	.15	−.09	.23*
Mira	.05	−.03	−.15	.05	−.13	.12	−.10	−.04

* : < 0.05

ENMG-variables:

1. MCV of median nerve
2. Motor distal latency of median nerve
3. Sensory distal latency of median nerve
4. MCV of ulnar nerve
5. Motor distal latency of ulnar nerve
6. CVSF of ulnar nerve
7. Sensory distal latency of ulnar nerve
8. MCV of deep peroneal nerve

The closest connection was found between the MCV of the arm nerves and the speed in the Santa Ana and the Bourdon-Wiersma tests. All the correlations were in the expected direction; five of them were significant.

The correlations between psychomotor measures and distal latencies were also in the expected direction but were not significant.

In general, the correlations between the ENMG and the Digit Symbol and the drawing speed in the Mira were lower than those between the ENMG and the Bourdon-Wiersma and the Santa Ana. This was expected as the Digit Symbol test can be assumed to measure more cortical learning function, and the drawing speed in Mira according to the factor analyses, seems to be a measure of "personal tempo" rather than a measure of maximum speed of performance.

The results seem to indicate that the disturbances in the peripheral nervous system account for the retarded speed of exposed workers, but the correlations, even the significant ones, are low. This could be expected as the PNS is only one of the factors contributing to performance speed.

The use of these multidimensional analyses increases knowledge

of behavioral effects of CS_2. By extending the investigation to other industrial poisons it is possible to learn more about the specificity of the symptoms and to find out if the behavioral changes brought about by other industrial poisons differ from those of CS_2. All this demands both interdisciplinary and international research co-operation.

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DEVELOPMENT OF BIOLOGIC STANDARDS FOR TRICHLOROETHYLENE

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Trichloroethylene (TCE) is one of the most widely used chlorinated aliphatic hydrocarbon solvents in American industry. Its chemical stability, non-flammability, and physical properties have made it popular as a vapor degreasing, dry-cleaning and general cleaning solvent.

To protect the workman against excessive TCE vapor exposure, an industrial Threshold Limit Value (TLV) of 100 ppm has been established[1]. This TLV is based upon the available experimental animal and human toxicology data, but that data base is deficient because it lacks documentation of TCE's effects on cognitive and behavioral tasks.

Another problem posed by the current industrial standard is the horrendous task of monitoring the breathing zone of all exposed workmen to assure that over-exposure to TCE does not occur. While periodic monitoring of the breathing zone is adequate in those industrial situations which are stable and unchanging, work areas with widely fluctuating TCE concentrations require more frequent or continuous monitoring of the breathing zone, a desirable, always expensive, and not always feasible procedure.

An attempt has been made to circumvent the necessity for frequent or continuous breathing zone monitoring by substituting a reliable biologic test with which a workman's exposure can be estimated by measuring his total body burden of TCE. Two biologic tests have been advocated: the measurement of the urinary excretion of the major metabolites of TCE and the measurement of TCE in the expired breath of the workmen[2-14]. Wide individual variation in metabolite excretion has marred the usefulness of the former test, while the latter has been used only in experimental situations.

This investigation was undertaken with a dual purpose: (1) the collection of sufficient TCE expired breath data with which to establish the basis for a biologic test; (2) the study of TCE's effects on cognitive and behavioral tasks.

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Table I. EXPOSURE OF HUMAN SUBJECTS TO TRICHLOROETHYLENE VAPOR: 20, 100, 200 PPM FOR FIVE CONSECUTIVE DAYS

Planned exposure	Subjects	Duration of exposure, hrs.	Vapor exposure, ppm (Mean ± S.D.)				
			Day 1	Day 2	Day 3	Day 4	Day 5
20 ppm, Non-Fluctuating	4 males	7½	20.4 ± 1.8	20.6 ± 2.3	20.0 ± 2.2	20.8 ± 1.8	19.9 ± 7.9
	3 males	3	20.8 ± 2.3	20.2 ± 1.9	20.8 ± 3.0	21.2 ± 2.3	19.3 ± 1.4
	3 males	1	20.7 ± 1.4	21.1 ± 2.2	18.8 ± 1.3	19.9 ± 0.8	19.5 ± 1.1
100 ppm, Non-Fluctuating	4 males	7½	99.2 ± 7.2	98.0 ± 3.8	98.1 ± 5.0	99.8 ± 6.8	100.9 ± 8.2
	3 males	3	97.7 ± 11.1	95.6 ± 3.5	98.4 ± 4.6	97.8 ± 7.8	100.8 ± 5.5
	3 males	1	99.1 ± 1.6	98.4 ± 1.6	97.5 ± 2.0	96.9 ± 5.9	95.6 ± 17.7
100 ppm, Fluctuating	4 males	7½	91.7 ± 53.1	101.9 ± 55.1	100.6 ± 51.4	99.73 ± 51.1	100.6 ± 51.2
	3 males	3	104.3 ± 60.0	100.9 ± 59.3	100.4 ± 56.4	93.7 ± 51.9	98.5 ± 55.5
	3 males	1	no exposure	114.4 ± 64.1	100.9 ± 58.3	99.73 ± 56.1	100.2 ± 52.4
200 ppm, Non-Fluctuating	4 males	7½	200.9 ± 7.4	197.1 ± 5.6	198.3 ± 8.3	198.8 ± 4.9	199.9 ± 6.7
	3 males	3	200.1 ± 7.2	194.0 ± 5.5	200.4 ± 8.0	198.5 ± 5.8	198.6 ± 7.8
	3 males	1	195.3 ± 8.0	200.2 ± 5.2	193.0 ± 10.3	198.6 ± 3.7	201.2 ± 5.5
100 ppm, Non-Fluctuating	4 females	7½	100.0 ± 3.3	100.0 ± 2.8	100.0 ± 3.2	100.0 ± 4.8	100.0 ± 3.5
	3 females	3	99.1 ± 3.8	99.4 ± 3.2	100.0 ± 3.7	99.9 ± 4.0	99.6 ± 2.8
	3 females	1	100.1 ± 2.2	99.8 ± 2.0	98.9 ± 2.5	97.9 ± 2.6	97.7 ± 3.2

EXPERIMENTAL PROCEDURE

Ten male and ten female volunteers were exposed to TCE vapor for one week at concentrations of 20, 100, and 200 ppm for periods of 1, 3, or 7½ hours each day. The vapor exposures were performed in a controlled-environment chamber and were performed with strict adherence to the ethical and technical requirements for human inhalation experimentation[15]. This included obtaining the informed consent of each subject after the nature of the procedure had been fully explained.

Subjects

The twenty healthy adults ranged in age from 19 to 46 years. Prior to exposure, each was given a comprehensive medical examination which included a complete history and physical examination with the clinical laboratory studies listed below. The subjects were paid $2.50 per hour.

Exposure Schedule

Table I details the exposure sequences for this series of experiments. The concentration excursions scheduled during the fluctuating TCE vapor experiment are presented in Table II.

Table II.
FLUCTUATING TCE VAPOR EXPOSURE SCHEDULE

Elapsed Time, min.	TCE, ppm	Event
0	50	7½ and 3 hour subjects enter
80	100	
140	200	
179.8	200	3 hour subjects exit
180	50	
247	100	
297	200	
330	50	1 hour subjects enter
357	100	
377	200	
389.8	200	1 hour subjects exit
390	100	
450	100	7½ hour subjects exit

Exposure Chamber

The experiments were conducted in a controlled-environment suite of three rooms: a 20 ft. x 20 ft. x 8 ft. testing room containing a 5 ft. x 4 ft. x 8 ft. toilet facility, and a 4 ft. x 5 ft. x 6½ ft. grounded audiometric booth. The air flow through the rooms to the exhaust was approximately 500 cubic feet/minute, which created a slight negative pressure within the suite. The ambient temperature in the testing facility was maintained at 72-74° F, while the relative humidity ranged between 45 and 55%. Trichloroethylene was introduced into the testing facility's atmosphere by sweeping the vapor from a large flask into the return air duct.

Analysis of Exposure Chamber Atmosphere

The trichloroethylene used in these experiments was shown by infrared analysis to be 99.9% pure. The concentration of TCE in the chamber atmosphere was continuously recorded by an infrared spectrometer equipped with a 20-meter path-length gas cell which was continuously flushed with air drawn from the test facility through a ¼-inch diameter polyethylene tubing. The absorbance at 11.75 μ was measured. A gas chromatograph equipped with a hydrogen flame ionization detector and an automatic sequential sampler provided the second independent analytical method. The infrared analytical data were monitored every second by an on-line Digital PDP-12 computer, which displayed the mean vapor concentration for each 30-second time interval of exposure. Calibration standards of TCE in pure air were prepared in saran bags and analyzed by both monitoring methods before and hourly during each experiment.

Breath Sample Collection and TCE Analysis

Alveolar breath samples were obtained from each subject prior to exposure and at the following post-exposure times: 1 minute, 30 minutes, 1 hour, 2 hours, 4 hours, and at 9 a.m. the morning following exposure. The samples were collected in 50-ml glass pipettes fitted with screw caps containing saran liners. One of the caps had a pre-drilled hole through which a gas-sampling syringe needle could be introduced to pierce the saran liner and enter the main pipette chamber. The subject flushed the pipette three times with his exhaled breath and after holding the fourth breath for 30 seconds, exhaled and collected the end-tidal portion of his expired breath.

Trichloroethylene was analyzed in the breath samples using a gas chromatograph equipped with a hydrogen flame ionization detector. Breath aliquots were injected directly onto a 1-foot x ⅛-inch stainless steel chromatographic column containing Poropak Q operating at 140° C.

Clinical Testing

The subjects underwent a training program of ten test sessions in the controlled-environment chamber during the week prior to the inhalation experiments. During this time they became proficient in the testing procedures listed below.

Prior to each exposure a repeat physical examination was performed on each subject. Twice a week the following clinical laboratory determinations were made: complete blood count, complete uninalysis, and a SMA-12 survey panel of clinical chemistries. These tests were repeated 72 hours following the final TCE vapor exposure.

At no time were the subjects informed as to the concentration of TCE vapor to which they were being exposed. However, they were aware that they were being exposed because the odor of TCE was readily detectable by all at the three concentrations studied.

After entering the controlled-environment chamber, the subjects were under continuous surveillance by medical personnel and all important chamber activities were video-taped by closed-circuit TV. During the experiments EKG lead II was continuously monitored by telemetry.

On the first and fourth day of each exposure week the following tests were performed by the 3- and 7½-hour subjects after they had been exposed for 2 and 5 hours, respectively: Flanagan coordination test, Flanagan arithmetic test, random number inspection test, 10- and 30-second time estimation tests, and the Marquette time estimation test[16].

A modified Romberg test and a heel-to-toe test were carried out by each subject just after his entry into the chamber and immediately before leaving. Spontaneous EEG and the visual evoked response (VER) were obtained on the 7½-hour subjects during the training week prior to exposure, days 1 and 4 of each exposure week, and during the post-exposure week. Prior to entering the chamber each exposure day and hourly during the exposure, each subject filled out a subjective response form which inquired as to the presence of headache, eye, ear or nose irritation, chest pain, abdominal pain, dizziness, and intensity of TCE vapor odor.

Each subject collected a 24-hour urine for the measurement of trichloroacetic acid and trichloroethanol. Serial alveolar breath samples were collected in the post-exposure period for TCE analysis. Venous blood samples were obtained via the chamber arm port during and following vapor exposures so that blood concentrations could be correlated with the results of the clinical testing.

RESULTS

Post-exposure TCE breath decay curves were constructed from the data obtained from the volunteers who were exposed to the three

vapor concentrations for three different intervals of time. A family of these breath decay curves is presented in Figure 1 where it can

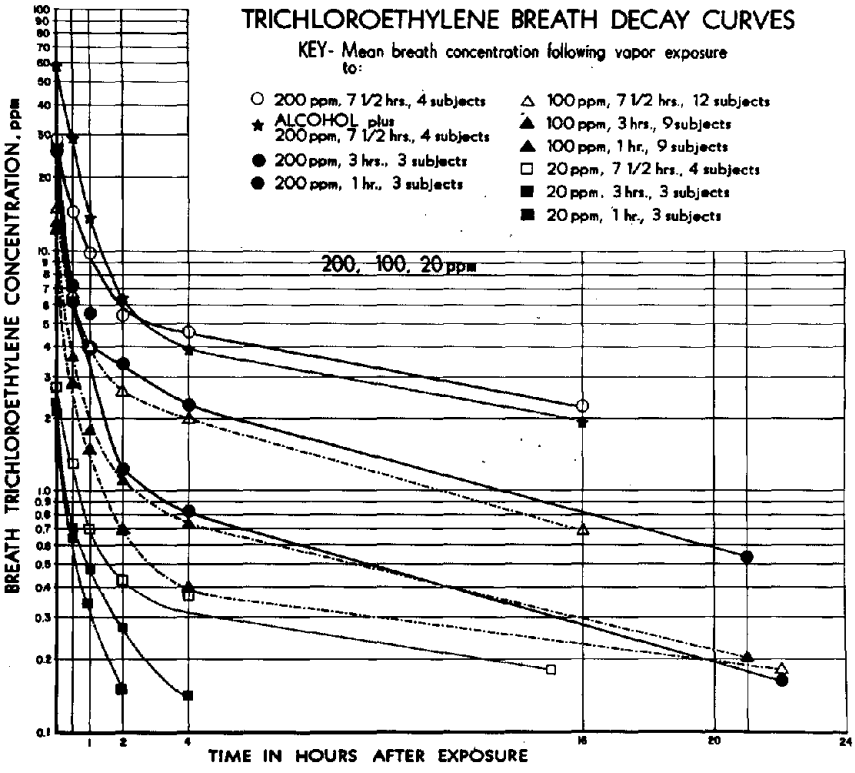

FIGURE 1. Trichloroethylene post-exposure breath decay curves obtained from adults who were exposed for periods of one hour, three hours, and 7½ hours to trichloroethylene vapor, 20 ppm, 100 ppm and 200 ppm.

be seen that they are in close agreement with those previously published[14]. It is apparent that breath analysis provides a method for establishing an unequivocal diagnosis of exposure and that analysis of breath in the immediate post-exposure period reflects the most recent vapor exposure which a person has experienced. Trichloroethylene breath analysis 8 to 24 hours following exposure is an accurate index of the time-weighted average vapor exposure. The complete set of breath decay curves obtained along with model uptake and excretion equations are to be detailed in a separate communication.

Trichloroethylene was detected in the blood of all subjects during and following each vapor exposure, but the methodology was more tedious and the results less accurate than those obtained by breath analysis. The measurement of the urinary metabolites of TCE was found to be an unsatisfactory index of the magnitude of vapor ex-

Table III. RELATIONSHIP OF TCE EXPOSURE TO TEST PERFORMANCE

Test	Male group test scores: Mean and range							Female group test scores: Mean and range		
	Control mean & range	TCE, 20 ppm 1st day	TCE, 20 ppm 4th day	TCE, 100 ppm 1st day	TCE, 100 ppm 4th day	TCE, 200 ppm 1st day	TCE, 200 ppm 4th day	Control mean & range	TCE 100 ppm 1st day	TCE 100 ppm 4th day
Flanagan Coordination	52.3 (16-70)	36.9 (23-56)	34.7 (10-58)	39.6 (−10→62)	39.31 (−2→55)	35.4 (19-58)	31.6 (−13→50)	69.9 (45-94)	65.3 (58-76)	61.7 (21-91)
Random Number Inspection	93.6 (82-114)	78.3 (62-93)	81.7 (68-104)	89.21 (70-124)	88.7 (66-128)	87.1 (84-101)	88.28 (78-105)	91.1 (67-131)	99.9 (74-134)	90.1 (62-120)

Table IV.
PAIRED t VALUES FOR COMPARISON OF MALE CONTROL VERSUS EXPOSURE TEST SCORES

Test		TRICHLOROETHYLENE CONCENTRATION											
		Monday 20 ppm t	df	Thursday 20 ppm t	df	Monday 100 ppm t	df	Thursday 100 ppm t	df	Monday 200 ppm t	df	Thursday 200 ppm t	df
Marquette test:													
	E/S	0.41	6	1.07	6	1.33	6	1.81	6	0.02	6	0.66	6
Sound stimulus	|E-S|	0.25	6	−0.09	6	−0.04	6	0.55	6	−0.23	6	0.06	6
	RxT	−0.91	6	−0.87	6	−0.77	6	0.46	6	−0.05	6	0.02	6
	E/S	0.43	6	0.45	6	−0.31	6	0.61	6	0.09	6	−0.62	6
Light stimulus	|E-S|	1.74	6	0.87	6	0.48	6	0.91	6	−1.66	6	0.33	6
	RxT	−0.00	6	−0.03	6	0.22	6	0.22	6	0.02	6	0.55	6
10 Second estimation		−3.29**	6	−0.30	6	1.19	6	2.25	6	0.79	6	0.44	6
30 Second estimation		−0.68	6	−1.17	6	−0.82	6	1.22	6	0.07	6	0.30	6
Arithmetic test		−0.78	6	−1.84	6	−4.75**	6	−2.05	6	0.85	6	−0.11	6
Coordination test		2.62*	6	3.89**	6	2.32	6	5.64**	6	3.24**	6	3.68*	6

posure because of the great individual variance and the cumulative effects of daily exposure. A detailed presentation of the clinical usefulness of blood TCE analysis and the measurement of urinary TCE metabolites will be the subject of a future communication.

There were no abnormalities observed in the battery of clinical laboratory tests during or following the vapor exposures.

The odor of TCE was detected by the subjects at each of the three concentrations studied. After one hour of exposure most of the subjects could no longer smell the odor of the solvent. The only untoward subjective response elicited was the complaint from the female subjects that they were unduly fatigued after exposure to TCE and that their sleep requirements were substantially increased.

Exposure to TCE, 100 ppm or greater for 7½ hours daily produced an alteration in the VER of one of three males and all female subjects. A definitive change in the spontaneous EEG was noted in one male subject exposed to 200 ppm. A detailed discussion of this segment of the experiment will be presented in a future communication.

Decrements in task performance were observed in two tests: Flanagan coordination and the random number inspection. These data are presented in Table III. No dose-response effect was observed. A paired t-test was employed to compare each subject's

Table V.

PAIRED t VALUES FOR COMPARISON OF FEMALE CONTROL VERSUS EXPOSURE TEST SCORES

		TRICHLOROETHYLENE CONCENTRATION			
		First day 100 ppm		Fourth day 100 ppm	
Test		t	df	t	df
Marquette test:					
	E/S	0.758	6	−1.81	6
Sound stimulus	|E-S|	−2.14	6	−1.38	6
	RxT	−3.85*	6	−1.47	6
	E/S	−1.08	6	−2.19	6
Light stimulus	|E-S|	−2.07	6	−0.44	6
	RxT	−0.20	6	1.54	6
10 Second estimation		−4.28**	6	−4.87**	6
30 Second estimation		−3.48*	6	−3.66*	6
Arithmetic test		−0.36	6	0.00	6
Coordination test		1.31	6	1.88	6
Inspection test		−5.72**	6	0.11	6

*Significant at P = .05 **Significant at P = .01

baseline performance with his performance during TCE vapor exposure (Tables IV and V). The decrement in performance of the 10-second estimation test and the arithmetic test which occurred on only one occasion were interpreted to be spurious.

COMMENTS

The decrements in performance of the coordination and the random number inspection tests would be worrisome if they were shown to be due to central nervous system depression. That there was no dose-response over the range of TCE studied suggests that a non-drug factor was responsible for the decrements observed. Volunteer subjects who have been carefully informed about the central nervous system depressant potential of TCE may give a biased performance when tested in a setting where the odor of TCE immediately informs the subjects that they are being exposed. The fact that no dose response decrement occurred with the male subjects who were exposed to three different TCE vapor concentrations requires that additional research be conducted to clarify this observation.

Salvini, et al., has reported that exposure to 110 ppm for 4 hours is sufficient to produce decrement in the performance of four tasks[17]. This observation prompted him to suggest that the current TLV does not provide an appropriate margin of safety. However, Salvini compared the effects of only one concentration of TCE to those at zero and may be criticized for having introduced subject bias into an experiment because of the obvious odor of TCE. The design of his experiments did not permit response to be correlated with dose. Additional experimentation will be required employing a better designed protocol so that the effect of 100 ppm vapor exposure to TCE upon cognitive tasks can be assessed accurately.

The alteration in the configuration of the VER's of some subjects exposed to TCE 100 ppm or greater for 7½ hours daily indicates that an exposure of this magnitude does have the potential for altering cerebral electrical activity. The significance of this alteration in light perception remains to be elucidated.

The TCE breath decay data obtained during this series of experiments is in agreement with the TCE breath decay data obtained in 1968 at a different location[14]. Breath analysis can be useful in estimating the time-weighted average TCE exposure. Even without detailed mathematical modeling, the data indicate that if the TCE vapor concentration has been steady a breath sample in the post-exposure period will accurately reflect the concentration inhaled when the exposure duration is known. If, however, the TCE vapor concentration fluctuated during exposure, the TCE in the breath in the early post-exposure period will be a reflection of the vapor

concentration to which the subject has most recently been exposed. In this situation one must wait until 6 or 8 hours post-exposure before the TCE breath concentration will accurately reflect the average vapor exposure.

Compounds with a distinctive odor present a formidable problem in proper experimental design, requiring that multiple concentrations be investigated so that a dose response relationship can be elicited. This study revealed that exposure to TCE, 100 ppm for 7½ hours daily produced an alteration in the visual evoked response of 4 of 6 subjects, and possibly increased the sleep requirements of the female subjects. Additional research will be required to determine whether an exposure of this magnitude exerts a detrimental effect upon manual coordination and random number inspection.

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PSYCHOLOGICAL EFFECTS OF TRICHLOROETHYLENE AND 1-1-1 TRICHLOROETHANE UPON MAN

M. Salvini, M.D.

Interest within our Institute at the University of Pavia in "behavioral toxicology" is extensive. In 1967 an industry which manufactured electrical parts started a new production line in a new section of the plant. During operations, workers in this new area were exposed to an atmosphere contaminated with trichloroethylene. Operations were planned to achieve a maximum production level with predicted amounts of waste materials and a number of rejected parts. Interestingly, during actual work operations, these predictions were not confirmed and errors in manufacture were more numerous than what was predicted. This resulted in decreased production. For an explanation of this phenomenon, worker efficiency was considered but excluded as a factor. With worker efficiency having been excluded, it was up to us to isolate the causes for decreased production. Several studies conducted to resolve this problem are described below.

Initially students in preventive medicine volunteered to be placed in this same work environment and performed for four continuous hours a series of psychological tests. Decreases in reasoning capacity were evident if the atmosphere of the work environment contained trichloroethane in an average concentration of 80-120 ppm (with a maximum level of 250 ppm). Initially it was thought that trichloroethane was the cause of the noted decrease in production, especially in those phases of the work cycle that placed demands on intellectual processes. Accordingly, three groups of volunteer subjects were tested during a five-month period where trichloroethylene and 1-1-1 trichloroethane were alternated in the production cycle. It was noted that production was improved when 1-1-1 trichloroethane was used. When research results were communicated to workers, immediate positive motivation, of course, was manifested and production reached a very high level right away. At this point the opportunity of publishing the results was recognized. Documentation of these findings with additional experimental data was considered desirable. For this reason, physicist Professor Riva, applied psychophysiologist Professor Binaschi, biologist Doctor Gazaniga, twenty-two volunteer students from the university, and I conducted inhalation studies of the effects of 1-1-1 trichloroethane and trichloroethylene on psychological functions in humans. (Figure 1)

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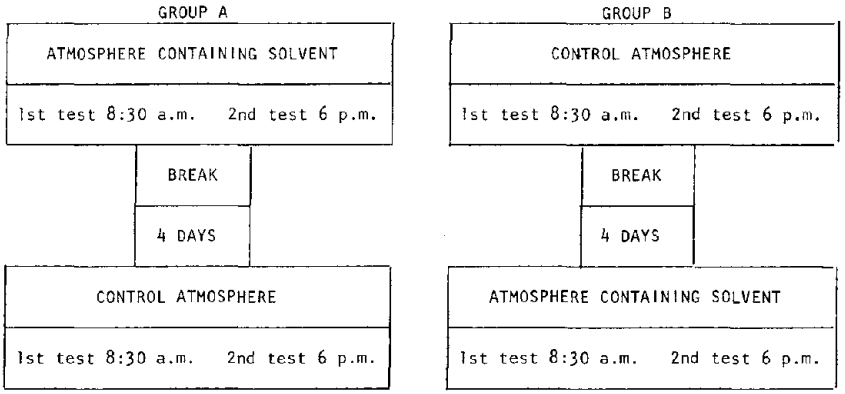

FIGURE 1. Experimental protocol used in 1,1,1 trichloroethane and trichloroethylene studies.

An inhalation study with 1-1-1 trichloroethane (methylchloroform) was completed and the results published.[1] Briefly the results indicated that in humans exposed to a vapor concentration of 450 ppm methylchloroform the psychophysiological functions that were measured were not affected. A reduction in performance that was observed was not statistically significant. In a test of visual perception an interaction between exposure to methylchloroform and mental fatigue was observed.

In a similar study the effect of trichloroethylene was evaluated[2]. Six male students were exposed to an average vapor concentration of 110 ppm trichloroethylene. Each subject was examined on two different days. On one day the subject completed a set of tests in an atmosphere containing trichloroethylene vapor and on another day in a controlled atmosphere which did not contain trichloroethylene. On each of the two days the tests were performed at 8 a.m. and were repeated at 6 p.m. These tests included measures of perception, manual dexterity, complex reaction time, and Wechsler Memory Scale. A crossed scheme analysis of variance was used to evaluate the results. A statistically significant decrease in task performance ability was observed in all measures. The greatest decrease was associated with the more complex tests (Tables I-V — Salvini, et al., Brit. J. Ind. Med., Vol. 28, 1971).

The Threshold Limit Value for trichloroethylene of 100 ppm appears to be very close to the average concentration capable of interfering with psychological efficiency even in the absence of other undesirable manifestations.

Due to the widespread use of trichloroethylene in industrial operations, it is important to evaluate the significance of these behavioral findings for worker safety implications.

Table I. PERCEPTIVE TEST — ANALYSIS OF VARIANCE

	SSQ	*D.F.*	S^2	*F*
Trichloroethylene (TCE)	28·12	1	28·12	27·84[3]
Mental fatigue (MF)	2·34	1	2·34	2·32
TCE × MF	15·31	1	15·81	15·65[2]
Learning (L)	35·02	1	35·02	34·67[3]
L × MF	15·75	1	15·75	15·59[2]
Subjects (S)	38·60	5	7·72	7·64[1]
S × MF	15·10	5	3·02	2·99
Error	8·11	8	1·01	·

[1]$p < 0.05$ [2]$p < 0.01$ [3]$p < 0.001$
SSQ = sum of square about the mean
D.F. = degrees of freedom
S^2 = estimate of variance
F = Snedecor's variance ratio

Table II. IMMEDIATE MEMORY TEST — ANALYSIS OF VARIANCE

	SSQ	*D.F.*	S^2	*F*
Trichloroethylene (TCE)	462·68	1	462·68	27·41[2]
Mental fatigue (MF)	85·58	1	85·58	5·51
TCE × MF	216·74	1	216·74	12·84[2]
Learning (L)	293·88	1	293·88	17·41[2]
L × MF	160·53	1	160·53	9·51[1]
Subjects (S)	347·75	5	69·55	4·12
S × MF	116	5	23·20	1·37
Error	135·01	8	16·88	·

[1]$p < 0.05$ [2]$p < 0.01$ [3]$p < 0.001$

Table III. COMPLEX REACTION TIMES (SPEED OF RESPONSES) — ANALYSIS OF VARIANCE

	SSQ	*D.F.*	S^2	*F*
Trichloroethylene (TCE)	119·72	1	119·72	26·14[3]
Mental fatigue (MF)	77·04	1	77·04	16·82[2]
TCE × MF	127·37	1	127·37	27·81[3]
Learning (L)	87·71	1	87·71	19·15[2]
L × MF	46·44	1	46·44	10·14[1]
Subjects (S)	162·80	5	32·45	7·11[1]
S × MF	105	5	21	4·59
Error	36·68	8	4·58	

[1]$p < 0.05$ [2]$p < 0.01$ [3]$p < 0.001$

Table IV. COMPLEX REACTION TIMES (REGULARITY OF RESPONSES) — ANALYSIS OF VARIANCE

	SSQ	*D.F.*	S^2	*F*
Trichloroethylene (TCE)	132·54	1	132·54	12·41[2]
Mental fatigue (MF)	19·65	1	19·65	1·84
TCE × MF	22·86	1	22·86	2·04
Learning (L)	18·48	1	18·48	1·73
L × MF	8·76	1	8·76	0·82
Subject (S)	108·95	5	21·79	2·04
S × MF	101·45	5	20·29	1·90
Error	85·41	8	10·68	

[2]$p < 0.01$

Table V. MANUAL ABILITY AND DEXTERITY — ANALYSIS OF VARIANCE

	SSQ	*D.F.*	S^2	*F*
(*a*) *Right hand*				
Trichloroethylene	480·11	1	480·11	25·47[2]
Learning	173·61	1	173·61	9·21[1]
Subjects	295·95	5	59·19	3·14
Error	75·42	4	18·85	
(*b*) *Left hand*				
Trichloroethylene	83·39	1	83·39	23·80[1]
Learning	37·79	1	37·79	10·41[1]
Subjects	401·85	5	80·37	22·14[2]
Error	14·51	4	3·63	
(*c*) *Right and left hands*				
Trichloroethylene	71·88	1	71·88	27·33[2]
Learning	68·88	1	68·88	26·19[2]
Subjects	308·65	5	61·73	23·47[2]
Error	10·51	4	2·63	

[1]$p < 0.05$ [2]$p < 0.01$

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EFFECTS OF TRICHLOROETHYLENE ON BEHAVIORAL PERFORMANCE CAPABILITIES

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To protect American workmen from the toxic effects of trichloroethylene (TCE) a Threshold Limit Value (TLV) of 100 ppm has been established[1,2]. Recently the adequacy of this workplace standard has been questioned[3,4]. In 1971, Salvini, Binaschi and Riva reported degradation of behavioral performance in six university students exposed to 110 TCE ppm for a single eight-hour work day[3]. As a result of "statistically very significant" performance decrements in complex reaction time, tachistoscopic perception, finger dexterity, and the Wechsler memory test, these authors concluded that a TLV of 110 ppm "appears to be very close to the average concentration capable of interfering with psychophysiological efficiency".

To investigate these TCE-induced behavioral performance decrements, the study described below was undertaken.

EXPERIMENTAL METHOD

One of the authors visited the laboratory of Salvini, Binaschi and Riva at the Institute of Preventive Medicine for Workers and Applied Psychology, University of Pavia, Italy to study the testing equipment and the methodology described by the Italian scientists[3,5]. Then an expanded version of the Salvini experiment was performed in which two TCE vapor concentrations were investigated instead of one. Three female and six male volunteers were exposed in groups of three in a controlled-environment chamber to TCE

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Dr. Richard D. Stewart and Staff are from the Department of Environmental Medicine and Biostatistics Unit, The Medical College of Wisconsin, Milwaukee, Wisconsin. Dr. H. Harvey Cohen and Mr. R. Struble are with the Behavioral Studies Laboratory, National Institute for Occupational Safety and Health. Mr. K. A. Busch is Chief, Statistical Services, DLCD, NIOSH.

vapor concentrations of 0, 50, and 110 ppm for eight hours. Each exposure day consisted of a 4-hour exposure in the chamber, a 1½-hour lunch period outside the exposure chamber followed by an additional 4 hours of exposure in the chamber. Behavioral performance tests were carried out twice each exposure day. Four non-exposure days elapsed between each TCE exposure.

Volunteer Subjects

The three females, designated Group I, were 26, 34 and 42 years of age and were all housewives. Group II consisted of males of ages 19, 22 and 29. One was a physician, while the other two were temporarily unemployed. Group III consisted of males ages 22, 23 and 28, all temporarily unemployed.

This investigation was performed with strict adherence to the ethical and technical requirements for human inhalation experimentation previously detailed, which included obtaining the informed consent of each subject after the objectives of the study and the nature of the procedure had been fully explained[6].

Exposure Schedule

In addition to the TCE vapor exposure of 110 ppm investigated by Salvini, et al., each group was exposed to 50 ppm, expanding the data base so that a dose-response relationship could be sought. Neither the subjects nor the investigators giving the behavioral tests were informed as to the TCE vapor concentration on any given day.

Exposure Chamber

Exposures to TCE were conducted in a controlled environment room 20 ft. x 20 ft. x 8 ft. in size, which contained a 5 ft. x 4 ft. x 8 ft. toilet facility and a 4 ft. x 5 ft. x 6½ ft. audiometric booth, both of which were ventilated with exposure chamber air. Exhaust air flow was approximately 500 cu. ft./min., creating a slight negative pressure within the chamber. The ambient temperature in the facility was maintained at 72°-74° F, while the relative humidity ranged between 45-55%.

Liquid TCE was pumped at a constant rate into a heated flask. A stream of air flowing through the flask swept the TCE vapor into the return air duct of the chamber. The proportion of fresh air used by the system was varied along with the TCE pumping rate to achieve vapor concentration control.

Analysis of Exposure Chamber Atmosphere

The TCE used in these experiments was 99.9% pure. The vapor concentration in the chamber atmosphere was continuously recorded

by an infrared spectrometer equipped with a variable path-length gas cell set at 5.25 meters. The gas cell was continuously flushed with air drawn from the test facility through a ¼-inch diameter polyethylene tubing. Transmittance at 11.74 μ was monitored every second by an on-line Digital PDP-12 computer, which recorded the mean vapor concentration for each 30-second time interval of exposure and the time-weighted-average exposure from the beginning of each session. A gas chromatograph equipped with a hydrogen flame ionization detector and an automatic sequential sampler provided a second independent method for analyzing the exposure chamber atmosphere. Calibration standards of TCE in pure air were prepared in saran bags and analyzed before and hourly by both instruments during each daily exposure.

Breath Sample Collection and TCE Analysis

Alveolar breath samples were obtained from each subject prior to exposure and at the following post-exposure times: 1 and 30 minutes, 1, 2, 4 and 16 hours[7]. Samples obtained prior to exposure and 1 and 30 minutes post-exposure were collected in saran bags. The remaining samples were collected in 35-ml glass tubes fitted with screw caps containing saran liners. One of the caps had a pre-drilled hole through which a gas sample could be withdrawn. The subjects flushed the tube three times, then after holding the fourth breath for 30 seconds, exhaled and capped the tube while finishing the exhalation.

A Varian Aerograph Model 2700 gas chromatograph equipped with a hydrogen flame ionization detector was used to determine TCE in the breath samples. The gas chromatograph (GC) was fitted with a stainless steel column, 3 ft. x ⅛ in., packed with 25% Apiezon L on Chromosorb W, AW, 45/60 mesh. The column was preconditioned at 220° C overnight prior to its use. The operating conditions of the GC were as follows: carrier gas (helium) flow rate, 40 ml./min.; column temperature, 105° C; injection port, 210° C; and detector 250° C. Standards at five concentrations to bracket the unknown levels were prepared with air as the diluent. One-ml aliquots were injected in duplicate and the concentration of TCE in unknowns was obtained by direct comparison of peak heights to those of the standards.

Medical Surveillance

Each subject was given a comprehensive medical examination three days prior to the first day of exposure. This examination was repeated the day following each individual's last exposure. Each examination included a complete history and physical examination with the following laboratory studies: complete blood count, SMA-

12 survey panel of clinical chemistries, urinalysis, 12-lead electrocardiogram, Orthorater visual performance profile (initial examination only) and a pregnancy test (initial examination on females only). Prior to each day's exposure, the subjects were given a repeat medical examination and were under continual surveillance by medical personnel while they were in the chamber. During all sessions spent in the environmental chamber, each subject's electrocardiogram (lead II) was continuously monitored by telemetry, and recorded at hourly intervals.

All subjects, when wearing glasses, had normal visual acuity. None of the female subjects was pregnant the day prior to her first TCE exposure.

Behavioral Performance Testing

Behavioral tests were carried out twice each day while the subjects were in the chamber: first upon entering the chamber for the morning session and then 1¼-hour prior to leaving the chamber during the afternoon session. Total time required for each test session was 1 hour and 5 minutes. The six tests used are described below in the order in which they were given. Because only one apparatus for each of the Salvini procedures was available, the three subjects exposed on any given day entered and exited the chamber at 15-minute intervals, and always in the same sequence.

Training for the test battery was carried out on the day prior to the first exposure. At that time each task was explained and one practice session was held.

Subjects were told that they would be exposed to TCE vapor, 110, 50 or 0 ppm, but they were not informed as to the vapor concentration on any given exposure day. Before starting the series of three exposures, they were encouraged to do their "best" on every task. They were given no feed-back on performance prior to the end of the exposures. Each test was always administered by the same individual.

As Salvini had done, all subjects exercised for 20 minutes at approximately 3 kcal./min. on Monark bicycle ergometers, midway through each session or twice per exposure day.

Complex Reaction Time

The subject was seated 24 inches from a 16 square inch wooden panel containing one central neon lamp and six peripheral neon lamps radially disposed 3.9 inches apart (Figures 1, 2). On the rear of the panel was mounted a "bird chirp" auditory signal. The subject's responses to visual or auditory signals were via two hand-held push-button devices and two-foot pedals. The incident room illumination was 8-foot candles.

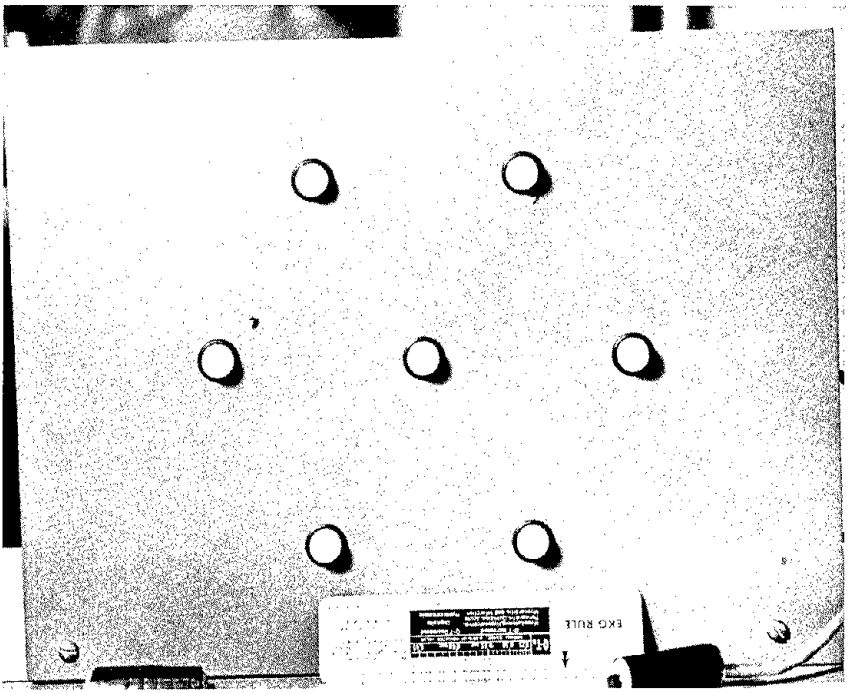

FIGURE 1. Panel of the Complex Reaction Time Test.

The task consisted of responding to 42 stimuli which were automatically and randomly presented with BRS-LVE solid-state programming equipment. Stimuli were 1/50 second in duration and were presented at the rate of one every 15 seconds. The total duration of this task, therefore, was 10½ minutes. The subjects were instructed to respond as quickly as possible so that reaction time to each stimulus presented, incorrect responses and missed stimuli could be recorded. When the three lamps on the right side of the panel lit, the subject was instructed to press the hand-held push-button device with his left thumb; when the three lamps on the left side lit he pressed the hand-held push-button with his right thumb. He responded to the central light by depressing the right foot pedal and to the auditory signal by depressing the left foot pedal. Reaction time in milliseconds was registered by a Hunter Manufacturing Company Model 220C Klockcounter (Figure 3).

Tachistoscopic Perception Test

In this test the subject viewed a series of 13 slides projected onto a screen (Figure 4). Each image contained a large square subdivided into nine equal squares. In the smaller squares either 4, 5,

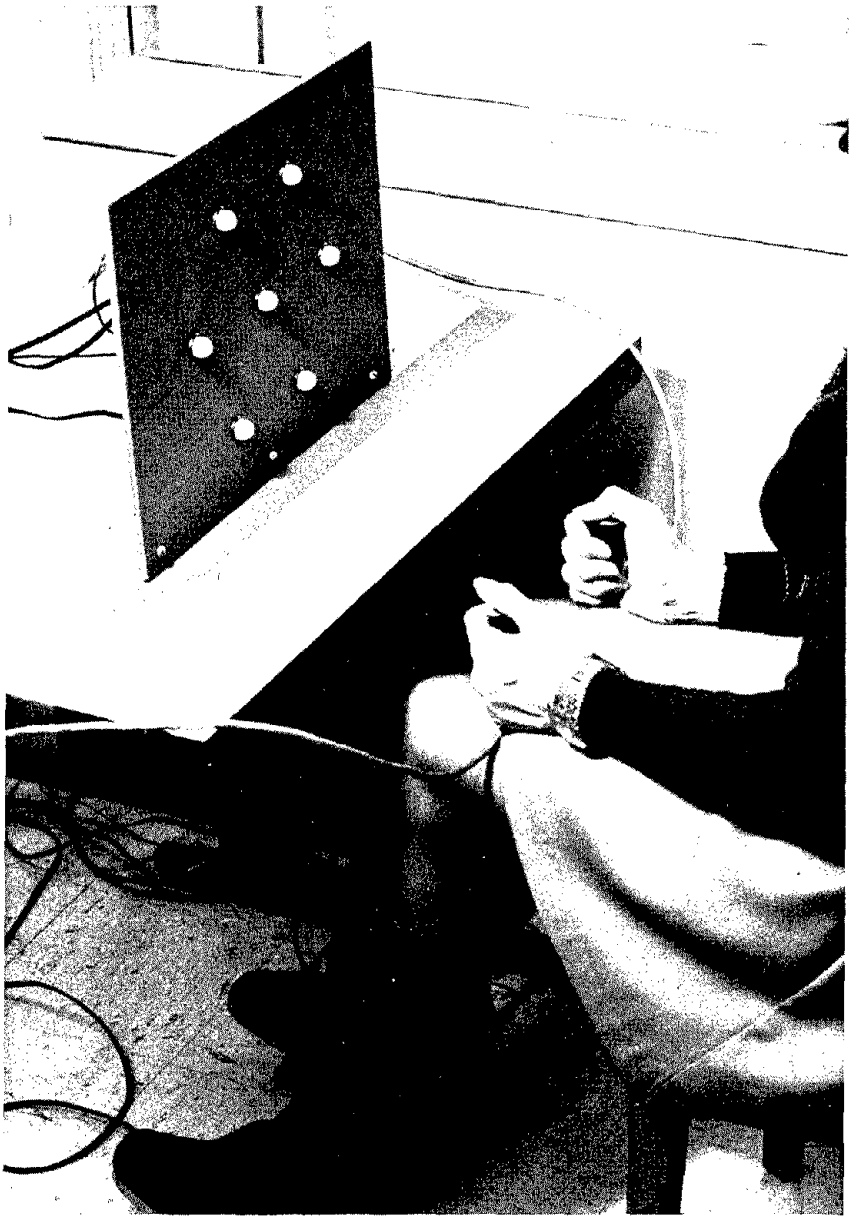

FIGURE 2. Subject performing the Complex Reaction Time Test.

or 6 black circles randomly appeared (one per square). The subject's task was to reproduce as accurately as possible the exact pattern of black circles he saw by drawing X's in the corresponding squares of an answer sheet. Stimuli were presented for 1/20 second

FIGURE 3. Investigator recording the reaction time for the 42 stimuli comprising the Complex Reaction Time Test. The subject performing the test can be seen in the controlled-environment chamber on the other side of the observation window.

by means of a Ralph Gerbrands Company Model 61168 Electronic Tachistoscopic Shutter with Timer and Kodak Custom Carousel 800 HC (low intensity) Projector. The projected image was 9 inches square and the viewing distance was 45 inches; the stimuli subtended a visual angle of 12°. The brightness of the test image at the screen was 500 foot candles. Room illumination was 40 foot candles at the screen.

Four slides contained four black circles, another four slides contained six black circles, and the remaining five slides contained five black circles all randomly placed within the nine inner squares. The 13 slides were rotated 90° to present a different randomly ordered group of slides for each test session.

The test was scored as follows: a one-point penalty was given for each black circle omitted and a ½-point penalty was given for each transposition.

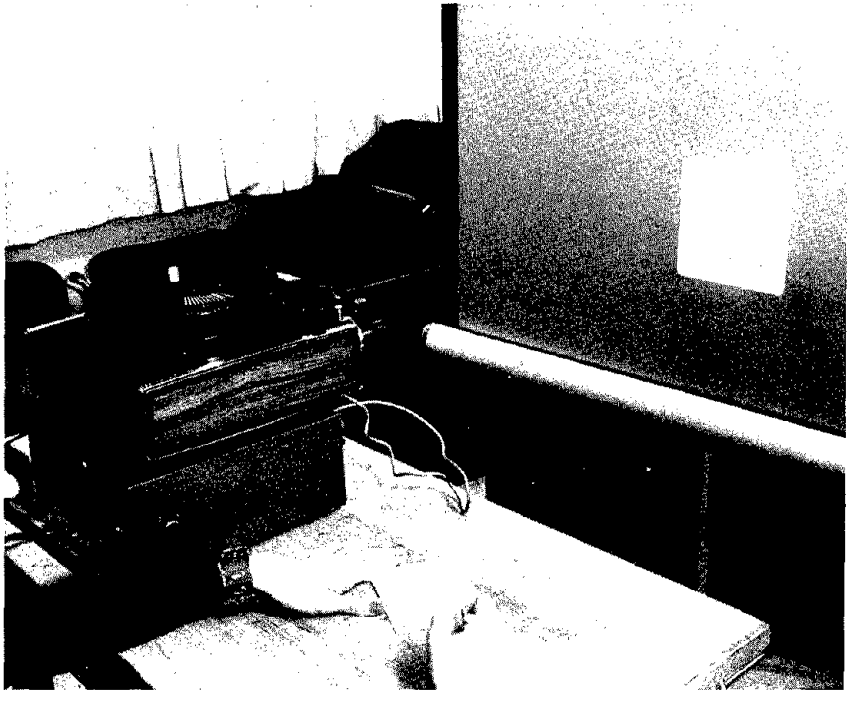

FIGURE 4. Subject performing the Tachistoscopic Perception Test.

Digit Span Test

Lists of successively increasing numbers of randomly generated digits were orally presented to subjects who were comfortably seated in the audiometric booth (Industrial Acoustics Company Model 401) located in the controlled-environment chamber (Figure 5). The subject's task was to repeat the numbers presented, first in a digits forward and then in a digits backward manner. The standardized instructions for the administration of this test were followed[8]. A different random order of digits was generated for each test session. The test score was the total number of digits correctly repeated forward plus the number correctly repeated backward.

Finger Dexterity Test

The subject's task in this test was to place as many sets of three metal pins in small round holes as he could in 9 minutes. For the first three minutes the task was performed with the right hand, then for three minutes with the left hand and, finally, for three minutes with alternate hands. A board with 100 holes, each large enough to

FIGURE 5. Subject seated in the audiometric booth performing the Digit Span Test.

hold four pins, was placed in front of the subjects. The subject was instructed to pick up three pins at a time and fill as many of the holes as possible within the allotted time (Figure 6, 7), starting in the farthest corner opposite to his hand. The test score was the total number of holes filled with three pins in the allotted time. Incident desk top illumination was 110 foot candles. The test device was purchased from the Lafayette Instrument Company, Lafayette, Indiana.

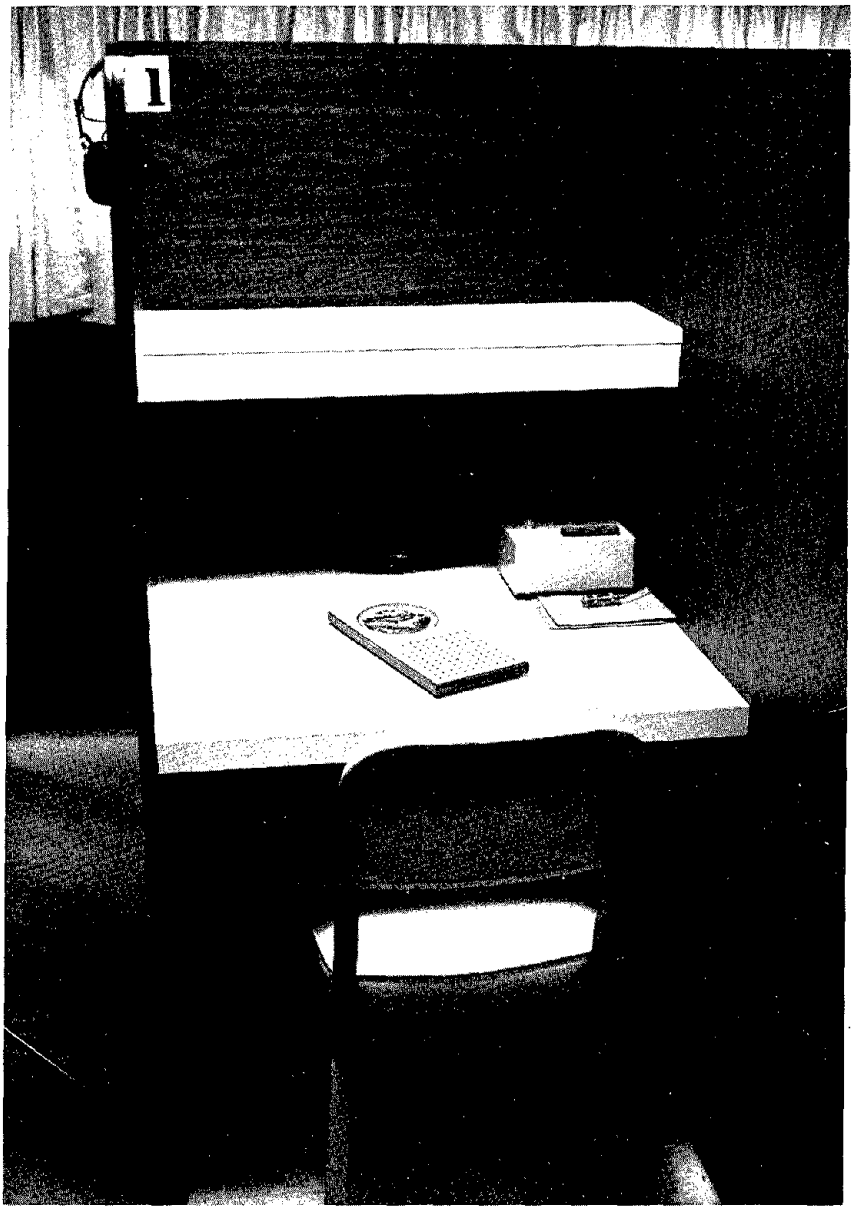

FIGURE 6. Carrel within the controlled-environment chamber where the Finger Dexterity Test was performed.

Flanagan Coordination Test

The Flanagan Coordination Test and the Digit Inspection Test were added to the test battery studied by Salvini so that the responses of the nine volunteers could be compared with those of other

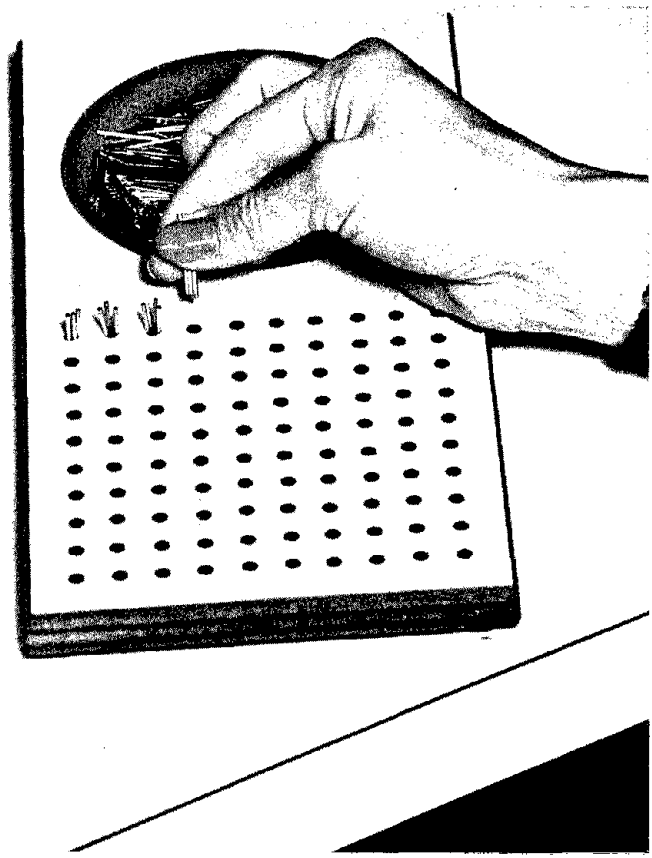

FIGURE 7. Subject performing the Finger Dexterity Test.

volunteers chronically exposed to TCE who had performed these two tests[9].

The Flanagan Aptitude Classification Test 7A, Coordination, published by Science Research Associates, Inc., 259 East Erie Street, Chicago, Illinois, is a paper-pencil test which requires a seated subject to trace a spiral pathway with a pencil (Figure 8). The standardized instructions for the administration of the test were followed. Forty seconds were allowed for the completion of each of the six spirals. The first two spirals were practice tests while the last four were scored. The test score was a function of the distance traced minus the number of times the edges of the spiral pathway were touched with the pencil.

Digit Inspection Test

This test was a measure of the speed with which a subject could

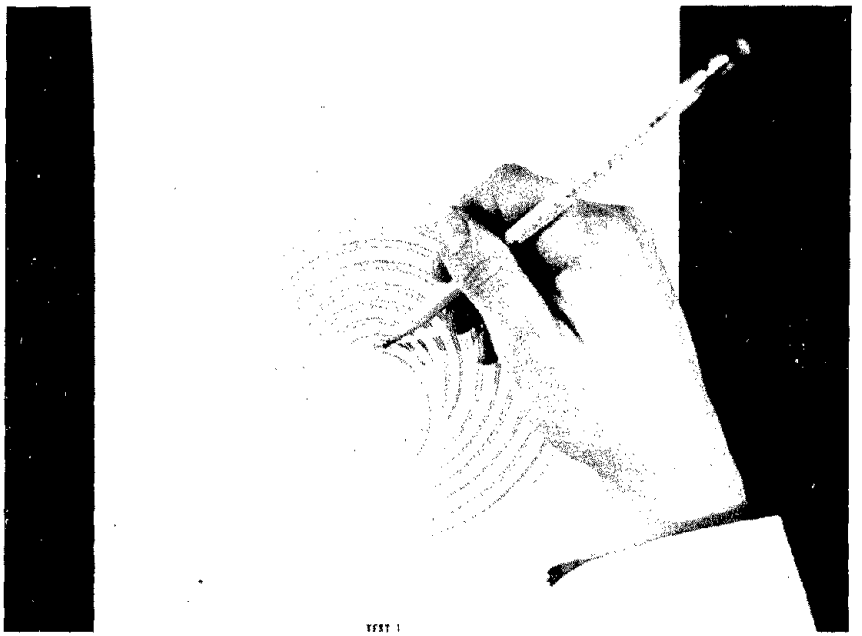

FIGURE 8. Flanagan Coordination Test.

detect the number "3" in rows of random numbers on an 8½ x 11" page (Figure 9). The subject, while comfortably seated at a desk, was asked to inspect each row of numbers beginning at the top of the page and mark as many 3's as possible in two minutes. The subject's score was the total number of "3's" struck. A different page of random numbers was used each test session.

Subjective Responses

All subjects were required to complete a subjective response form immediately after entering the chamber and hourly thereafter until four hours post-exposure. The form inquired as to the presence of headache; nausea; dizziness; abdominal pain; chest pain; eye, nose, throat irritation; and solvent odor (absent, mild, moderate or strong). The subjects were instructed to record any untoward response on this form.

RESULTS

The time-weighted average TCE vapor concentrations in the chamber for each four-hour session are listed in Table I. These concentrations were within ± 4% of that planned, with the exception of the second exposure of Group II, when the morning session averaged 12% above the planned 50 ppm concentration.

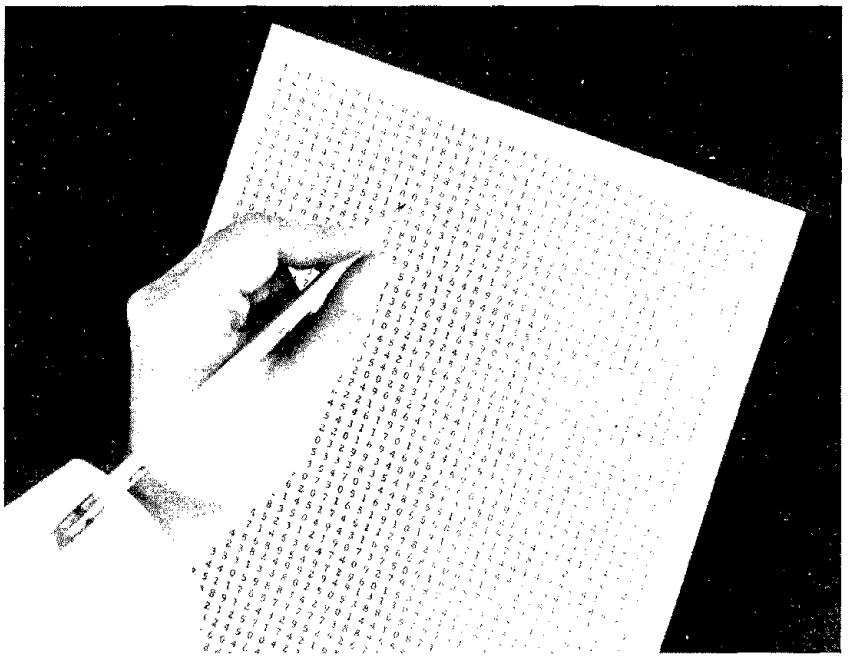

FIGURE 9. Digit Inspection Test.

The post-exposure TCE breath decay curves are plotted in Figure 10. No TCE (detection limit of 0.01 ppm) was present in the pre-exposure breath samples.

Pre-exposure physical and medical examinations to determine the health of each subject prior to the series of exposures and prior to each individual exposure revealed no significant abnormalities. The physical and medical examinations conducted the day after the last exposure revealed no significant change, indicating that all subjects had remained in good health. The subjective responses reported during the three separate days of exposure are summarized in Table II. An entry, "⅓ slight", in the headache row indicates that one of three subjects noted a slight headache some time during the exposure or in the first four hours thereafter. A dash indicates that no subject gave a positive response during any of the time periods.

Data Analysis

The design of the Salvini experiment was such that an analysis of variance procedure was most appropriate to assess the significance of the variables which had the potential for influencing the six behavioral performance tests. These variables were: (1) the concen-

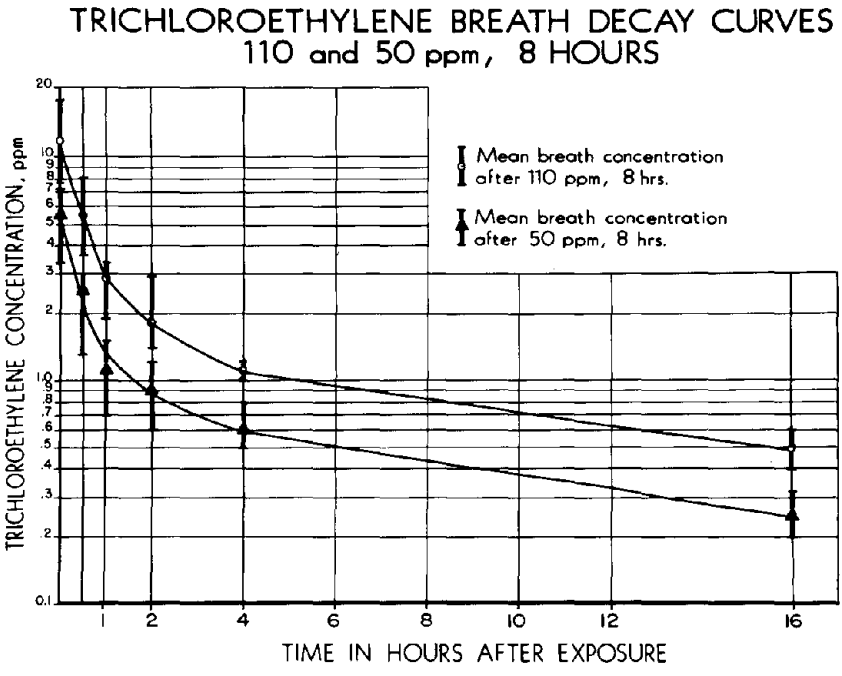

FIGURE 10. The concentration of trichloroethylene in the expired breath was monitored following each vapor exposure. Note the separation between the two exposure concentrations. These data are in close agreement with those previously published [11,12]. These breath data confirm that vapor exposure to 50 ppm and 110 ppm did occur.

tration of TCE vapor; (2) the day of the exposure sequence on which a test was performed; (3) the time of day the test was performed; (4) individual differences between the nine subjects in their ability to perform a test; (5) the differences in test performance ability among the three groups, and (6) combinations of interaction factors.

The analysis procedure for each of the behavioral tests was to construct analysis of variance tables for each group of three subjects. Next, a final analysis of variance table was constructed so that variation attributable to groups, subjects in groups, TCE vapor concentration, day sequence, residual interactions, am-pm effect, and finally, the interactions of am-pm effect with groups, TCE vapor concentration, day sequence and residual interactions could be assessed. Considering each of the three groups separately, the TCE

vapor concentration source is confounded (inseparable) with the day sequence source. This means the differential effects due to TCE vapor concentration and day sequence can not be independently examined in each group. By nature of the experimental design for the three groups as a composite, the variation due to TCE vapor concentration and day sequence can be tested for significance. Assuming negligible interactions with groups, F-ratios in each analysis of variance table are used to judge whether the indicated source is a significant or non-significant source of variation.

Day sequence represents differences between day 1, day 2 and day 3. Performance on some of the behavioral tests improves with practice or training, and day sequence reflects this learning effect. The source TCE vapor concentration measures differential effects due to the three vapor concentrations. Each of the TCE vapor concentration and day sequence sources is represented by 2 degrees of freedom in each analysis of variance table. The TCE x day sequence interaction, reflecting pattern changes in TCE mean responses across day 1, 2, and 3, would normally be represented by a 4 degree of freedom source of variation. However, this particular interaction is mathematically separated into two parts of 2 degrees of freedom each. Due to the design of the experiment, one part is confounded with the three groups while the other part (also confounded with groups and with interactions among groups, TCE and day sequence) is labeled residual interactions. These interactions were presumed to be negligible. The TCE vapor concentration and day sequence sources are assessed appropriately by the residual (1) mean square in each analysis of variance table.

The am-pm source represents differential responses measured between the morning and afternoon. Since this is a within a day effect it can be considered to measure the influence of fatigue. Inter-

Table I. ACUTE 8-HOUR EXPOSURE TO TRICHLOROETHYLENE VAPOR

Exposure day	Session	Vapor exposure, ppm (Mean ± S.D.) Group I	Group II	Group III
1	4-hr. Morning	0	110 ± 2	51 ± 4
	4-hr. Afternoon	0	112 ± 3	49 ± 1
2	4-hr. Morning	113 ± 4	56 ± 4	0
	4-hr. Afternoon	114 ± 2	50 ± 1	0
3	4-hr. Morning	49 ± 2	0	110 ± 2
	4-hr. Afternoon	49 ± 2	0	110 ± 2

Table II. SUBJECTIVE RESPONSES TO TRICHLOROETHYLENE VAPOR EXPOSURE

	Group I Exposure sequence			Group II Exposure sequence			Group III Exposure sequence		
	0 ppm	110 ppm	50 ppm	110 ppm	50 ppm	0 ppm	50 ppm	0 ppm	110 ppm
Subjective responses:									
Headache	2/2 slight	.....	.....	.....	.....	.....	.....	1/3 slight	1/3 slight
Nausea	.....	.....	.....	.....	.....	.....	1/3 slight	.....	1/3 slight
Dizziness	.....	.....	.....	.....	.....	.....	2/3 slight	1/3 slight	1/3 slight
Abdominal pain	.....	.....	.....	.....	.....	.....	1/3 slight	.....	.....
Chest pain	.....	.....	.....	.....	.....	.....	1/3 slight	.....	1/3 slight
Eye, nose, and throat irritation	2/2 slight	.....	.....	.....	.....	.....	1/3 slight	2/3 slight	1/3 slight
Other untoward responses	.....	.....	.....	.....	.....	.....	2/3 drowsy	.....	2/3 drowsy
Odor	2/2 mild	3/3 strong to mild	2/3 moderate to mild	3/3 strong to mild	3/3 moderate to mild	.....	3/3 strong to mild	.....	3/3 strong to mild

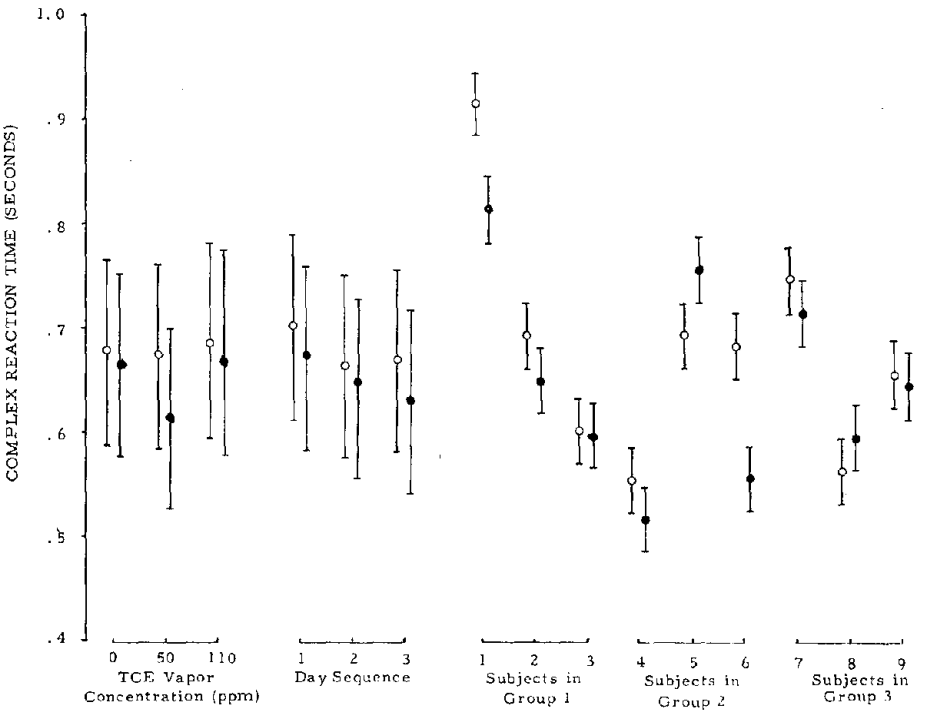

FIGURE 11. Complex reaction time mean responses and 95% Confidence Intervals.

Table III. FINAL ANALYSIS OF VARIANCE FOR COMPLEX REACTION TIME

Sources	DF	Sum of squares	Mean square	F ratio	
Groups	2	2.8388	1.4194	0.5015	NS
Subjects in groups	6	16.9807	2.8301	86.5474	$P < 0.001$
Day sequence	2	0.5772	0.2886	0.4781	NS
TCE	2	0.4415	0.2207	0.3656	NS
Residual interactions	2	0.0401	0.0200	0.0332	NS
Residual (1)	12	7.2436	0.6036	18.4587	
am pm	1	0.5027	0.5027	1.9576	NS
am pm x group	2	0.2117	0.1059	0.4123	NS
Day sequence x am pm	2	0.0399	0.0200	0.1938	NS
TCE x am pm	2	0.2587	0.1294	1.2539	NS
Residual interaction x am pm	2	0.3263	0.1632	1.5814	NS
Subject x am pm in group	6	1.5410	0.2568	7.8532	$P < 0.001$
Subject x am pm x session in group	12	1.2386	0.1032	3.1559	$P < 0.001$
Residual (2)	2214	72.4283	0.0327		

actions involving am-pm depict am-pm pattern changes associated with changes in the indicated factors. For example, if the day sequence x am-pm interaction is significant, as it was in the inspection test, this indicates that the relation of the am response to the pm response is not the same for each of days 1, 2 and 3. Thus, the analysis of variance table summarizes the relative influence of the many sources of variation.

The mean test scores for each of the behavioral tests are presented in Figures 11-16. The white circles designate the am mean scores while the black circles represent the pm scores.

Complex Reaction Time:

The mean complex reaction times for the 42 stimuli comprising each test are presented in Figure 11. In those instances in which the subject failed to respond to a stimulus, the average reaction time for the remainder of the stimuli was inserted so that a balanced set of data could be subjected to statistical analysis.

Trichloroethylene exposure did not appear to be a significant factor in the number of errors committed or missed stimuli. The number of errors recorded for the nine subjects were as follows: TCE 0 ppm, 16 errors; TCE 50 ppm, 17 errors; and TCE 110 ppm, 16 errors. The number of missed signals were as follows: TCE 0 ppm, 7; TCE 50 ppm, 1; and TCE 110 ppm, 6.

Table III presents the final analysis of variance for the complex reaction time response. Here are indicated the significant effects for subjects in groups and the subject x am-pm x session in group interaction. The interaction indicates that the am-pm response pattern across the three days is not the same for all subjects and this is seen in Figure 11. Effects due to TCE vapor concentration, day sequence, and am-pm effect are not judged significant.

Tachistoscopic Perception Test:

The mean performance scores are presented in Figure 12. Analysis of variance (Table IV) indicates that the only significant source of variation is the subjects in groups. This variability among subjects is evident upon inspection of Figure 12. Variation among subjects is also significant within each of the three groups (analysis not presented). TCE vapor concentration, day sequence, am-pm effect and corresponding interactions are not significant.

Digit Span Test:

The mean digit span responses are presented in Figure 13 and the analysis of variance in Table V. The digit span test has an addi-

tional forward-backwards variable which is labeled F B. Subjects in groups is a significant factor. Analysis of groups (not presented) shows that subjects was a significant source only in group 2. The backwards-forwards effect is highly significant with the forward mean response of 7.333 and the backward response of 6.000. Table V also calls attention to a significant day sequence effect. Figure 13 also illustrates this improvement in mean responses from day 1 to day 3 (averaging am and pm). TCE vapor concentration was not a significant source of variation and Figure 13 reveals mean responses of about equal magnitude for three TCE vapor concentrations. The forward-backward response pattern was not the same for all TCE vapor concentrations as shown in Table VI. The forward-backward differential decreases as TCE vapor concentration increases. All other sources of variation indicated in Table V are judged to be not significant.

Finger Dexterity:

The finger dexterity performance data are presented in Figure 14. Confidence intervals for subject means are large because the measure of variation used to determine interval length is differences between right and left hands. The analysis of variance for the finger dexterity (Table VII) reveals significance for subjects in groups, day sequence, and am-pm effect, but not for TCE vapor concentration or any other sources. Subjects differ significantly in groups 1 and 2 but not group 3 (analysis not presented). The effect of learning upon performance is illustrated by the improvement trend from day 1 to day 3 in Figure 14. The pm mean is consistently higher than the am mean, a significant am-pm effect confirmed in Table VII.

Flanagan Coordination Test:

The mean performance data are illustrated in Figure 15 and analysis of variance results are presented in Table VIII. The coordination test resulted in several significant sources of variation, namely: subjects in groups, day sequence, TCE vapor concentration, the am-pm x TCE interaction and am-pm x residual interactions. Table IV gives individual means for subjects in each of three sessions (days 1, 2 and 3). Subjects 5 and 6 have low test scores in session 1 (TCE, 110 ppm), which explains the significance of the interaction, day sequence and TCE vapor concentration factors. When interactions are significant the data should be separated into groups and attempts made to assess factors. However, TCE vapor concentration and day sequence cannot be assessed in groups 1, 2 and 3 because of the aforementioned confounding. The low scores could be due to a genuine central nervous system depressant effect

Table IV. FINAL ANALYSIS OF VARIANCE FOR TACHISTOSCOPE PERCEPTION

Sources	DF	Sum of squares	Mean square	F ratio	
Group	2	313.343	158.171	1.266	NS
Subjects in groups	6	749.541	124.924	25.6155	$P < 0.001$
Day sequence	2	38.620	19.310	3.214	NS
TCE	2	4.232	2.116	0.352	NS
Residual interaction	2	1.150	0.575	0.096	NS
Residual (1)	12	72.112	6.009		
am pm	1	3.375	3.375	0.7112	NS
am pm x group	2	1.861	0.931	0.1962	NS
am pm x subjects in groups	6	28.472	4.745	0.9730	NS
am pm x TCE	2	18.361	9.180	1.8825	NS
am pm x day sequence	2	1.583	0.791	0.1623	NS
am pm x residual interaction	2	1.444	0.722	0.1481	NS
Residual (2)	12	58.523	4.876		

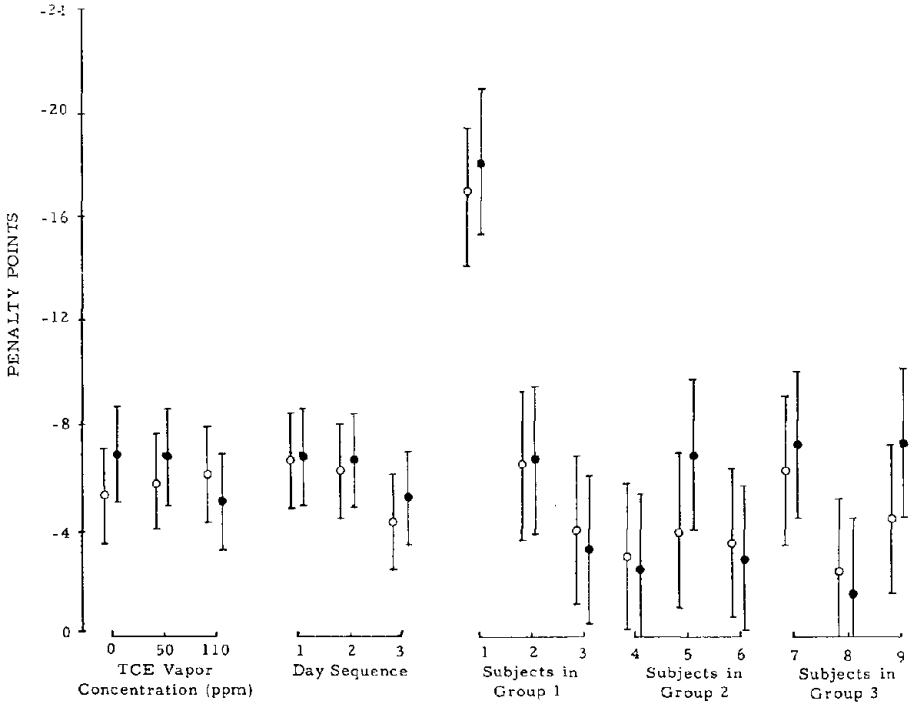

FIGURE 12. Tachistoscopic perception mean responses and 95% Confidence Intervals.

Table V.
FINAL ANALYSIS OF VARIANCE FOR DIGIT SPAN TEST

Source	DF	Sum of squares	Mean square	F ratio	
Groups	2	38.38852	19.1942	3.1745	NS
Subjects in groups	6	36.27816	6.0463	10.1245	$P < 0.001$
Sessions in groups	6	10.44484			
TCE	2	0.72222	0.3611	0.4171	NS
DS	2	9.55555	4.7777	5.5190	$P < 0.025$
Residual interactions	2	0.16666	0.0833	0.0963	NS
Residual (1)	12	10.38852	0.8657		
—	—	—	—	—	
am pm	1	0.33332	0.3333	0.5582	NS
am pm x group	2	2.16707	1.0835	1.8144	NS
FB	1	47.99929	47.9992	80.37	$P < 0.001$
FB x group	2	0.50076	0.2503	0.4193	NS
A x FB	1	0.92633	0.9263	3.7053	NS
A x FB x G	2	1.24032	0.6201	2.4806	NS
A x TCE	2	2.16667	1.0888	1.1361	NS
A x DS	2	2.16666	1.0888	1.1361	NS
A x Residual interaction	2	3.66666	1.8333	1.9129	NS
FB x TCE	2	4.05566	2.0278	6.3488	$P < 0.025$
FB x DS	2	0.22222	0.1111	0.3478	NS
FB x Residual interactions	2	2.05566	1.0278	3.2149	NS
A x FB x TCE	2	0.68488	0.3424	0.5733	NS
A x FB x DS	2	2.74033	1.3701	2.2942	NS
A x FB x residual interaction	2	0.24166	0.1208	0.2023	NS
A x P in groups	6	2.49964	0.4166	0.6976	NS
FB x P in groups	6	6.83333	1.1389	1.9071	NS
A x FB x P in groups	6	1.50002	0.2500	0.4186	NS
P x S x A in groups	12	11.50040	0.9584	1.6048	NS
P x S x FB in groups	12	3.83339	0.3194	0.5348	NS
Residual (2)	12	7.16594	0.5972		

Table VI. DIGIT SPAN MEAN RESPONSES

	TCE Vapor Concentration		
	0 ppm	50 ppm	110 ppm
Forward	7.555	7.222	7.222
Backward	5.722	5.944	6.333

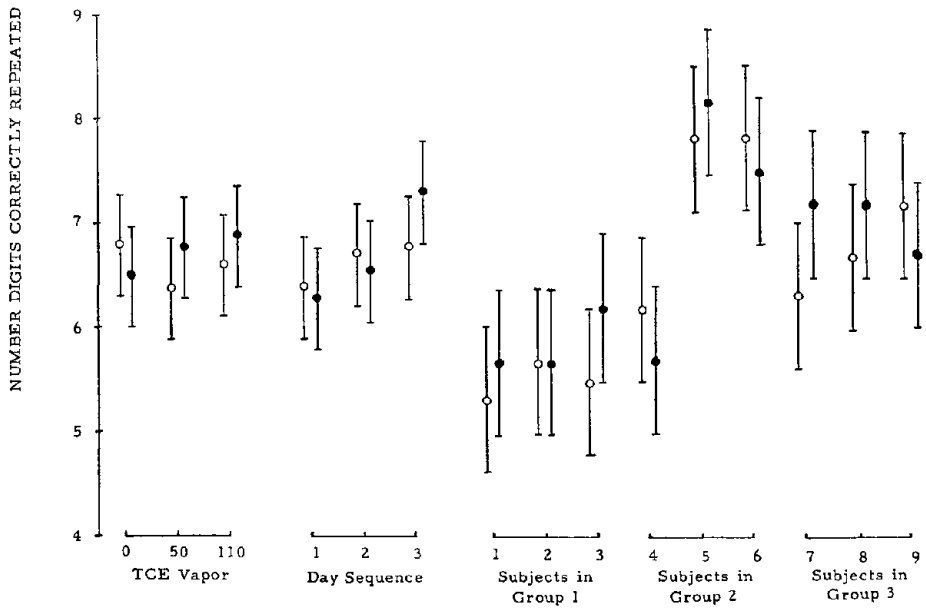

FIGURE 13. Digit span mean responses and 95% Confidence Intervals.

Table VII. FINAL ANALYSIS OF VARIANCE FOR FINGER DEXTERITY

Source	DF	Sum of squares	Mean Square	F ratio	
Groups	2	723.185	361.092	2.8114	NS
Subject in groups	6	1541.278	128.439	6.2261	$P < 0.005$
Day sequence	2	372.074	186.037	16.9881	$P < 0.001$
TCE	2	4.463	2.232	0.2038	NS
Residual interaction	2	0.074	0.037	0.0034	NS
Residual (1)	12	131.416	10.951		
am pm	1	171.259	171.259	21.3327	$P < 0.005$
am pm x group	2	15.403	7.701	0.9593	NS
Day sequence x am pm	2	0.963	0.481	0.0671	NS
TCE x am pm	2	10.907	10.453	1.4564	NS
Residual interaction x am pm	2	5.629	2.814	0.3921	NS
am pm x subject in group	6	48.167	8.028	0.3892	NS
am pm x subject x session in group	12	86.139	7.178	0.3479	NS
Residual (2)	54	1114.000	20.629		

of the solvent in more sensitive individuals or they could simply be low scores on the initial part of the learning curve. Statistically, TCE vapor concentration is a significant source. However, had TCE exerted a depressant effect, test scores would be expected to be lower or as low in the afternoon. Such was not the case. Differences between subjects is also obvious in Figure 15. Day sequence reveals a generally increasing score when days 2 and 3 are compared to day 1, all of which indicates that a test requiring multiple training sessions to reduce learning effect probably should not have been included in the test battery.

Digit Inspection Test:

Mean performance data for the digit inspection test are presented in Figure 16. Significant performance difference among subjects is evident and a learning trend is discernible. The analysis of variance (Table IX) reveals significant sources for subjects in groups, day sequence, residual interactions, the am-pm x TCE in-

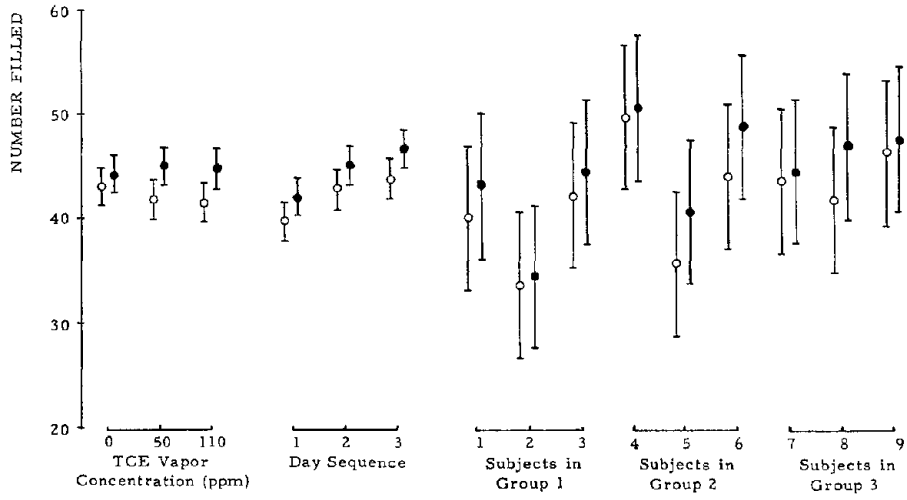

FIGURE 14. Finger dexterity mean responses and 95% Confidence Intervals.

Table VIII. FINAL ANALYSIS OF VARIANCE FOR COORDINATION TEST

Sources	DF	Sum of squares	Mean square	F ratio	
Group	2	853.000	426.500	0.4353	NS
Subjects in group	6	5878.333	979.72	21.4	$P < 0.001$
Day sequence	2	757.000	378.500	4.2382	$P < 0.05$
TCE exposure	2	1159.000	579.500	6.4889	$P < 0.025$
Residual interactions	2	277.325	138.663	1.5527	NS
Residual (1)	12	1071.666	89.306		
am pm	1	88.166	88.166	1.3105	NS
am pm x group	2	120.333	60.166	0.8943	NS
am pm x subjects in group	6	403.668	67.278	1.4670	NS
am pm x TCE	2	373.000	186.500	4.0666	$P < 0.05$
am pm x day sequence	2	39.000	19.500	0.4252	NS
am pm x residual interaction	2	444.000	222.000	4.8407	$P < 0.05$
Residual (2)	12	550.332	45.861		

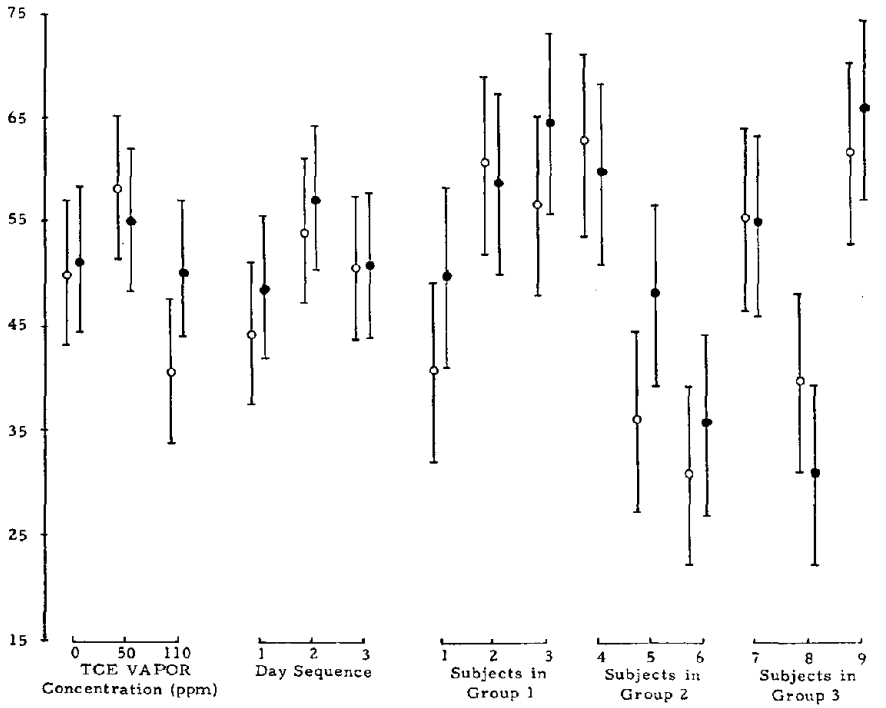

FIGURE 15. Coordination test mean responses and 95% Confidence Intervals.

Table IX. FINAL ANALYSIS OF VARIANCE FOR INSPECTION TEST

Sources	DF	Sum of squares	Mean square	F ratio	
Group	2	231.4455	115.7225	0.2145	NS
People in groups	6	3237.2200	539.5367	45.7072	$P < 0.001$
Day sequence	2	868.7777	434.3888	20.6761	$P < 0.001$
TCE exposure	2	128.1111	64.0555	3.0489	NS
Residual interactions	2	940.5571	470.2785	22.3846	$P < 0.001$
Residual (1)	12	252.1080	21.0090		
am pm	1	7.4074	7.4074	0.3933	NS
am pm x group	2	46.9259	23.4629	1.2458	NS
am pm x subject in group	6	113.0010	18.8335	1.5955	NS
am pm x TCE	2	102.9259	51.4630	4.3597	$P < 0.05$
am pm x day sequence	2	345.3704	172.6852	14.6291	$P < 0.001$
am pm x residual interactions	2	26.7037	13.3519	1.1311	NS
Residual (2)	12	141.6498	11.8042		

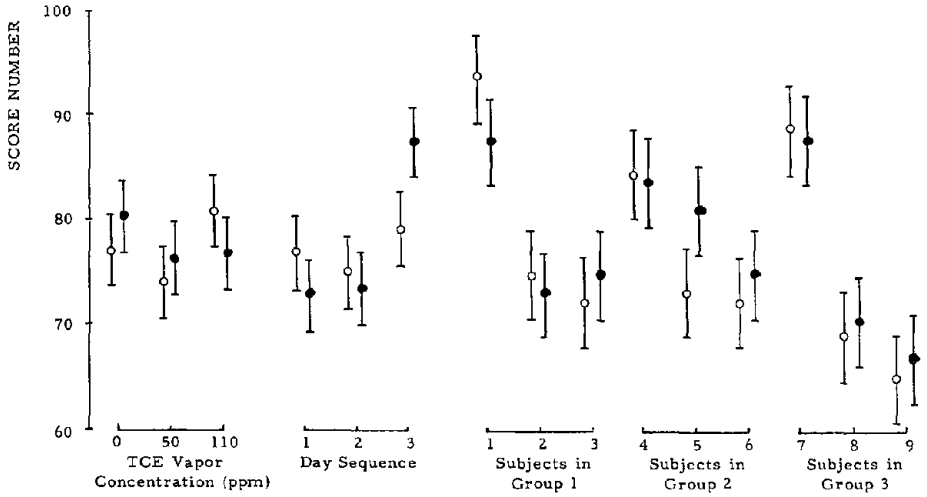

FIGURE 16. Digit inspection test mean responses and 95% Confidence Intervals.

teraction and the day sequence interaction. The am-pm x TCE and am-pm x day sequence interactions are significant. Due to the overall experimental design, TCE vapor concentration and day sequence cannot be assessed for each of the groups, as they should be when residual interactions are significant.

Summary of Behavioral Testing:

In each of the six behavioral tests administered variability among subjects was highly significant. This clearly suggests that experiments of this type must be designed to include more subjects to minimize the effect of subject variation thus permitting more accurate assessment of the factor of interest (TCE). Four of the six tests produced evidence of learning as measured by day sequence (Digit Span, Finger Dexterity, Coordination and Digit Inspection). The am-pm differential (day fatigue) was significant for only one test (Finger Dexterity). TCE vapor concentration was an insignificant source of variation in five of six tests. TCE vapor concentration was statistically significant in the coordination test, a test which was also influenced by other interaction effects.

COMMENTS

This investigation failed to corroborate the Salvini, Binaschi, and Riva report that an acute 8-hour exposure to TCE 110 ppm resulted in a decrement in the performance of four behavioral tests. There are three technical reasons which could account for the differences in results between the two laboratories: questionable statistical analysis of the data, an inaccurate TCE vapor exposure, or poor experimental execution.

In the opinion of the authors, technical aspects of the analysis of variance tables in the Salvini, Binaschi and Riva paper (source of variation breakdown and choice of some F-ratios) appear to be incorrectly performed. It is unfortunate that the Salvini paper fails to present mean test scores and other summary statistics because this lack of basic statistical information makes it impossible to either compare results (mean scores, magnitude and direction of observed differences) between papers or to make an independent assessment of the Salvini data.

There is some question about the adequacy of analytical control in the Salvini TCE vapor exposures. Intermittent atomization of liquid TCE in a small chamber monitored intermittently with a gas sampling syringe through a small port in the entrance door sets the stage for an exposure which could feature wide unmeasured fluctua-

tions in TCE vapor concentration. The failure to use a second independent analytical procedure with which to check the accuracy of the primary instrumentation coupled with the failure to monitor the TCE in the breath or blood of the subjects in the post-exposure period prevents a retrospective review of the accuracy with which the vapor exposures were measured.

The experimental design employed by Salvini and his co-investigators would have been stronger had it included exposure to two concentrations of TCE instead of a single one-time exposure to 110 ppm. At 110 ppm the chloroform-like odor of TCE vapor is strong and distinctive, precluding the performance of even a single-blind experiment. The use of a single concentration also precludes the establishment of a dose-response relationship. It is conceivable that highly suggestible individuals could be induced to perform poorly once they were made aware of the CNS depressant effect of TCE.

Another troublesome feature of the experimental design of the Italian scientists is the 1½-hour lunch break during which time the subjects were free to leave the laboratory setting. Had the subjects imbibed wine, the blood stream concentrations of TCE would have been elevated dramatically[10-12]. Blood alcohol concentrations of 0.05%, concentrations easily achieved while drinking wine, could result in a doubling of the TCE blood stream concentration, converting the physiological effect of TCE 100 ppm to that of vapor concentration much higher.

In our study, the Flanagan coordination test and the random number inspection test were added to the Salvini battery of four tests for comparison purposes because they had been used in other TCE studies[11-12]. In retrospect the decision to do so was poor since the repetitive use of either test should be preceded by an adequate training period if the learning effect on performance is to be minimized. The experimental design of the Salvini experiment precluded this. Two of the nine subjects who were exposed in an initial session to 110 TCE ppm had low coordination test scores. This could represent a definite CNS depressant effect of TCE or a low score on a normal learning curve or a biased subject response to exposure. This finding merits additional study, preferably in individuals who have been adequately trained so that the influence of learning on test scores can be eliminated as a variable.

It was of extreme interest to observe the wide variation in subjective responses reported by the subjects (Table II). Note that two reported headache and lightheadedness the first time they were placed in the controlled-environment chamber, though they were not exposed to TCE. This reemphasized the role of suggestion in

creating subject bias and the danger of studying a single TCE concentration.

Salvini and his co-investigators were critical of the human response data of Stopps and McLaughlin because they had failed to simulate an 8-hour working day when exposing subjects to TCE for fewer than 8 hours[14]. Continuing with the same reasoning, Salvini, et al., may be admonished for drawing conclusions regarding TCE effect without having simulated a workweek which is essential when evaluating the effect of a solvent which builds up in body tissue with repeated, daily 8-hour exposures[4, 11]. Therefore, the judgment regarding the adequacy of the present TLV to protect workmen from TCE-induced behavioral performance decrement must be held in abeyance until data from individuals repeatedly exposed are available.

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BEHAVIORAL EFFECTS OF METHYLENE CHLORIDE AND CARBON MONOXIDE AS ASSESSED BY SENSORY AND PSYCHOMOTOR PERFORMANCE

G. Winneke, Ph.D.

INTRODUCTION

A number of experiments conducted in our laboratory during the past three years have demonstrated that inhaled vapors of Methylene Chloride (MC) exert a depressant effect on behavioral functions in animals and man (SCHLIPKOTER et al., FODOR and WINNEKE, WINNEKE [1-3]. Accidental and experimental observations of STEWART and colleagues[4] have clearly shown, furthermore, that there is a marked increase of carboxyhemoglobin concentrations in blood after the inhalation of MC-vapors at concentrations between 500 and 1000 ppm.

These findings, which have been fully confirmed and extended in our laboratory by FODOR et al.,[5] in rats, give rise to the question, whether the behavioral effects observed under the influence of MC-vapors might reasonably be explained by endogenous CO production or not. In order to answer this question, behavioral data for both these agents must be compared by functionally the same or at least similar behavioral criteria. Such a comparison of effects - as well as considerations on the adequacy of the present TLV for Methylene Chloride - is the topic of this paper.

A short description of the physicochemical, toxicological and industrial "properties" of both these agents will be given first.

Methylene Chloride

Methylene Chloride is a colourless, noninflammable liquid of high volatility and a sweetish odor. It is a good solvent for alkaloids, bitumen, crude rubber, oils, waxes and many organic compounds. According to BROWNING[6] it has the following uses:

(1) constituent in paint removers
(2) refrigerant in air conditioning
(3) degreasing and cleaning fluid, for the cleaning of electrical motors
(4) "stretching" solvent in the artificial silk industry
(5) in the extraction of oils, fats, perfumes and drugs

It has a depressant effect on the CNS and leads to narcosis at concentrations of about 2 Vol.% within 30 minutes. It has in

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fact been used as a narcotic, but no longer, since the margin between narcotic and lethal dose is narrow; fatal cases from occupational exposure have been reported only rarely, MOSKOWITZ and SHAPIRO[7]. The recommended TLV is 500 ppm in most western countries, e.g. in the U.S., in Western Germany and in Great Britain, whereas it is 144 ppm in the GDR, 14 ppm in the USSR and only 6 ppm in Hungary, Documentation of MAC in CSSR[8]. According to STEWART and colleagues[4] a one hour exposure to 500 ppm gives rise to COHb-values between 3 and 5%.

Carbon Monoxide

CO is a non-smelling, non-irritating gas, which is formed through incomplete burning. It is therefore a common constituent in the atmosphere of many industrial and non-industrial settings. Its toxicity is mainly due to its high hemoglobin-affinity, thereby reducing the oxygen-carrying capacity of the blood and consequently the oxygen-content of other tissues. This is especially critical in tissues with a high oxygen-demand, first of all for the brain and the heart.

Conflicting behavioral data have been reported for COHb-levels between 3 and 10%, some studies showing detrimental effects at measured or computed COHb-levels around 5% e.g. SCHULTE[9], BEARD and WERTHEIM[10], BEARD and GRANDSTAFF[11], others showing no effects whatsoever, even at COHb-levels between 10 and 20%, e.g. STEWART et al.,[12]. The TLV for CO has been set at 50 ppm in many western countries.

The data from the experimental studies to be described below have been used to answer the following two questions:

(1) Can the present TLV of 500 ppm for Methylene Chloride be considered safe, from the point of view of behavioral toxicity?

(2) Is COHb-formation responsible for the behavioral effects observed during and after inhalation of MC-vapors?

METHODS

Performance Measures

The behavioral measures used in our studies were: An auditory vigilance task, visual flicker fusion frequency and a battery of psychomotor tasks.

Vigilance Task

The auditory Vigilance Task was a modified version of a task originally employed by LOEB and BINFORD[13]. Ss listened by earphone to a train of pulses of white noise (40 dB, 2.0 secs apart and of 0.36 secs duration). At random intervals with a probability

of p=0.03 slightly less intense pulses (i.e. by 4.8 or 3.8 dB) were inserted and these had to be detected and reported by pressing a key. Omission errors (percentage of signals missed per 15 minutes) as well as response latencies were taken as performance measures. Error percentages were transformed to arcsine values for statistical treatment. The whole test consisted of 4 to 5 observation periods of 30 to 45 minutes duration each; between any two observation periods an interruption of about 5 minutes duration was interposed for CFF-determination.

Critical Flicker Fusion

Visual Critical Flicker Frequency (CFF) was determined binocularly by means of an electronic flicker device (Bettendorf Corp., Type 40236), brightness of flicker light and on-off ratio being held constant. The descending presentation was employed throughout the experiment, since ascendingly determined values were found to be rather insensitive in a former study by WINNEKE et al.,[14], and the averaging of ascendant and descendant CFF seems highly objectionable by GINSBURG[15, 16]. Each CFF value is an average of eight single descending CFF determinations.

Psychomotor Variables

The choice of psychomotor tests and their interpretation according to factorial structure were partly based on the systematic studies of FLEISHMAN[17].

Tapping

Speed of hand movements without eye-hand-coordination was assessed in the Tapping Test. Ss tapped at maximum speed without arm rest and the average number of the contacts during 4 test periods of 15 sec. duration each was evaluated.

Two Plate Tapping

Speed of arm movements with only little eye-hand coordination was tested in the Two-Plate Tapping-Test. Ss tapped two plates, 40 cm apart, as fast as possible and the average number of tappings during 4 testing periods of 20 sec. duration each was determined.

Steadiness

The static Steadiness Test assesses the static control of hand and arm: S held a stylus as steady as possible in an opening avoiding contacting the edges and the average number of contacts (EC) as well as the cumulated contact time (ECT) of four test periods of 30 sec. duration each were measured.

Hand Precision

Coordination and precision of quick aiming movements under paced working conditions were assessed in the Purdue Hand Precision-Test (PHP). Ss tapped with a stylus on three targets which appeared in succession on a disk rotating at 50 rpm, trying not to hit the edges. Scores were the number of hits (PHP-Hits), the number of errors and the cumulated contact time of errors (PHP-ECT), all taken as averages over three periods of 60 secs. duration each.

Pursuit Tracking

Pursuit Rotor Test was used for testing visual-motor control of larger muscle groups during visual pursuit tracking. Ss were instructed to keep the tip of a stylus in contact with a small target placed on a disk rotating at 50 rpm. Score was the average "time-on-target" during 40 seconds from three trials.

Speed of Reaction

Visual Reaction Times without (RT-) and with (RT+) gross motor reactions and choice reaction times with five alternatives (RT-5) were measured. Ss were required to switch off a light-signal as quickly as possible by pressing the appropriate button with the right index finger, which was placed directly over the appropriate button (RT-) or 25 cm apart (RT+). In the RT-5 condition one of 5 lights was switched off by pressing one of five compatible buttons. The time between light-on and light-off was measured in milliseconds and an average over at least 30 single presentations was computed.

A principal components analysis on psychomotor control-scores resulted in 4 independent factors accounting for 80% of the total variance, Factor I "Steadiness", Factor II "motor speed with little visual motor coordination", Factor III "control precision" and Factor IV "visual reaction time". Some additional psychomotor tests actually used in one MC-experiment - like the Purdue Pegboard, the Minnesota Rate of Manipulation and dotting and dynamic steadiness - have not been described here, since they were not included in all our experiments and can't be used, therefore, for our present purpose of comparing behavioral effects of Methylene Chloride and CO.

Experimental Procedure

Five experiments were performed. In each experiment Ss were tested according to an entirely balanced repeated measurement design[18]: each S was tested single blind either in the morning or in the afternoon under all experimental conditions including control.

The interval between any two experimental sessions was 7 days. Figure 1 illustrates the temporal structure of the five experiments.

In *experiments I, II and III* (first line) behavioral effects of three concentrations of MC - 300, 500 and 800 ppm - on auditory vigilance and CFF were studied in 20 female volunteers. Four vigilance sessions of 45 minutes duration each were separated by CFF-determinations. In *experiment IV* behavioral effects of 800 ppm of MC were assessed by means of a comprehensive battery of psychomotor tests in 18 female volunteers. The vigilance task in this experiment served mainly to bridge the gap between onset of exposure and the beginning of psychomotor testing. *Experiment V* dealt with behavioral effects of 50 and 100 ppm of CO after 5 hours of exposure; all 18 Ss were non-smokers, 9 males and 9 females. The COHb-values given below refer to estimations according to the equation given by COBURN, et al.,[19].

All these experiments took place in the same environmental chamber measuring about 3 x 6 x 3 m high. MC (99.5% purity) was metered into the chamber after being vaporized and then mixed with clean air to the desired concentration. CO was metered into the chamber from a compressed gas cylinder. Atmospheric concentrations of both these agents were measured, as continuously as possible, MC by means of gas chromatography (FI-Detector), CO by means of infrared absorption. Air samples were drawn via

	TEMPORAL STRUCTURE OF EXPERIMENTS (SCHEMATIC)											
Experiments I II III CH_2Cl_2	CFF I	Instruction	VIGILANCE I	CFF II	VIGILANCE II	CFF III	VIGILANCE III	CFF IV	VIGILANCE IV	CFF V		
Experiment IV CH_2Cl_2	CFF I	Instruction	VIGILANCE I	CFF II	VIGILANCE II	CFF III	PSYCHO-MOTOR AND COGNITIVE TASKS			CFF IV		
Experiment V CO	CFF I	Instruction	PSYCHO-MOTOR TASKS I	CFF II	VIGILANCE I	CFF III	VIGILANCE II	CFF IV	VIGILANCE III	CFF V	PSYCHO-MOTOR TASKS II	CFF VI
COHb% 50 ppm	0,7			2,4		3,4		4,0		4,7		5,1
COHb% 100 ppm	0,7			4,2		6,2		7,6		9,0		10,0
MINUTES	0			77		127		177		227		277
	MINUTES AFTER ONSET OF EXPOSURE											

FIGURE 1. Temporal structure of Methylene Chloride (CH_2Cl_2) and Carbon Monoxide Experiments.

Teflon- or Polyethylene-tubes from about 20 cm above Ss' head. Temperature and humidity were found to be 22° Centigrade and 44% respectively, on the average, with no significant differences between conditions.

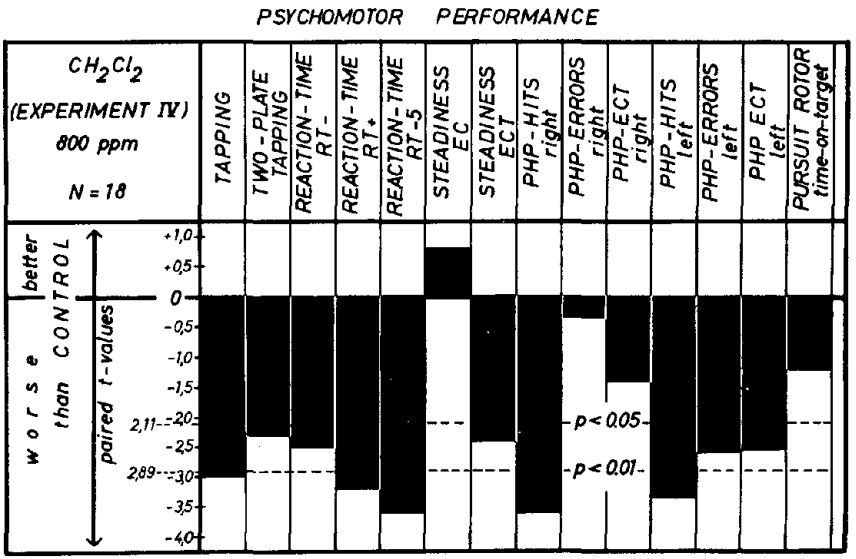

FIGURE 2. Change of psychomotor performance after about 4 hours of exposure to 800 ppm Methylene Chloride vapor (CH_2Cl_2). Impairment relative to control-values = downwards.

Psychomotor Performance

Psychomotor data will be given first (Figure 2). These columns represent improvement (upwards) or impairment (downwards) of psychomotor performance after 4 hours of exposure to 800 ppm MC as compared to performance under control-conditions. The ordinate represents paired t-values, i.e. the ratio of mean differences to within Ss' variance. Motor speed and speed of reaction are impaired under MC-influence as well as control precision, especially for left-hand performance.

Though, of course, we do not know yet, what COHb-values might result from 4 hours of exposure to 800 ppm MC it is interesting to note, that no such effects could be observed after 5 hours of exposure to 50 or 100 ppm of CO (Figure 3).

Comparing this figure to the last one illustrates clearly the differences in performance-depressing potency between MC and CO, especially with regard to the 100 ppm-condition (lower part of Figure 3. No clearcut deviation from zero can be seen - although COHb-values must have been around 10% on the average.

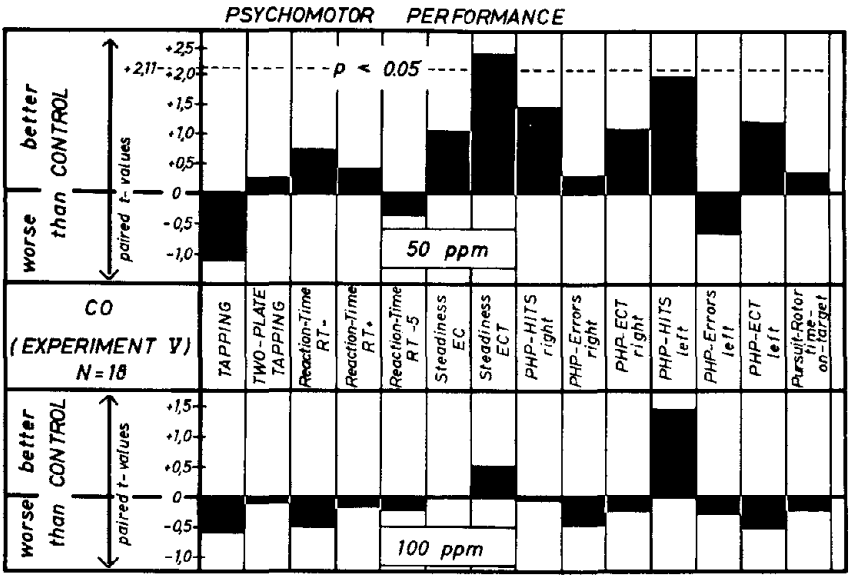

FIGURE 3. Change of psychomotor performance after about 5 hours of exposure to 50 ppm (upper part) and 100 ppm (lower part) of Carbon Monoxide (CO). Impairment relative to control-values = downwards.

Flicker Fusion Performance

The effects noted from psychomotor criteria become even more impressive for visual CFF-data (Figure 4).

The ordinate in this figure represents deviations from initial value - in flashes/sec - whereas the abscissa is duration of exposure. MC-data (left part of figure) exhibit relatively clearcut exposure-effects, whereas CO-data (right part) do not. Control data in this figure were collected from experiments I, II and III, 300 ppm-values from Experiments II and III, 500 ppm values from Experiments I and III and 800 ppm from Experiment II. Statistical treatment by analysis of variance was done separately for every experiment. As for statistical significance, it may be mentioned that CFF-depression due to MC was given even for 300 ppm at the 5% level. Values for 300 and 500 ppm are indistinguishable, whereas those for 800 ppm are markedly depressed. These data thus support the results of a former pilot study by WINNEKE et al.,[14], in which CFF-depression due to 3 hours of exposure to 500 ppm MC was found to be statistically significant, also. As for CO-data (right side), dose-response relationships are not apparent, nor are differences between conditions statistically significant.

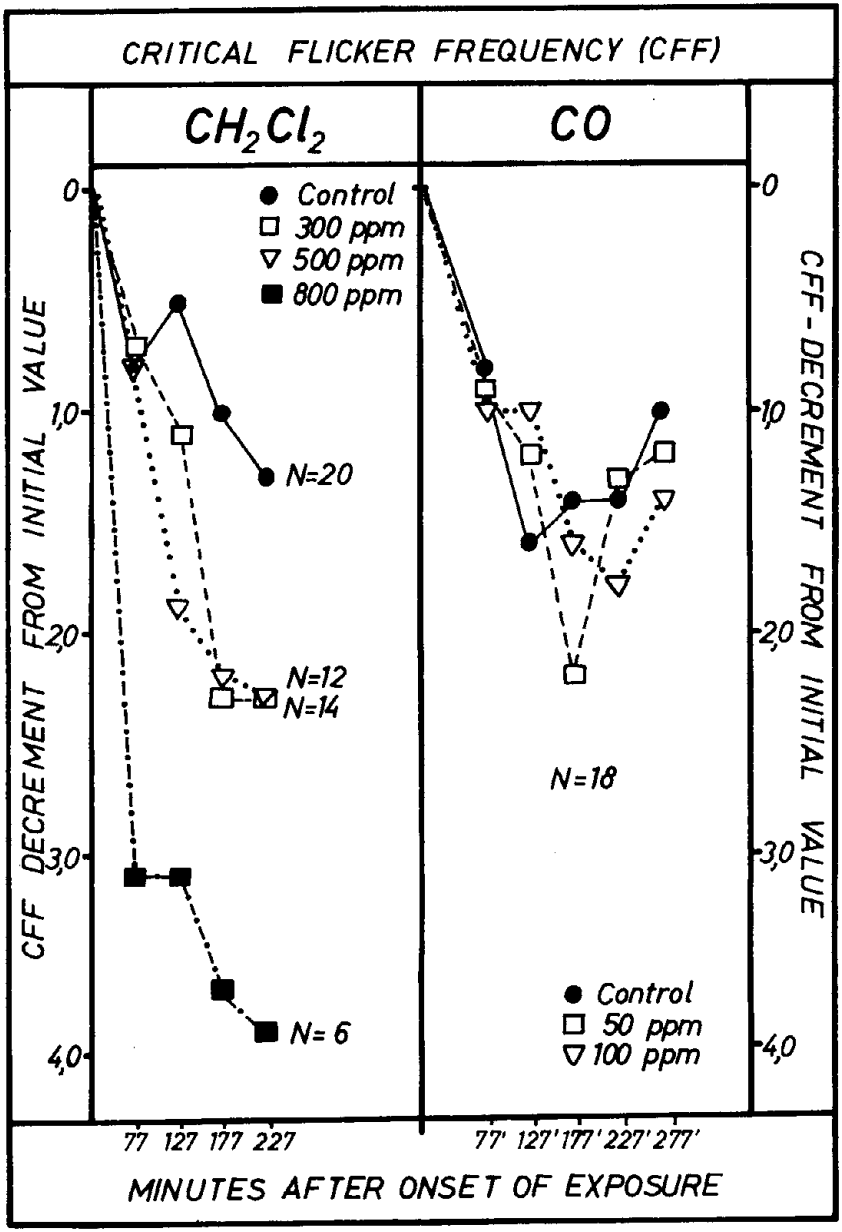

FIGURE 4. Decrement of critical flicker frequency (CFF) from initial value under Methylene Chloride (CH_2Cl_2; left part) and Carbon Monoxide (CO; right part).

Vigilance Performance

As for detection-efficiency in our auditory vigilance task these same tendencies became apparent. Figure 5 helps to illustrate the temporal structure of this task.

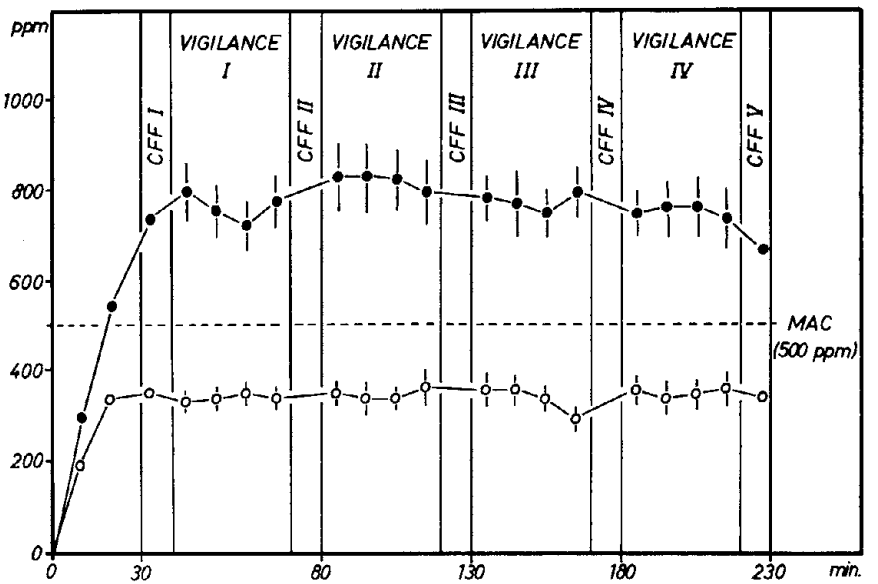

FIGURE 5. Temporal structure of the auditory vigilance task and Methylene Chloride concentrations for 300 ppm (lower curve) and 800 ppm (upper curve). Vertical bars represent standard errors of the respective means.

The vigilance task of 4 observation intervals of 45 minutes duration each was interrupted by CFF-determinations. Superimposed are the concentration-curves of 300 and 800 ppm of MC, with vertical bars representing the standard errors of the respective means.

Mean percentages of omission errors within 45 minutes observation-time for MC and CO-experiments are given in Figure 6.

This graph again illustrates what has been shown before: A significant impairment of performance under the influence of MC (left part), even at concentrations as low as 300 ppm, and no such performance decrement under CO (right part). It should be mentioned that COHb-values under the influence of 100 ppm of CO should have risen from about 4 to about 9% when performing the vigilance task. There is an obvious inversion of dose-response relationship between 300 and 500 ppm of MC, because one experiment (Experiment III) failed to show significant performance decrement for 500 ppm MC, whereas another one (Experiment I)

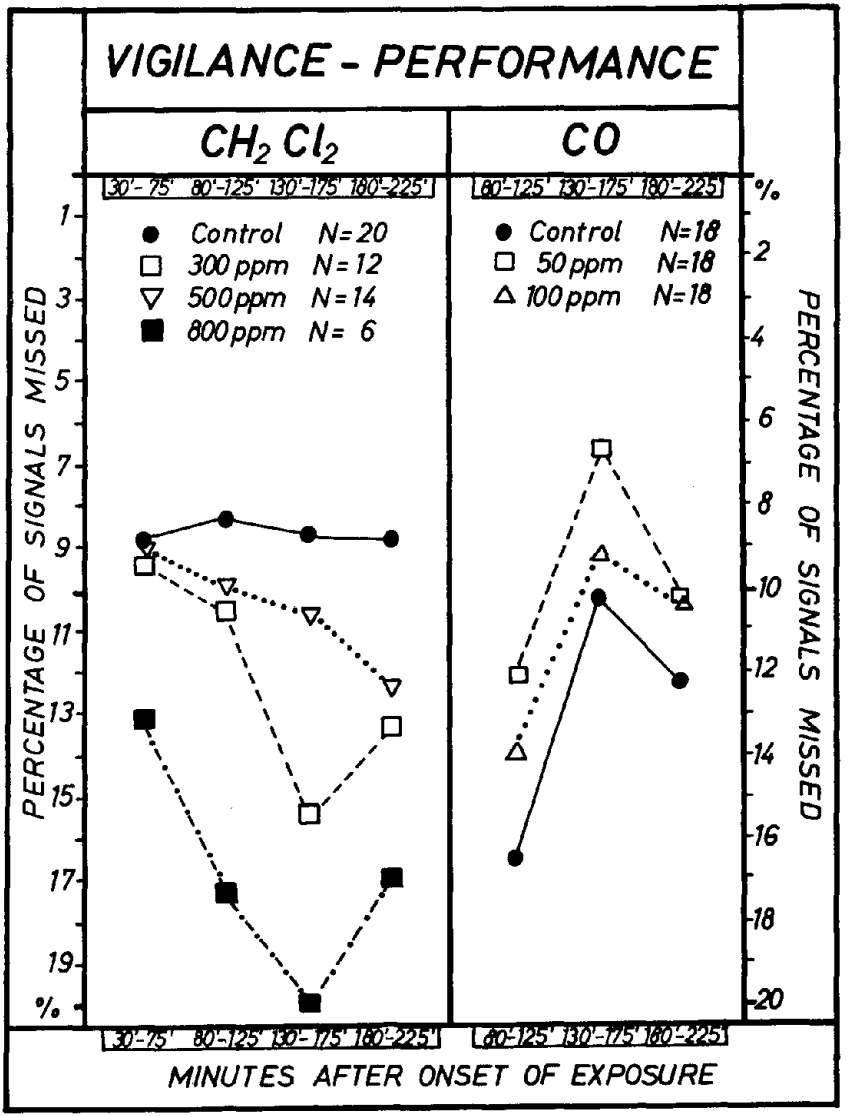

FIGURE 6. Vigilance performance (percentage of signals missed during 45 minutes of observation time) under Methylene Chloride (CH_2Cl_2; left) and Carbon Monoxide (CO; right).

did (see Table I). Thus, the pooled data for 500 ppm from both experiments are lower than those for the 300 ppm-condition.

Before discussing our findings with regard to biological and practical significance - especially concerning the present TLV of 500 ppm - I would like to summarize, what has been found in our MC-experiments from the point of view of statistical significance (Table I).

Table 1. SUMMARY OF SIGNIFICANCE-LEVELS FROM ANALYSIS OF VARIANCE-TESTS FOR MC-EXPERIMENTS (? means $p > 0.10$)

Experiment	Conditions	CFF	Vigilance	Motor
0 n = 12	0/500 ppm	$p < 0.01$	not tested	not tested
I n = 8	0/500 ppm	?	$p < 0.05$[1]	not tested
II n = 6	0/300/800 ppm	$p < 0.05$	$p < 0.05$[1]	not tested
III n = 6	0/300/500 ppm	$p < 0.06$	?	not tested
II + III n = 12	0/300 ppm	$p < 0.01$	$p < 0.05$[1]	not tested
IV n = 18	0/800 ppm	?	$p < 0.10$[1] $p < 0.05$[2]	$p < 0.05$ $p < 0.01$

[1]Omission errors (arc sine-values).
[2]Reaction times (M Sec.).

Two remarks first: The concentrations given are the *ideal* values - not those actually measured. Mean MC-concentrations, as measured by gas chromatography, as well as standard errors of these means, were as follows: 317 ppm (± 19), 470 ppm (± 27), 751 ppm (± 56). Experiment O was a pilot study on the effects of only 3 hours of exposure to 500 ppm in 12 Ss[14]; CFF-depression turned out to be the only significant result among a number of cognitive and perceptual tasks. This experiment has been mentioned above.

Insignificant results are symbolized by question marks in the table. Although some insignificant results did in fact occur - as can be seen - the bulk of evidence indicates, that low concentrations of MC do in fact impair CFF as well as psychomotor and vigilance performance. Of particular interest is the combination of Experiments II and III - showing detrimental effects of MC at an exposure level as low as 300 ppm. These changes are clearly not

directly related to COHb-level, since exposure to CO alone did not alter these behavioral measures to any significant degree.

DISCUSSION

Although the biological meaning of both CFF-depression and vigilance-decrement is by no means fully understood it may be taken for granted that both these measures can be regarded as indicating a lowered degree of CNS-activation. The evidence on this point comes from psychophysiology as well as from experimental psychology and psychopharmacology. It has been shown for example, that mental strain lowers the flicker threshold[20], that CNS-depressants (barbiturates) depress the flicker threshold[21] and at the same time impair vigilance performance, whereas CNS-stimulants - like e.g. amphetamine - both raise CFF and improve vigilance performance. From psychophysiological work with cortical evoked potentials (HAIDER et al.,)[22] (WILKINSON et al.,)[23], it is known, that high detection efficiency in vigilance tasks is correlated with high amplitudes of cortical evoked potentials and vice versa.

Taking into account the known narcotic properties of MC, it seems logical to describe the state induced by low concentrations of this agent as a state of reduced cortical arousal or - more precisely - as a pre- or sub-narcotic state. In such states Ss' intake of information from the environment is reduced and the probability of missing important signals is increased. Whether or not this is related to accidents still remains to be shown unequivocally. If, however, we add our finding that precision and speed of psychomotor performance are impaired at MC-levels in the neighborhood of the present TLV it seems justified to conclude, that the adequacy of this exposure limit should be seriously questioned. This conclusion rests on behavioral toxicity with signs and symptoms of CNS-depression alone. COHb-production, as demonstrated by STEWART et al.,[4] is another, independent case in point showing that the toxicity of MC probably has been underestimated for workmen, in general, and even more so for workers with cardiovascular impairment.

As for the obvious differences in the performance-inhibiting properties between CO and MC, the question may be raised, whether this might actually be due to the fact that CO is a nonsmelling gas, whereas MC-vapors can be detected at concentrations above 214 ppm according to LEONARDOS et al.,[24]. Ss might have thus been able to identify exposure conditions by odor in the MC-experiments but not under CO. Since we have asked our Ss about any odor-experiences by questionnaire, however, we can say that this was probably not the case (Table II).

Table II. NUMBER AND PERCENTAGE OF PERSONS IN MC-EXPERIMENTS REPORTING ODOR-EXPERIENCES UNDER CONTROL- AND EXPOSURE-CONDITIONS

(Significance means "significance of response-change")

	0 ppm	300 ppm	500 ppm	800 ppm
Number of Odor Responses (Exp. I, II, III, IV)	5/38	4/12	2/14	8/24
Percentage of Odor Responses (Exp. I, II, III, IV)	13%	33.3%	14%	33.3%
Significance of Response-change (relative to respective controls)	-	1.0[1]	0.2[1]	3.58[1]

[1]McNEMAR — x^2; $x^2_{0.025}$ (df = 1) = 3.84 (one-tailed).

Since odor-responses were not markedly elevated under conditions of exposure as compared to control-conditions, significance ($p<0.05$) only for 800 ppm, we may conclude that our MC-experiments were truly single-blind and that the differences in behavioral toxicity between CO and MC are more real than spurious. In explaining our MC-data, an interaction between endogenous CO and MC cannot be completely ruled out, however.

At our present state of knowledge no simple answer can be given to the question, why some authors have demonstrated significant vigilance decrement for low levels of CO-exposure, e.g. GROLL-KNAPP et al.,[25]; HORVATH et al.,[26] whereas our vigilance data did not exhibit any impairment whatsoever. Maybe, that even slight changes in methodology - e.g. signal characteristics, temporal structure - result in a change in functional significance of given tasks. More basic research seems necessary to clarify the biological significance of different behavioral vigilance tasks in relation to neurophysiological indicators of vigilance.

This is equally true for other psychomotor or sensory indicators, often used in behavioral toxicity studies. The present approach in this field is purely eclectic, which is almost inevitable and even fruitful at this early developmental stage of behavioral toxicology. In future studies however, close collaboration of neighboring scientific fields and different laboratories will be necessary, if a firm body of knowledge shall eventually be established, which will allow valid recommendations for standards of exposure based on behavioral toxicity criteria.

SUMMARY

Behavioral toxicity data for Methylene Chloride (CH_2Cl_2) and Carbon Monoxide (CO) have been collected in several independent human exposure studies. The following performance measures were used: Visual critical flicker frequency (CFF), auditory vigilance and psychomotor performance (reaction time, hand precision, steadiness, tapping and pursuit tracking).

Impairment of performance as a sign of CNS-depression was clearly shown after only 3 to 4 hours of exposure to Methylene Chloride vapors at concentrations as low as 300 ppm. No comparable effects could be demonstrated after 5 hours of exposure to 50 or 100 ppm of CO.

Two conclusions may be drawn from these results: (1) Endogenous CO-production, as demonstrated by STEWART et al., (1972) in man after exposure to Methylene Chloride vapors, cannot be considered responsible for the behavioral signs of CNS-depression observed; (2) The present TLV for Methylene Chloride of 500 ppm must be checked, since behavioral impairment obviously occurs at lower concentrations.

Such behavioral deficits, characterized mainly by vigilance decrement and CFF-depression, are typical for subnarcotic states and may be considered conducive to accidents.

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WORKER EXPOSURE TO PESTICIDES

SESSION III

Chairman and Keynote Speaker

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PESTICIDES SEMINAR KEYNOTE ADDRESS

Kingsley Kay, Ph.D.

I should first note that organic mercury and lead compounds, some of which are pesticidal, will be dealt with at the Metals seminar tomorrow. The pesticide seminar will cover organic pesticides recognized to have neurological effects - organophosphates, carbamates and chlorinated hydrocarbons. The experts of the panel may however choose to present new findings on other pesticidal chemicals found to have produced behavioral deficits.

I am sure many of you have wondered why this first seminar on the behavioral toxicology of pesticides is being held in 1973 though the chemicals concerned came into large scale use over 25 years ago. From the inception of their use it has been known that organophosphates, carbamates and chlorinated hydrocarbon pesticides were effective because they were neurotoxic. However, indications of their potential neurotoxicity to animals and man were in considerable measure devaluated under the pressure that was exerted by the agricultural and industrial sector to make these neurotoxic chemicals widely available for pest control.

As to the possibility of behavioral deficits resulting from their use, it was literally not conceived, even by health authorities in the early days, that the levels of human exposure in manufacture, formulation and application might produce such effects. The result was that early research on the organophosphates and carbamates emphasized alterations in the target enzymes, acetylcholinesterase and pseudocholinesterase. Early chlorinated hydrocarbon research featured such aspects of toxicity as storage of the chemicals in fat depots and effects on the liver, since the compounds were known to be liver poisons. Then, it was discovered in 1954 by Ball and associates[1] that chlorinated hydrocarbon pesticides increased the activity of esterases in vivo, esterases capable of metabolizing parathion. The increase in esterase activity was sufficient to reduce the toxicity of parathion in rats some tenfold. A few years later Frawley and associates[2] showed that EPN and malathion were potentiating by virtue of their competition for available esterases. Meanwhile almost no research was undertaken on the neurotoxicity or behavioral aspects although it was abundantly evident from accumulating clinical observations that these chemicals were strongly neurotoxic to man. Massive occupational overexposures occurred continually and as these highly toxic materials were permitted to be used in and around the home, poisoning cases of various degrees of severity presented at the

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clinical level with neurotoxic signs characteristic of the different chemical classes of pesticides.

It gradually became clear in the 1950-1960 period that lower than acute doses of chlorinated hydrocarbon pesticides had neurotoxicological significance. For instance convulsions were reported in rats fed for one week on diets with 100 ppm aldrin[3]. Hyperexcitability, sensitivity to noise, irritability and intense fighting instinct were observed among laboratory animals on low level chlorinated hydrocarbon dosing. In 1959, vector control personnel spraying dieldrin in the tropics showed typical signs of chlorinated hydrocarbon neurotoxicity[4], and Khairy[5] reported gait changes in rats on 100 ppm dietary DDT. By 1960, Narahashi and Haas had shown the effect of DDT on axonic action potentials[6].

In regard to organophosphates, Vandekar[7] showed in 1957 that some organophosphates had an anesthetic effect and in 1961, Russell et al[8]., produced behavioral changes when brain cholinesterase was depressed by 50 percent with systox (0,0-diethyl-s-ethylmercaptoethanol thiophosphate). The field of behavioral toxicology of pesticides developed from that time onward to an extent that has made it appropriate to organize this seminar.

Apart from advances in knowledge of behavioral toxicology of pesticides, attention of the Panel is again directed to the fact that Eastern European and USSR research workers have for some years been examining effects of low dosages of toxic materials on conditioned reflexes in animals. It has been reported that such effects may occur in the absence of observable somatic changes. As noted this morning, the findings from such studies became the basis for many official threshold limit values[9] in that part of the world, but in America, toxicity evaluation of pesticides has been based on evidence of pathological, physiological functional and biochemical changes. It may be that consideration of behavioral changes induced by pesticides would provide evaluations more rational in terms of health protection and it is hoped that some guidance in this regard may result from today's discussions.

In a recent Ciba Foundation study group covering chemical influences on behavior, Boyland[10] noted that some alkylating agents are also neurotoxic. This may be significant to the seminar deliberations since carcinogenicity has recently been established in laboratory animals for some chlorinated hydrocarbons-DDT[11, 12], αisomer of benzene hexachloride[13, 14] and polychlorinated biphenyls[15]. Some carbamate pesticides have also been incriminated[12]. Organophosphates have been shown to be alkylating agents in vitro[16-19] though apparently of the non-radiomimetic type since mutagenic and carcerogenic effects have never been reported. It has been postulated that their rate of metabolism by esterases in

the body is so fast that the cellular events from alkylation of nucleic acids (and proteins) to neoplasia cannot occur[20]. Chemosterilization is one of the new approaches to insect control. Thiotepa and the oxygen analogue tepa exemplify two chemosterilants of current interest which have been shown to be mutagenic though apparently metabolized rapidly[21]. The possibility that chemosterilizing agents may influence behavior will surely have to be considered before this new approach to insect control is widely adopted. Another potentially significant situation is that well over 100,000,000 gallons of creosote were used in the United States last year[22]. Since creosote contains polycyclic aromatic carcinogens and cocarcinogenic phenols the question arises as to possible neurotoxic effects.

Another area of concern for the seminar is the possibility of behavioral deficits from interaction of pesticides with drugs and chemicals absorbed or ingested at home, on the job and from the general environment. For instance, the chlorinated hydrocarbon pesticides have been found to reduce the half-life of antipyrine in exposed workers[23]. This class of pesticides has been shown to stimulate the metabolism of endogenous steroids, for example, hydroxylation of estradiol-17β[24]. The organophosphates, as they deplete esterase activity, can be expected to reduce metabolism of some exogenous and endogenous substances, introducing the possibility of intensification of action of chemicals with neurological potentialities. It will be necessary also to take into account that cold[25, 26], high protein diets[27, 28], coffee and tea[29], alcohol[30, 31] and countless other exogenous substances are enzyme inducers. Even impairment of dieldrin microsomal enzyme induction has been brought about by ascorbic acid deficiency[32]. In view of these disclosures it would seem possible that threshold levels for some substances may have to take into account associated factors that may very well be geography-related.

In the past few years various strictures have been placed on the use of pesticides, notably those of the chlorinated hydrocarbon class which are stored in body fat. It is important to consider whether these restrictive actions may have an impact on the behavioral toxicological outcome as the chlorinated hydrocarbon levels in depot fat and brain decline. Of course, chlorinated hydrocarbon pesticides will continue to be used in the tropics and these compounds are being manufactured and formulated for export in increasing amounts[22]. Workers so employed must therefore receive special health consideration. In the United States it is now the case that the non-persistent organophosphates and carbamates are replacing the persistent types and have to be employed more frequently because they are non-persistent. The inherent danger to

health of applicators in this trend has been recognized by EPA and USDA. A special safety educational program, Project Safeguard, has been initiated.

Various questions will have to be considered in light of this change in chemicals. For example, is there the possibility that brain acetylcholinesterase may be cumulatively depleted in some exposed workers without typical clinical signs as was found with certain strains of rats and mice[33]? Will genetic differences such as occur for pseudocholinesterase[34] prove significant in the behavioral aspect? Will the stimulation of the use of organophosphates and carbamates result in the introduction of new examples having delayed neurotoxicity[35, 36]?

Our Panel today is made up of distinguished experts in the various areas of the subject which have been touched on in these necessarily brief opening remarks. It is therefore appropriate that I should call upon our experts without further comments on my part.

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COLLABORATIVE STUDY OF NEUROBEHAVIORAL AND NEUROPHYSIOLOGICAL PARAMETERS IN RELATION TO OCCUPATIONAL EXPOSURE TO ORGANOPHOSPHATE PESTICIDES

David L. Mick, Ph.D.

The Pesticide Community Studies, presently located in 13 states of the U.S., have been looking into the relationships between human health and pesticide exposure during the last 5-6 years. Each project has compiled data concerning pesticide exposure in conjunction with the health status of individuals using pesticides in their occupations. Individuals with minimal exposure serve as controls.

METHODS

Each participant received a physical, electrocardiogram, chest x-ray, urinalysis, and a battery of blood biochemistry tests. The results of these tests have not isolated any health effect that can be ascribed to being caused by chronic pesticide exposure. However, some of the individuals in the exposed group have reported some health effects that *they* have attributed to pesticide exposure. Most of the symptoms — headache, upset stomach, nausea, irritability, anxiety, etc. — could not be scientifically established as resulting from pesticide exposure. But the possibility of a cause and effect relationship stimulated our present study of attempting to assess some of the neurological functions of a group primarily exposed to organophosphate (OP) insecticides. There are reports in the literature relating the effects of OP exposure on man and these will be discussed in the papers that follow.

Our study involved about 60 participants, ½ of whom are defined as exposed to OP insecticides and the other ½ have essentially no exposure and therefore serve as the control group. The groups are being matched for age, education, and sex.

The exposed group consists of farmers and CPA's who have been actively engaged in the application of OP insecticides for at least 2 consecutive weeks and the last usage must have been within 2 weeks of entering the testing program. The participants in the control group are farmers who are not actively engaged in using pesticides.

The products most often used by the exposed group include

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Dasanit, Diazinon, Dursban, Dyfonate and Thimet. Pesticides other than OP insecticides are often used by the exposed group; this fact will be considered when analyzing and interpreting the data.

RESULTS

Biochemical

Blood samples from each subject are analyzed for plasma and RBC cholinesterase at the time of administering the neurological tests. The RBC cholinesterase is considered a more specific indicator of OP insecticide exposure and the analyses to date showed no significant difference between the exposed and control groups. We are not unhappy with this finding because we are primarily interested in chronically exposed individuals who do not exhibit any observable or measurable clinical manifestations as a result of pesticide exposure.

Neurobehavioral and Neurophysiological

The neurobehavioral and neurophysiological parameters of this collaborative study are presented in the following papers: "Behavioral Effects of Occupational Exposure to Organophosphate Pesticides" (Dr. Levin); "Neurological and Behavioral Aspects of Occupational Exposure to Organophosphate Pesticides" (Dr. Rodnitsky and "Electrodiagnostic Study of Pesticide Toxicity" (Dr. Kimura).

BEHAVIORAL EFFECTS OF OCCUPATIONAL EXPOSURE TO ORGANOPHOSPHATE PESTICIDES

Harvey S. Levin, Ph.D.

Behavioral effects of exposure to organophosphate compounds have been investigated in both human[4] and animal[6] subjects. Acute organophosphate toxicity in man has been extensively described[9, 20, 21] as have symptoms subsequent to chronic occupational exposure[8, 12]. On the other hand, relatively few studies have considered behavioral effects of chronic organophosphate exposure in workers who have not sought medical treatment for their complaints[10, 19]. The purpose of this report is to describe preliminary findings with respect to the effect of chronic exposure to organophosphate pesticides on proprioceptive feedback performance, memory, language, anxiety, and depression in agricultural workers.

Motor deficits including tremor, muscular twitching, and muscular fasciculation have been found to result from acute organophosphate poisoning[21]. In their study of subclinical effects of organophosphate compounds, Metcalf & Holmes[19] reported that chronically exposed workers showed coordination deficits on neurological examination and were impaired in rotary pursuit performance.

In contrast to these findings for motor disturbance, sensory defects have been cited only infrequently. Two of the parathion sprayers studied by Arterberry and his associates[1] were found to have paresthesias. Percutaneous injection of an anti-cholinesterase was reported by Bowers, Goodman & Sim[4] to produce somatosensory disturbance in a normal volunteer. In view of evidence indicating that proprioceptive cues contribute significantly to motor skills such as tracking[13, 15], tapping[15], and pushing a button within a specified distance[16], assessment of somatosensory function in exposed workers suspected of motor deficit was undertaken. Implication of organophosphate poisoning in crop duster accidents[26, 30] prompted selection of a proprioceptive feedback task which incorporates features of manual control systems found in aircraft and agricultural machinery. Recent studies have demonstrated the sensitivity of the task to neurologic impairment[5, 17] and age related decline in performance[18].

Acute organophosphate poisoning may produce memory impairment[25] with similar findings in chronically exposed workers who develop toxicity[12]. Complaints of forgetfulness were elicited from

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53% of the exposed workers and 20% of the control subjects interviewed by Metcalf & Holmes[19]. Although the authors reported that the Benton Visual Retention Test and a story recall task confirmed their interview findings, the test data were not described in detail. On the basis of these studies, a test of memory was given under experimentally controlled conditions.

Expressive language defects including slurring, difficulty in repetition, and paraphasic disturbance have been reported to occur in patients with organophosphate toxicity[12, 25]. More subtle linguistic defects have not received attention from other investigators and these were considered in the present study.

Prominent among the psychiatric sequellae of organophosphate toxicity are anxiety and depression[8, 12]. Acute organophosphate poisoning has been shown to produce these symptoms[25]; they have also been produced in normal volunteers following administration of an organophosphate anticholinesterase[4]. In the latter study, severity of psychiatric disturbance was directly related to degree of whole-blood cholinesterase inhibition. Convergent with these findings, Rowntree and his colleagues[24] reported that administration of an organophosphate compound exacerbated depressive symptoms in patients with manic depressive illness. Central cholinergic "dominance" has been recently postulated[14] as an etiologic factor in depression. Given these lines of evidence for psychopharmacologic effect of cholinesterase inhibition, tests of anxiety and depression were included in the protocol. Although extent of regional use of organophosphate compounds does not appear to be related to incidence of major psychiatric disorders as reflected by hospital admissions[27], it was deemed important to examine the possibility of subclinical disturbance temporally related to occupational exposure.

METHOD

SUBJECTS

Workers were included in the exposed group if they had used an organophosphate compound for a period of at least two weeks and had last used the compound within two weeks of the testing date. Index measures of amount of individual exposure, e.g., acreage sprayed, will be reported at the completion of the study. The original design of the study restricted participation to farmers and other agricultural workers. However, adverse weather conditions which delayed planting and spraying necessitated the inclusion of commercial pesticide applicators. To date, 13 exposed male workers including 5 applicators have been tested. Mean age of the exposed group was 38.4 years (SD=8.8) while their mean educational level was 12.3 (SD=1.4).

Farmers evaluated prior to the beginning of the spraying season and farmers who hired other workers to do their spraying served as control subjects in the study. It was elected to risk confounding the exposure variable with time of testing and variables related to whether a farmer did his own spraying in order to control for occupational background. Undetermined practice effects on the experimental tasks combined with problems inherent in recalling participants for a second session militated against a within subject design. At the time of the Workshop, 16 control workers were tested (mean age=47.5 years, SD=8.1; mean educational level=10.4, SD=2.1). Although the plan of the study was to individually match exposed subjects and control subjects on age and education, this was not feasible at the time of this report.

APPARATUS AND PROCEDURE

Testing was conducted in the Neuropsychological Laboratory, Department of Neurology, University Hospitals, Iowa City. The research assistant who served as the experimeter was given only minimal information concerning the participants in order to approximate "blind" testing conditions. A number of control subjects were scheduled for testing during the spraying season for the same purpose.

Proprioception

The equipment used to measure approximation of the index finger within a specified distance to an electronic proximity detector (Distansense 3B.S.) has been described elsewhere[5, 17]. The pulse generated by the detector increased with increasing proximity of an approaching push button. A spring, which resisted forward travel of the push button, and facilitated its return to resting position, provided proprioceptive feedback. Range of finger movement was specified at 2 mm. Excursions of the push button in either direction beyond this range activated a tone and were recorded on a Rustrak event recorder (Model 292-4). The tone served to inform the subject that his finger was "out of range" while providing no information concerning the direction or extent of his error. Trial duration (30 sec) was controlled by a calibrated Hunter timer.

The subject was blindfolded and seated in front of the apparatus with the manipulandum conveniently located. He was told that pushing the button out of bounds would activate the tone and that his task was to keep the tone off. Each trial began only after the subject initially pushed the button within the specified range. Two trials were given for each of three spring intensities - 10g, 100g, and 300g. The order of presentation of the pressure intensities

was 10g, 100g, 300g, 300g, 100g, and 10g for all subjects. In view of the finding that the two hands do not differ in performance on this task[17], testing was restricted to the preferred hand.

Memory

Memory was assessed by a verbal recall task developed by Peterson & Peterson[22]. The tape recorded stimulus material consisted of a consonant trigram[29] presented 3 sec after a ready signal and immediately followed by a three digit number. The subject was told to immediately repeat the number and begin counting backward in cadence with a metronome. The purpose of this distracting procedure was to prevent auditory rehearsal of the letters. After an interval of varying duration (3,6,9,12,15 or 18 sec), the subject was told to stop counting and retrieve the trigram. The intertrial interval was 15 sec. Eight trials were given for each of the six delay intervals; each interval was represented within each block of six randomly ordered trials.

Language

Linguistic competence was evaluated by tape recorded administration of the Sentence Repetition subtest of the Multilingual Aphasia Examination[3]. This test required the subject to repeat sentences of progressively increasing length. The protocol of 14 sentences was presented at a rate of 130 words per minute and included examples of varied linguistic constructions. In an attempt to increase the sensitivity of the test to subclinical impairment of receptive language, an alternate form was presented to each subject under a condition of compressed speed (216 words per minute). Speech compression was achieved by a Lexicon Varispeech-I with preservation of pitch, intonation, etc. A similar procedure has been shown to reduce speech comprehension in normal subjects[11].

Anxiety and Depression

Anxiety was measured by the Taylor Manifest Anxiety Scale[28] which consists of 50 true-false items derived from the Minnesota Multiphasic Personality Inventory. Aspects of anxiety covered by items on the Scale include tension, inability to concentrate, fear, restlessness, insomnia, gastrointestinal complaints, increased sweating, and heart rate. Clinical research has generally supported the reliability and validity of this instrument[7].

Depressive symptoms were screened by the Beck Depression Inventory[2] which consists of 21 multiple choice items read by the subject and the examiner. Manifestations of depression covered by the Inventory include sadness, self-depreciation, guilt, suicidal ideation, social withdrawal, work retardation, insomnia, loss of

appetite, and loss of libido. Beck[2] has presented evidence to support the validity of his test.

RESULTS

Age and Education

Statistical analysis of the age and education variables indicated the exposed group to be significantly younger ($t=2.86$, $p < .01$) and better educated ($t=3.02$, $p < .01$) than the control workers.

Proprioception

Time out of range or error time was the dependent measure. A perfect performance on a trial corresponded to an error time of 0 ms whereas complete failure to maintain the push button within range corresponded to an error time of 30 sec. The average score of a subject for the 2 trials given at each intensity was used in the analysis of variance (ANOVA). One subject in each group could not be tested because of technical difficulties.

Mean error time as a function of feedback intensity is shown for each group in Figure 1. Standard deviations at 10g, 100g, and 300g were 4225 ms, 2343 ms, and 1318 ms in the control group and 3348 ms, 3622 ms, and 2005 ms in the exposed group. The impression gleaned from Figure 1 that increasing feedback intensity tended to reduce error time was confirmed by the test of the main effect for intensity ($F\ 2, 54=16.15$, $p < .001$). Contrasts between the error time at 10g and the average error time at 100g and 300g were performed to examine the effect of intensity separately for each group. This test was significant in both the control group ($t=3.88$, $p < .002$) and the exposed group ($t=3.09$, $p < .02$). The suggestion in Figure 1 of more accurate performance by the exposed subjects was not supported by the test of the main effect for groups ($F\ 1, 25=1.17$, $p > .05$). The apparent group X intensity interaction was also nonsignificant ($F\ 2, 54=.70$).

Memory

Number of trigrams correctly recalled at each delay interval was determined for each subject. These scores were subjected to ANOVA. The mean scores, expressed as a percent correct of the eight trigrams presented at each interval, are given in Figure 2. Percent of recall was inversely related to the duration of the delay interval ($F\ 5, 45=10.13$, $p < .001$). Although there was a directional trend for more accurate recall by the exposed group, it was not confirmed by the test of the main effect for groups ($F\ 1, 27=1.36$, $p > .05$). A test of the groups X delay interaction was also nonsignificant ($F\ 5, 135=.50$).

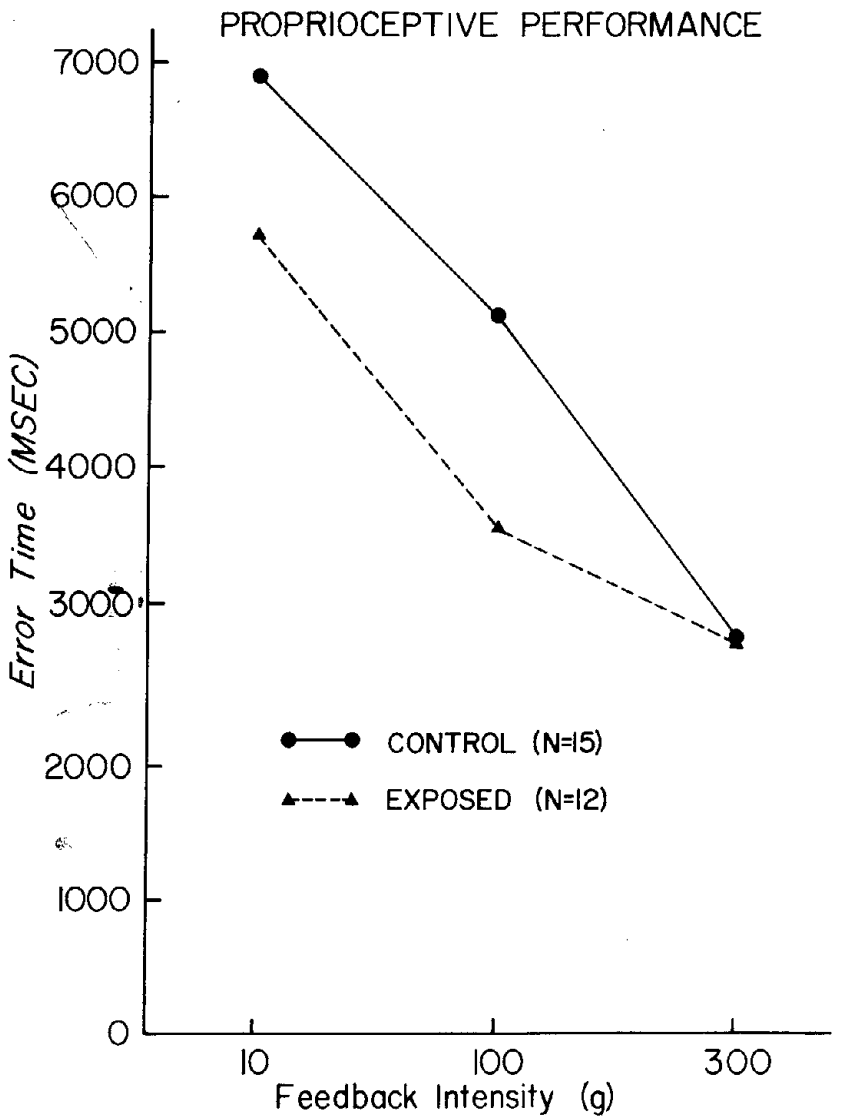

FIGURE 1. Mean error time as a function of feedback intensity for exposed and control groups.

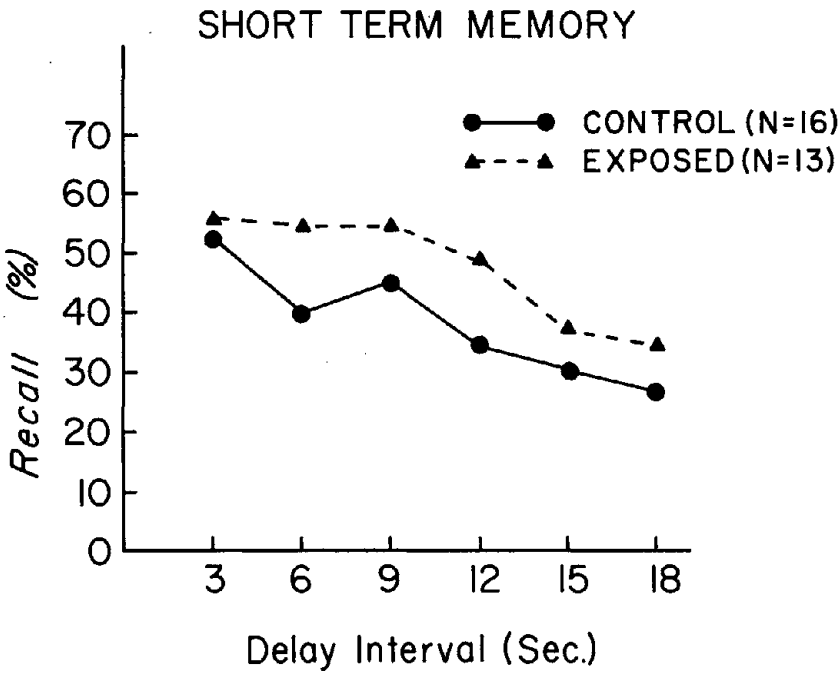

FIGURE 2. Percent of recall of trigrams as a function of duration of delay for exposed and control groups.

Language

Number of sentences correctly repeated served as the performance measure, a score of 14 indicating a perfect performance. This score was then corrected for age and educational level[3] and used in the statistical analysis. At the normal presentation rate sentence repetition by exposed subjects (mean=12.7, SD=1.4) was comparable to the control group (mean=12.5, SD=2.2). Performance of the two groups was also similar in the compressed speech condition with both the exposed subjects (mean=8.4, SD=2.3) and the control subjects (mean=8.4, SD=1.7) evidencing impaired repetition. ANOVA of the scores yielded a highly significant effect for presentation rate ($F\ 1, 29=134$, $p < .0001$). The groups did not differ in accuracy of repetition ($F\ 1, 27=.01$). Consideration of the impairment in sentence repetition produced by speech compression indicated a decline of 34% in the exposed group and a 33% decrement in the control group. The group X presentation interaction was nonsignificant ($F\ 1, 27=.11$).

Anxiety and Depression

Positive responses to the Taylor Scale were computer tallied

with a maximum score of 50 indicating an extremely high level of anxiety. Application of the t-test revealed greater anxiety in the exposed subjects (mean=12.9, SD=9.2) than the control group (mean=7.6, SD=5.1) according to a one-tail test ($t=1.89$, $p < .05$). Arrangement of the data into a histogram (Figure 3)

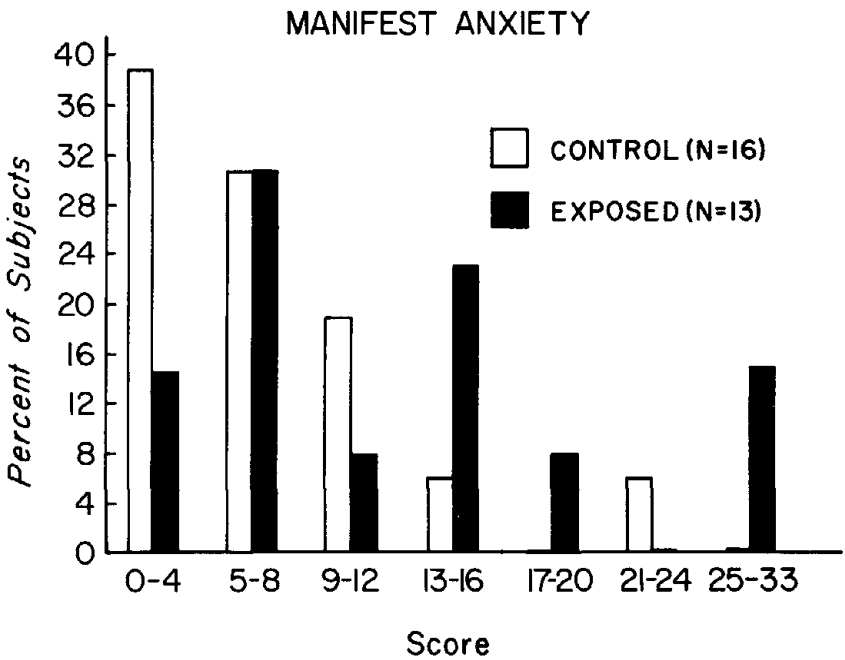

FIGURE 3. Distribution of Taylor Manifest Anxiety scores in exposed and control groups.

reveals that low anxiety scores tend to be more characteristic of control subjects whereas the scores of the exposed group are skewed in the pathological direction. Two exposed subjects (15%) and no control subject produced anxiety scores within the range found in psychiatric patients[7]. Of the exposed subjects with the two highest scores, one was a farmer while the other was a commercial pesticide applicator.

Protocols for the Beck Depression Inventory were scored for total points, a high score indicating marked depression. The mean depression score for the exposed group was 4.1 (SD=3.0) which did not significantly differ from the control group mean of 4.3 (SD=3.2) according to the t-test. Neither group contained subjects whose scores could be considered indicative of clinically significant depression.

DISCUSSION

Preliminary findings on the proprioceptive feedback task have failed to indicate any deficit in the exposed participants. The non-significant trend toward more precise regulation of the push button by exposed subjects is most parsimoniously explained by their younger age. A recent gerontological study of proprioceptive feedback disclosed a decrement in performance with increasing age[18] In any case, there was no suggestion of sensory defect as has been suggested by studies of organophosphate toxicity.[1, 4].

Memory impairment has been reported in chronically exposed workers who were not obviously ill at the time of testing[19]. Administration of the Peterson recall task under controlled conditions failed to reveal a significant difference between exposed and control groups. Consistent with previous work[22], accuracy of recall was inversely related to delay interval.

A similar picture emerged for the sentence repetition test; exposed workers did not significantly differ from control subjects. Speech compression markedly impaired performance, a finding consistent with a previous study[11]. Thus, speech defects which have been described in case study of organophosphate toxicity[12, 25], were not detected in this investigation.

Personality testing disclosed greater anxiety in the exposed subjects than in the control group with two of the exposed subjects scoring in the clinically significant range. This tentative finding is consistent with both case reports[8, 12, 23] and a study by Bower et al[4]., which showed increased anxiety in normal subjects following percutaneous injection of an anticholinesterase. However, concurrent assessment of depression in the present study failed to produce a significant group difference. Depression scores of both groups were within the limits of normal variation.

This ongoing investigation is progressing towards individual matching of control and exposed workers on variables of age and education. The findings reported here will be supplemented at the completion of the project.

SUMMARY

Workers exposed to organophosphate pesticides and control subjects were compared on tests of proprioceptive feedback, memory, linguistic competence, anxiety, and depression. The only significant group difference obtained for these measures was on the anxiety test, indicating greater anxiety in the exposed participants. These tentative results were interpreted with caution in view of the younger age and higher educational level of the exposed workers at the time of this report.

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NEUROLOGICAL AND BEHAVIORAL ASPECTS OF OCCUPATIONAL EXPOSURE TO ORGANOPHOSPHATE PESTICIDES

Robert L. Rodnitzky, M.D.

The problem of identifying the possible deleterious effects of exposure to pesticides can be broadly divided into two major categories. First, those effects which are immediately apparent to family members or co-workers on the basis of casual observation or to a physician on the basis of a careful physical examination — that is, the so-called "clinical" effects; and secondly, those which become manifest under laboratory conditions only and are of such minor functional significance as to escape notice in the course of an individual's daily activities or the so-called "subclinical" effects.

As previously mentioned, among agricultural workers exposed to organophosphate pesticides in the course of their daily activities, the incidence of apparent, that is *clinical*, toxic effects is rather low. On the other hand, it has been recently suggested that in at least one area, namely that of neuromuscular function and transmission, *subclinical* abnormalities can be found with great regularity in exposed subjects, notwithstanding the absence of any clinically apparent signs and in the face of normal cholinesterase levels in these same individuals[1].

This establishes the fact that in the *peripheral* nervous system the lowest end of the spectrum of toxic abnormalities is well below our threshold for recognition by standard clinical techniques. The question must then be raised whether this general principle holds true in the *central* nervous system as well. Does the individual who has been minimally exposed to organophosphate compounds and who is by standard neurological and psychological examination "normal" suffer from minor derangements of function which might only be brought to light by more sensitive functional assays than those ordinarily employed. Also, have the subclinical abnormalities of the peripheral nervous system, which are already known, been fully explored?

These are the questions to which we have addressed ourselves in our current study. We have, accordingly, compiled a battery of tests designed to enhance the sensitivity of commonly employed clinical measures of function such that previously undetected "subclinical" abnormalities might be uncovered. There are an infinite number of behavioral, psychomotor, neurological and neurophysi-

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ological parameters which might be logically included in such a study. From amongst this myriad of possibilities we have chosen to screen for those abnormalities which: a) appear rather consistently, in gross form, in acute intoxication; b) might be predicted on the basis of the known neurophysiologic functions of acetylcholine and acetylcholine esterase; c) might be related to occupational accidents such as tractor accidents on the farm or crop duster crashes.

Our study is a multi-disciplinary effort which has brought together expertise in the fields of clinical neurology, clinical neurophysiology, psychology, and toxicology.

Prior to discussing some of the individual components of our test battery, I should point out that our study is still in progress. Because most of our exposed subjects are farmers our testing schedule is at the mercy of the vicissitudes of weather and planting conditions and as a result of recent flooding in the midwest we have less exposed subjects at this point in time than we had anticipated. Suffice it to say that the results presented today are to be interpreted only as possible trends which may or may not be borne out once the study is completed.

SUBJECTS

The subjects used in this portion of the study consisted of pesticide exposed workers and controls as outlined in Dr. Mick's paper. At this stage, they are not yet age matched, a fact that may have an effect on some of the preliminary results reported today. The mean age of 12 exposed subjects was 38.4 years, and that of 16 control subjects was 47.5 years.

METHODS

Neurological Examination

Each subject undergoes a standard clinical neurological examination in an effort to detect evidence of overt neurologic dysfunction. It is not expected that overt toxic neurological abnormalities will be found in the exposed group since these subjects are, by history, minimally exposed, but the possibility of neurologic deficit being present as a result of a previous unrelated illness, in both the control and exposed groups must be noted. Any such neurological handicap, if present, might render subsequent neurological and behavioral tests inaccurate.

Structured Interview

Each subject is engaged in a structured interview of our own design. This consists of questions intended to uncover specific

subjective target symptoms which might be related to organophosphate exposure. As in the accurate determination of any medical history, open ended questions are used rather than place the subject in the position of choosing an answer in an either-or situation. The importance of this approach when inquiring about subjective complaints in exposed subjects has been recently emphasized[2]. Thus, when inquiring into the possibility of muscle cramps, the subject is not asked, "Have you had muscle cramps?" but rather, "Do your muscles bother you?" He must then spontaneously avow the specific target symptom being sought after. A much more accurate incidence of each target symptom is obtained in this manner.

The symptoms inquired about in this study include blurring of vision, wheezing, excessive salivation, abdominal cramps, nausea, diarrhea, muscular cramps, muscular weakness, irritability, fatigue, drowsiness, insomnia, anorexia, decline in memory, confusion, difficulty in concentration and headache. If the subject's response is judged positive for any of these target symptoms, further inquiries regarding the severity, quality and duration are made. Based on this information the severity of each target symptom is graded on a 1 to 4 scale. A "symptom severity score" is then compiled by totalling the points obtained in each of the target symptom categories.

Vigilance Task

This test is a simple reaction time in which there is random variation in the preparatory interval between 1.0, 2.8, 5.0, 7.5, and 10.5 seconds. The subject is seated comfortably in front of a display which consists of a single centrally placed light which is the test stimulus. On the table in front of him is a single push button which he is instructed to depress with the preferred index finger. At a randomly variable interval after depressing the push button the test stimulus is given and the subject must release the push button as soon after the onset of the test stimulus as possible. Each subject completes 12 trial blocks, each of which contain 5 trials representing one of each of the five possible preparatory intervals.

Difficulty in concentration is a common complaint among those acutely exposed to organophosphates and the successful execution of this task requires the constant attention of the subject since the test stimulus occurs at unpredictable intervals. Tasks of similar experimental design[3] have demonstrated deficient vigilance in other patient groups known to be unable to concentrate and persevere on a task.

Information Processing Time

This task is a complex reaction time in which the preparatory signal also provides the rule governing the subjects' response to the test signal. It is an adaption of a technique which has been felt to be a sensitive index of signal processing time[4]. The subject is seated comfortably before a display panel consisting of four lights, 2 of which are arranged in a vertical array and 2 of which are arranged in a horizontal array, i.e., there is a top, a bottom, a right and a left light. The subject depresses two push buttons, one with each index finger, and is instructed to release either the right or left sided push button in response to the appropriate test stimulus. There are 3 separate but similar experiments which comprise this part of the battery. In all 3 experiments, either the top or the bottom light serve as the ready signal and either the right or left sided light serves as the test stimulus.

Experiment (a)

The subject is told in advance that in each trial the top light will be the ready signal. It is followed in 100 milliseconds by either the right or left sided light. The subject is to respond in corresponding fashion, that is by releasing the right push button in response to the right sided light and the left sided push button in response to the left sided light. There are 18 trials consisting of 9 right and 9 left sided stimuli presented in random order.

Experiment (b)

The subject is told in advance that the bottom light will serve as the ready signal. Again, it is followed, in 100 milliseconds by either the right or left sided light. The subject is instructed to make a non-corresponding response, that is by releasing the right sided push button in response to the left light and the left sided button in response to the right light. Again, there are 18 trials consisting of 9 right and 9 left sided stimuli presented in random order.

Experiment (c)

The subject is told that in the remaining trials, either the top light or the bottom light is to serve as the ready signal. As in the first two parts of the experiment the ready signal is followed in 100 milliseconds by either the right or left sided light. In each trial in which the top light serves as the ready signal the subject is to make a corresponding response and in those trials in which the bottom light serves as the ready signal he is to execute a non-corresponding response. There are 4 possible combinations of stimuli and the subject is given 9 trial blocks, each contain all

four stimulus combinations randomly ordered. In each experiment the subject is instructed to move as soon as he can but to be particularly careful not to make any errors. Prior to each experiment 4 practice trials are given.

This technique has been previously shown[4] to be useful in separating information processing time from total execution time. In experiment (c), since the ready signal also provides the "rule" which will govern the response, the subject's reaction time is a reflection of the time required for such processing as long as the interval between the "rule" and the response is kept short (100 milliseconds), so that the rule is not likely to be fully processed prior to the time the test stimulus is given.

RESULTS

Neurological Examination

To date, none of the exposed or control subjects have been found to have any significant neurological deficit which was felt to be related to exposure or which might render subsequent tests inaccurate. A host of minor neurological deficits such as unilateral ptosis, ruptured biceps tendon and mild carpal tunnel syndrome were noted. Minimal clinical and EMG evidence of carpal tunnel syndrome was particularly frequent and felt to be a common sequela of occupational trauma among exposed and control farmers.

Structured Interview

A symptom severity score was compiled for each subject. The subjects were divided into three groups; control, exposed farmers and exposed commercial pesticide applicators. The results are shown in Table I. It can be seen that there is a preponderance of higher symptom severity scores among the two exposed groups. Among the 12 exposed subjects, 3, including 2 of the commercial applicators obtained scores exceeding the highest score obtained by any of the controls. The number of subjects is not sufficient at this time for adequate statistical analysis but the tabulation of scores appears to indicate a trend toward higher scores in the exposed groups, particularly the commercial applicators.

Vigilance Task

The results of this task are shown in Figure 1. At each of the five preparatory intervals the difference in reaction time between the control and exposed groups was not significant. While not significantly different the exposed group had slightly faster reaction times at each preparatory interval.

Table I. SYMPTOM SEVERITY SCORES DERIVED FROM A STRUCTURED INTERVIEW

Controls (n=16)	Exposed farmers (n=7)	Applicators (n=5)
Points	Points	Points
7	9	11
5	1	8
4	1	3
3	0	2
2	0	0
2	0	
1	0	
1		
1		
1		
0		
0		
0		
0		
0		
0		

Information Processing Time

The results of experiment (c) are shown only (Figure 2). The first two experiments serve to acclimate the subject to the test design and further establish the fact that the subject has no independent difficulty in responding under the short (100 millisecond) preparatory interval condition. Experiments (a) and (b) have thus far served both these purposes as expected. On the complex task, no subject has made more than 3 errors in 36 trials. Incorrect responses were not included in the tabulation of mean reaction time. In Figure 2 it can be seen that under both corresponding and non-corresponding conditions the control group had paradoxically slowed reaction times ($p < .05$) than the exposed groups. As expected, each group displayed slower reaction time under the non-corresponding condition. In interpreting these results it is noteworthy that the two groups are not yet age matched, the mean age of the control group being 9 years greater than that of the exposed group.

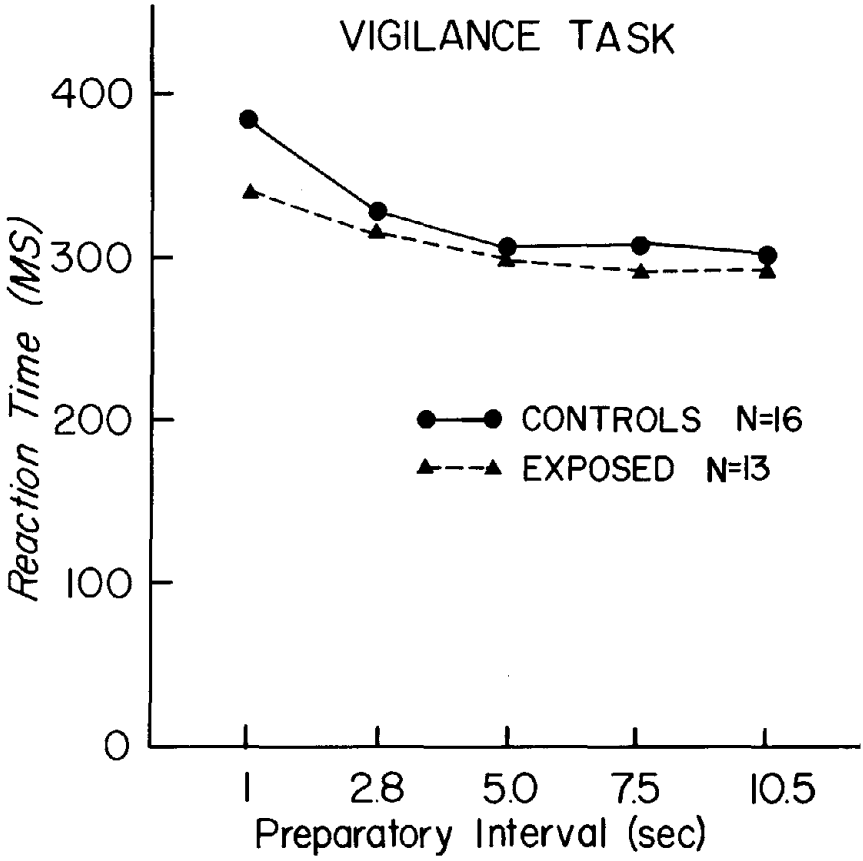

FIGURE 1. Comparison of performance of exposed and control subjects in a simple reaction time task utilizing variable preparatory intervals.

DISCUSSION

While the current results are preliminary and require corroboration by greater numbers, some interesting observations may be made. The absence of findings on the clinical neurological examination is not surprising in a group that has suffered only minimal exposure. This finding could be predicted by the fact that it is rare for such occupationally exposed subjects to seek medical attention except subsequent to overt accidental exposure. Subjective complaints however are not uncommon and most farmers and applicators apparently simply accept these minor symptoms as their lot in life. The preliminary results of our structured interview indicate that the high incidence of these nondisabling symptoms can be accurately uncovered if meticulously and objectively sought after.

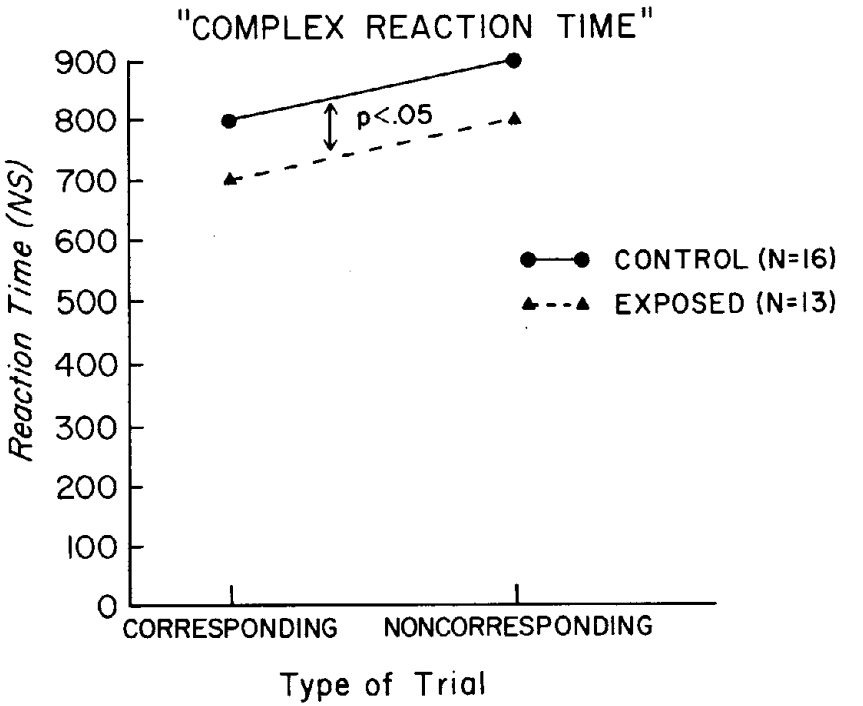

FIGURE 2. Comparison of performance of exposed and control subjects in a complex reaction time task intended as a reflection of information processing time.

While acutely exposed subjects frequently complain of difficulty in concentration[5], we have not been able to document such difficulty in our examiner paced vigilance task. Previous attempts[6] at documenting this deficit utilizing a subject-paced vigilance task have not been successful.

The complex reaction time task included in our battery reveals a paradoxically better performance by the exposed group. The most logical explanation for this finding is the age difference of 9 years between the groups, since this task is known to be highly age sensitive[4]. A less viable, but interesting, explanation is that the minimally exposed subject is actually at a pharmacologic advantage when compared to non-exposed subjects. This concept is not unknown. In rats[7], it has been shown that when organophosphate ingestion is used to produce only a very minimal reduction in brain acetylcholine esterase levels, behavioral efficiency actually can be increased above that of control animals. If further, more substantial lowering of brain acetylcholine esterase is produced, however, efficiency declines once the enzyme level falls below a certain critical

level. In the face of a more likely explanation for the superior performance of our exposed subjects to date, this latter hypothesis remains conjectural and rather unlikely.

SUMMARY

Clinical neurological signs, the incidence of specific target symptoms, vigilance and information processing time have been assessed in subjects who were occupationally exposed to organophosphate insecticides. Preliminary results have not suggested the presence of clinical neurological deficit in these exposed subjects, nor has there been an indication of impaired vigilance when compared to control subjects. A slight tendency toward a greater number of subjective symptoms was noted in the exposed group. An assay of information processing time revealed a significant group difference, the exposed subjects performing more efficiently than controls. Corroboration of these results awaits the accumulation of greater numbers of subjects and appropriate age matching.

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ELECTRODIAGNOSTIC STUDY OF PESTICIDE TOXICITY

Jun Kimura, M.D.

Acute poisoning due to organophosphorus compounds has followed their use as insecticides. Most symptoms are probably attributable to an excess of acetylcholine, which is the transmitter substance at the neuromuscular junction. Neurological symptoms may be varied but a disorder of motor function is one of the chief clinical manifestations of severe toxicity. This is at least in part because a variety of pesticides containing organophosphorus compounds interfere with normal neuromuscular transmission. Since electromyography is known to provide a sensitive measure of neuromuscular function in various neurological diseases, such as myasthenia gravis, it is hoped that this technique may also be useful in detecting subtle changes of pesticide toxicity which may be present in the absence of clinical symptoms. Although electromyography is now routinely used in most medical centers, few studies[2, 3] have dealt specifically with its use as a diagnostic aid for early detection of pesticide toxicity.

The purpose of this paper is to describe electromyographic techniques used at the University of Iowa in our study of pesticide exposed subjects. We also wish to report some of our preliminary observations, although our study is not yet completed.

MATERIALS AND METHODS

Selection of pesticide exposed subjects and normal controls was discussed in an earlier paper by Dr. Mick. Thus far 21 pesticide exposed subjects and 21 age matched controls were studied. The mean age of the exposed group was 38 (age range 23 to 52) and that of the control was 43 (age range 22 to 57).

All the electromyographic tests were carried out in a warm room with the patient lying on a bed equipped with mechanical devices to hold the arm and hand in a reasonably fixed position. The following tests were carried out in each exposed and control subject:

(1) Motor Nerve Conduction Velocity

The median nerve was stimulated by supramaximal electric shock at the wrist and elbow using surface electrodes and muscle action potentials were recorded with a surface electrode placed over the thenar muscles and a reference electrode located on the

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dorsum of the thumb. Similarly, muscle action potentials were elicited from the abductor digiti minimi after supramaximal stimulation of the ulnar nerve at the wrist and elbow. The evoked potentials were displayed on a dual trace oscilloscope after conventional amplification using a two channel amplifier. The latencies were measured from the stimulus artifact to the beginning of the initial deflection of the evoked potential. Distances between the stimulating points at the wrist and elbow were measured along the course of the nerves and motor nerve conduction velocities were calculated in the conventional manner.

(2) Antidromic Sensory Conduction Velocity

The median and ulnar nerves were electrically stimulated in the same manner as described above for motor nerve conduction velocity. The antidromic potentials were recorded bipolarly with surface electrodes from the index finger for the median sensory nerve conduction and from the small finger for the ulnar nerve. The latencies were measured from the stimulus artifact to the peak of the evoked potential. Distances between the recording site and the stimulating point at the wrist and between the two stimulating points at the wrist and elbow were measured and divided by appropriate latencies to give antidromic sensory nerve conduction velocities.

(3) Measurement of Neuromuscular Excitability

Percutaneous shocks were delivered in pairs to the median nerve at the wrist with interstimulus intervals between the conditioning and test stimuli systematically varying from 1 to 1000 msec (Figs. 1, 2). Pairs of stimuli were separated by a time interval of 30 sec. or longer to minimize interaction between successive trials. Conditioning and test stimuli were of equal intensity and were adjusted individually to give supramaximal responses.

Summated action potentials in response to paired stimuli were recorded from the thenar muscles using surface electrodes, amplified by conventional means and fed into a magnetic tape recorder for later display on a storage oscilloscope. The positions of the recording electrodes were carefully selected, one over the belly of the thenar muscles and the other on the dorsum of the thumb so that the initial negative peak of the recorded muscle action potential was maximal in amplitude. The hand and the arm were held firmly to a board to minimize movement artifacts. Care was taken not to interfere with circulation by strapping the hand too tightly.

In addition to the amplitude of the conditioning (1st) and

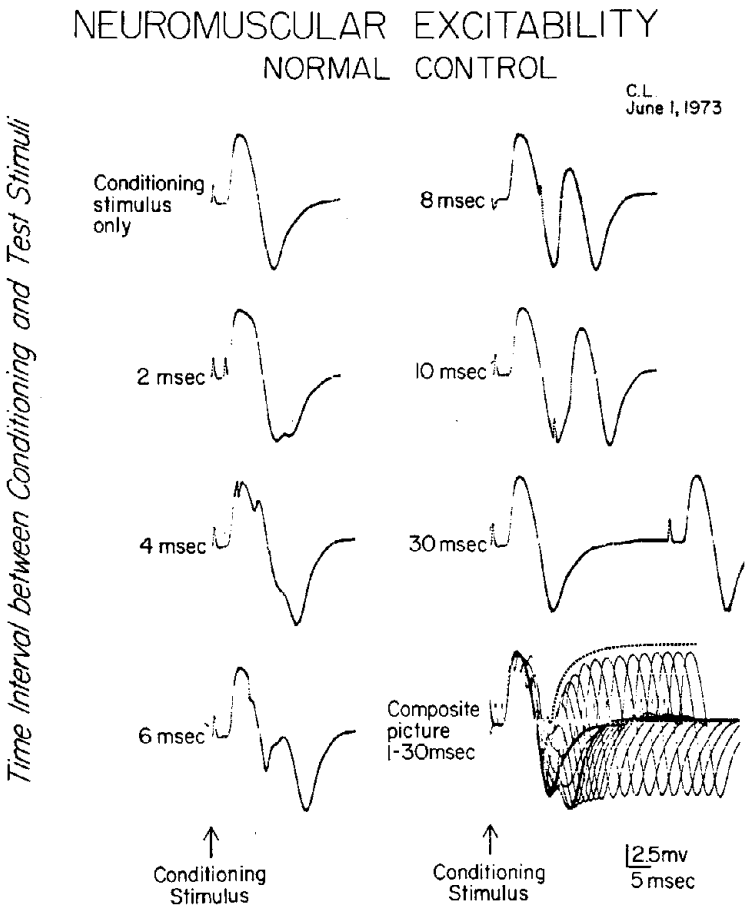

FIGURE 1. **Normal Control.** Except for the top tracing on the left and the bottom tracing on the right, paired shocks were delivered to the median nerve at the wrist with time intervals ranging from 2 to 30 msec between conditioning (arrow) and test stimuli. The action potentials were recorded from the thenar muscles. The top tracing on the left was obtained by a single stimulus. The bottom tracing on the right was obtained by superimposing the responses obtained by 20 paired shocks with interstimulus intervals ranging systematically from 1 to 30 msec. Note the response to the first shock of each pair appeared in the same spot on the photo and superimposed exactly on top of the other first responses. The responses to the second shock of each pair were displaced successively according to the interstimulus interval of each pair. An imaginary line connecting the peaks of successive second responses represents the time course of neuromuscular excitability change following the conditioning stimulus.

test (2nd) responses, the area covered by each summated potential of the pair was obtained by means of a full wave rectifier and an electronic integrator and displayed on two digital panel meters. To establish recovery curves of neuromuscular excita-

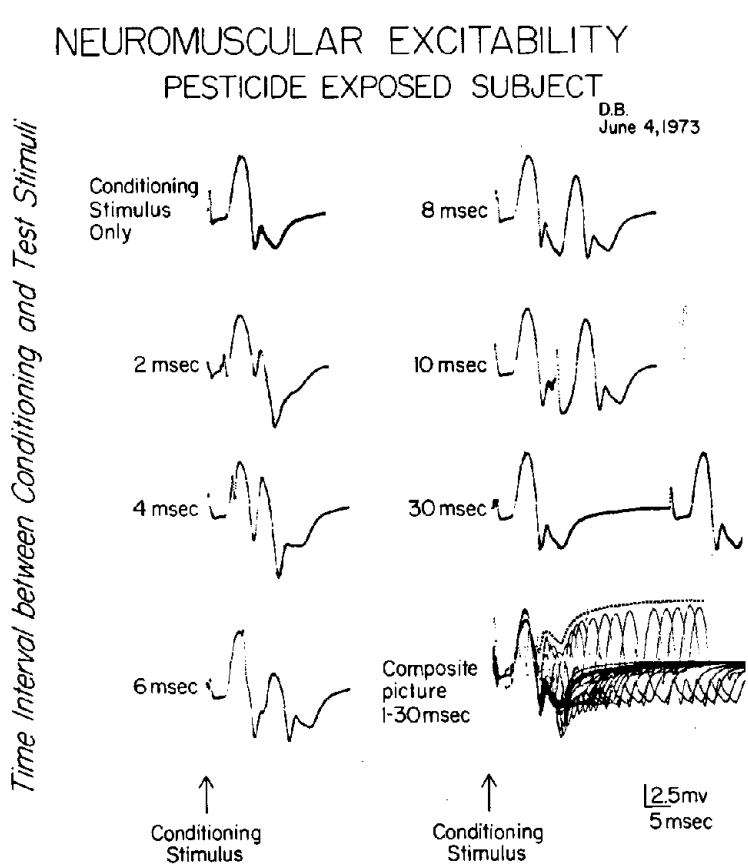

FIGURE 2. **Pesticide-exposed subject.** The arrangement of the tracings is the same as in Figure 1. Note that the muscle action potential evoked by a single stimulus in the top tracing on the left was double peaked with a small second negative phase not seen in the control shown in Figure 1. The recovery curve of neuromuscular excitability shown in the bottom tracing on the right also showed a small initial hump not seen in the control. The time course of this relative potentiation coincides exactly with that of the second negative peak of the conditioning response and probably results from temporal summation between conditioning and test responses.

bility, the size of the test (2nd) response thus determined was converted into a percentage of the size of the corresponding conditioning (1st) response. To visualize the time course of the neuromuscular excitability change following a conditioning stimulus, we have found it helpful to superimpose a number of paired responses obtained by paired shocks of various interstimulus intervals. When this is done, as shown in Figures 1 and 2, the response to the conditioning (1st) shock of each pair appears in the same spot on the photo and superimposes

exactly on top of the other first responses. The responses to the test (2nd) shock of each pair are, on the other hand, displaced successively according to the interstimulus interval of each pair. An imaginary line connecting the peaks of successive test (2nd) responses represents the time course of neuromuscular excitability following the conditioning (1st) stimulus.

RESULTS

On the basis of the medical history and neurological examination, the carpal tunnel syndrome was suspected in 2 of 21 pesticide exposed subjects and 4 of 21 controls included in this study. The results of the electrodiagnostic tests in these subjects were compatible with the diagnosis and consisted of an increased terminal latency of the median motor nerve conduction and a slowing of the median sensory nerve conduction velocity across the wrist. In another pesticide exposed subject, there was electrophysiological evidence of a mild polyneuropathy involving the motor and sensory nerve conduction of both the median and ulnar nerves. He was subjectively asymptomatic and neurological examination was essentially negative. No specific etiology of his polyneuropathy could so far be determined.

The motor and sensory nerve conduction velocities for the remainder of the exposed subjects were within the limits which are regarded as being normal in our laboratory. The mean values and standard deviations for the pesticide exposed subjects are presented in Table I and compared to the control values. Statistical analysis (t-test) revealed no significant difference ($P > 0.05$) between the pesticide exposed subjects and the controls in regard to the latency or velocity of the motor and sensory nerve conduction.

The action potential of the thenar muscles in response to median nerve stimulation averaged 9.3mv (range:4.0 to 19.0mv) in amplitude for the pesticide exposed subjects. The corresponding value for the control subjects was 10.4mv (range:4.6 to 17.5mv). The difference between the two groups was not statistically significant ($P > 0.05$).

In 11 of 21 pesticide exposed subjects and in 17 of 21 controls, the configuration of the summated action potential of the thenar muscles after median nerve stimulation was a smooth diphasic wave, which consisted of an initial negative phase followed by a less prominent positive phase (Fig. 1). In the remaining 10 exposed and 4 control subjects, however, there was a second negative phase which was much smaller than the initial negative phase. In these subjects, the second negative phase was constant and did

Table I. CONDUCTION VELOCITY AND TERMINAL LATENCY (Mean ± S. D.)

	Pesticide-exposed Subjects	Control
Median motor		
Elbow to wrist (M/S)	55.6 ± 3.6	59.0 ± 6.4
Terminal latency (msec)	3.7 ± 0.5	4.0 ± 0.9
Median sensory		
Elbow to wrist (M/S)	58.2 ± 8.1	58.6 ± 6.9
Wrist to finger (M/S)	46.3 ± 3.7	39.2 ± 6.9
Terminal latency (msec)	3.3 ± 1.3	4.2 ± 0.8
Ulnar motor		
Elbow to wrist (M/S)	62.3 ± 6.0	64.1 ± 9.4
Terminal latency (msec)	2.8 ± 6.4	3.0 ± 5.9
Ulnar sensory		
Elbow to wrist (M/S)	65.0 ± 6.1	62.6 ± 8.7
Wrist to finger (M/S)	42.2 ± 3.3	39.3 ± 5.1
Terminal latency (msec)	3.4 ± 0.3	3.6 ± 0.6

not seem to vary in amplitude or shape with repeated trials (Fig. 2).

The results obtained by the conditioning and testing technique have not yet been sufficiently analyzed to be reported quantitatively at this time. It may be stated, however, that the time course of the recovery curve of neuromuscular excitability was very predictable in normals and varied little from one individual to another. In a majority of control subjects, the test response was absent or very small at time intervals of 1 to 5 msec between shocks. It then recovered gradually reaching its original excitability at about 30 msec (Fig. 1). In pesticide exposed subjects so far studied the time course of recovery did not seem to deviate detectably from the control curve.

In those subjects who had double peaked muscle action potential in response to single stimulation, however, the recovery curve of the test response was characterized by a small initial hump at time intervals of 4 to 7 msec between shocks. The period of this relative potentiation of the test response coincided exactly with the second negative peak of the conditioning response (Fig. 2).

DISCUSSION

Studying patients with myasthenia gravis, Roberts and Wilson[2] described three features of abnormal electromyographic patterns

associated with overtreatment with carbamates, neostigmine or pyridostigmin. These were: (1) low amplitude potentials, (2) repetitive activity and (3) depression after voluntary activity. According to these authors, these patterns were observed before there was any clinical evidence of overtreatment. More recently, Jager, Roberts and Wilson[3] extended their investigation to examine workers exposed to high concentration of organophosphorus and organochlorine pesticides. They reported that approximately one-half of those exposed to organophosphorus and organochlorine compounds in a formulating plant showed electromyographic responses similar to those myasthenic patients overtreated with anticholinesterase. These features were not observed in any of their control subjects. Thus they concluded that electromyography provided a sensitive and objective method of nerve and muscle function in pesticide workers who were apparently in good health.

Our preliminary observations with pesticide exposed subjects using similar electromyographic techniques so far have revealed no significant alteration in motor or sensory nerve conduction or in amplitude of the evoked muscle action potential. In agreement with the original observation by Jager et al[3]., double peaked configuration of the evoked potential was seen more commonly in pesticide exposed subjects when compared to controls. It is to be noted, however, that a few controls also showed the same pattern of muscle evoked potentials in our series. Whether this is a relatively specific finding seen in association with pesticide exposure, therefore, remains to be seen. Jager et al[3]., postulated that this change in configuration of evoked potentials was due to repetitive muscle discharges but the exact underlying pathophysiology is not known.

In our series the amplitude of the evoked potential varied from one subject to the next over a much wider range than reported by the previous authors[3] in both exposed and control subjects. This may be in part because, in our study, the evoked potential was recorded from a group of thenar muscles innervated by the median nerve. This is in contrast to recording from the adductor pollicis muscle after stimulation of the ulnar nerve. Nevertheless, we are impressed with the spectrum of the amplitude variability in both exposed and control subjects and believe that the amplitude criterion is not very useful when individual cases are being examined for possible pesticide toxicity.

Our preliminary results thus seem to indicate that ordinary electromyographic techniques are in general of limited value in evaluating minor pesticide toxicity. It is our opinion that a further search for more sensitive diagnostic methods is needed in order to detect subclinical abnormalities that may be present in the absence

of overt symptomatology. If diligently sought after, utilizing sensitive techniques, minor changes in the neurophysiological spheres may be present in what has previously been considered insignificant pesticide exposure.

SUMMARY

Several lines of electromyographic technique were applied to pesticide exposed subjects to study early signs of pesticide toxicity. The preliminary results indicated that motor and sensory nerve conduction velocity, and the amplitude of the evoked muscle action potentials were within the limits of normal range in exposed subjects. In 10 of 21 exposed subjects, the summated action potential of the thenar muscles after median nerve stimulation consisted of a prominent initial negative phase and a smaller second negative phase. This finding, however, may not be very specific to pesticide toxicity as the same pattern was found in 4 of 21 control subjects. It is our opinion that ordinary electromyographic techniques are of limited value in detecting early signs of pesticide toxicity. A further vigorous search for more sensitive neurophysiological methods is needed in order to evaluate subclinical abnormalities due to minor pesticide exposure.

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ANTICHOLINESTERASE PESTICIDES OF ORGANOPHOSPHORUS TYPE: ELECTROMYOGRAPHIC, NEUROLOGICAL AND PSYCHOLOGICAL STUDIES IN OCCUPATIONALLY EXPOSED WORKERS

A. Jusic, M.D.

Most organophosphorus insecticides exert a generalized cholinergic action by inhibiting central and peripheral cholinesterases. In order to find a physiological monitor of early intoxication in occupationally exposed workers we started systematic electromyographic, neurologic and psychologic investigations.

METHODS

Subjects

Two main groups were selected, a group of 35 seasonal workers working in pesticide application and a group of 10 factory workers employed in pesticide production.

The first group consisted of truck-drivers, workers handling bags containing pesticides for use in aircraft in crop dusting or workers who had sprayed trees with pesticides during the last year. All examined workers were in good health, aged between 18 and 50 years, and were selected strictly according to the Law on Safety at Work. Alcoholics were excluded. The first examination was performed before the workers were exposed to pesticides.

The second group was composed of full-time factory workers engaged in the production of pesticides. Two of them were diabetics and two were alcoholics. They worked with different pesticides for the same period of time many months before the first testing. This group although small and randomly selected was used for comparison because of their past chronic occupational exposure to pesticides and would be helpful in evaluating the phenomenon of tolerance to pesticides.

Both groups were reexamined a month and two months after the first examination.

Electromyographic

A critical need in electromyographic testing was the development of a technique that would permit maximal constancy of

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recorded muscle signals in spite of voluntary contraction movements. The method would be as painless as possible and at the same time give considerable information.

For detecting muscle action potentials surface electrodes were used. Needle electrodes were used for nerve stimulation (Figure 1). We preferred needle electrodes to surface ones because with them supramaximal stimulation intensities were obtained with lower voltage. Stimulation and movement artefacts were also minimized. The analysis was always done on two nerves and two muscles: on the median nerve and opponens pollicis muscle, on the ulnar nerve and abductor digiti minimi. A well known finding with myasthenic patients is that negative results from only one muscle have a much more limited value than negative results obtained from several muscles. The myasthenic features, as well as other neuromuscular insufficiencies may not necessarily be present in all muscles. The detection in adductor pollicis region described by Jager, Roberts and Wilson[1] was missed because of the interference of innervation zones and muscle functions and because of the greater mobility of the recording electrode at this location. The position of the median detection electrode was checked by thumb opposition. The ulnar recording place was the point of the ulnar antithenar arch, the test movement the abduction of the fifth finger. The voluntary move-

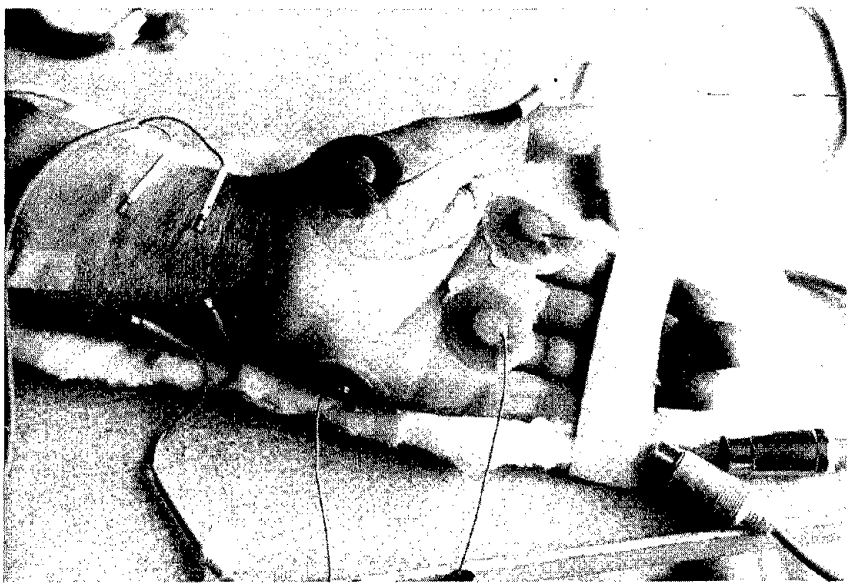


FIGURE 1. Electromyographic configuration for nerve stimulation and for detection of muscle action potentials.

ments during the testing were the following: resisted thumb opposition and resisted abduction of the fifth finger.

The basic outlines of the test battery are from Harvey and Masland[2], Lambert[3], and Desmedt[4]. Our test battery comprised the following stages in sequence:

(1) single stimulus determination of initial amplitude of direct muscle action potential at m.opponens pollicis and m.abductor digiti minimi,
(2) serial stimulations at 3/s for about 2 seconds,
(3) maximal contraction of the opponens pollicis or abductor digiti minimi by the subject for ten seconds that was resisted by the examiner,
(4) five seconds after the voluntary contraction the subject was given another series of 3/s stimuli in order to test for posttetanic activation,
(5) four minutes after voluntary contraction the third series of 3/s was given in order to test for posttetanic exhaustion.

All serial stimulations were first done on the median nerve - opponens pollicis and afterwards on the ulnar nerve-abductor digiti minimi muscles. Muscle action potentials were monitored with an oscilloscope and photographed. Attention was paid not only to amplitude but to the details of the shape of the potential.

The results in 40 healthy subjects were compared with the results obtained with the same technique in 6 myasthenic patients. The difference was impressive. Significant amplitude changes were observed only in the myasthenic group.

Neurologic

To perform all clinical examinations as uniformly as possible a standardized test form, partly based on the Mayo clinic record, was developed containing questions covering neurological anamnesis, and neurological data. In both cases special attention was paid to autonomic functions and to muscarinic and nicotinic side effects of cholinesterase inhibitors. Skin temperature was measured at different points with a biological thermometer, (Disa).

Work Environment and Occupational History

A questionnaire pertaining to work conditions and to present and past exposures was developed. A record of work habits was also obtained.

Psychologic

The following tests were used:

(1) Benton visual retention, multiple choice test, Form G for elimination of workers with I.Q. less than 80,

(2) Benton visual retention test, Form C for visual perception, memory and two-dimensional constructive praxis,
(3) Benton constructional praxis tasks for three-dimensional constructive capabilities,
(4) Benton-Spreen-Turdiu word fluency test for detection of minor language dysfunctions. All the tests mentioned were used at the reexamination but in different order.

Additionally the Turdiu personality questionnaire for detection of manic-depressive shifts of mood was used, as well as the Luscher-Color test for evaluation of emotional states and kind or level of neuroticism.

RESULTS

Electromyographic

The results in typical control subjects and myasthenic patients are shown in Figures 2 and 3.

Muscle action potentials were recorded as follows: Trace a, before the resisted voluntary activity; Trace b, five seconds after stopping voluntary activity; Trace c, three minutes later. Signals were recorded with a fast oscilloscope sweep in order to compare not only the amplitude of the potential but also the shape. Significant differences in the shape would indicate the involvement of different motoneuron pools. The constancy of amplitude and shape in the derivation of normal controls, Figure 2, is quite clear.

The results obtained in patients with myasthenia gravis were different, Figure 3. A decrease in potential amplitude, "fade" effect under a., "posttetanic potentiation", increase of amplitude under b, and "posttetanic exhaustion," decreases in amplitude under c, are significant.

The derivations done in exactly the same way in occupationally exposed workers did not differ significantly from the results presented in Figure 2. A difference in amplitude and shape was found if the results of different sessions were compared, Figure 4. However, this was noted also in the controls.

The muscle action potentials in each column were recorded at different times but in the same subject and on the same muscle. With the method described it is quite clear that the comparison of the muscle potential amplitudes which are recorded during different electromyographic sessions may be misleading.

Neurologic

In a number of exposed workers transient symptoms such as headache, nausea, colic, hypersalivation or hyperhydrosis were noted. In most of them, there was another possible explanation for the pathogenesis of these symptoms and signs.

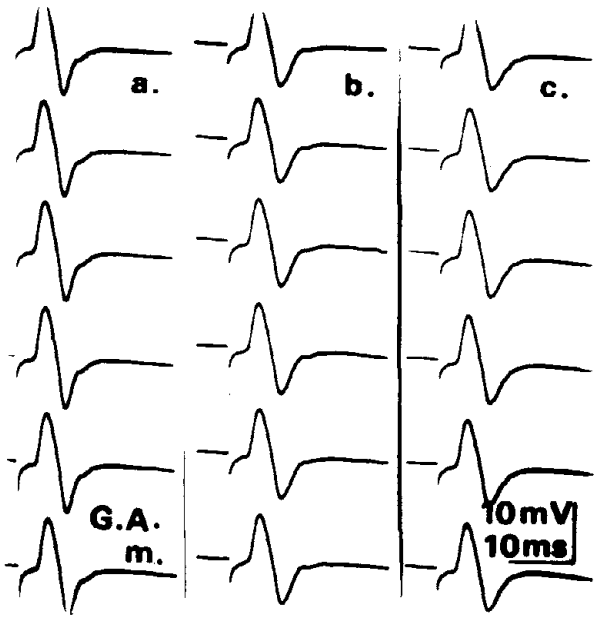


FIGURE 2. Muscle action potentials obtained in controlled subjects.

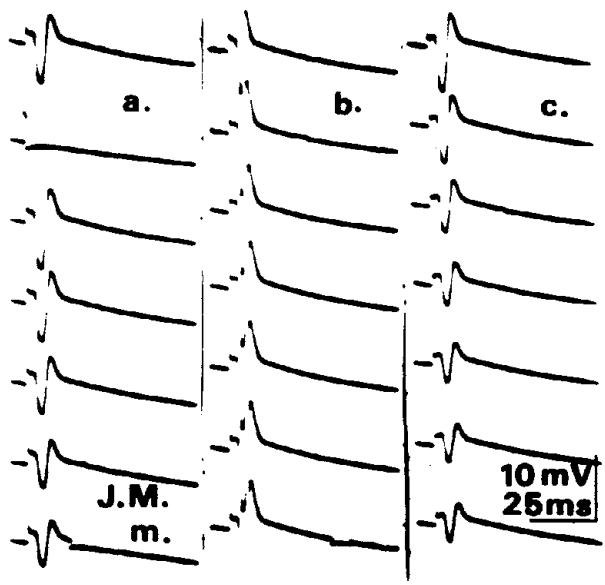


FIGURE 3. Muscle action potentials obtained in patients with myasthenia gravis.

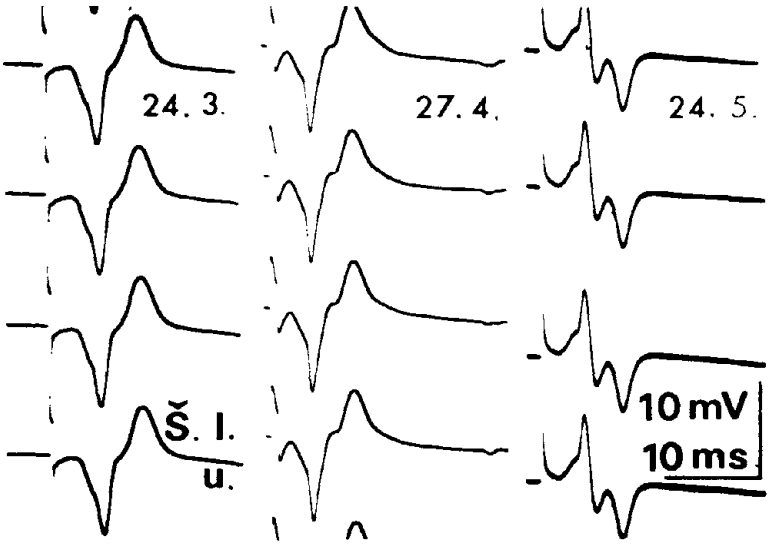


FIGURE 4. Comparison of muscle potential amplitudes recorded during different electromyographic sessions.

Psychologic

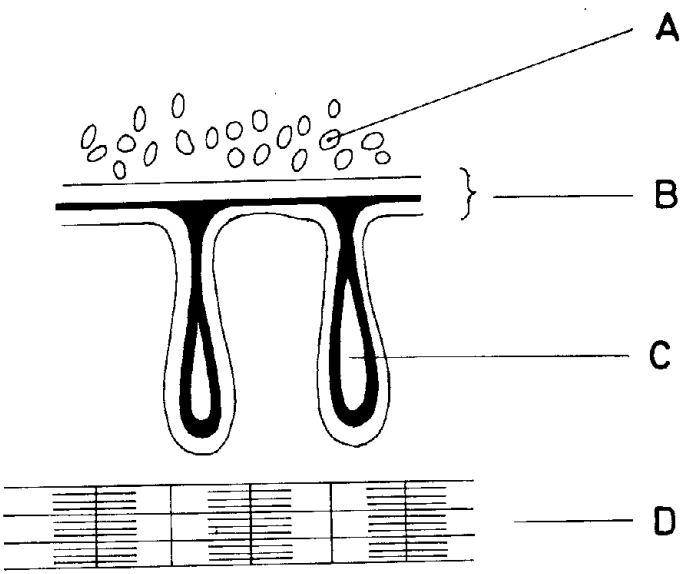
Statistically significant changes were found only in the visual retention test (Model $C_{10''}$ and $E_{10''}$) and in the constructional praxis tasks (Model II and III) with better results in the control examination. The better results should be due to the fact that the testing situation during the control examination was already known, so that the workers reacted more precisely and quickly.

DISCUSSION

Our interest was mainly focused on neuromuscular synapse functions, (Figure 5).

On the neural side there are vesicles with acetylcholine (A). The synaptic space proper consists of axoplasmic-sarcoplasmic interface (B). On the sarcoplasmic side there are junctional folds, which enlarge the receptor side of the neuromuscular junction (C). Under the nerve impulse the vesicles secrete acetylcholine into the synaptic space and acetylcholine binds transiently to muscle membrane. Consequently, permeability changes occur. The muscle membrane is depolarized and electrical action potentials appear. The enzyme cholinesterase hydrolyses acetylcholine and thus enables the membrane to repolarize. Insufficient depolarization, as well as prolonged depolarization may bring about a neuromuscular block.

Neuromuscular transmission can be disturbed in different ways,



A vesicles with Ach

B axoplasmic - sarcoplasmic interface
(synaptic „cleft")

C junctional fold

D myofibrils

FIGURE 5. Schematic drawing of a typical neuromuscular junction.

through different agents, presynaptically or post-synaptically. The electromyographic testing proved to be of significance in making a differential diagnosis of the type of neuromuscular block. Two main types of neuromuscular block are distinguished: (1) non-depolarizing block characteristic of myasthenia gravis, and (2) depolarizing block as in Decamethonium application. In nondepol-

arizing block muscle action potentials to direct nerve stimulation decrease in amplitude following twich or tetanic stimulation. A twich stimulation of the nerve a few seconds after tetanization results in a significant increase in potential amplitude. During the typical depolarizing block there is no decrease either in twich stimulation or in the tetanic one. There is also no increase a few seconds after tetanization or voluntary activity. With the increasing dose of a depolarizing drug a depolarizing block can change into a nondepolarizing one. This is the so called phenomenon of dual block of Wylie and Churchill-Davidson[6]. At this stage a significant increase in the muscle potential amplitude following the injection of cholinesterase inhibitor Tensilon may be observed. This is again a finding typical of myasthenic nondepolarizing block.

In the workers exposed to pesticides no reliable signs indicative of nondepolarizing block were found. The depolarizing block may be discussed after examinations are performed on a larger group, with isometric muscle strength correlations. This type of block would fit better with low cholinesterase activity levels. Again experience with myasthenia gravis can be mentioned. The myasthenic patients can have very low cholinesterase activities due to treatment with cholinesterase inhibitors such as Mestinon or Prostigmin. At the same time they can have electromyographic results and clinical findings normalized if compared with the results obtained before the therapy was started.

What part the development of tolerance to low acetylcholine levels plays in the results obtained in our pesticide workers is a question which remains to be answered. The same question has been considered by Chippendale and collaborators[6] who performed experiments on albino rats. One of our future concerns would therefore be to standardize the scopolamine challenge test in humans. Testing, with short acting cholinesterase inhibitors, such as Tensilon, as it is done in the treatment of myasthenia gravis could also be of some significance.

The anticholinesterase hypothermia described by Meeter, Wolthuis and Benthem[7] in rats was observed in one of our patients. It might be also indicative of occupational intoxication with organophosphorus pesticides.

The fact that most of our results do not differ from normal may be due to low or short duration exposures. The test may not be sensitive enough or may not measure what is expected to change under the influence of pesticides. Perhaps the pesticides do not affect the functions measured. Nevertheless, the examinations must be extended to a larger group.

SUMMARY

Comparisons were made between control subjects, myasthenic patients and occupationally exposed workers.

A neurologic questionnaire and a questionnaire pertaining to the working process were developed.

In this preliminary study significant differences were noted only in the results of the first two groups. Psychological tests showed no significant differences between the results before and after exposure, except for better performance in visual retention test and in constructional praxis tasks. This is probably due to familiarity of the testing situation and practice effects.

Further development of electromyographic neuromuscular synapse testing is suggested.

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PESTICIDE INTERACTIONS IN ANIMALS

Florence K. Kinoshita, Ph.D.

An important area of pesticide toxicology concerns the possible toxic interactions that might result from exposure to combinations of pesticides. It might be expected that with a knowledge of the pharmacological action of the individual components of a combination, the toxicity resulting from a combination could be predicted. This has been the case with some combinations. However, with other combinations of pesticides, the resultant toxicity has not been that which could be predicted. It has been found that the toxicity of some combinations is greater than or less than that which would be expected on the basis of the toxicities of the individual compounds.

One type of pesticide interaction which is of great concern is the potentiation of the toxicity of one cholinergic organophosphate insecticide by another. This type of toxic interaction was discovered in 1957[1] when it was found that the administration of the cholinergic organophosphate insecticide, EPN (O-ethyl O-*p*-nitrophenyl phenylphosphorothioate), simultaneously with another organophosphate insecticide, malathion [[S-[1,2-bis(ethoxycarbonyl) ethyl] O,O-dimethyl phosphorodithioate]], resulted in the potentiation of the acute toxicity of malathion. Since this first demonstration of potentiated toxicity by the administration of these two organophosphate insecticides, many other combinations have been shown to have potentiated toxicity[2]. It was also found that the simultaneous administration of two cholinergic insecticides would not necessarily demonstrate potentiated toxicity. In 1961, Hagan, Jenner and Fitzhugh[3] demonstrated that the administration of Delnav® [2,3-*p*-Dioxane S,S-bis (O,O-diethyl phosphorodithioate); dioxathion] 4 hours prior to the administration of malathion resulted in greater potentiation of malathion toxicity than if the two pesticides were administered simultaneously.

Malathion, as shown in Fig. 1, is metabolized to an active anticholinesterase agent by a microsomal oxidation reaction in the liver. In the reaction the sulfur is replaced by oxygen. The detoxification of malathion involves the subsequent hydrolysis of the carboxylester linkage. It has been found by Murphy and DuBois[4] and Cook *et al*[5], that the potentiation of malathion toxicity by EPN results from the inhibition of the hydrolysis of the carboxylester linkage by EPN. Once the mechanism involved in potentiation was elucidated, it was thought that a study of the inhibitory effects of

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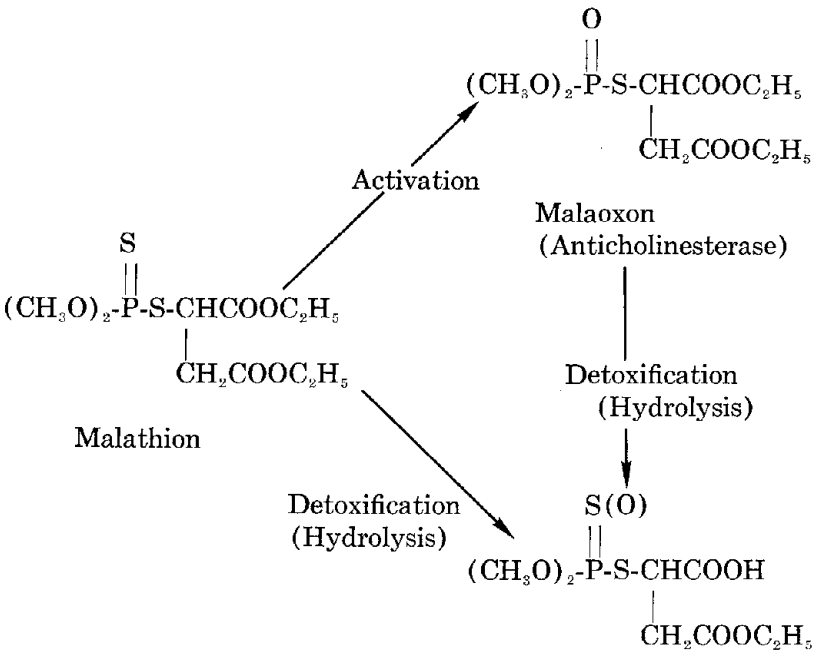

FIGURE 1. Metabolism of malathion.

the organophosphate insecticides on non-specific or ali-esterases would be a more worthwhile predictive tool than acute toxicity studies done with combinations. While the stimulus for studying the potentiation of the toxicity of cholinergic organophosphates was the possible exposure by humans to more than one member of this class of compounds, it should be kept in mind that other chemicals, including drugs, are metabolized by aliesterases. Any compound, such as EPN, which can inhibit aliesterases could not only potentiate the toxicity of another organic phosphate, but could also affect the detoxification and, therefore, the pharmacologic action and toxicity of any chemical that is detoxified by aliesterases.

With this in mind, some of our recent effort has been directed toward determining the effects of exposure to organophosphate insecticides on aliesterase[6]. Methods were developed which would allow the determination of inhibition of aliesterase activity *in vivo* using diethylsuccinate and tributyrin as substrates. Diethylsuccinate and tributyrin are ester-containing compounds which are hydrolyzed by carboxyesterases. Diethylsuccinate is that portion of the malathion molecule which is rapidly hydrolyzed.

Initially a study was done in order to develop a quantitative procedure for the measurement of aliesterase inhibition by organophosphate insecticides[6]. In this study two known potentiators of malathion toxicity, EPN and Delnav, were fed in the diet to rats

at various concentrations for periods of time up to 13 weeks. The rates of hydrolysis of diethylsuccinate and tributyrin were measured using liver and serum from the treated animals. In these studies it was found that dose-related inhibition of the hydrolysis of diethylsuccinate and tributyrin occurred. The inhibition of the hydrolytic enzymes for a particular feeding level of either EPN or Delnav was maximal at the end of 1 week of treatment. The inhibition of acetylcholinesterase activity in the liver, serum and brain of the treated animals was also determined. It was found that while both EPN and Delnav caused inhibition of the hydrolysis of diethylsuccinate and tributyrin at a feeding level of 1 or 5 parts per million (ppm), there was little inhibition of acetylcholinesterase in any of the tissues examined. While 25 ppm of either EPN or Delnav caused nearly complete inhibition of diethylsuccinate and tributyrin hydrolysis, there was only 50% inhibition of acetylcholinesterase in the liver and serum of Delnav treated animals and 38% and 14% inhibition in the liver and serum, respectively, of EPN treated animals. These studies demonstrated that the inhibition of aliesterases could be caused by lesser *in vivo* concentrations of organophosphate insecticides than those which cause the inhibition of acetylcholinesterase.

Following these initial studies a series of organophosphate insecticides which included parathion (O-O-Diethyl-O-*p*-nitrophenyl phosphorothioate), Systox® (demeton; O,O-Ethyl O-2[ethylthio]-ethyl phosphorothioate), Di-Syston® (disulfoton; O,O-Diethyl S-2-[ethylthio]ethyl phosphorodithioate), Phosdrin® (mevinphos; 2-Carbomethoxy-1-propen-2yl dimethyl phosphate), Guthion® (azinphosmethyl; O,O-Dimethyl S[4-oxo-1,2,3-benzotriazin-3[[4H]] ylmethyl] phosphorodithioate), Folex® (tributyl phosphorotrithioite), and TOTP (triorthotolyl phosphate), were fed to female rats for a period of 1 week in order to compare their effects on the hydrolysis of diethylsuccinate, tributyrin and acetylcholine[7]. At least three dietary levels of each organophosphate were used. The dietary level that would cause 50% inhibition of the enzyme was calculated by plotting the logarithm of the dietary concentrations of the pesticide against the per cent inhibition of the particular enzyme. The results of this study are shown in Table I.

All of the organophosphates used in this study caused inhibition of the aliesterases at some dietary level. There were, however, differences in the susceptibility of the aliesterases to the different organophosphates. Most of the compounds did not cause markedly different inhibition of the aliesterases and acetylcholinesterase. However, some of the compounds, such as, Folex and TOTP,

Table I. DIETARY LEVELS OF ORGANOPHOSPHATE INSECTICIDES THAT PRODUCE 50% INHIBITION OF ALIESTERASE AND ACETYLCHOLINESTERASE ACTIVITY

Organophosphate	Diethylsuccinate hydrolysis		Tributyrin hydrolysis		Acetylcholinesterase		
	Liver	Serum	Liver	Serum	Brain	Liver	Serum
Parathion	3.4	7.6	1.8	7.0	12.5	17.0	8.4
Systox	2.6	12.0	0.7	13.0	3.4	14.0	5.0
Di-syston	2.1	8.5	0.6	9.0	5.2	14.5	6.0
Phosdrin	10.5	35.0	3.1	30.0	23.0	28.0	17.0
Guthion	25.1	102.0	10.0	100.0	37.0	47.0	40.0
Folex	1.0	3.7	3.1	3.9	430.0	38.0	37.0
TOTP	7.0	17.0	22.0	21.0	580.0	310.0	240.0

Dietary Level to Produce 50% Inhibition of Enzyme Activity (ppm). From Su *et al.*[7]

were much more potent inhibitors of aliesterases than of cholinesterase.

As part of this study an attempt was made to correlate the amount of inhibition of the aliesterases with the potentiation of acute malathion toxicity. Another group of rats was fed dietary levels of the same organophosphates for 1 week and the LD-50 of malathion was determined using these treated animals. The dietary levels used were those that did not produce inhibition of acetylcholinesterase, but which inhibited the hydrolysis of diethylsuccinate and tributyrin. It was found that when the hydrolysis of diethylsuccinate was inhibited by more than 20%, there was an increase in the toxicity of malathion[7].

Another type of interaction that can occur with pesticide combinations or with combinations of pesticides and drugs or other chemicals is one which does not always lead to potentiated toxicity. It has been known for several years that many chemicals, including drugs and the chlorinated hydrocarbon insecticides, can cause the induction of hepatic microsomal enzymes. In 1954[8] it was shown that the pretreatment of rats with the chlorinated hydrocarbon insecticide, aldrin (1,2,3,4,10,10-hexachloro-1,4,4a,5,8,8a-hexahydro-1,4-*endo-exo*-5,8-dimethano-naphthalene), protected against the toxicity of parathion. Since that time several studies have shown that induction of the hepatic microsomal enzymes can protect against the toxic effects of other organophosphate insecticides[9, 10]. An effect on the toxicity of organophosphate insecticides by hepatic microsomal enzyme inducers is expected, since the phosphorothioate insecticides can be detoxified, as well as activated, by microsomal

enzymes[11]. It has been found by several investigators that the phosphorothioates may also be directly detoxified by cleavage without prior activation to the oxygen analog[12, 13]. The enzyme catalyzing the direct cleavage of these compounds is a microsomal enzyme and is inducible. This would correlate with the protection that is afforded by pretreatment with an inducing agent such as aldrin. In a series of experiments which were done in our laboratory, the acute toxicity of several organophosphate insecticides was determined after pretreating male rats with 50 mg/kg of the hepatic microsomal enzyme inducing agent, phenobarbital, daily for 5 days. The results of this study are shown in Table II.

Table II. EFFECT OF PRETREATMENT OF MALE RATS WITH PHENOBARBITAL ON THE ACUTE TOXICITY OF ORGANOPHOSPHATE INSECTICIDES

Organophosphate insecticide	Control LD_{50} (mg./kg.)**	Phenobarbital pretreated* LD_{50} (mg./kg.)**
Parathion	2.5	7.3
EPN	7.3	75.0
Systox	1.4	5.0
Di-syston	2.1	17.0
Ethion	25.9	302.6
Delnav	17.2	118.7
Malathion	619.4	949.9

*Phenobarbital administered i.p. for 5 days at a dose of 50 mg./kg./day.
**LD_{50} calculated by the Probit Method of Finney.
From DuBois and Kinoshita[14].

The acute toxicity of this series of organophosphate insecticides was reduced by prior treatment with this enzyme inducing agent[14]. The same type of results have been obtained using DDT (dichlorodiphenyltrichloroethane) or dieldrin (1,2,3,4,10,10-hexachloro-6,7-epoxy,1,4,4a,5,6,7,8,8a-octahydro-1,4-*endo-exo*-5,8-dimethanonaphthalene) as the pretreatment/inducing agent[15]. However, other data have shown that the toxicity of other organophosphates such as octamethyl phosphoramide (OMPA) may not be changed or in fact, may be enhanced, by pretreatment with phenobarbital, depending upon the species used for the study[14]. Also, it has been shown by Murphy[16] that while phenobarbital reduces the toxicity of Guthion, methylcholanthrene, another hepatic microsomal enzyme inducing agent, will cause an increase in its toxicity.

SUMMARY

The studies presented here demonstrate that interactions can

occur with combinations of pesticides and/or drugs. It should also be kept in mind that other chemicals occurring in the environment could also interact with pesticides. The result of the interaction can be a lessening or an enhancement of toxicity. The use of quantitative biochemical methods and the elucidation of the mechanisms involved in interactions provide a greater opportunity to evaluate the potential hazard of combinations of pesticides and other chemicals than the use of standard toxicity tests with all possible combinations.

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WORKER EXPOSURE TO METALS

SESSION IV

Chairwoman and Keynote Speaker

Dr. Eula Bingham
University of Cincinnati Medical Center

METALS SEMINAR KEYNOTE ADDRESS

Eula Bingham, Ph.D.

The first book on industrial toxicology or hygiene was written in 1453 by Ulrich Ellenbog, although it was not printed until 1543. It seems to me that his description of occupational poisoning resulting from metals is relevant for us as we begin our discussion of metals and behavioral toxicology.

"This vapour of quicksilver, silver and lead is a cold poison,
for it maketh heaviness and tightness of the chest,
burdeneth the limbs and oftimes lameth them
as often one seeth in foundries where men do work with large masses
and the vital inward members become burdened therefrom"
Ulrich Ellenbog — 1473

And while some of you may think of mercury and mercurial compounds in relation to their diuretic effects, the classical works on occupational disease present mainly the neurological symptoms. As a matter of interest the first textbook on occupational diseases published in this country leaves little doubt as to why we need to talk about metals and behavioral changes. The following quotation is one of the earliest accounts of occupational poisoning due to mercury, which originated in South America.

". . . sooner or later even the strongest mitayos
succumbed to mercury poisoning,
which entered into the very marrow of their bones
and made them tremble in every limb . . . . ."
Juan de Solorzano Pereira
. . . . . in charge of the
quicksilver mines at
Huancavelica—1616

These interesting bits of information about mercury were gleaned from a very well written history of quicksilver by Dr. Leonard Goldwater. It really should be a best seller to those of us in toxicology.

Mercury undergoes interconversions in our environment, i.e. ambient, industrial, and personal. Table I provides a point of reference for us; perhaps too simplified for our chemists but not for most of us. You see the metallic mercury or elemental mercury, the phenylmercury, and bivalent inorganic mercury.

Data on the consumption of mercury provides us with the clues where occupational exposures occur (Table II).

Now if we look at the signs of chronic, low level exposure to mercury, such as we usually encounter in occupational exposures, we see that neurological signs of exposure are most prevalent. The

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occurrence of albuminuria is frequently the only kidney impairment noted. The other classical signs of poisoning that are listed in Table III may not be present.

Table I. ENVIRONMENTAL INTERCONVERSIONS OF MERCURY

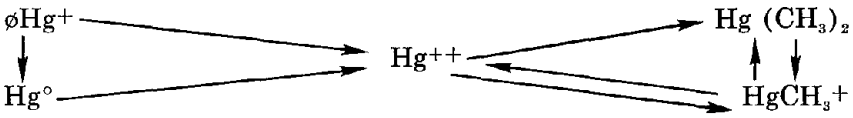

Table II. CONSUMPTION OF MERCURY

1. Elemental — Hg° (largely recycled)	2. Non-elemental (dissipated)
chlor-alkali industry	Hg^{++} — plastics
medical and electronic	organic — Hg — paints
dental	agriculture
	pulp and paper
	pharmaceutic
2140 T/y	750 T/y

Table III. CHRONIC INORGANIC MERCURY EXPOSURE

Signs of poisoning	Physiological or biochemical changes observed in poisoning
Tremors	
Restricted visual field	
Numbness	
Lassitude	
Weakness	Generalized binding to various reactive substances — e.g. SH groups — (inhibitors) Inhibition of Na & K ATPase
Insomnia	
Nephropathy, Proteinuria — ?	
Gingivitis, Stomatitis, Excessive salivation — ?	

Table IV presents the most frequently occurring signs and symptoms in Minimata disease[1]; a disease associated with the intake of organic mercury.

Table IV. FREQUENCY OF OCCURRENCE OF VARIOUS SYMPTOMS AND SIGNS IN MINAMATA DISEASE (%)

Symptoms	Fetal	Children	Adults
Mental disturbance	100	100	71
Ataxia	100	100	94
Impairment of the gait	100	100	82
Disturbance in speech	100	94	88
Hearing impairment	4.5?	67	85
Constriction of visual fields		100?	100
Disturbance in chewing and swallowing	100	89	94
Brisk and increased tendon reflex	82	72	34
Pathological reflex	54	50	12
Involuntary movement	73	40	27–76
Primitive reflex	73	0	0
Impairment of superficial sensation	?	?	100
Salivation	72	56	24
Forced laughing	27	29	

Some classical signs of lead poisoning are listed in Table V, and it should be noted that only when anemia is associated with lead poisoning do we have reasonably convincing evidence of a biochemical effect.

It is interesting that we, who have been so busy measuring biochemical and physiological changes, can report to you several investigators who are in search of signs or symptoms.

The point of these last three figures is obvious: with occupational exposures to Hg or Pb, the major target organ is the nervous system. Most toxicologists have ignored this until recently. It appears to me that we are long overdue for a workshop on this topic. Our breakthroughs may be slow in coming, but consider that after all the economic pressure we have had for investigating mercurials as diuretics, there is no good correlation between degree of SH inhibition and diuretic potency, after 30 years of research.

Next, let's turn our attention to metal interactions or interrelations. It is true that I can't give you any examples associated with the nervous system, however, it does not seem too far fetched to suggest they could occur. The first example is taken from experiments by Parizek[2] from the Czechoslovakian Academy of Sciences in Prague. While some of you may know that it has been reported that inorganic selenium or selenomethionine decreases the toxicity of Hg, I found this experiment presented in Table VI intriguing.

Table V. CHRONIC LEAD POISONING

Signs	Physiological or biochemical expressions of poisoning
Anemia	Inhibition of heme synthesis stippled cells
Colic	
Nephropathy (Gout)	
Neurological impairments encephalopathy paralysis foot and wrist drop delirium, etc.	
None	Changes not clearly associated with signs
	Fanconi syndrome
	Coproporphyrins
	ALA-D

Table VI. ADULT FEMALE RATS (30 animals in each group)

	20 μmole kg	mortality 24 hours
solvents	$ZnCl_2$	0%
(oil)	$CdCl_2$	0%
	$HgCl_2$	0%
	0	3%
dimethylselenide	$ZnCl_2$	0%
200 μmole/kg b.w.	$CdCl_2$	7%
	$HgCl_2$	100%

Now turning to lead: some interesting relationships between lead and copper have been demonstrated by a group of investigators in our department led by Dr. Harold Petering[3].

These investigators had read the literature carefully. In 1958 Rubino et al., published data which showed that there was an elevated copper content of red blood cells of patients suffering from lead poisoning, which was correlated with an increase in RBC pro-

toporphyrin content. At about the same time Iodice and associates found that ALA-D was depressed in the livers of rats and in the blood of ducklings fed a copper deficient diet, although a function for copper in ALA-D activity has not been established.

In 1969, Alloway working with Davies at the University of Wales compiled data on swayback in lambs dropped by ewes pasturing on grasslands which were normal in their copper and molybdenum content but high in lead. The data clearly show that the lambs had sera low in copper and that swayback was a common occurrence in such lambs, conditions which are usually attributable to a nutritionally induced copper deficiency. They concluded that there was a correlation between the lead in the grass and the apparent copper insufficiency found in the lambs. Following this lead, Klauder in our laboratory began to examine the interaction of dietary copper and the administration of lead orally to male rats. Using the same model mentioned above, in which the copper content of the diet was controlled, Klauder *et al.*,[3] (Table VII) found that lead toxicity in rats was increased by a dietary deficiency of copper.

More and more we shall probably uncover interrelationships among metals acting in the whole animal. Only if we are aware that such things can occur will we recognize them in our experiments.

Table VII. EFFECT OF LEAD ON COPPER METABOLISM IN MALE RATS

	Low Cu	Normal Cu	Low Cu + Pb 0.5%	Normal Cu + Pb
Hematocrit %	48.5	51.4	36.2	51.2
Hemoglobin g %	12.2	13.2	8.1	12.3
Serum Cu μg %	6.0	57.2	7.5	12.4
Serum Zn μg %	138.0	124.0	98.0	121.0
Ceruloplasmin mg %	6.7	29.1	2.8	10.7

L Cu = 0.5 ppm in diet
N Cu = 2.5 ppm in diet

Data from:
D. S. Klauder, L. Murthy, and H. G. Petering
Trace Substances in Environmental Health VI
1973: p. 131-135

As a means of shifting from metals to the behavioral and neurophysiological aspects of this session I would like to present a three- or four-minute film that demonstrates remarkably well the behavioral changes induced by one organometallic compound, tetramethyllead. (Scenes from this film are shown in Figures 1, 2, 3 & 4).

FIGURE 1. Fine continuous tremors of the whole animal after TML administration.

FIGURE 2. Spontaneous convulsions after TML administration.

FIGURE 3. Convulsions initiated by tactile stimulation after TML administration.

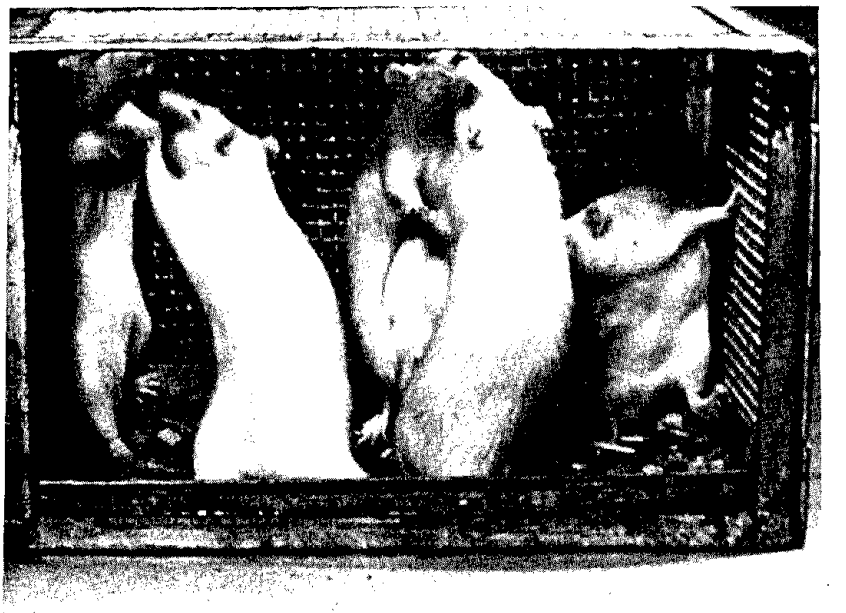

FIGURE 4. Aggressive boxing stance after TML administration.

The signs of illness have been described by us previously[4, 5] and include: one to three days after the administration of the compounds, tetraethyllead or tetramethyllead, the animals become hyperirritable. Fine continuous tremors of the whole animal develop (1). The time of the appearance of tremors varies with the dosage of the trialkyllead chlorides but not with that of tetramethyllead or tetraethyllead. Convulsions sometimes occur spontaneously (2). They may be initiated, at times, by auditory or tactile stimulation (3). Upon being annoyed, the animals become aggressive, rear on their hind legs, mouth open, emitting cries, and pawing the air (4). As they subside, the animals sink back upon their haunches and exhibit postural difficulties. This state of hyperexcitability may persist for days, depending upon the dosage. The animals gradually recover their usual behavior or become weaker and die.

When I first started to work in the building over on Eden Avenue, where most of us have been accused at one time or another of looking at the world through metal-colored glasses, I was shown around the animal facility. I had come from an endocrine lab where we carried rats around in our lab coat pockets and made pets of them. I was astounded when I saw these rats exposed to a metallo-organic compound at very low levels of exposure.

The effects are not subtle as you have seen. The challenge to you who are behavioral toxicologists occurs when we use 1/10 or 1/100 of the dosage. What are the tests you will use? How can we quantitate the changes in behavior? Should we observe not on day 5 but on day 1 or at 1/10 or 1/100 the level of exposure? What do the behavioral changes mean in relation to function and ultimately to a biochemical lesion? What are the implications of behavioral changes to worker safety and health?

These are the challenges Behavioral Toxicologists face today.

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EARLY DETECTION OF MERCURIALISM

John Wedig, Ph.D.

The early detection of adverse or toxic symptoms from industrial exposure to elemental mercury presents a challenge. There have been a number of reviews concerning the toxic effects of elemental mercury over the past years which attribute many toxic and adverse behavioral signs and symptoms to this metal.

A major problem in the industrial environment is that values for a specific biologic material (mercury) being measured for a biological threshold limit may not necessarily correlate with the values for exposure based on the analysis of work environment air. For instance, an employee working in a mercury cell chlor-alkali plant is being exposed to this metal 24 hours a day when you consider his microenvironment. His personal hygiene and work habits exert a great influence on the extent of his total exposure.

In the report of the International Committee on Mercury in Stockholm in 1968, there is the statement, "Contamination of skin or work clothes with mercury compounds could cause heavy exposure to mercury vapor by inhalation."[1] This type of contamination can result in a higher concentration of mercury vapor in the microenvironment around an employee than the concentration found in the breathing zone in the general work environment. Table I illustrates the concentration difference in the microenvironment vs. the general environment. The data for the general work environment of the cell room were obtained from a continuous automatic mercury vapor monitor in one of our chlor-alkali plants. We see a minimum of 0.06 and a maximum of 0.11 mg Hg per cubic meter of air for breathing zone air in the cell room in October. Comparing these values with the air near the outer clothing, gloves, hands, etc., we see a range of approximately 2 to 6 fold. If the contaminated clothing is worn home, the exposure continues. Even if the clothing is changed at the plant after work, the residual mercury on the skin can serve as a continual source of exposure during the remainder of the day and while sleeping. Years ago, I am sure that fresh work clothing was not furnished at the beginning of each shift; nor was a shower taken at the end of the shift. Vostal[2] has reported finding pools of mercury in a washing machine in the house of a person employed in work which resulted in contamination of the skin and clothing with liquid mercury. "Moonlighting" could also increase the total exposure to elemental mercury. Danziger and Possick[3] published a paper concerning the carrying home of mercury on con-

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Table I. MERCURY VAPOR CONCENTRATIONS IN AIR NEAR CONTAMINATED CLOTHING AND SKIN
(October 24-26, 1972)

Locker room	Mg mercury/ Cubic meter of air
General room atmosphere	0.03-0.04
Air near	
1. Outer clothing furnished by company and laundered daily; worn one shift before measurements	0.1 -0.2
2. Gloves	0.08-0.2
3. Hands (before washing)	0.5 -0.6
4. Clean hands (washed)	0.04-0.08
5. Sweater (employee in mercury recovery area)	0.2 -0.5
6. Rubber coated shoes (inside)	0.02-0.05
(outside)	0.10-0.5
7. Cotton undershirt worn approximately 6 hours in cell room. Person had no known contact of outer clothing with liquid mercury nor salts of mercury.	0.01
8. Cell room, breathing height — October	0.06-0.116
— November	0.02-0.08

taminated clothing, as did Wood, Weiss and Weiss[4]. However, in the published literature there are no estimates of total exposure to mercury when working in an environment contaminated with mercury.

In the United States, a major source of mercury exposure is the chlor-alkali plant where elemental mercury functions as a flowing cathode in an electrolytic process to produce chlorine and caustic. We have been operating this type of plant since 1898 and have not observed the classical toxic and behavioral signs and symptoms attributed to elemental mercury over-exposure. A large majority of our plants' populations have had 10-30 years of service, while some have worked there for up to 42 years. Total urinary mercury levels in some of these people have been as high as 500 μg/l. The actual level and length of exposure may have been higher than this urinary value reflects in relation to time weighted average exposures in the work environment air.

Inorganic mercury classically produces a triad of effects: (1)

oral cavity disorders — stomatitis, gingivitis, (2) involuntary movement disorders — intention tremors, (3) psychological disturbances — shyness, irritability, insomnia, impaired judgment, and memory defects. The primary effects appear to be related to the central nervous system. In order to detect these early preclinical signs of mercury intoxication, specific tests have to be devised and validated. A positive correlation and dose-response relationship should be established between these parameters and mercury concentrations either in the urine or blood. Several studies of this nature have been performed.

Figure 1 shows the major medical findings of Smith et al.[5] for chlor-alkali workers exposed to mercury. The weight and appetite loss were not actually measured — they represented a yes or no answer on a medical history. Krause et al.[6] have shown that there is no correlation of actual measured weight changes with the extent of exposure to mercury of employees in the chlor-alkali industry.

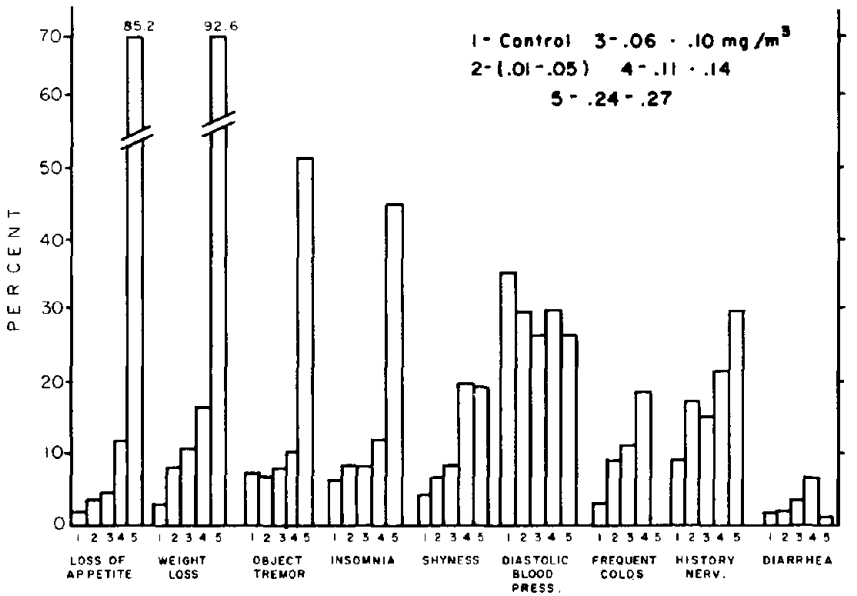

FIGURE 1. Percentage prevalence of certain signs and symptoms among workers exposed to mercury in relation to degree of exposure (from Smith, et al., 1970).

Chaffin et al.[7] performed an extensive evaluation of chronic mercury exposures using electromyographic and psychomotor function tests in chlor-alkali workers. They stated that "Among the list of symptoms found in the *medical history* and *clinical neurological examination* data summary, special attention was directed to those

of irritability, sleep disturbances, anorexia and weight loss, because significant correlation had been found in a previous study with regard to these symptoms." These findings were not substantiated even though one exposed group of 23 people had a mean total urinary mercury concentration of 787 μg/l with a standard deviation of 471. The medical records of these workers were examined from the past 3-10 years and no significant weight changes were found.

Kazantzis[8] had reported on the use of tremor measurements in the early diagnosis of chronic mercurialism at the International Conference in Stockholm in 1968.

Chaffin et al.[7] found a correlation of tremor frequency with blood and urine mercury concentrations. Thus, tremor measurements may be an effective means for detecting early reversible adverse effects of mercury exposure. This testing, coupled with urinary or blood mercury determinations, could serve as an effective means to monitor employees exposed to mercury.

The body has protective as well as detoxification mechanisms to prevent elemental mercury from gaining entrance into the brain once it has been absorbed. The blood brain barrier appears to protect this organ from "mercury" depending upon the rate of absorption into the body. Magos[9] has shown that the brain absorbs 10 times as much elemental mercury as it does inorganic mercury salt when given an equivalent dose. This has been demonstrated for both ingestion and inhalation. Clarkson et al.[10] studied the equilibrium of mercury vapor with blood. At some rate of absorption of elemental mercury vapor by inhalation, the elemental mercury may be converted to ionic mercury before reaching the brain. If the rate of absorption exceeds the rate of oxidation, elemental mercury will penetrate the blood brain barrier and accumulate in the brain. The data of Smith et al.[11] appears to confirm this, Table II.

Table II. SUMMARY TISSUE ANALYSES
Hg CONCENTRATION, μg/g (dry weight)

	Control	0.1	0.5	1.0
Brain:				
Medulla	0.1	0.2	24	55
Cerebellum	0.4	0.6	11	64
Occipital	0.2	0.4	15	84
Frontal	0.3	0.6	12	87

From Smith et al. (1971)

The mercury content in the cerebellum of control monkeys was 0.4 μg/g (dry wt. basis) compared with mercury contents of 0.6,

11 and 64 μg/g for monkeys exposed to 0.1, 0.5 and 1.0 mg of mercury vapor per cubic meter of air for 6 hours a day, 5 days a week for 5 years for the 0.1 and 0.5 mg/M^3 groups and 3½ years for the 1 mg/M^3 group. The approximate 20-fold increase in cerebellar mercury concentration with only a five-fold increase in exposure suggests an overloading of the oxidative mechanism resulting in accumulation in the brain. However, at low levels of exposure this oxidative system probably serves as a protective mechanism to prevent accumulation of mercury in the brain. Similar differences were found in the medulla, occipital and frontal portions of the brain. These mercury determinations were performed by UV absorption using a mercury vapor meter and measured total mercury.

The differences in accumulation of mercury in the brain with different dose rates and forms of mercury suggest the possibility of a difference in the form excreted in the urine. The cold flameless atomic absorption procedure for the determination of mercury in urine can be adopted for determination of elemental, stannous chloride-reducible and total mercury. Urine samples from chlor-alkali employees have been analyzed for elemental, stannous chloride-reducible and total mercury. The final analytical step is the same for all three — elemental mercury is swept from the diluted pretreated urine sample into a gas stream and measured by cold flameless atomic absorption. Pretreatment of the aliquots provides the feature to distinguish among the three in urine.

We normally find only a small amount of elemental mercury in urine samples. This would conform to the findings of Clarkson et al.[10] that elemental mercury is quickly oxidized to Hg^{+2} once it reaches the blood stream. Thus, after being exposed to a high vapor concentration of elemental mercury, it should be found in blood or urine for only a short time. Figure 2 illustrates total mercury vs. ionic plus elemental mercury in urine samples. The curve is essentially flat below 0.3 mg total mercury per liter of urine. This graph indicates that at rates of exposure to elemental mercury vapor which lead to a urinary excretion of total mercury less than 0.5 mg/l, the body binds the mercury such that it is not reducible by stannous chloride. When the exposure to elemental mercury is such that the total urinary mercury exceeds approximately 0.5 mg/l, then the quantity of stannous chloride reducible mercury increases. This possibly represents the concentration at which an adverse behavioral toxic effect may occur.

In conclusion, our experience in the operation of six mercury cell chlor-alkali plants has demonstrated that no adverse effect on the health of an employee has been found even though total urinary mercury concentrations in some employees have exceeded 0.5 mg/l.

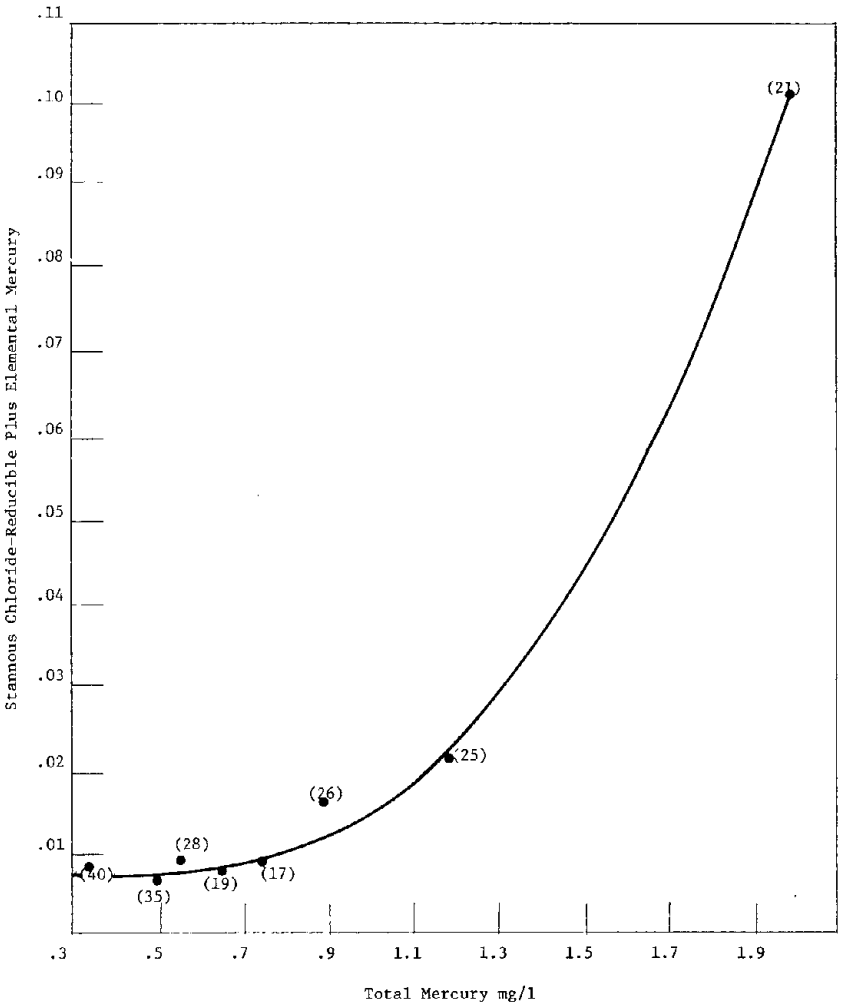

FIGURE 2. Total versus stannous chloride-reducible plus elemental mercury in urine. Group averages, number of samples per group in parentheses.

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BEHAVIORAL AND NEUROLOGICAL EVALUATION OF WORKERS EXPOSED TO INORGANIC MERCURY

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This is a summary of an 18-month study to determine the neurological and psychomotor changes associated with chronic exposure of workers to metallic mercury vapor. The study is based on the well recognized fact that chronic exposure to mercury can result in complex alterations to a person's physiological state. Primary effects are to the central nervous system. These manifest themselves in varied symptoms and signs, as well as in altering the person's performance capabilities. If the chronic exposure is incurred as part of the person's job, occupational health and safety problems can exist, depending upon both the amount of exposure (dose) and the length of time exposed.

A current management problem is to prevent mercurialism by means of adequate monitoring of mercury levels in both the air and in the exposed workers. This will assure that even the earliest signs of deterioration in the person's health or performance are detected and prevented from progression. The challenge, therefore, is to devise the means to recognize preclinical evidence of overexposure in order that appropriate corrective action can be taken before permanent damage is incurred.

To date, the thrust of most past research has relied on correlations between either clinical findings and exposure levels, or tissue pathology following controlled exposure of animals. These have contributed to the setting of safe exposure levels for toxic substances. A review of the literature regarding the effects on humans and animals exposed to inorganic mercury discloses widely differing answers to such basic questions as:

- How long can a person be safely exposed to a given level of mercury?
- What are the most prevalent symptoms and signs related to chronic exposure to inorganic mercury?
- If some adverse mercury related symptoms or signs are

This is a summary report of a study performed as part of contract HSM-099-71-62 for the National Institute for Occupational Safety and Health, Department of Health, Education and Welfare. NIOSH Project Officers were Charles Xintaras, Barry L. Johnson and Edward J. Fairchild, Jr., II. Other key contributors from the University of Michigan included Bertram D. Dinman, Ralph G. Smith, David H. Zontine and Shashikant Kelkar.

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manifested in an individual, which are reversible, and on what basis may a person be returned to exposure?
- How frequently should a person be evaluated for the potential adverse effects of mercury?
- What are the population variations (individual tolerances and sensitivities) that can be expected with given levels and durations of exposure to mercury?

RESEARCH SIGNIFICANCE

The research reported herein is one attempt to compile the information necessary to gain more definite answers to these questions. It represents a new approach, in that people's health status are monitored by non-invasive, quantitative tests of specific neuromuscular and psychomotor functions. In this study, the test results are correlated with the person's: current blood and urine mercury burdens; length of time exposed to mercury; age; height; weight; and cigarette smoking rate. Retests of some people whose exposures to mercury had been reduced for a period of time are also reported. This allowed the consideration of the reversibility of some of the positive findings.

It is demonstrated that several of the neuromuscular and psychomotor tests developed and used in this study are sensitive to the effects of mercury. They can, it is believed, be used to assess the degree of individual response to given doses of mercury. These tests are proposed as a major means of diagnosing and controlling the effects of mercury and possibly other related neurotoxic substances before a person's health or safety is jeopardized. Large response variations in the population are documented in this and other studies. Thus, these tests are believed to be a reasonable and necessary requirement in the control of neurotoxic substances.

It is now realized that it is economically impractical to set an air or biological standard unreasonably low because of the most sensitive person in the working population. Yet without objective knowledge about a person's health status, and thus his individual response to the toxic agent, this must be the rule to provide the greatest health protection for all. This project demonstrates that if the working population as a whole is to be free of any adverse subclinical changes in their neuromuscular or psychomotor functions, a low level of urine or blood mercury is necessary as an action level. This is defined as that point where quantitative functional tests of health status are instigated on a routine basis. A person would be removed from exposure when the combination of the periodic biological tests and the health status tests indicate deterioration in his health or a decrement in his ability to perform

safely. If such status tests also determine that a number of people in a certain work area are showing adverse effects, the atmospheric level may be high and the operation would require immediate corrective action.

With further research and development it is proposed that such a procedure could become feasible for control of many environmental agents. Indeed, it is analogous to the noise control procedure, wherein a person's hearing acuity must be routinely tested for adequate health protection. Developing such a health status monitoring procedure for toxic agents can satisfy the primary objective of maintaining the individual worker's health. A secondary concern is complying with the standard based on indirect indices of atmospheric and biological concentrations of the toxic agent.

EXPERIMENTAL DESIGN

Subjects

This study involved 142 male volunteers from four different plants. Seventy-seven of these people were working in jobs where daily exposure to inorganic mercury was incurred, thus resulting in them being identified for this study as the *Exposed Group.* The remaining sixty-five people were classified as *Controls,* based on the participating plant's industrial hygiene and safety personnel designating that they were not currently, nor had they been recently (within six months) working in areas where mercury exposure could take place. Age matching was requested and was best achieved in the last two plant groups visited.

The first three plant groups (Groups I, II, and III) were engaged in chloralkali manufacturing, while the fourth group (Group IV) worked in a patented process for the manufacturing of magnetic materials. Groups I and II were revisited during the study period. This provided the opportunity to collect some retest data on people who had demonstrated marked reductions in their urine mercury levels as a result of changes in climatic conditions, industrial hygiene controls, and/or jobs which reduced their exposure to mercury. In total, 198 tests were conducted by three investigators during seven site visits.

Independent Variables

The major objective of this study was to gather the necessary data to indicate how specific neurological and psychomotor functions correlate with a person's blood or urine mercury levels at the time of testing. Thus, the *current blood and urine mercury levels* are the two major independent variables. The laboratory method for determining the value of each was that of flameless atomic absorption.

There are numerous other independent factors which may enter into the determination of the dependent measures. The following were selected as having possible relevance.

1. *Height (stature) and Weight* were included based on the assumption that these might indicate, a state of physical fitness, a change in the muscle loading during the weight holding test, or a change in the motion dynamics required during the eye-hand coordination test.
2. *Age* was included based on its potential of indicating a state of physical fitness, or a slowed simple decision and motor performance capability.
3. *Smoking* was included based on its potential for reducing the person's endurance capacity (through the CO effect on oxygen affinity and binding for hemoglobin). The heavy smoker may also absorb more mercury vapor by the increased ventilation required of him when physically active.
4. *Duration of Exposure* was included based on the assumption that those people exposed for a short period may biologically react entirely different than those exposed over many years.

A summary of these subject characteristics is presented in Table I, based on the first visit to each plant.

Dependent Variables

This study involved the establishment of a set of quantitative non-invasive tests of a person's neuromuscular and psychomotor functions which from past data could be expected to be sensitive to the adverse effects of inorganic mercury. The following primary test measures were administered to 142 people, 77 of whom were exposed to metallic mercury vapor in their jobs.

- *Surface Electromyography* — Earlier tests by us had indicated that the power spectrum of the EMG was significantly altered in persons having elevated mercury body burdens. A separate study by others using clinical needle EMG's on persons having elevated mercury burdens confirmed changes which would correspond to the surface EMG power spectrum changes reported by us.
- *Forearm Tremor* — Earlier studies by others have indicated tremor to be one of the most sensitive functional manifestations of mercury intoxication.
- *Localized Muscle Fatigue* — Earlier studies indicated that "fatigue" is a general symptom of mercury intoxication, thus a specific submaximal weight holding endurance test was devised using surface EMG frequency changes as a measure of the resulting fatigue rates.
- *Discrete Positioning Eye-Hand Coordination* — Other studies have indicated that eye-hand coordination in manipulative tasks is adversely affected by mercury. A test

Table I. SUMMARY OF INDEPENDENT VARIABLES FROM FIRST VISIT STUDIES

Plant	I			II			III			IV			ALL (I, II, III, & IV)		
Group	Control	Exposed	Combined	Control	Exposed	Combined	Control	Exposed	Combined	Control	Exposed	Combined	Control	Exposed	Combined
Sample size	15	20	35	14	23	37	26	24	50	10	10	20	65	77	142
Height, inches	70.2 (3.08)	69.5 (2.42)	69.8 (2.70)	69.3 (3.15)	70.6 (2.98)	70.1 (3.7)	69.7 (2.68)	69.5 (2.66)	69.6 (2.64)	69.3 (2.16)	69.7 (2.67)	69.5 (2.37)	69.7 (2.74)	69.8 (2.71)	69.8 (2.72)
Weight, pounds	172 (18.7)	174 (38.0)	173 (30.8)	166 (32.4)	172 (31.2)	169 (31.3)	177 (23.6)	184 (26.0)	181 (24.9)	179 (20.2)	183 (19.9)	181 (19.6)	173 (24.6)	178 (30.2)	176 (27.8)
Age, years	31.2 (7.0)	27.2 (7.3)	28.9 (7.4)	49.9 (6.4)	41.5 (7.4)	44.7 (8.0)	33.6 (9.6)	36.4 (8.9)	35.1 (9.3)	37.9 (6.45)	36.9 (6.67)	37.4 (6.41)	37.1 (10.39)	35.7 (9.47)	36.4 (9.89)
Dur. of Exp., months	—	30.8 (26.7)	—	—	93.2 (85.4)	—	—	25.2 (16.1)	—	—	90.6 (49.1)	—	—	55.9 (60.5)	—
Cigarette, packs/day	0.29 (0.49)	0.0 (data on 6 subjects only)	0.21 (0.48)	0.79 (0.55)	0.83 (0.68)	0.81 (0.63)	0.42 (0.56)	0.42 (0.56)	0.42 (0.56)	0.45 (0.60)	0.20 (0.34)	0.33 (0.49)	0.52 (0.58)	0.48 (0.60)	0.50 (0.59)
Blood Mercury, μg/100 ml	2.17 (1.10)	6.25 (3.66)	4.45 (3.47)	5.89 (2.71)	17.11 (5.77)	12.74 (7.32)	0.90 (0.45)	3.97 (3.04)	2.56 (2.72)	0.93 (0.38)	4.51 (2.54)	3.49 (2.70)	2.49 (2.51)	8.56 (6.90)	5.97 (6.23)
Urine, μg/liter	68 (53)	288 (240)	197 (216)	152 (100)	787 (471)	547 (486)	18.3 (22.1)	143 (104)	84 (99)	7.11 (3.26)	129 (69.7)	71.5 (79.8)	62.6 (78.1)	374 (399)	240 (342)

Note: Numbers in parentheses indicate Standard Deviation.

of this functional capacity was selected which also relates to general industrial skill levels.

A secondary test battery was also developed to augment the findings of the primary battery. This included the following tests which were subsequently administered to 87 people, (47 of whom were exposed to metallic mercury vapor):

- Simple reaction time
- Two-choice decision time
- Four-choice decision time
- Finger tapping speed
- Toe tapping speed
- Blind toe pointing accuracy
- Pencil flipping speed

A standard clinical neurological status evaluation was administered to 32 people in the study. The measures included:

- History of neurological symptoms
- Mental status
- Cranial nerve evaluation
- Sensory modality status
- Motor system functions
- Cerebellar functions
- Involuntary movements
- Deep tendon reflexes
- Automatic functions

Retesting of some of the people was accomplished after a four- to six-month period using all of the above tests.

DATA COLLECTION

The following is a general description of events at each site visit:

1. Set up test apparatus in quiet area of plant (usually Medical Department).
2. Coordinate scheduling of volunteers with plant nurse or medical technician to achieve an approximate interval of between 30 to 45 minutes for each volunteer.
3. Ask each volunteer to read and sign a statement regarding the purposes, risks, and data confidentiality of the study.
4. Have each volunteer fill in a standardized medical questionnaire, and have him designate a physician to whom his personal test results should be sent.
5. Obtain a urine sample in an acid washed bottle.
6. Attach EMG surface electrodes in a monopolar configuration to the skin over the belly of the biceps brachii of the dominant arm.
7. Instruct the volunteer in the eye-hand coordination test procedure and have him perform the test four times while recording his performance.

8. Strap a 15-pound weight and tremor measuring device to his wrist and ask the person to hold the weight for a period of five minutes, while recording both the tremor and EMG signals.
9. Perform the eye-hand coordination test a fifth time immediately after the subject has set the weight down.
10. Perform neurological examination (performed in only two of the four plant groups).
11. Conduct psychomotor testing (performed in only two of the four plant groups).
12. Obtain a blood sample using 10 ml. vacutainers with potassium oxalate preservative.

Tremor and EMG

Both the forearm tremor and biceps surface EMG were obtained simultaneously. To accomplish this a four-channel FM tape recorder (Hewlett Packard Model 3970) having a bandwidth from DC to 312 Hz. (at 15/17 IPS) was utilized. A specially constructed preamplifier (10^{11} ohms input impedance, 5K Hz. response) was used to amplify the signals with known gains. The EMG electrodes were Hewlett Packard Model 14057. The tremor transducer was specifically constructed to detect changes in a force load of less than two ounces. An oscilloscope was used to monitor both the EMG and tremor signals during the tests to assure that peak clipping did not occur.

Procedure

The data were collected by the following procedure:

1. The subject was shown the test apparatus with an explanation of the procedure to be employed.
2. The subject was seated and his dominant arm was supported by an elbow rest which allowed a 90° elbow angle with the forearm unsupported.
3. Three surface electrodes were applied to his dominant arm.
   - Ground Electrode, over the medial epicondyle of the elbow.
   - Reference Electrode, over the lateral epicondyle of the elbow.
   - Active Electrode, over the anterior center of the biceps brachii when contracted to give a 90° elbow angle.
4. A padded wrist cuff was strapped around the wrist to which both the force sensing transducer and a 15-pound load were attached.
5. The subject was instructed to pick up the load by flexing his arm to a 90° elbow angle and hold it there for five minutes, or until he was fatigued if this occurred before five minutes had elapsed.

During the five-minute holding test both the EMG and tremor signals were recorded continuously, along with 15-second timing comments. A picture of a typical test set-up is depicted in Figure 1.

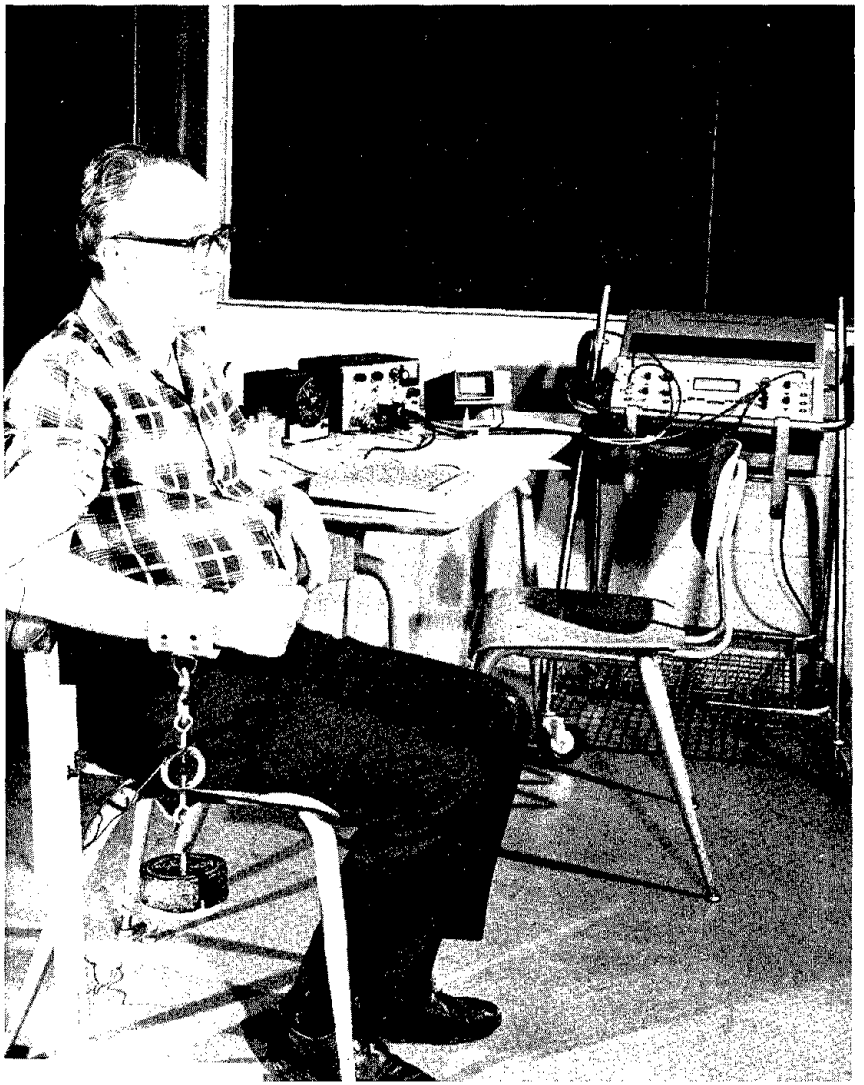


FIGURE 1. Typical test setup for EMG and tremor measurements.

Eye-Hand Coordination

As noted in earlier sections, elevated blood and urine mercury levels due to chronic exposure to inorganic mercury correlate with deteriorations in specific neuromuscular and psychomotor capabilities. These specific dysfunctions strongly indicate that more general

psychomotor skills may also be adversely affected by relatively low mercury body burdens. It was of interest to determine the effect of mercury exposure on a person's ability to repeatedly and accurately move their hand to specific locations in space. In other words, *discrete positioning eye-hand coordination* was evaluated as a function of the population parameters described in Table I, (i.e., blood and urine mercury levels, length of time of occupational exposure to mercury, height, weight, cigarette smoking rate and age).

The discrete positioning eye-hand coordination test chosen for the study was developed by Poock (1968). It was chosen due to its test-retest repeatability ($r=.89$), its correlation with other motor functions ($r=.50$), and its correlation ($r=.35$) with the performance demonstrated by industrial people on various hand motions (i.e., reaches, grasps, and moves) common to many industrial manual tasks (Poock, 1968).

The test is composed of a directionally varying maze of 119 holes (⅛ inch diameter) in an eight-inch square steel plate. The subject is asked to repeatedly insert a stylus in each hole following a sequence dictated by a painted line connecting each hole. Figure 2 depicts a person performing the test.

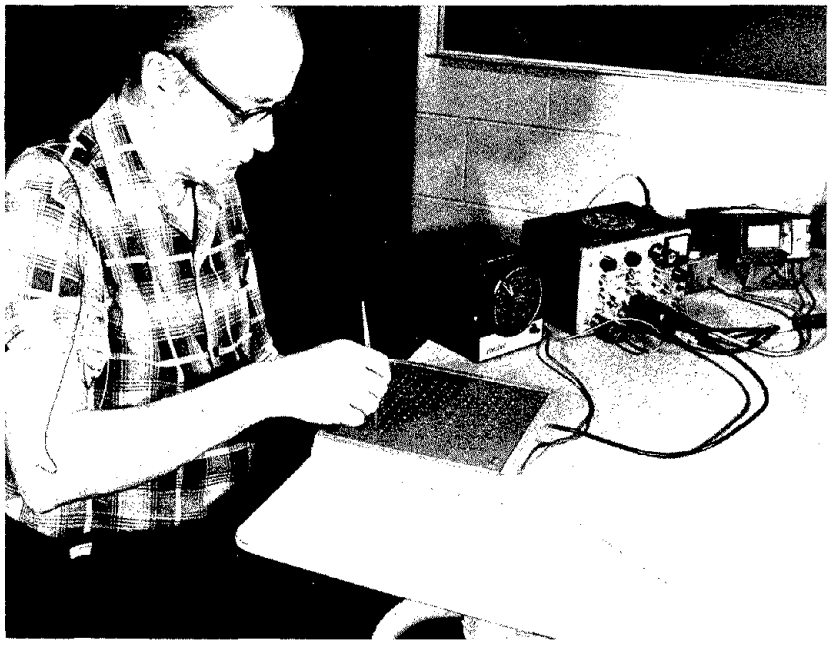


FIGURE 2. Eye hand coordination test setup.

The output from the test was recorded by two methods. An electrical stop clock (minimum reading 0.01 second) was used to measure the elapsed time from the withdrawal of the stylus from the first hole to insertion into the last hole. This measure produced the gross performance time on the test, which was the performance measure used by Poock (1968). A second performance measurement, which was developed for this study, greatly augmented the sensitivity of this test. This method utilized a second thin steel plate which was placed directly behind the first. A contact microphone attached to this plate, that was relatively acoustically isolated from the maze plate and frame, allowed the recording of the insertion of the stylus through the maze holes. To accomplish this a special signal conditioning amplifier was constructed to shape the "tapping" signals, produced by the stylus hitting the second sounding plate, into discrete changes in signal level. These were then recorded on the same Hewlett Packard FM tape recorder used to record the EMG's and tremor signals described earlier.

Procedure

The subject was acquainted with the test equipment. He then was asked to sit down and the maze plate was positioned directly in front of his body (i.e., in the mid-sagittal plane). If the subject was left handed the maze was rotated to provide the same motion pattern as for a right handed individual. He was asked to grasp the stylus about two inches from the contacting end, and, without allowing his hand to touch the maze, attempt several stylus insertions. He then was given an opportunity to move his chair forward or rearward to his own preference. Following this, he was given these test instructions:

1. Follow the painted line when moving between holes.
2. Perform the test as quickly as possible.
3. Do not skip any holes.
4. Completely insert the stylus into each hole.
5. Do not rest your hand or forearm on either the test equipment, table or arm of the chair while performing the test.

The subject then attempted four trials. After the five-minute weight holding exertion, he immediately performed a fifth trial. His performance data on both the fourth trial *before exertion* and the fifth trial immediately *after exertion* were used for this study.

Psychomotor Test Battery—
Equipment and Procedure

For the purposes of this experiment, decision time (or reaction time) is defined as that time which elapsed between the onset of a visually displayed digit and the initiation of a response as measured

by the subject's pressing a button with his finger. This definition is consistent with traditional and current experiments involving simple and choice reaction times.

For the Tests 1 through 5, the subject was seated such that he could observe a visually displayed number about 18 inches from his eyes and 1 inch by 1½ inch in size (see Figure 3).

FIGURE 3. Decision test and experimental layout.

Test No. 1: Two Choice Decision Time, Trial #1, (Figure 3). In this first test, he was told that either a number 2 or a number 3 would appear with equal probability. As soon as the number appeared and he identified it, he was to push a correspondingly numbered button located between him and a visual display. Preceding the appearance of the number, his left forefinger was placed on top of the number 2 button, his right forefinger was placed on top of the number 3 button. It thus required only the slightest movement of either finger to indicate a response to the visual stimulus. Each subject was given five trials during which no data were collected. Thereafter, he was given twenty trials with the numbers 2 and 3 randomly appearing and with an intertrial interval for all Tests 1-5 ranging from 1 to 6 seconds randomly.

Test Number 2: Two-choice Decision Time, Trial #2. Following Test Number 1, the subject was given a rest period of about 30

seconds. Thereafter, an additional 20 trials occurred as a replication to Test Number 1, with the learning trials omitted.

Test Number 3: Four-choice Decision Time, Trial #1. In this test, the subject was told that the numbers 1, 2, 3, and 4 would appear equally often and he must again push the appropriate button that corresponded to the number which appeared visually. The index and middlefinger of the right hand were placed on top of buttons 3 and 4. The subject was given five learning trials before the 20 trials during which data were collected.

Test Number 4: Four-choice Decision Time, Trial #2. After a 30-second rest period, the previous test, Number 3, was replicated with the learning trials omitted.

Test Number 5: Simple Reaction Time. To determine simple reaction time only the Number 3 appeared on the visual display but with a random inter-trial interval of up to 6 seconds. The subject placed his dominant finger on the button Number 3 and was told to press that button as soon as he saw the number appear in the visual display window. After five learning trials, 20 additional trials followed during which data was collected. This test was not replicated.

Test Number 6: Finger Tapping (Figure 4). Following Test Number 5, the subject was given a 2-minute rest period. The subject's dominant hand and arm were then rested on the table top in front of where he was sitting. With the hand and fingers in an unbended condition, the subject was able to rest his index and middle fingers on a 1½ inch diameter button. This button was raised above the plane on which the arm and hand rested about ½ inch (see Figure 4). The subject was then told that his task was to tap on the button as fast as he could, alternating between his middle and index fingers. He was asked to keep his fingers and hands as straight as possible. He was told that each trial would last about 10 seconds and that there would be several trials. Initially, he was given one trial to learn the task. After he began tapping, the recorder was turned on and data were collected for 5 seconds after which he was told to stop tapping. Data were collected for a total of three trials and he was given about 10 seconds between trials. The data consisted of a time history of tapping over the five second period. These same data were collected for all tapping tests.

Test Number 7: Toe Tapping (Right Foot) (Figure 5). Following Test Number 6, the subject rotated his chair 90° until he could comfortably rest the heels of both feet on the floor while easily reaching 2 side-by-side 1½ inch response buttons with his large toes. The buttons were elevated one inch off the floor with one quarter inch clearance between them. The subject was asked to tap the

FIGURE 4. Finger tapping.

button on the right as fast as he could with his right toe, keeping the toe as straight as possible. The left foot was allowed to be placed anywhere comfortable to the subject. Similar to Test Number 6, each trial lasted 10 seconds during which only five seconds of data were collected. Following one learning trial, he was given three subsequent data collection trials spaced about 10 seconds apart.

Test Number 8: Toe Tapping (Left Foot) (Figure 5). In the identical fashion as the previous experiment, the subject was then asked to perform the tapping tests with his left toe operating on the left button in front of him. He was free to place the foot not being used in any position comfortable to him.

Test Number 9: Toe Tapping (Alternating) (Figure 6). After about 30 seconds rest, the subject was then asked to position his feet so that he could comfortably reach the left and right buttons before him with his left and right large toes respectively. He was then asked to alternate tapping between his left and right toes as

FIGURE 5. Single foot tapping.

fast as possible for a period of 10 seconds for each trial. After one learning trial, he performed three subsequent trials with 10 second rest periods between each trial.

Test Number 10: Pencil Flipping. Using a standard unsharpened Number 2 wooden pencil, the subject was asked to use his thumb, middle and index fingers and rotate the pencil 180°. The pencil was initially placed in a vertical position. After it was rotated 180°, the subject was to tap it on the table top once. This he repeated after each 180° rotation. He was also asked to make the rotations in one direction only. The total number of rotations was 25. He was to do this as fast as possible without dropping the pencil. The time to complete 25 rotations of 180° was recorded. If the pencil

FIGURE 6. Alternating foot tapping.

was dropped, the subject picked it up and continued while the stop watch continued running.

The psychomotor test battery described above was first used in the revisit of plant Group II. The second time the psychomotor tests were used was for the first visit to plant Group III. The third time a battery of psychomotor tests was administered was in the retesting of 11 subjects from plant Group II after they had been away from the mercury exposure for from four to six months. Whenever reference is made to combined data in subsequent analyses, it is the data from plant Groups II and III which have been used. No new tests were added to the battery used in the study of plant Group III; however, there were some deletions. First, since plant

Group II data indicated very high correlations between replications of decision tasks, it was not considered necessary to repeat the two- and four-choice decision tasks. Instead of two sets of data with twenty trials per set, the plant Group III data have only one set of twenty trials for the two- and four-choice decision tasks.

RESULTS

Forearm Tremor

By performing a power spectra analysis on the forearm tremor for the first 15 seconds of the weight holding test, it was possible to quantify each worker's tremor frequency distribution and amplitude. The resulting values were then correlated (by step-wise regression) with the worker's biological data presented in Table I. This procedure indicated that the average tremor frequency increased significantly for persons having elevated urine mercury burdens, as depicted graphically in Figure 7. A similar graph results when blood mercury levels are plotted; although it is not quite as well correlated.*

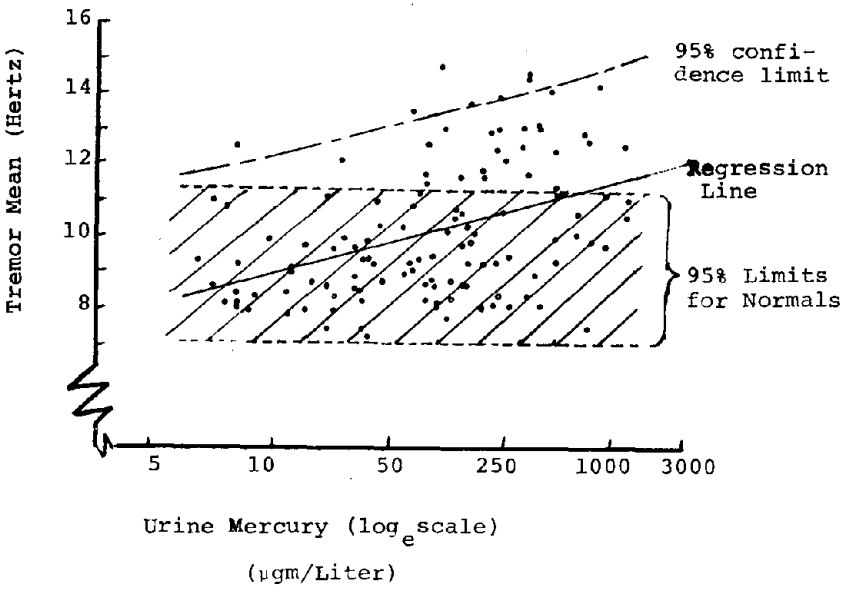
Thirty-two people were retested after being at reduced exposure for from four to six months. During this time, their urine mercury levels reduced from the initial mean level of 562 μg/L. to a mean of 347 μg/L. It was found that their mean tremor frequencies also decreased significantly from a mean of 11.4 Hz. to a more normal mean of 9.3 Hertz. This, then, indicated a reversal in the effects of mercury on tremor frequency.

Surface EMG

A power spectra analysis was also performed on the EMG's obtained from the biceps brachii of each worker during this same 15 seconds of weight holding. Once again the resulting power spectra statistical moments were regressed onto each person's biological data presented in Table I. From this, it was determined that as body mercury levels increased the bandwidth of the electromyogram was larger with both the low and high frenquency power increased. Figure 8 depicts these data as plotted against the log of the urine mercury levels.

When retesting 24 of these workers after they had been for four to six months in a greatly reduced exposure their EMG frequency bandwidths (standard deviations) were significantly reduced from 31.7 Hertz to 28.6 Hertz. This corresponded to a lowering in urine mercury levels from a mean of 599 μg/L. to a mean of 292 μg/L.

*Shashikant Kelkar, a Ph.D. candidate at the University of Michigan, is currently evaluating differences between blood and urine mercury levels as indicators of health status.



Prediction Equation for Regression Line:

$$\text{Tremor Mean (Hz)} = 7.8 + 0.53 * \text{LOG}_e(\text{Urine})$$

Correlation Coefficient = 0.45

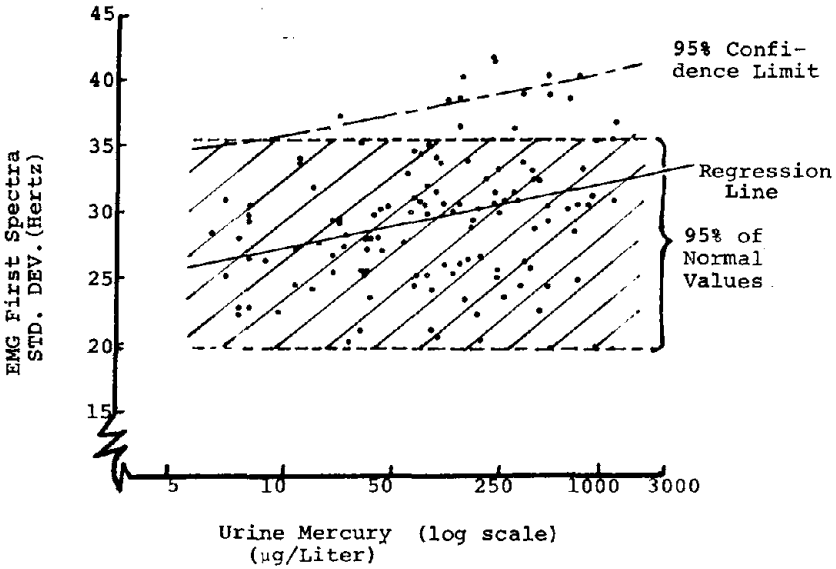
FIGURE 7. Regression of tremor frequency mean and urine mercury level in both exposed and unexposed populations.

Muscle Fatigue

Frequency shifts in the surface EMG were monitored during the five-minute weight holding test. The rate of these shifts with time was taken as the indicator of localized muscle *fatigue.* These rates for each worker were then correlated with his biological data in Table I. The results of this analysis disclosed that a greater height or weight tended to correlate with a higher rate of fatigue, but current urine or blood mercury levels did not correlate with these same fatigue rates.

Eye-Hand Coordination

The discrete positioning eye-hand coordination test consisted of 119 holes, one-eighth inch in diameter, arranged in a maze pattern in an eight-inch square steel plate. By inserting a pointed stylus in each hole, a timing impulse was obtained electronically. By continuously recording each timing impulse, it was possible to construct a hole-to-hole movement time distribution for each worker



Prediction Equation for Regression Line:

EMG First Spectra STD. DEV. (Hz) = 24.87 + 1.05* LOG_e(Urine)

Correlation Coefficient = 0.34

FIGURE 8. Regression of surface EMG bandwidth (standard deviation of power spectra) and urine mercury levels in both exposed and unexposed workers.

as they moved the stylus through the 118 discrete moves in the maze. The statistical moments describing each worker's hole-to-hole motion time distribution were regressed onto their biological data in Table I. This resulted in the finding that the workers who had elevated blood and urine mercury levels also tended to be slightly more erratic in their movements between the 119 holes. This effect was slightly more pronounced when these same workers had preceded the test with the physical activity of holding a 15-pound weight in the dominant hand. Figure 9 depicts the standard deviations of the motion times in the eye-hand coordination test as plotted against the log of urine mercury levels.

For the retest situation, the reduced exposure period decreased their mean urine mercury levels from 599 µg/L. to 292 µg/L. and also resulted in improving their eye-hand coordination. This improvement consisted of about a 5% faster mean time with 5% lower standard deviation.

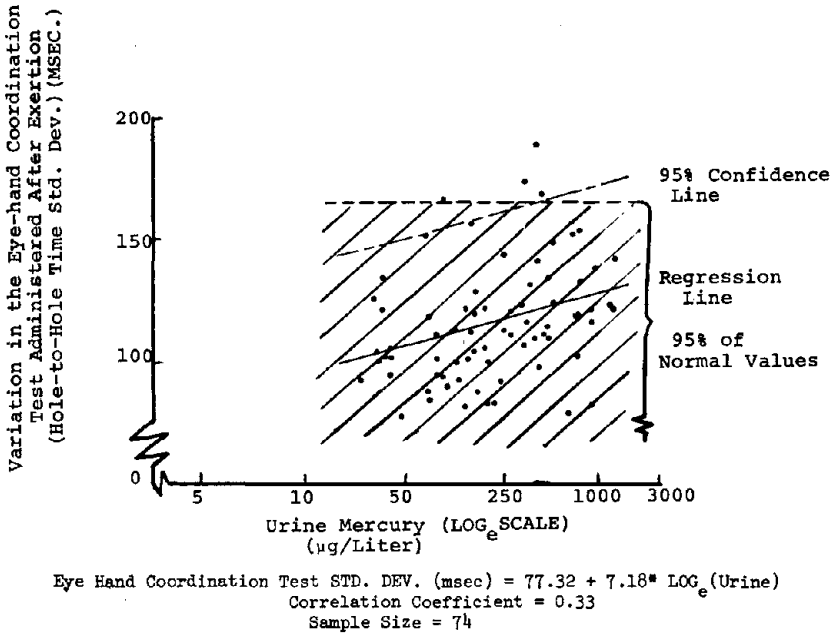


FIGURE 9. Regression of eye-hand coordination test - variability in performance and urine mercury levels in exposed population.

Tapping Performance

One of the secondary psychomotor tests that became most significantly correlated with increased mercury body burdens was the worker's tapping performance. Rates for finger tapping, right foot tapping, left foot tapping, and alternating foot toe tapping were all significantly decreased in those people having elevated mercury burdens. This result was based on the testing of the 87 workers comprising plant groups II and III, as depicted earlier in Table I. When 11 of the workers having initially high urine mercury levels of 596 µg/Liter were removed from exposure for from four to six months, their mean urine mercury levels decreased to 95 µg/Liter. Their tapping rates increased an average of about 40 percent showing a considerable reversal effect. The dramatic reversal in right toe tapping was particularly striking for all 11 workers on the retest (Figure 10).

Other Psychomotor Test Results

It was also found that those people having elevated mercury burdens tended to be slower in responding to simple forced-choice tasks and simple reaction time tests. Unfortunately, a lack of consistency between people in these tests resulted in the statistical significance tests being inconclusive. It would appear though that further research is warranted.

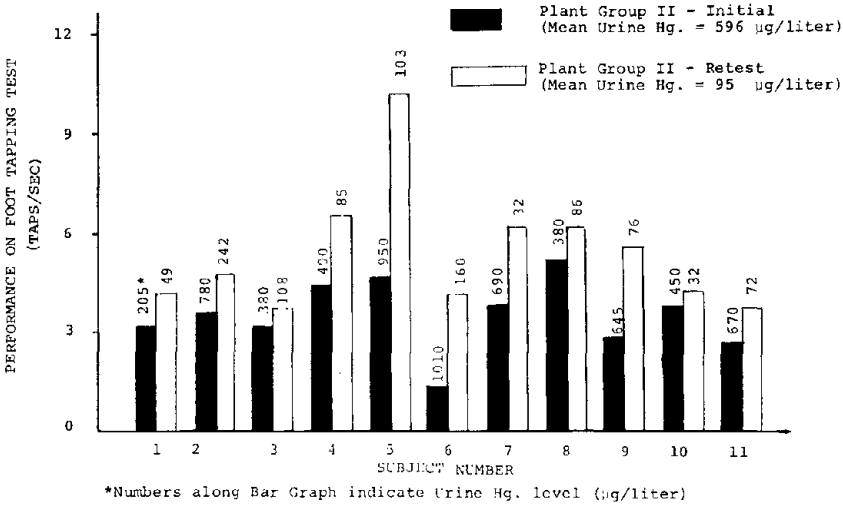


FIGURE 10. Test/Retest comparison of right foot tapping rates.

Clinical Neurological Findings

The 32 workers who volunteered for a clinical neurological examination provided data which could be compared to other clinically based studies. Most of these 32 workers had been exposed to mercury in the past or present. Of these, eleven who were found to have very high mercury burdens were removed from exposure for from four to six months, and were then re-examined by the same neurologist. The most positive results were found when testing for dermatographia (skin writing) and eye-lid fasciculations. The presence of these abnormalities in those people having currently elevated urine and blood mercury levels was significant. However, these signs did *not* remit in those people after removal from exposure in spite of the fact that their mean urine mercury levels decreased from an initial value of 596 µg/L. to 95 µg/L.

Cerebellar functioning and involuntary movements were evaluated by observing intention tremors, dysmetria and dysdiadokokinesia. Especially noted were tremors about the mouth, tongue, and other facial and head tremors as these are the more classical symptoms of mercury poisoning. Of the 32 people initially tested, 12 had at least one type of these abnormalities. In the retest group, four who had the tremors initially showed complete clearing of the cerebellar deficits.

CONCLUSIONS AND RECOMMENDATIONS

The following conclusions and associated recommendations have resulted from this investigation:

Conclusion 1

The following neuromuscular and psychomotor functions are significantly correlated with varying levels of blood and urine mercury burdens:

A. The frequency of forearm *tremor* increases with elevated blood and urine mercury levels.
B. The bandwidth of the *surface EMG* increases with elevated blood and urine mercury levels.
C. The speed of hand and foot *tapping* decreases with elevated blood and urine mercury levels.
D. The ability to consistently move the hand quickly to discrete positions *(eye-hand coordination)* becomes more varied with elevated blood and urine mercury levels.

Recommendation 1:

The above functional measures should be further developed and evaluated in field tests to routinely assess the health status for individuals repeatedly exposed to inorganic mercury.

Conclusion 2

The retesting of the above measures of tremor, EMG, tapping ability, eye-hand coordination, and simple response time tests were performed after a four- to six-month period of reduced exposure. This resulted in the conclusion that these decrements in functional capabilities were reversible in the workers removed from exposure (mean urine mercury level about 600 μg/Liter).

Recommendation 2:

This conclusion further substantiates the feasibility of these subclinical functional measures as having a potential utility for assuring the health of a person who is occupationally exposed to inorganic mercury. It is therefore proposed that a longitudinal demonstration project be established to assess the utility of these tests in controlling industrial mercury intoxication.

Conclusion 3

Based on Conclusion 1, it is postulated that the cerebellum is probably the neurological structure most associated with chronic inorganic mercury loading. This is consistent with other tissue distribution studies. The clinical evidence of an increased prevalence of eyelid fasciculation and dermatographia among workers demonstrating relatively high mercury body burdens (averaging 787 μg/Liter of urine) suggests a more diffuse neurological involvement. This same group of workers failed to demonstrate remission of these findings after four to six months removal from exposure, wherein the decrease in burden reached 95 μg/Liter in urine.

Recommendation 3:

The development and evaluation of additional quantitative

neurological tests should be promoted to better establish the pathology of long term chronic exposures to inorganic mercury.

Conclusion 4

It is concluded that the simple variable, "length of time exposed to occupational mercury", is not a good predictor of the effects on a person's health, even when combined with knowledge of the person's current urine and blood mercury burdens.

Recommendation 4:

Future studies of this type should include either routine atmospheric or biological sampling records to provide detailed information regarding the worker's history of exposure.

Conclusion 5

It would appear that health status testing of behavioral and neurological measures should be considered for any person routinely exposed to mercury when their concentrations exceed approximately 100 μg/Liter. In other words, this could be considered the *Health Evaluation Action Level* for mercury. It was at this level that a significant number of people had changes in their neuromuscular indices of tremor and EMG. It should also be noted that the psychomotor tests were shown to be affected at higher urine levels; this could be due to the higher variability in the population's responses on these tests.

Recommendation 5:

The large individual variations in response to given levels of urine and blood mercury burdens (documented in this and other studies) suggest that routine health evaluations be instituted and evaluated to determine future alternatives in protecting the individual's health. Based on the data of this study, a biologically based action level of 100 μg of mercury per liter of urine is recommended. On exceeding this level, a person would be routinely examined to determine if any deterioration in his health status has taken place. The outcome of these functionally based health evaluations would be of value in determining if the individual should be removed from exposure, or simply be retested at more frequent intervals. The basis for this latter more important decision now requires further investigation.

Conclusion 6

It is concluded from the standardized five-minute weight holding test that localized muscle fatigue per se, as determined by EMG frequency changes is probably not associated with increased mercury body burdens. It must be noted, however, that the performance on the eye-hand coordination test was more adversely affected by

mercury when the test was performed after the standard exertion period.

Recommendation 6:

A standardized exertion may be helpful in differentiating the effects of mercury on certain psychomotor test parameters and, therefore, its use in the development of better performance testing should be pursued. Furthermore, additional evaluations of muscle fatigue as affected by elevated mercury body burdens should be undertaken using measures other than the EMG frequency shift. For example, abnormal strength decrements may be found after a submaximal exertion by those people having elevated body burdens if denervation or decreased muscle fiber contractibility has occurred due to the mercury.

Conclusion 7

It is concluded at this time that *neither* blood nor urinary mercury level is better correlated with a person's functional changes, especially in the tremor and EMG. The fact that blood and urinary mercury burdens were correlated with $r = .79$, is similar to other reports in the literature. This indicates a potential for either one to become the dominant correlator with health status measures. This did not happen, however, and hence this conclusion.

Recommendation 7:

In performing this study, it was not possible to control the sampling times during the day or week, although they were reasonably random in time. Hence, it is recommended that future studies of this type attempt to be more specific as to when the samples are taken. This could possibly increase the correlations with the health status indicators and help determine whether urine or blood measures should be favored.

SUMMARY

This is primarily a cross-sectional study involving both neurological and psychomotor skill assessments of 142 male industrial workers from four different plants. None of the workers reported being clinically ill from mercury at the time of the study. Of the 142 workers in the study, 77 were routinely exposed to inorganic mercury vapor in the course of their jobs, while the remaining 65 served as unexposed controls drawn from the same plants.

The primary health status indicators employed in the study were: forearm tremor, surface electromyography and discrete positioning eye-hand coordination. The following specific changes in these were significantly correlated with elevated levels of both blood and urine mercury burdens:

- Mean frequency of forearm tremor increased.

- Surface EMG bandwidth increased.
- Discrete positioning hand motions became more erratic.

When the workers displaying these effects were placed in a reduced exposure for from four to six months the responses returned to more normal population levels.

Some psychomotor tests were also included in the study. These indicated that finger and toe tapping rates were significantly decreased in workers having elevated blood and/or urine mercury levels. When workers with these adverse findings were removed from exposure for four to six months their tapping rates returned to more normal levels. Other additional psychomotor tests also suggest that forced-choice decision times could be sensitive to chronic exposure to inorganic mercury.

Thirty-two of the workers volunteered for a clinical neurological examination. Though this was not a large enough sample to perform adequate statistical analysis, it was determined that in a group of workers having elevated urine mercury burdens (averaging 787 μg/L. of urine), the prevalence of both eyelid fasciculations and dermatographia was much higher than expected in a normal population. It was also found that the symptoms did not remit after these people were removed from exposure for from four to six months.

The statistical findings of the study are used as the basis for a recommendation that specific quantitative neurological and psychomotor tests, such as those described in this study, could be utilized and evaluated in various industrial medical departments to routinely assess the health of workers exposed to inorganic mercury. It is shown that such a health evaluation program should be developed due to the large variation in individual tolerance to mercury documented in this study.

This study has greatly contributed to the knowledge required to define the mercury control procedures which are necessary and reasonable. The challenge now is to further develop and implement health evaluation tests that can be routinely administered when mercury intoxication is suspected. With these tests it is proposed that effects due to toxic materials other than mercury could be controlled in a manner that would provide the maximum protection of the individual's health and safety.

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PERIPHERAL NERVOUS SYSTEM IN LEAD EXPOSED WORKERS

Anna Maria Seppäläinen, M.D.

Last year we published a paper[1] on subclinical lead neuropathy in cases with lead poisoning or excessive absorption (viz. lead in blood usually 80 μg/100 ml or more). Among these patients of whom 31 men had clinical lead poisoning and eight men excessive or increased absorption at the time of the study, we could very often show neurophysiological signs of neuropathy. Twenty of our subjects had a previous episode of lead poisoning. About half (17) of the cases came from two lead scrap smelters, seven from two storage-battery factories, five were engaged in ship-breaking and the last five in welding and repair of sulphuric acid containers or were mixing red lead into crystal glass mass. The conditions had been poor in lead scrap smelters and in one of the accumulator factories; the concentration of lead in the air being some 10 to 20 times the threshold limit value of 0.2 mg/m^3; of the other working places we do not have regular measurements. Use of personal protective equipment was common.

A detailed electroneuromyographic examination was carried out on all these subjects as well as on age-matched control persons. This consisted of the measuring of maximal motor conduction velocities (MCV) of median, ulnar and deep peroneal nerves, the distal latency of the median nerve and the conduction velocity of slower motor fibers (CVSF) of the ulnar nerve. All the subjects with lead exposure had a needle electromyographic examination of four to eight muscles.

The subjects were given a neurophysiological score based on neurophysiological findings in such a way that a normal person would get a score of 0. Fig. 1. shows the distribution of NF scores in lead workers A with a first episode of poisoning (clinical classes I-III) or excessive absorption (classes IV-V) and lead workers B subjects with one or more previous episodes of lead poisoning.

There is tendency among lead workers A with the higher scores to be in the classes with more severe poisoning, but no such relationship exists among lead workers B, who tend to have higher scores as a group. The lowest NF scores in high poisoning classes are mainly from workers in the PVC plastics plant with a rather short but intensive exposure; they had mainly abdominal symptoms.

The conduction velocities of the lead workers showed a statistically significant difference when compared to age-matched controls (Table I). The variables which best separated lead workers from

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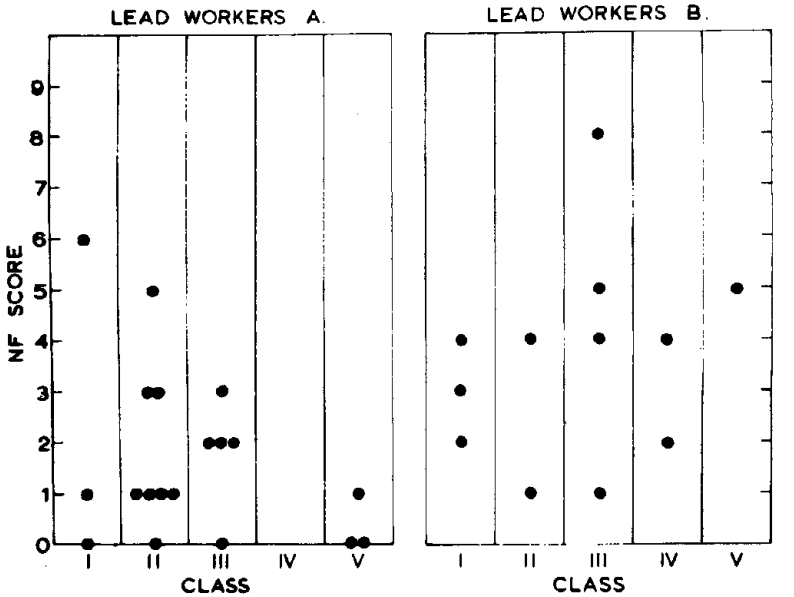

FIGURE 1. NF score and present lead effect.
Lead workers A = first episode of excessive absorption or poisoning.
Lead workers B = subjects with one or more previous episodes of lead poisoning.

Table I. NERVE CONDUCTION VELOCITY FINDINGS IN 39 LEAD WORKERS AND 39 AGE-MATCHED CONTROLS

Variable	Mean ± S.D. Exposed	Controls	T	P
Age (yr.)	36.1 ± 11.7	35.5 ± 11.5		
MCV (m/s) of the median nerve	55.3 ± 3.7	58.6 ± 3.7	4.00	< 0.001
Distal latency (ms) of the median nerve	4.6 ± 0.6	4.0 ± 0.5	5.10	< 0.001
MCV (m/s) of the ulnar nerve	54.0 ± 5.2	56.7 ± 3.4	2.84	< 0.01
CVSF (m/s) of the ulnar nerve[1]	39.0 ± 8.0	46.6 ± 3.7	4.90	< 0.001
MCV (m/s) of the lateral popliteal nerve	48.4 ± 5.1	50.7 ± 3.6	2.30	< 0.025

[1]Data from 32 exposed subjects and 32 controls.

controls were the CVSF of the ulnar nerve and the distal latency of the median nerve (discriminant function analysis). These findings are consistent with slight damage in the peripheral nerves, most likely segmental demyelination, which has been described in experimental animals exposed to lead (Fullerton 1966[2], Schlaepfer 1969[3]).

There was no relationship between the NF score and any isolated laboratory test, such as lead in blood, urinary ALA or hemoglobin, which might suggest that the mechanism of nervous damage in lead poisoning is different from that of hematopoietic effect. Thus, we cannot feel sure of preserving the nervous system intact, even if the blood-forming system is unimpaired.

LEAD EXPOSURE AND PERIPHERAL NERVES

This year we did a further study of lead exposure and the peripheral nervous system. We were especially interested in seeing if any effect upon nerves could be shown in workers with lead exposure but without episodes of clinical poisoning. We were able to study 28 employees of a storage-battery factory, where the workers' exposure has been monitored through frequent medical check-ups. For example, the lead in blood has been measured four times a year for the last three or four years. We chose the workers whose lead in blood never exceeded 70 μg/100 ml and in whom no clinical signs of lead poisoning had been noticed during the period of employment. We took a careful history of previous jobs to reveal possible previous lead exposure. Fifteen of the workers had worked in lead exposure for less than four years, but all had had at least one year's exposure. Only six men had lead exposure for more than six years, the longest exposure being 23 years. Most of these employees were now working in cleaner areas of the storage-battery plant, the lead concentration in the workroom air being under normal conditions less than the threshold limit value of 0.2 mg/m^3. They were working with the electrical parts, assembling the batteries or working in the warehouse.

The lead workers as well as an equal number of age and sex matched control persons were given several nerve conduction velocity studies, namely the MCV of the median, ulnar, deep peroneal and posterior tibial nerves, CVSF of the ulnar nerve and sensory conduction velocity (SCV) of the median and ulnar nerves. (All the controls did not have SCVs).

RESULTS

As with the findings in cases with lead poisoning we could also show statistically significant differences in certain conduction velocities when compared to controls. MCVs of the ulnar and median nerves were slower among lead-exposed, $p < 0.01$, Fig. 2. In the

MCVs of the nerves in the lower extremities no such difference could be detected, Fig. 3. This is also different than what is found in workers with solvent exposure; CS_2 exposed workers had significant slowing in the leg nerves, not in the MCVs of arm nerves. Sensory conduction velocities were also of similar range in exposed and controls, Fig. 4. (In regard to the SCV of the median nerve one should notice that the number of controls is much less than that of the exposed.)

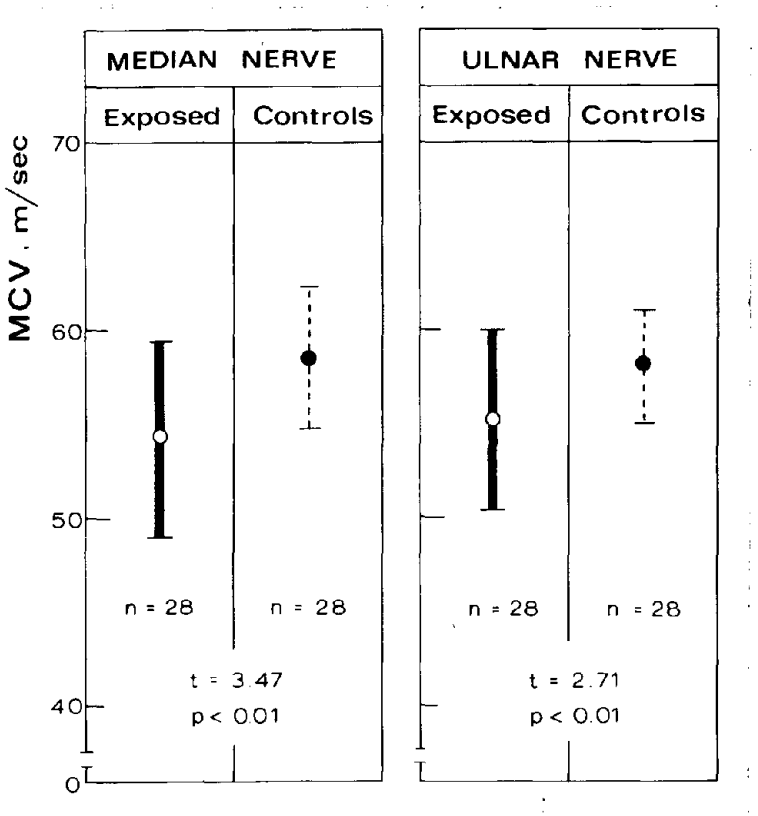

FIGURE 2. The means ± SD of the maximal motor conduction velocities (MCV) of the median and ulnar nerves among lead exposed and controls.

The CVSF of the ulnar nerve is of special interest; this was an important factor in separating lead poisoning cases from the controls. In regard to the CVSF of the ulnar nerve, the difference between lead-exposed and controls was statistically highly significant ($p < 0.001$). Fig. 5 shows a box diagram of a scattering of individual CVSF findings in lead-exposed and age-matched controls and the patients noted in our lead poisoning paper and their controls. Our present lead-exposed workers are between the poisoning cases

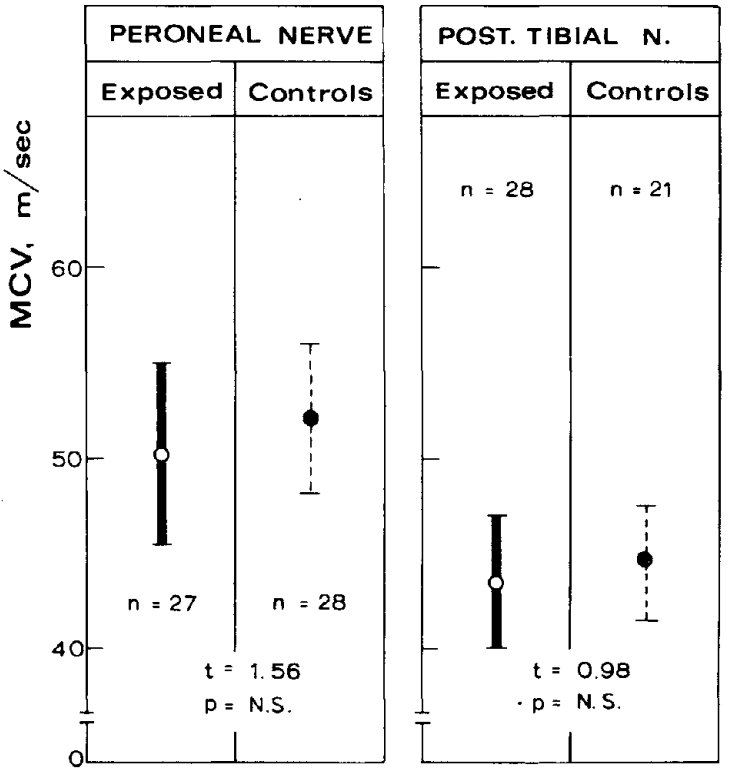

FIGURE 3. The means ± SD of MCVs of the deep peroneal and posterior tibial nerves among lead exposed and controls.

and the controls as can be seen from the general distribution and the means.

To study the dose-response (dose-effect) relationship I looked for the range of lead in blood values of individual workers and abnormal conduction velocity findings. Ten out of 28 lead-exposed had slight slowing of some conduction velocities and four had borderline findings. Neuropathic signs need a long time to develop and they also often stay long even when the level of lead in blood goes down. Therefore I thought it more appropriate to take the range of lead in blood values from 1969 to the present (the workers with shorter exposure had, of course, a shorter follow-up time). Fig. 6. shows the lead in blood variations of individual subjects. The males with lead in blood at times up to 70 μg/100 ml had almost all abnormal findings. Individual variations in susceptibility exist: one subject with normal CVs and lead in blood at times up to 70 μg, had lead exposure for 19 years. There were also several cases with abnormal findings in the 60 μg range.

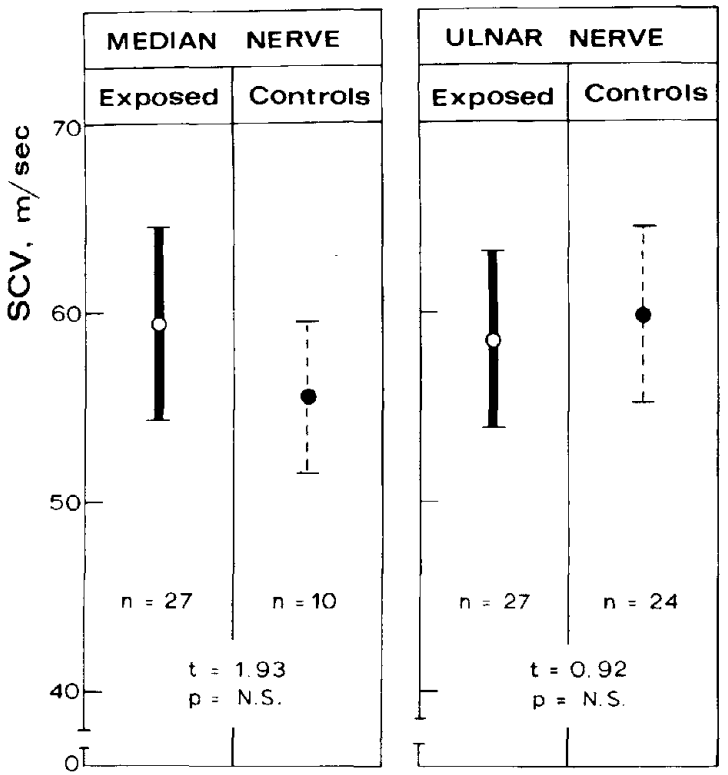

FIGURE 4. The means ± SD of sensory conduction velocities (SCV) of the median and ulnar nerves among lead exposed and controls.

Females seem to be more susceptible than males, at least, abnormal findings are seen with lower blood concentrations in this limited series.

CONCLUSIONS

Lead in blood in concentrations less than 80 μg/100 ml can exert a toxic effect on the peripheral nerves. Although the change is slight, it represents an effect upon the nervous system. I thought that the effect would be a reversible one, but I am not as certain any more. Most of the higher values in the diagram were measured in 1969 or 1970. Now in 1973 these people still have slight abnormalities, although the lead in blood is now close to 50 μg/100 ml. Body burden may play a part here.

A few of the workers complained, on confrontation, of paresthetic feelings and tiredness, especially of the legs. However, none has needed sick leave for these symptoms. I do not want to call these findings lead poisoning, but I am convinced that they are an indica-

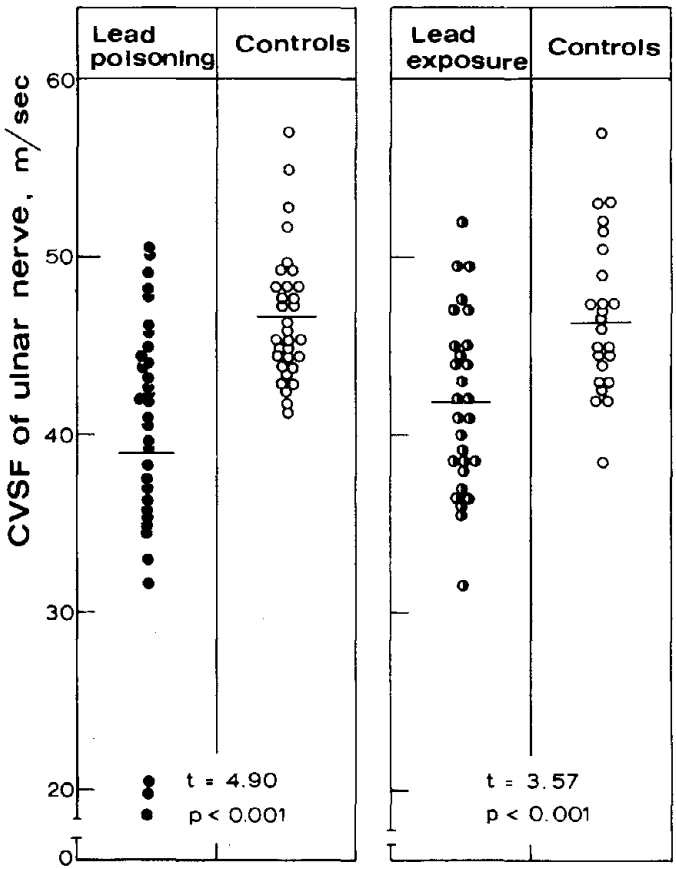

FIGURE 5. Conduction velocity of the slower motor fibers (CVSF) of the ulnar nerve among 32 patients with lead poisoning and age-matched controls + CVSF of the ulnar nerve among 28 workers with lead exposure and age-matched controls. Horizontal lines represent the means.

tor of toxicity; they indicate an effect (and possibly a harmful one) of lead upon the peripheral nerves.

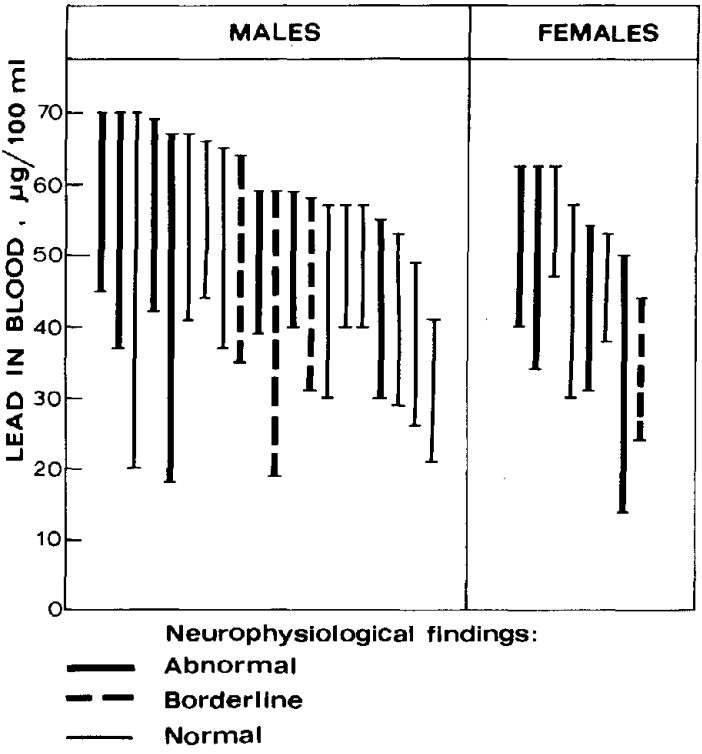

FIGURE 6. The range of lead in blood during last four years in lead-exposed workers and neurophysiological findings.

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EVALUATION OF BEHAVIORAL FUNCTIONS IN WORKERS EXPOSED TO LEAD

Ben B. Morgan, Jr., Ph.D. and John D. Repko, Ph.D.

Many of the contaminants which are currently under investigation by behavioral toxicologists are recent bi-products of technology and our modern society. However, the potential hazards associated with the use of lead have been recognized for centuries; the Roman author Horace called it a "pestilential and noxious metal" (see Hoover & Hoover, 1950). This awareness, that lead is potentially dangerous, was accelerated during the 1800's by the findings that lead could be absorbed through the skin and by inhalation, as well as by ingestion. Publications during this period described certain of the health and symptomatological effects of lead exposure (see Hunter, 1969, p. 330; Garrison, 1929, p. 440). Today, literally thousands of articles are available concerning the use of lead and its associated health hazards (Repko, Corson, Morgan, in preparation).

The majority of the available literature, however, deals with the etiology of plumbism and the physiological, biomedical, pharmacological, and symptomatological effects of lead. In addition, most studies have been concerned with small groups of subjects, typically those with acute lead poisoning. Most studies are descriptive in nature and deal only with the symptoms or clinical signs associated with lead absorption. Very few studies have systematically investigated the effects of lead on human performance or psychological processes; few have been concerned with the effects of long-term, low-level exposures to lead in adults; and few have measured large samples of workers or attempted to relate body-burden of lead to the behavioral functions of adult workers.

Thus, the primary purpose of the current study is to provide definitive data concerning the effects of inorganic lead on the behavioral functions(performance) of workers exposed to lead. In addition, the study has been designed to identify possible behavioral tests which may be used as early-warning indicators of the effects of occupational exposure to lead, to provide definitive data from a large sample of adults concerning the relationships among the five most common clinical indices of body-burden of lead, and to determine the relationships among body-burden of lead and various

This is a preliminary report of a study performed by the University of Louisville, Performance Research Laboratory under Contract HSM 99-72-123 for the National Institute for Occupational Safety and Health. NIOSH Project Officers were Barry L. Johnson and Charles Xintaras.

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aspects of performance, particularly at low-levels of blood lead.

The research reported here is scheduled to be conducted over an 18-month period from 29 June 1972 through 31 December 1973. At the completion of the study, performance-test measurements and clinical determinations will have been obtained individually for at least 300 lead-exposed and 100 non-exposed (control) workers. At the time of this writing data collection and analysis is only about 50% completed. Thus, the major purpose of this paper is to describe the test battery, the performance measures, the testing procedures, and the data analyses which are being employed in this study; later publications will present the complete results and conclusions of the study.

METHOD

Subjects

As indicated above, the subjects for this study are to include at least 300 workers who have been exposed to inorganic lead at their job sites (experimental subjects), and at least 100 workers who have not been exposed to lead (control subjects). If possible, all subjects are to be drawn from the same or similar industries and the control group is to be matched to the experimentals in terms of age, sex, work histories, education, etc.

As of this date, 195 exposed workers (including five pilot subjects) and five control subjects have been tested. These individuals were volunteers from among workers in two automobile storage battery manufacturing plants. They were selected strictly on a voluntary basis, and each subject was paid for his participation in the study.

General Procedure

A total of 12 behavioral tests and a comprehensive personal-data questionnaire (not discussed in this report) have been selected for use in this research. Each instrument was chosen on the basis of its potential usefulness in measuring a behavioral function which might be expected to change as a result of increases in the body-burden of lead. A review of the literature dealing with the behavioral and biologic effects of lead provided a list of symptoms which typically occur in cases of lead exposure (Repko, *et al.*, in preparation). Tasks which appear to be sensitive to such symptomatological changes were then selected for use in the test battery.

In the data collection which has been completed, the performance testing was conducted (in, or near an entrance to, the plant) within space provided by the company in which the subjects worked. On the day prior to testing, each subject was given a copy of the personal-data questionnaire, which was to be filled out prior to the time of testing, and a plastic container, in which he was to collect a urine

sample (first voiding of the day) to be returned to the experimenters at the time of testing. Five subjects were scheduled to report for testing at the same time and the tests were scheduled so that all five individuals could be tested concurrently; about two hours were alloted for the testing of each group of subjects, and between two and four groups were tested during each day of testing. During the testing period each subject was required to complete the necessary consent and release forms. Performance testing was then conducted, and a blood sample was collected in a 10 ml heparinized, lead-free Vacutainer. Detailed descriptions of the behavioral tests and performance measures are given in the following sections.

Behavioral Tests

The core of the test battery consists of five tasks from the multiple-task performance battery (MTPB) of the Performance Research Laboratory (see Alluisi, 1969; Alluisi & Chiles, 1967; Chiles, Alluisi, & Adams, 1968; Morgan & Alluisi, 1972). These tasks provide measurements of watchkeeping, vigilance, and attentive functions, sensory-perceptual functions, memory functions, and time-sharing functions. For the purpose of the current research project, the use of the MTPB was modified so that reliable measurements could be obtained during a 1-hr. period of testing; thus, about half of the total testing period was required for the performance of these tasks.

In addition to the functions assessed with MTPB tasks, other psychological, sensory, and psychomotor functions were assessed by the use of tests of the following performances: (*a*) visual acuity, (*b*) auditory acuity, (*c*) tremor, (*d*) muscular strength, endurance and recovery, (*e*) eye-hand coordination, (*f*) immediate recall (memory), and (*g*) mood or affect. These seven tasks were scheduled so that each of five subjects could be tested on each test during the second hour of the testing period. Table I provides a summary of all 12 tests in terms of the functional category assessed by the test, the specific name of the test, and the approximate time required for its administration.

Tasks of the MTPB. — The MTPB tasks used in this study are essentially identical to those employed previously in investigations of the effects of alcohol and altitude on behavior and in the selection of air traffic controller trainees (cf. Chiles & Jennings, 1970; Jennings, Chiles, & West, 1972). The tasks are displayed on each of five identical operator panels (one for each member of a 5-man worker group) similar to the one shown schematically in Figure 1. All tasks except the Code-lock solving are used in the current research.

The first of the watchkeeping tasks is presented by a pair of warning-lights, one green and one red, located on the extreme left

Table I. SUMMARY OF TESTS INCLUDED IN THE COMPREHENSIVE BEHAVIORAL TEST BATTERY

Test area	Specific instrument	Functional category tested	Time required
Signal detection	Warning-lights, blinking-lights, and probability monitoring (MTPB)	Watchkeeping vigilance, and Attentive	10 min.
Pattern discrimination	Target identification (MTPB)	Sensory-perceptual	20 min.
Mental arithmetic	Arithmetic computations (MTPB)	Cognitive and memory	20 min.
Visual acuity	Bausch & Lomb Ortho-Rater	Sensory	5 min.
Auditory acuity	Maico audiometer	Sensory	10 min.
Tremor	SAM hand steadiness test	Psycho-physiological	5 min.
Muscular strength, endurance, & recovery	SER apparatus	Psycho-physiological	10 min.
Eye-hand coordination	Michigan eye-hand coordination test	Psychomotor	5 min.
Immediate recall (memory)	Digit-span	Cognitive	5 min.
Subjective feelings	MAACL	Psychological	5 min.

of the panel. This task requires that the worker respond to the lighting of a red light or the extinguishing of a green light. Located on the extreme right of the panel is a pair of vertically arranged amber lights which flash alternately at an over-all rate of two flashes per second. This task, blinking-lights monitoring, requires that the subject respond to a stop of the alternation of the two amber lights by pressing the button underneath the two lights. The third watchkeeping task, probability monitoring, is displayed along the top of the operator's panel and consists of four semi-circular scales, each with a pointer which normally rests at zero. The critical signal is an introduction of a bias which shifts one of the pointers by approximately 20 scale units (1/5 of the scale) to the right or to the left.

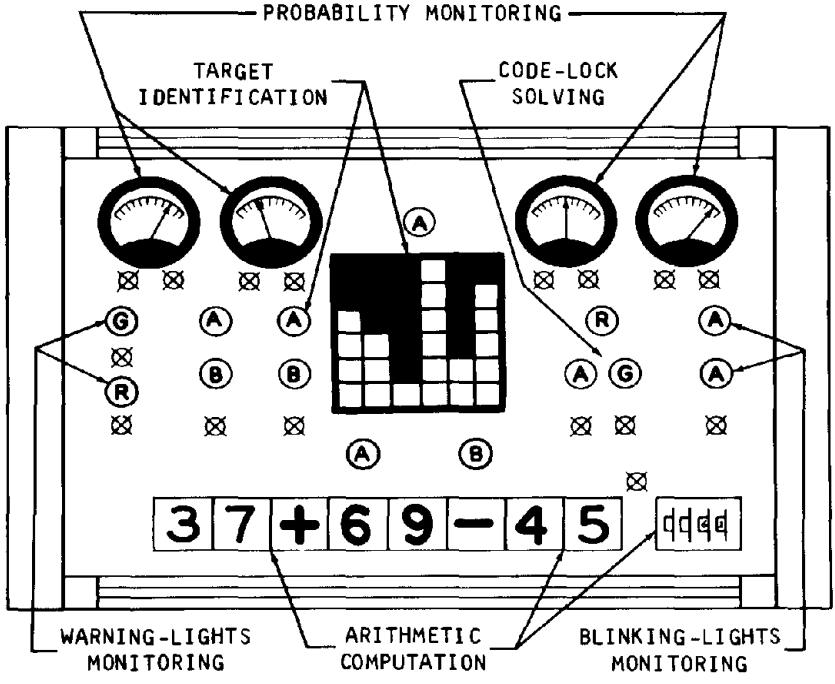

FIGURE 1. Schematic diagram of the front view of an MTPB operator panel. Letters in circles represent indicator lights, A—amber, B—blue, G—green, and R—red; the smaller circles with crossing diagonals represent push buttons.

The worker is required to detect this shift and press the appropriate button under the meter in question. Critical signals are presented at an overall rate of 72 signals per hour for each of these watchkeeping tasks. Inter-signal intervals are scheduled randomly and independently for each task .

The target identification task (TID), is presented to the worker by a 4-inch square array of 36 close-butted lights which form a 6 by 6 matrix used to present "metric histoforms." Each problem consists of the sequential presentation of two metric histoforms for 5 and 2 sec., respectively. The worker is required to report whether the two histoforms are the same or different; he responds by pressing the appropriate push button located to the left of the TID display. Knowledge of results is provided by a blue indicator light which is illuminated above the correct response button just prior to the presentation of the next problem. The task is force-paced at a rate of two problems per minute. An amber light on each panel provides a 30-sec. warning or "ready" signal prior to the beginning of the first problem.

The arithmetic computations (MATH) task requires that a

worker add two, 2-digit numbers and then subtract from that sum a third 2-digit number. The answer is indicated by manipulation of four decade thumb switches, located immediately to the right of the numerical indicators, and a push button just to the left and slightly above the switches. If the answer is correct, a blue indicator light is lit as the problem is removed and prior to the presentation of the next problem. The task is force-paced at a rate of three problems per minute during the task presentation. The beginning of this task presentation is also signaled by an amber light wihch is lighted 30 sec. prior to the first problem.

In this modified version of the MTPB, workers are required to time-share the various tasks so that they are responsible all of the time for the three watchkeeping tasks (warning-lights, blinking-lights and probability monitoring), but only part of the time for each of the other tasks (target identification and arithmetic computations). Thus, the relative demands on performance during a 1-hr. period are varied from low to medium to high depending on whether the watchkeeping tasks are presented alone or with one of the other tasks.

The basic 1-hr. task program is shown in Table II. As indicated, there are 5 min. of preliminary instructions about the tasks of the

Table II.
BASIC 1-HOUR TASK-PERFORMANCE SCHEDULE

Performance task	5-Minute interval in each 1-hour period											
	1	2	3	4	5	6	7	8	9	10	11	12
Blinking-lights monitoring	(Instructions)	X	X	X	X	X	(Instructions)	X	X	X	X	X
Warning-lights monitoring		X	X	X	X	X		X	X	X	X	X
Probability monitoring		X	X	X	X	X		X	X	X	X	X
Target identification			X	X	X	X						
Arithmetic computations								X	X	X	X	X
Level of demand		Low	Med.	Med.	Med.	Med.		Low	High	High	High	High

MTPB, followed by 5 min. of low-demand performance (monitoring tasks only) and 20 min. of medium demand performance (monitoring and target identification). This sequence of testing is then repeated for a second 25 min. in which the arithmetic computations task is used in place of the target identification task so as to provide for 20 min. of high-demand performance.

Visual acuity test. — The near and far visual acuity of each

worker is determined separately for both right and left eyes. As shown in Figure 2, these measures are obtained individually for each subject by means of a Bausch and Lomb Ortho-Rater, Type 71-21-31. Tests of near acuity of the right eye, near acuity of the left eye, far acuity of the right eye, and far acuity of the left eye (Ortho-Rater slides N-2, N-3, F-4, and F-5, respectively) are counterbalanced in order to control for effects due to practice. Standard procedures for use of the Ortho-Rater are employed throughout this test. Since this is a test of absolute visual acuity, testing is performed without visual correction. The average duration of this test is approximately 2 min.

FIGURE 2. Visual acuity test.

Auditory acuity test. — Two indications of auditory acuity are determined for each worker. Initial threshold values are acquired by means of a Maico Audiometer, Model F-1, for both left and right ears at frequencies of 500, 1000, 2000, 4000, and 8000 Hz (the earphones are "Calibrated Audiometric Headset Noise Barriers", Model M-7, which are fitted with Maico, Circumaural, Air Seal cushions to attenuate the ambient noise levels of the testing situation). A single tone-decay test is then made on the ear using the frequency at which the highest threshold value is recorded. As shown in Figure 3, these tests are made individually for each subject using the standard procedures for audiometric testing.

Tremor test. — Tremor is measured in conjunction with muscular strength, endurance and recovery in the following manner: tremor

FIGURE 3. Auditory acuity test.

is tested in both the preferred and non-preferred hands; strength, endurance, and recovery (SER) are tested for the preferred hand only; finally, an additional tremor test is performed with each hand. Both tasks are performed at a single testing station and as little time as possible is allowed to elapse between tasks.

The test of tremor is similar, if not identical, to the SAM Arm-Hand Steadiness Test developed by the Department of Psychology, AAF School of Aviation Medicine (see Melton, 1947, pp. 501-557). The basic dimensions of the apparatus, including stylus and target, were adapted from Model CM103A4 of the SAM Arm-Hand Steadiness test, and the procedures in administering the test are the same as those employed by Melton (1947).

As shown in Figure 4 the worker is seated in the chair and positioned with his preferred shoulder directly in front of the apparatus so that he has room to fully extend his preferred arm. He is required to insert the stylus in a hole ¼ in. in diameter and hold it there for 1 min. This procedure is repeated for the non-preferred hand, at the end of which the worker performs the SER tasks. Immediately upon the cessation of the SER tasks, the tremor task is repeated in exactly the manner and order (preferred hand, non-preferred hand) in which it was performed before.

Strength, endurance, and recovery task. — The apparatus for this task is a portable ergometric system which provides for a dynamic input from the worker and a dual output of information. This system is one of a series of similar devices used in the research

FIGURE 4. Tremor test.

of Dr. Lee S. Caldwell of the Experimental Psychology Division of the U.S. Army Medical Research Laboratory (AMRL), Fort Knox, Kentucky (Caldwell, 1963).

As shown in Figure 5, the worker is seated in the chair so that the hand-grip dynamometer is on his preferred side. The hand-grip

FIGURE 5. Strength, endurance, and recovery test.

is adjusted at approximately the height of the worker's knee and at a depth such that his elbow forms a 90° angle. Each worker's SER is then recorded according to Caldwell's (1963) procedure: the subject is required to pull initially with maximum strength; exactly 1 min. later, he is required to pull, for as long as possible, a load equal to 50% of the original maximum strength; this is followed by a 1-min. rest and a final maximum strength response.

Eye-hand coordination task. — The test of eye-hand coordination used in this study was employed originally by Poock (1967) at the University of Michigan, and has been employed by Chaffin and his co-workers at the University of Michigan in their studies of the behavioral effects of the occupational exposure to low-levels of mercury (Chaffin, Dinman, Miller, Smith, & Zontine, 1973). The equipment made for this study by the Performance Research Laboratory was based on specifications of the Michigan Eye-Hand Coordination test furnished by Dr. Donald Chaffin.

The apparatus for this task consists of a hole plate (on which interconnecting lines form a maze pattern which the workers are required to follow in performing the task), a sounding board, a microphone, a tape recorder, and a stylus.

As shown in Figure 6, the worker is seated facing the apparatus and required to grasp the stylus in his preferred hand. He is then instructed to insert the stylus in each of the 119 holes in the hole plate in the order indicated by the black lines, proceeding as quickly as possible without missing any holes; this procedure is then repeated

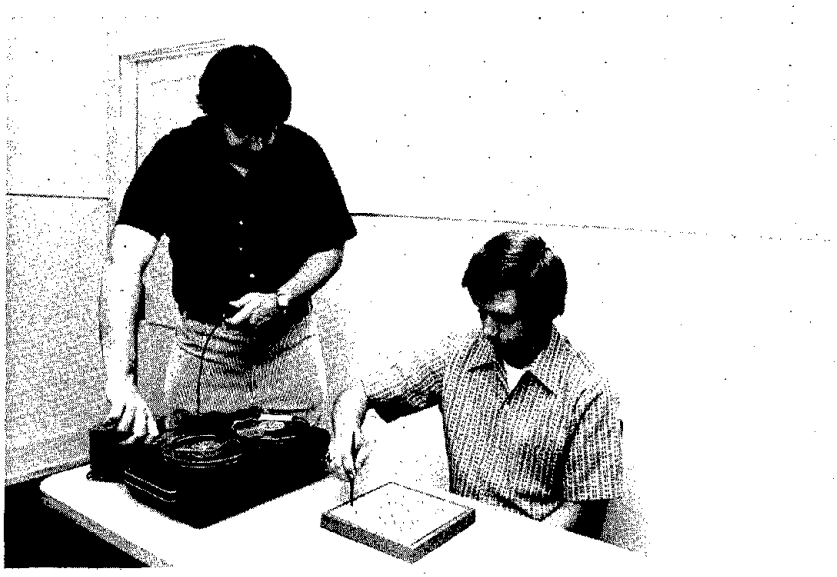

FIGURE 6. Eye-hand coordination test.

for the non-preferred hand. The sound of the stylus striking the sounding plate beneath the hole plate is recorded on a tape recorder and later scored by use of a digital computer.

Digit-span test. — Each worker is individually instructed to listen to, and then repeat, a sequence of single-digit numbers read aloud by the experimenter. The experimenter begins by reading a series (Trial 1) of three numbers. If the worker is able to correctly repeat the series, a sequence of four numbers is then presented. For every correct repetition of a series by the worker, a subsequent sequence is presented, in which the number of digits is increased by one unit to a maximum of nine digits. Whenever the worker answers incorrectly, a second series (Trial 2) is presented which contains the same number of digits as occurred in the previous (incorrect) trial. If the second trial of a series is repeated correctly, the procedure continues, until two trials of a given series are missed. The average duration of the test is approximately 2 min.

Multiple affect adjective check list. — The Multiple Affect Adjective Check List (MAACL), developed by Zuckerman and Lubin (see Zuckerman, 1960; Zuckerman, Lubin, Vogel, & Valerius, 1964) and published by the Educational and Industrial Testing Service, San Diego, California, is also employed in this study. It consists of a list of 132 adjectives, of which each worker is required to check those words which describe how he feels at the time of testing. Although no time limit is given, the MAACL is usually completed within 5 min.

RESULTS AND DISCUSSION

As indicated above, data collection is approximately 50% complete and does not include a completed sample of control workers. Two hundred workers have been tested at two separate industrial locations. Because the data of the first five workers represent pilot data, and five of the remaining 195 workers are non-exposed workers, only 190 of the 200 are lead-exposed. Basic demographic summaries of the exposed workers by sex, race, and work-shift are given in Table III for each industrial sample. (At the completion of data collection from the exposed workers, a control sample of workers will be selected on the basis of these characteristics, as well as three additional parameters; namely, highest formal education, length of work service, and age.)

From the data of Table III, it is clear that to date, the majority of exposed workers voluntarily participating in the study are white males drawn primarily from the first daily work-shift, between the hours 0800 to 1600. In addition, a preliminary summarization of the data regarding length of service has produced a bi-modal distribution with respect to this parameter; the majority of workers have been

employed (and exposed to lead) for periods less than five years (N=77) or greater than 20 years (N=39).

Table III.
SEX, RACE, AND WORK-SHIFT DISTRIBUTION OF CURRENT INDUSTRIAL SAMPLE

Sample	Sex		Race		Work shift			Total
	Male	Female	Negro	White	First	Second	Third	
Group A	72	8	14	66	37	33	10	90
Group B	95	15	3	107	81	24	5	110
Total	167	23	17	173	120	57	15	190

Clinical Determinations

The information presented below provides a preliminary summarization of certain of the clinical data. Presented in Table IV are the

Table IV. MEANS AND STANDARD DEVIATIONS (SD) OF THREE CLINICAL EVALUATIONS
(Blood lead, Urine lead, and Urine Coproporphyrin)

Measure	Controls (N=5)		Group A (N=80)		Group B (N=110)		Combined A & B (N=190)	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Blood lead	25.40	3.85	59.53	18.02	61.18	16.19	60.48	16.96
Urine lead	31.20	15.79	95.08	66.39	86.59	53.29	90.14	59.10
Urine coproporphyrin	13.60 (Median=13.0)	10.31	53.63 (Median=34.5)	59.63	68.99 (Median=53.5)	70.30	62.72 (Median=41.5)	66.33

means and standard deviations of the blood lead, urine lead, and urine coproporphyrin determinations for five controls and the two groups of lead-exposed workers (Groups A and B are presented separately and combined); because of the obviously skewed nature of the distribution of urine coproporphyrin, the medians of those values are also presented for each sample. These data indicate that with each of these measures, relatively minor differences were obtained between the two lead-exposed samples (considering, the relatively large standard deviations.

A histogram of the distribution of blood lead values is presented in Figure 7[1]. Examination of these data indicates that the blood

[1]These values represent the observed blood lead rather than expected blood-lead. This latter value is recommended by a recent National Academy of Sciences (1972) publication because it takes into account normal and observed hematocrits according to the formula:

$$\text{Expected Blood Pb} = \frac{\text{Observed Blood Pb}}{\text{Observed Hematocrit}} \times \text{normal hematocrit for age and sex.}$$

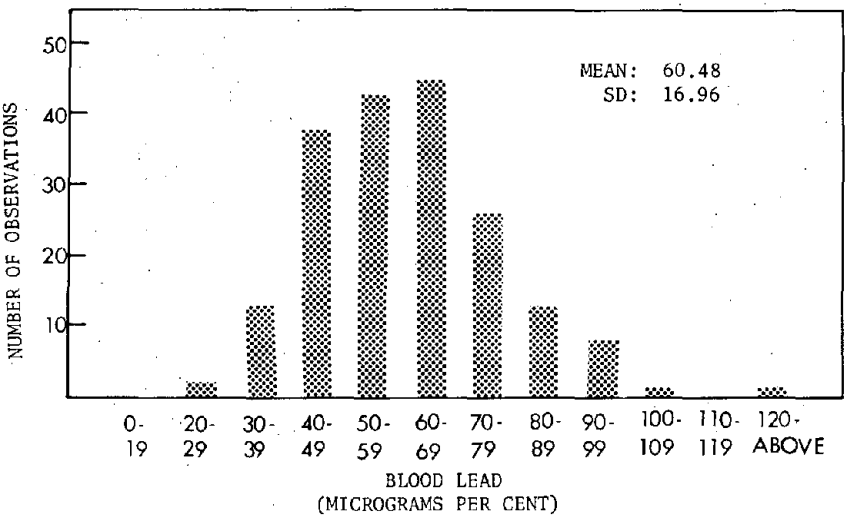

FIGURE 7. Frequency distribution of observed blood-lead levels for the total worker sample (N=190).

leads are skewed in the direction of the "acceptable range," between 40 and 80 micrograms percent (for a discussion of diagnostic levels, see Lane, Hunter, Malcolm, Williams, Hudson, Browne, McCallum, Thompson, deKretser, Zielhuis, Cramer, Barry, Goldberg, Geritie, Vigliani, Truhaut, Kehoe, and King, 1968, p. 501). The incidence of workers showing blood-lead levels in either excessive or dangerous amounts (i.e., above 80 micrograms percent) includes approximately 12% of the total sample tested.

In addition to clinical determinations involving blood and urine lead and urinary coproporphyrin, measures of the amount of urine aminolevulinic acid (ALA) and blood aminolevulinic acid dehydrase (ALA-D) were also determined. Table V presents the intercorrelations for these five clinical measures. As expected, most of these measures correlate well. These intercorrelations compare favorably with those found in the literature, particularly those cited by Ziel-

Table V. INTERCORRELATIONS OF FIVE CLINICAL MEASURES (df=193)

	Observed blood lead	Urine lead	Urine ALA	Urine coproporphyrin
Urine lead	0.399**			
Urine ALA	0.461**	0.572**		
Urine coproporphyrin	0.408**	0.530**	0.700**	
Blood ALA-D	−0.192**	−0.132	0.270**	−0.184*

*$p<0.025$; **$p<0.005$

huis (1971), in his review of clinical tests for lead intoxication, and by Cramer and Selander (1965).

Performance Measures

The modified MTPB provides 36 non-independent measures of general performance. These are derived from separate measures of (*a*) the speed of responding to the three watchkeeping tasks (red and green warning-lights, blinking-lights, and probability monitoring), (*b*) the percentage of correct detections and number of false responses to probability-monitoring signals, (*c*) the percentage correct and percentage attempted of arithmetic computation and target identification problems. Ten of these measures represent the average performance of workers during the entire period of presentation of a given task (with and without the time-sharing of other tasks) during the 1hr. test period. These 10 measures are regarded as the primary MTPB performance measures.

Of the 26 secondary measures, 18 represent performances on the watchkeeping tasks at the different levels of work load provided by the addition of other tasks. That is to say, the performances on each of the watchkeeping tasks during low-demand performance (*i.e.*, watchkeeping alone) as well as during medium- and high-demand performances (*i.e.*, watchkeeping with target identification and with arithmetic computations) were determined separately.

The remaining eight secondary measures were derived from the performances of the workers on the active tasks. Since performance on these tasks is somewhat more difficult than the performances on the watchkeeping tasks, the first half of their 20-min. presentation (see Table II) may represent acquisition or learning effects. In order to account for these possible effects, measurements were obtained separately during the first and second 10 min. of the task presentation. From the worker's point of view, however, there was no break between the first and second 10-min. presentations.

In addition to the MTPB performance measures, 44 other measures of sensory, psychomotor, and psychological functions were obtained. These included four measures of visual acuity, 12 of auditory acuity, eight of tremor, five of muscular strength, endurance, and recovery, 10 of eye-hand coordination, one of immediate recall (memory) and four of mood or affect.

A preliminary factor analysis of the 80 performance-test and five clinical measures was performed; this analysis is being developed for use when data from all 400 workers have been obtained. On the basis of 50% completion of the final sample, a total of 22 factors were extracted which accounted for approximately 81% of the total variance. Although the specific factors have not been identified,

these preliminary results indicate that measures associated with speed and motor coordination (especially tremor) account for a great deal of that variance.

The factor analysis also provided the intercorrelation of all 87 variables. For purposes of this report, several of these preliminary intercorrelations are presented below. Table VI presents the coefficients of correlation relating the blood lead and blood ALA-D measures to nine behavioral measures. These two clinical measures are selected for presentation at this time because the remaining tests (i.e., urine lead, urine coproporphyrin, and urine ALA) account for only about 2% of the total significant correlations, and therefore, bear little relationship to any of the behavioral measures.

The data given in Table VI show that while the observed blood lead correlates significantly with several MTPB response-time measures, it does not correlate with others. Similarly, blood ALA-D correlates significantly with the measures from the SER and tremor

Table VI. COEFFICIENTS OF CORRELATION RELATING TWO BLOOD DETERMINATIONS TO NINE BEHAVIORAL MEASURES (df = 193)

Behavioral measure		Observed blood lead	Blood ALA-D
Response time	(MTPB blinking lights)	0.205**	0.013
Response time	(MTPB blinking lights —medium)	0.234**	0.056
Response time	(MTPB blinking lights —high)	0.200**	−0.074
Muscular force	(Original strength—S_1)	0.080	−0.202**
Muscular force	(Secondary strength—S_2)	0.098	−0.166**
Muscular control	(Tremor: pre-test, 1st trial)	0.092	−0.194**
Muscular control	(Tremor: post-test, 1st trial)	0.043	−0.189**
Speed	(Eye-hand, total response time)	0.218**	−0.194**
Speed	(Eye-hand, mean inter-hole time)	0.224**	−0.190**

*$p<0.025$; **$p<0.005$

tests but not with the MTPB measures. Both clinical determinations correlate significantly with measures of eye-hand coordination.

At this stage of the research project it is too early to hypothesize, but it is interesting to observe that these significant correlations can be grouped into distinct categories depending upon the primary place of neurological innervation. For example, the observed blood lead correlates with the measure of response time for blinking lights. In this regard, blinking lights is a watchkeeping or vigilance task, the response to which is most likely mediated by the central rather than peripheral nervous system. On the other hand, strength and tremor, measures which correlate with blood ALA-D measures, are innervated primarily by the peripheral nervous system. These observations do not mean to imply that one's performance is innervated by a single division of the nervous system to the exclusion of the other. Obviously, both divisions are utilized, but a distinction can be made on the basis of *primary* involvement. A further observation from these data is that more complex tasks, such as eye-hand coordination, which involve complex integration of peripheral and central functioning do produce significant correlations with both the observed blood-lead and blood ALA-D.

An additional observation from the correlations given in Table VI (as well as others not reported here) is that the clinical measures are based on individuals whose blood lead values are less than 80 micrograms percent. Only 12% of the workers included in this sample of workers exceeded that criterion level. These data suggest, therefore, that some behavioral changes do occur as a result of low levels of lead in the system; but at this writing, it is too early to precisely define the range of behavioral dysfunction.

One final observation supported by the current data is that behavioral dysfunction related to increasing body-burden of lead may show up not only in the absolute level of performance, but also in terms of variability in performance. For example, the data in Figure 8 represent the performances of the workers on the blinking-lights task for the three work-load or demand levels of performance. It is clear from these data that as the blood lead levels increase, there are also large increases in performance variability. Moreover, as the workload of the worker is increased the absolute level of response time increases as well. These data suggest that only during periods of high-demand performance is a worker's capacity to work increasingly degraded by increases in the body-burden of lead.

The preliminary conclusions and observations reported herein must be confirmed by further analysis and research. Because of the enormity of the project, only a sampling of the data was selected for this report. Future statistical analyses will be computed with

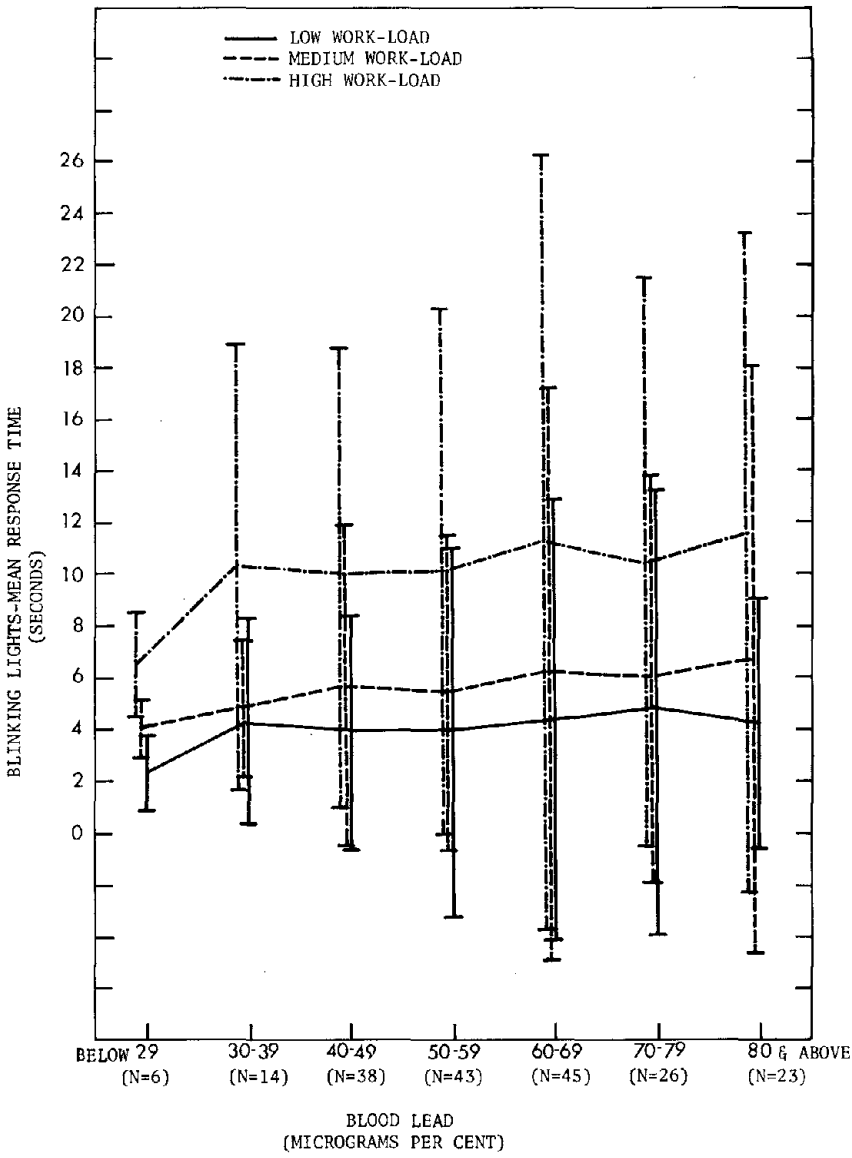

FIGURE 8. Mean response time (and standard deviation) in detecting blinking-light signals during period of low, medium, and high work-loads of performance as a function of blood-lead level (values in parentheses indicate the number of observations in each blood-lead group).

each of the obtained measures in order to test for significant differences between the exposed and non-exposed workers and between identifiably different body-burdens of lead. Other analyses will be computed in order to determine the relationships between the measures obtained with the behavioral test battery and associated pharmacological, physiological, psychophysiological, biomedical, and psychological variables. Reports of the results of these tests will be forthcoming in future publications.

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EFFECTS OF POLYVALENT CATIONS ON SYNAPTIC TRANSMISSION IN FROG NEUROMUSCULAR JUNCTION AND FROG SYMPATHETIC GANGLION

G. P. Cooper, Ph.D. and R. S. Manalis, Ph.D.

Since some of the manifestations of lead poisoning are the result of effects on the neuromuscular system,[1, 2] experiments were performed on the neuromuscular junction of the frog and on the sympathetic ganglion of the frog to determine how lead and other polyvalent cations might influence the process by which information is transferred from the motor nerve ending to the muscle fiber and from preganglionic nerve endings to postganglionic cells. The effects of lead on neuromuscular transmission have not been examined previously. However, synaptic transmission in the superior cervical ganglion of the cat has been shown by Kostial and Vouk[2] to be affected by lead. Perfusion of the ganglion *in vivo* with lead nitrate in concentrations as low as 4.8 micromolar depressed or blocked transmission. Since lead did not alter the response of the ganglion to perfused acetylcholine but did reduce the release of acetylcholine from presynaptic nerve endings, they concluded that lead interfered mainly with the presynaptic release of transmitter.

The experiments on the neuromuscular junction described below show for the first time some of the effects of lead on intracellularly-monitored events underlying synaptic transmission. These results demonstrate presynaptic and postsynaptic effects of lead, with the presynaptic effects being the most prominent. In our extracellular studies of synaptic transmission in sympathetic ganglia the comparative effects of polyvalent cations have been examined. Of the cations thus far examined cadmium and lead have proved to be the most potent in blocking transmissions.

NEUROMUSCULAR JUNCTION

Experiments were performed on the isolated sciatic nerve-sartorius muscle of the frog *(Rana pipiens)*. Preparations were mounted in a suitable chamber which permitted the temperature to be controlled and maintained at 15° C and which allowed easy introduction and removal of physiological solutions. The chamber was mounted on the mechanical stage of a compound microscope having a total magnification of 400X so that individual endplates could be identified visually. Standard microelectrode techniques were used for

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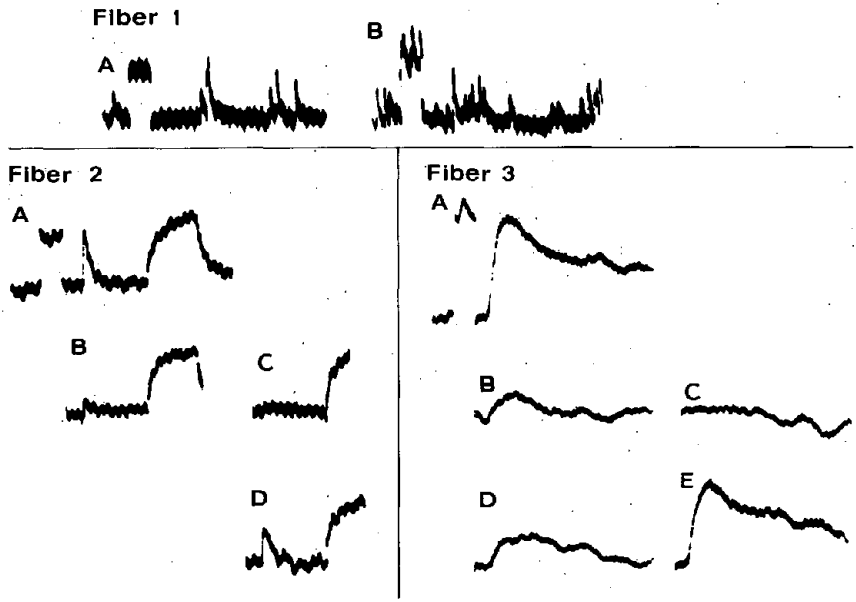

FIGURE 1. Effect of lead on neuromuscular transmission in the frog. Fiber 1: **A**, miniature endplate potentials recorded in normal Ringer solution; **B**, miniature endplate potentials after the preparation was exposed to 0.1 mM $PbCl_2$-Ringer for a few minutes. Note that the frequency of miniature endplate potentials increased in the presence of lead. The resting potential was −80 mV, and it was unaffected by lead. Although not indicated in this figure, the effect of lead on the frequency of miniature endplate potentials was found to be reversible. The calibration pulse in **A** and **B** is 1 mV high and 50 msec long. Fiber 2: **A**, from left to right, calibration pulse that is 2 mV high and 50 msec long; endplate potential evoked by sciatic nerve stimulation recorded from a curarized preparation (the d-tubocurarine chloride concentration was 2.26 μg/ml); electrotonic potential in response to the injection of a constant outward current pulse (not shown) of about 5 nanoamperes, thereby allowing the input resistance of the muscle fiber to be determined (it was 400 KΩ in this fiber). **B** and **C**, responses in the presence of 0.01 mM $PbCl_2$-Ringer (the curare concentration was unchanged). The endplate potential fell to zero (**C**) within thirty seconds after the preparation was first exposed to lead. **D**, responses four minutes into the recovery period. Lead was no longer present in the Ringer solution while the curare concentration remained unchanged. Note that the electrotonic potential remained unchanged throughout (**A-D**), indicating that the membrane resistance was not altered by lead. The resting potential was −85 mV, and it was unaffected by lead. Fiber 3: **A**, endplate potential, evoked by sciatic nerve stimulation, recorded from a curarized fiber. Recordings were made at a faster sweep than were those from fiber 2; the calibration pulse is 2 mV high and 2 msec long. **B**, **C**, responses in the presence of 0.01 mM $PbCl_2$. The endplate was completely blocked (**C**) within forty-five seconds after first exposing the preparation to lead. **D** and **E**, responses after 1 and 5 minutes recovery, respectively. The curare concentration was constant throughout (**A-E**). The resting potential was −82 mV, and it was unaffected by lead.

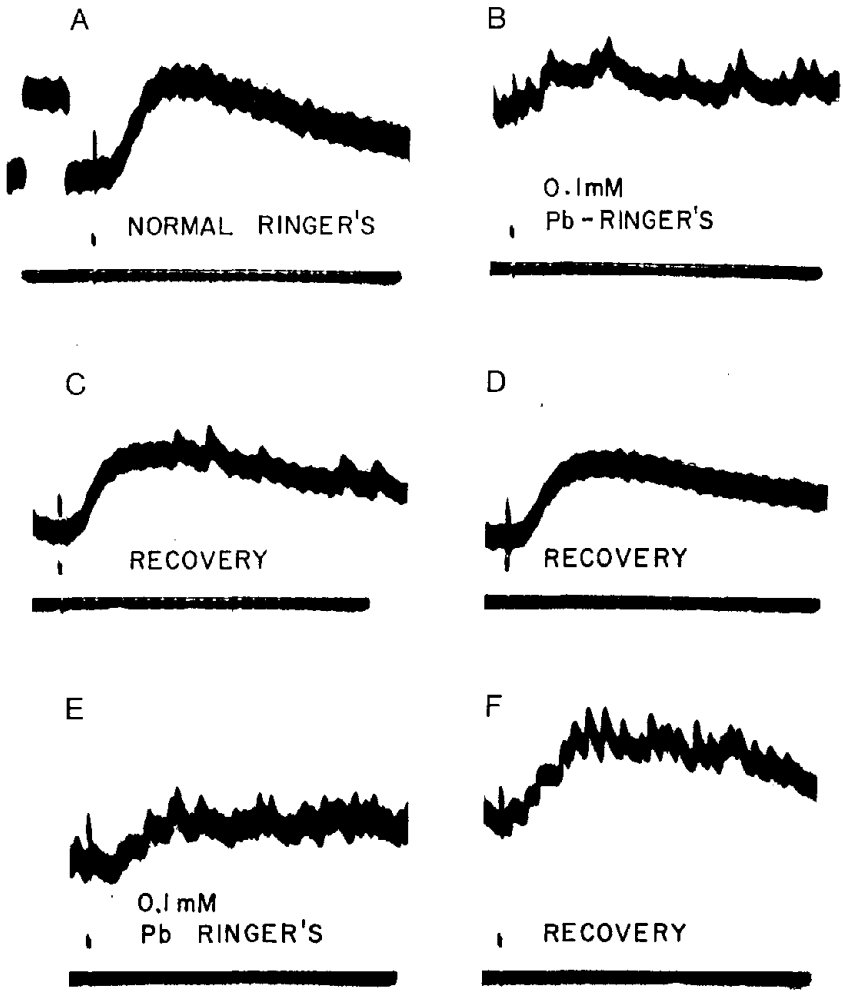

FIGURE 2. Effect of lead on ACh potential and on MEPP's in the frog neuromuscular junction. Each oscillogram (**A-F**) shows two traces, the bottom one shows the iontophoretic current released to produce the ACh potential recorded by the intracellular voltage microelectrode (upper trace). **A** shows the control response to the iontophoresis of ACh. **B** shows that the ACh potential was lowered in the presence of 0.10 mM $PbCl_2$. Note also that many miniature endplate potentials are present in **B**. Oscillograms **C-D** were taken after the preparation was washed with normal Ringer solution. The miniature endplate potentials were no longer present, and the ACh potential almost returned to its control value. The lead exposure was repeated in **E**. **F** was taken from the early part (second minute) of the recovery period at which time the ACh potential had recovered but the frequency of the miniature endplate potentials was still elevated. The charge delivered from the iontophoretic micropipette remained constant throughout. The vertical deflection of the calibrating pulse in **A** (upper trace) equals 2 mV and 400 nA; the horizontal deflection equals 2 ms.

voltage measurement, membrane polarization, and for the iontophoresis of acetylcholine (ACh). Control measurements were made while the preparation was bathed in normal Ringer solution (composition: 111 mM NaCl, 2.5 mM KCl, 2.0 mM $CaCl_2$, 4 mM Tris-maleate). All Ringer solutions were maintained at a pH of 6.9. Following the control period, lead chloride was added to the control solution. The concentration range of Pb^{2+} in the various experiments was 0.01 to 0.10 mM. To record endplate potentials in the absence of muscular contraction, the nerve-muscle preparations were bathed in a Ringer solution which contained curare.

Transmitter release at chemical synapses occurs in two manners: (a) spontaneous release which occurs in the absence of action potentials at the motor nerve terminals, and (b) phasic or synchronous release which occurs following the arrival of an action potential at the nerve terminal. Our results show that these two forms of transmitter release are oppositely affected by the same agent, lead.

The effect of lead on the frequency of miniature endplate potentials (spontaneous release) is shown in Fig. 1 (Fiber 1). Oscillogram A was taken during the control period with the voltage microelectrode inside a muscle fiber at the endplate region. When 0.10 mM Pb^{2+} was added to the normal Ringer solution, the frequency increased within less than a minute by about three times (B). In other experiments it was not uncommon to observe an increase of several hundred times in the presence of 0.10 mM Pb^{2+} (4). This effect of lead was reversible within 10 to 15 minutes after washing the preparation with the control Ringer solution (see Fig. 2). Concentrations of lead below 0.02 mM did not significantly increase the frequency of the miniature endplate potentials.

When 2.26 μg/ml of d-tubocurarine chloride was added to the normal Ringer solution, subthreshold endplate potentials were recorded (Fig. 1, Fibers 2 and 3). In Fiber 3, oscillogram A shows the control response to motor nerve stimulation recorded with an intracellular microelectrode. When 0.02 mM Pb^{2+} was added to this modified Ringer solution the endplate potential decreased immediately (B) and fell to zero within one minute (C). When this preparation was washed with control Ringer solution, the endplate potential reappeared, thereby showing that this effect of lead is also reversible. In such experiments the endplate potential could have decreased for any of the following reasons: (a) decreased membrane resistance caused by lead, (b) curariform action of lead on the endplate membrane, or (c) a decrease in the phasic release of transmitter. To investigate the possibility that lead lowered the membrane resistance of the muscle fiber, a second intracellular microelectrode was used to pass a small but constant outward current. The IR drop across the muscle membrane appears as a depolarizing electro-

tonic potential which follows the endplate potential in the oscillograms recorded for Fiber 2. In A the control endplate potential follows the calibrating pulse. Oscillograms B and C were taken in the presence of 0.02 mM Pb^{2+}. Note that the endplate potential was completely blocked (as it also was in Fiber 3) while the electrotonic potential was essentially unchanged (the small change in the shape of the electrotonic potential seen in B certainly cannot account for the large reduction in the endplate potential).

To test for the possibility of a postsynaptic blocking effect of lead, the endplate membrane was activated directly by the iontophoretic application of ACh[5]. When ACh is applied by iontophoresis through a micropipette, the resulting depolarization is referred to as an ACh potential. The ACh potential was shown to be quite insensitive to lead. Concentrations of lead lower than 0.10 mM had no significant effect on the ACh potential (Fig. 2). Moreover, the actual oscillograms show that when the concentration of lead was sufficient to reduce the ACh potential, there was a concomitant increase in the frequency of miniature endplate potentials (Fig. 2B, E). In fact, when ACh was applied in a more refined manner the ACh potential was shown to be reduced by only 5 percent[4]. This indicates that when ACh is released from the nerve terminal either spontaneously or phasically, it is released into the synaptic cleft in a concentration so high that the relatively few receptors bound by

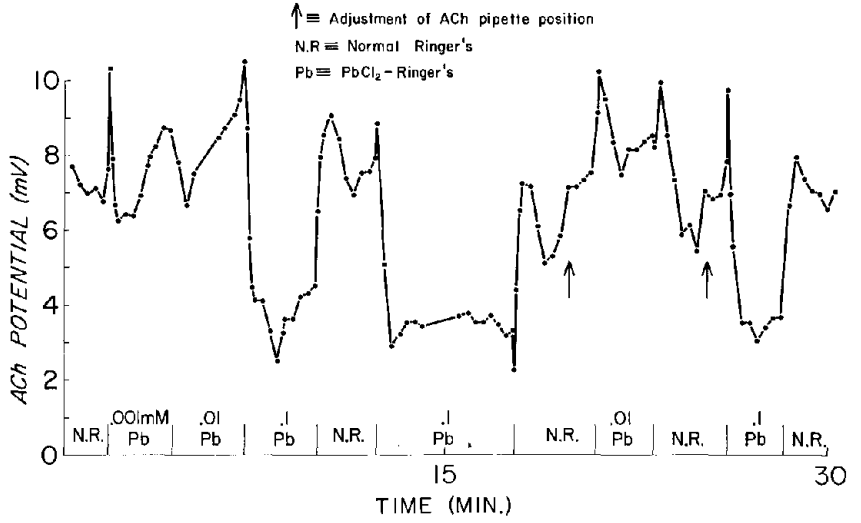

FIGURE 3. The effect of different doses of lead on the ACh potential in the frog neuromuscular junction. Obvious reductions in the ACh potential occurred only in the presence of 0.10 mM Pb^{2+}. The transient shifts in the ACh potential were related to solution changes.

lead do not result in an obvious reduction in the activation of the endplate membrane.

Since we have just shown that lead applied in a concentration of 0.10 mM has only a weak curariform action and since, in the presence of this same concentration of lead, miniature endplate potentials are not greatly reduced in amplitude while their frequency is increased, the reduction of the endplate potential in the experiments described above (Fig. 1) must be due to the reduction in the amount of transmitter released from presynaptic terminals. Additional experiments in which the endplate potential was reduced in the presence of high Mg^{2+}/low Ca^{2+} Ringer solution confirm that lead reduces the endplate potential by decreasing the phasic release of transmitter, presumably through a mechanism similar to magnesium. Lead, however, is about 1000X more potent on a molar basis than is magnesium. Of other polyvalent cations studied, lanthanum has been shown to have presynaptic effects at the frog neuromuscular junction very similar to those presented here[6, 11].

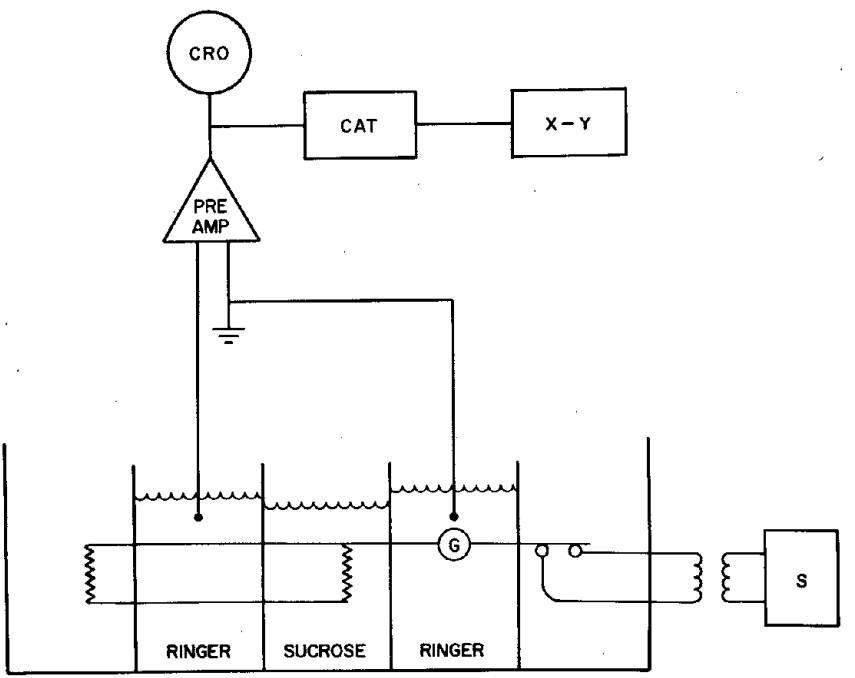

FIGURE 4. Diagrammatic representation of recording chamber, stimulating and recording equipment and ganglionic preparation. Abbreviations: **S**, stimulator; **G**, ganglion; **Preamp**, differential input preamplifier; **CRO**, cathode ray oscilloscope; **CAT**, computer of average transients (Technical Measurement Corporation); **X-Y**, x-y plotter.

SYMPATHETIC GANGLION

In other experiments the effects of lead and seven other polyvalent cations on synaptic transmission in the 9th or 10th sympathetic ganglion of the bullfrog *(Rana catesbeiana)* have been studied. The data presented here are from preliminary experiments done in collaboration with Mr. Mark Houser and Mr. Manuel Lowenhaupt.

The ganglion, along with its pre-and post-ganglionic trunk, was removed from the frog and mounted in a multi-compartment chamber. The chamber, preparation, and recording arrangement are shown diagrammatically in Figure 4. The ganglion was continuously perfused with frog Ringer solution or Ringer solution to which had been added 0.001 to 0.1 mM chloride salts of Pb^{+2}, Hg^{+2}, Cd^{+2}, Zn^{+2}, La^{+3}, Ba^{+2}, Sr^{+2}, and Mg^{+2}. All solutions were maintained at room temperature and a pH of 6.9. The preganglionic trunk was stimulated once per second with single supramaximal pulses. Ag: AgCl:agar-Ringer electrodes were used for recording between the

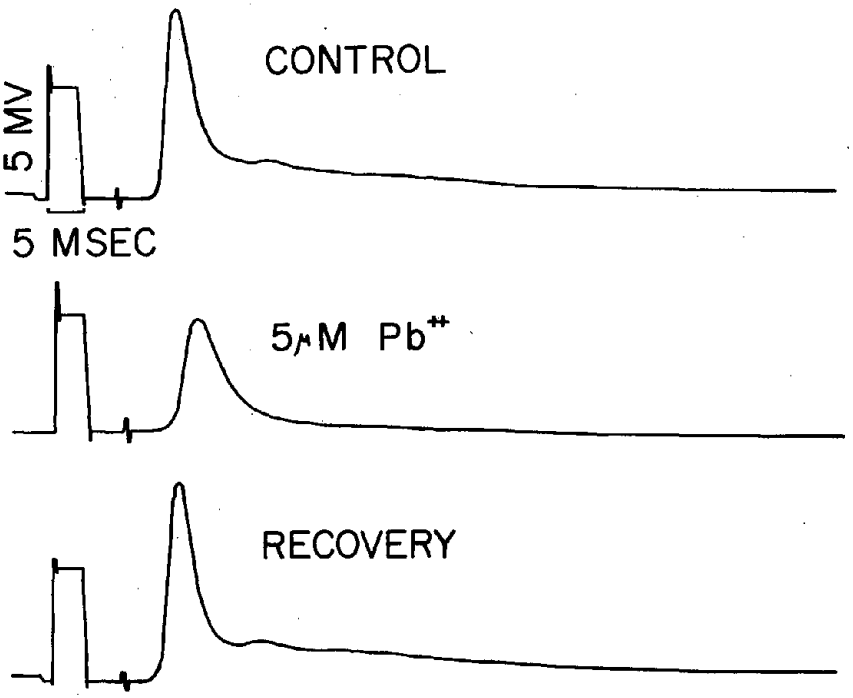

FIGURE 5. Effect of 0.005 mM lead chloride on the amplitude of the ganglionic response. Each trace is the average of 50 sweeps. The top record was taken while the ganglion was perfused with ordinary frog Ringer solution; the middle record was made after perfusing the preparation for 30 minutes with Ringer solution containing lead; the bottom trace was made 30 minutes after returning the preparation to lead-free Ringer solution. Calibration factors were the same for all three traces.

ganglionic Ringer pool and the postganglionic Ringer pool across a sucrose gap. Usually 30 to 50 individual ganglionic responses were electronically averaged and the average response recorded on an X-Y plotter.

Ganglionic responses were recorded first with the ganglion bathed in control Ringer, then after a 30-minute exposure to Ringer solution containing one of the polyvalent cations, then again after a recovery period in control Ringer. Recordings made in a representative experiment with lead are shown in Figure 5. In this instance perfusion of the ganglion for 30 minutes with 0.005 mM $PbCl_2$ reduced the amplitude of the ganglionic response by about 40 per cent (middle trace). The response recovered fully after perfusing

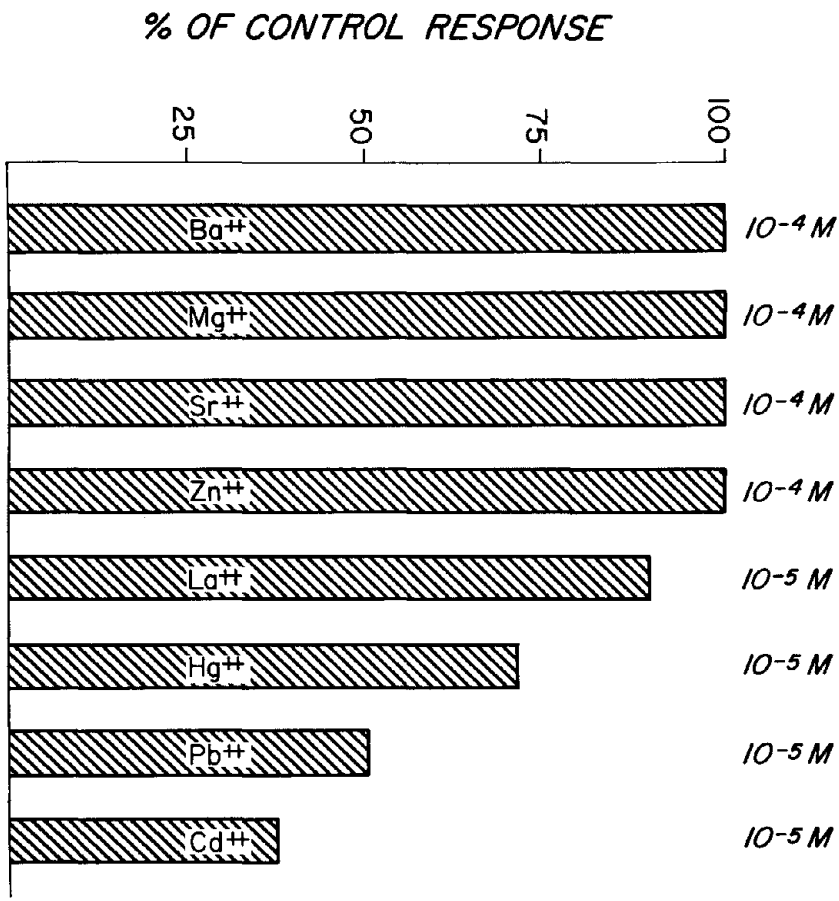

FIGURE 6. Ganglionic response amplitude after perfusing ganglia for 30 minutes with chloride salts of barium, magnesium, strontium, zinc, lanthanum, mercury, lead, and cadmium. Each value was the mean of 5 different observations. Note that the concentrations of Ba, Mg, Sr, and Zn were ten times greater than those of La, Hg, Pb and Cd.

the ganglion for 30 minutes with control Ringer solution (bottom trace).

As shown in Figure 6, responses of the ganglia were depressed, in order of increasing potency, by La^{+3}, Hg^{+2}, Pb^{+2}, and Cd^{+2}. For lead, which has been studied more extensively thus far than the other cations, the amplitude of the ganglionic response is an inverse linear function of the logarithm of lead concentration. The threshold for the lead effect under the conditions of this experiment is approximately 0.001 to 0.003 mM. As shown in Figure 6, Ba^{+2}, Sr^{+2}, Zn^{+2}, and Mg^{+2} had no detectable effects at a concentration of 0.01mM. The effects of Cd^{+2}, Pb^{+2}, and La^{+3} — but not those of Hg^{+2} — could be reversed by increasing the calcium concentration of the Ringer solution to 10 mM.

Our experiments with sympathetic ganglia have not progressed to a point which would allow us to make any definite conclusions concerning the site and mode of action of polyvalent cations. However, as pointed out above, Kostial and Vouk[3] determined that lead interfered with synaptic transmission in the cat superior cervical ganglion by reducing the presynaptic release of acetylcholine. They also found that the effects of lead could be reversed by elevating the calcium concentration. From these data and from our data derived from experiments on the neuromuscular junction, we might safely assume that lead interferes with synaptic transmission in the frog sympathetic ganglion by reducing the amount of acetylcholine released from presynaptic terminals. With respect to the other cations studied, we cannot be as certain about the mechanism of action. Since adding calcium to the Ringer solution quickly reverses the effects of lanthanum and cadmium, as it does the effects of lead, we can assume as a working hypothesis that the mode of action of lanthanum and cadmium is similar to that of lead. On the other hand the effects of mercury were not reversed by elevating the calcium concentration, so that its mode of action may be different. Clearly, only additional experiments can provide more definitive solutions to these problems.

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BLOCK OF NEUROMUSCULAR TRANSMISSION BY METHYL MERCURY

J. N. Barrett, Ph.D., D. Botz, Ph.D. and D. B. Chang, Ph.D.

The isolated frog neuromuscular junction is a useful preparation for studying the mechanisms of action of neural poisons because it has been studied extensively and because available evidence suggests that synapses in the central and peripheral nervous systems share many anatomical and physiological features.[1, 2] Certain cations (e.g., lanthanum, beryllium, copper, cobalt, manganese, magnesium and lead) have been shown to block synaptic transmission presynaptically, by preventing the release of acetylcholine from the motor nerve terminals[3-7]. Mercuric ions not only reduce the amount of transmitter released, but also act postsynaptically to reduce acetylcholine-induced depolarization of the muscle fiber[8].

Methyl mercury causes severe damage to the central nervous system, but the molecular and cellular mechanisms involved in this damage are largely unknown. This paper describes how methyl mercury blocks transmission at the frog neuromuscular junction. The neuromuscular synapse is not the first part of the nervous system to be severely damaged by low levels of methyl mercury, but the same basic processes that are blocked by methyl mercury at this peripheral synapse are likely to occur in central synapses as well.

METHOD

The cutaneous pectoris muscle and a 1 cm length of its attached nerve were dissected from the chest wall of frogs *(Rana pipiens)* and placed in a chamber containing frog Ringer solution (NaCl 116 mM, KCl 2.5 mM, $CaCl_2$ 1.8 mM, Na_2HPO_4 1.08 mM, NaH_2PO_4 0.43 mM, dextrose 5 mM, pH 7.3). In the experiments in which methyl mercury chloride was added to the Ringer solution care was taken to insure that the added methyl mercury did not change the pH by more than 0.1 pH unit. Bath temperature was controlled by a Peltier-effect thermoelectric device (Cambion 3954-01). The free end of the muscle nerve was sucked into a fine polyethylene tube containing the Ringer solution. Suprathreshold stimulation of the nerve by current pulses applied through the tube elicited nerve action potentials.

The electrical potential inside single muscle fibers was recorded using fine glass micropipettes (tip diameter less than 0.5 micron)

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filled with 1 M KCl. These were inserted into the end-plate (junctional) region of single muscle fibers using a micromanipulator (MP 1 Narashigi Co.). A silver wire connected the solution in the pipette to a unity-gain, high input impedance preamplifier (Picometric 181). The electrical signal from this preamplifier was amplified and displayed on a storage oscilloscope (Tektronix 564).

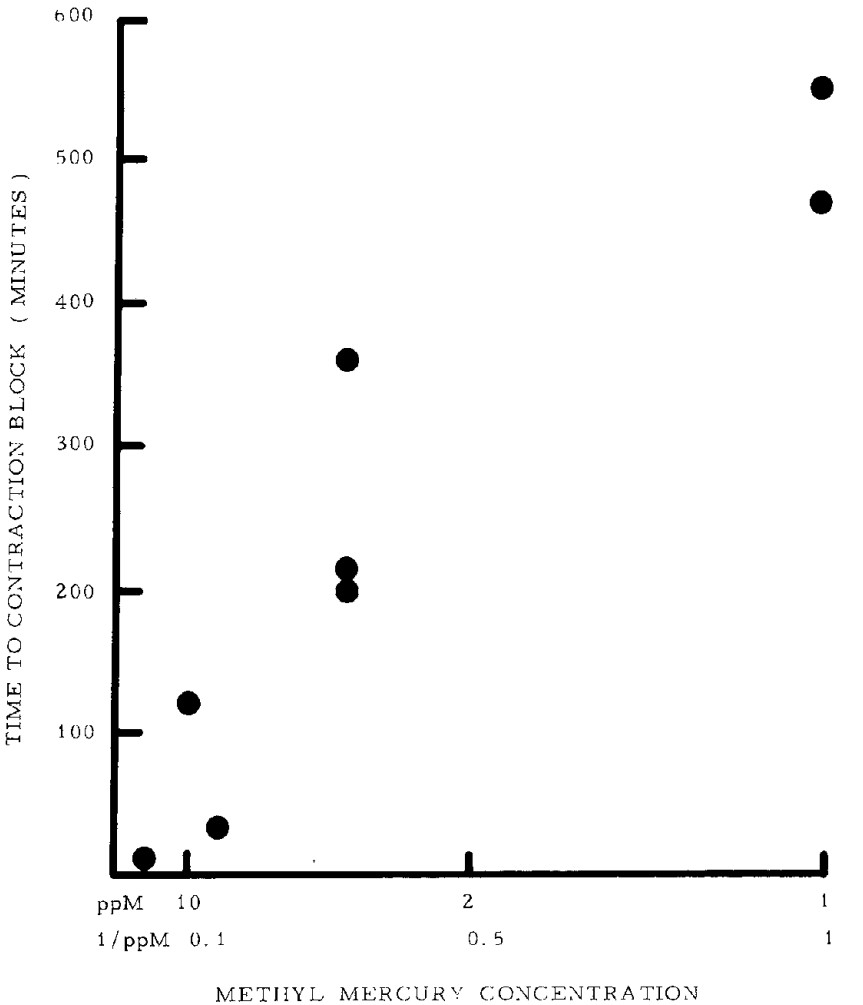

FIGURE 1. Effect of methyl mercury concentration (abscissa) on the incubation time (ordinate) required to block muscle contraction in response to nerve stimulation (22°C). The abscissa is linear with the reciprocal of the methyl mercury concentration in parts per million by weight (ppm).

RESULTS

Block of nerve-induced muscle contraction

Dose-dependence of time to block

In normal Ringer solution the muscle contracts in response to suprathreshold nerve stimulation. Addition of methyl mercury to the bath Ringer solution blocked this contraction after a delay time dependent on the methyl mercury concentration (Fig. 1). The higher the concentration (smaller values of the reciprocal of the concentration, 1/c), the shorter the exposure time required to produce block. There is considerable scatter in the data, but the time to block appears to be approximately a linear function of the reciprocal of methyl mercury concentration over the concentration range studied (1-25 ppm). The block of nerve-evoked muscle contraction could not be reversed by washing the preparation for up to 24 hours in normal Ringer solution.

Temperature dependence of time to block

The time required to produce contraction block was much greater at lower incubation temperatures. The incubation times giving contraction block in 10 ppm methyl mercury varied with a Q_{10} of 2 to 3 over a temperature range of 0 to 22° C.

The temperature dependence of nerve action potential evoked release of acetylcholine was investigated using a laser temperature jump technique to change the bath temperature rapidly.[9] Using this technique we found that exposure to methyl mercury (1 to 10 ppm) did not detectably alter the great temperature dependence of evoked release at the nerve-muscle junction. This is consistent with the hypothesis that the methyl mercury does not act directly on the temperature-sensitive transmitter release mechanism, but instead acts on the presynaptic nerve membrane as discussed later.

Persistence of muscle contraction in response to direct stimulation

Even after the muscles exposed to methyl mercury failed to contract in response to nerve stimulation at an intensity more than ten times greater than the original nerve threshold, an electrical shock applied directly to the muscle would produce contraction. This indicates that the initial block of contraction is not due to action of methyl mercury on the muscle action potential mechanism or on the contractile mechanism itself. At concentrations of methyl mercury exceeding 10 ppm direct muscle stimulation also failed to produce contraction following incubation times of one hour or more at 22° C.

Disappearance of end-plate potential and increased spontaneous potential frequency

Normal Ringer solution

Suprathreshold nerve stimulation in normal frog Ringers before

methyl mercury exposure normally produces a 15-30 mV synaptic potential at the muscle end-plate region, leading to a propagated muscle action potential and subsequent muscle contraction[10] Such contraction dislodges the recording electrode, so continuous intracellular recording during nerve stimulation was not possible until the methyl mercury poisoning had progressed to the stage of blocking

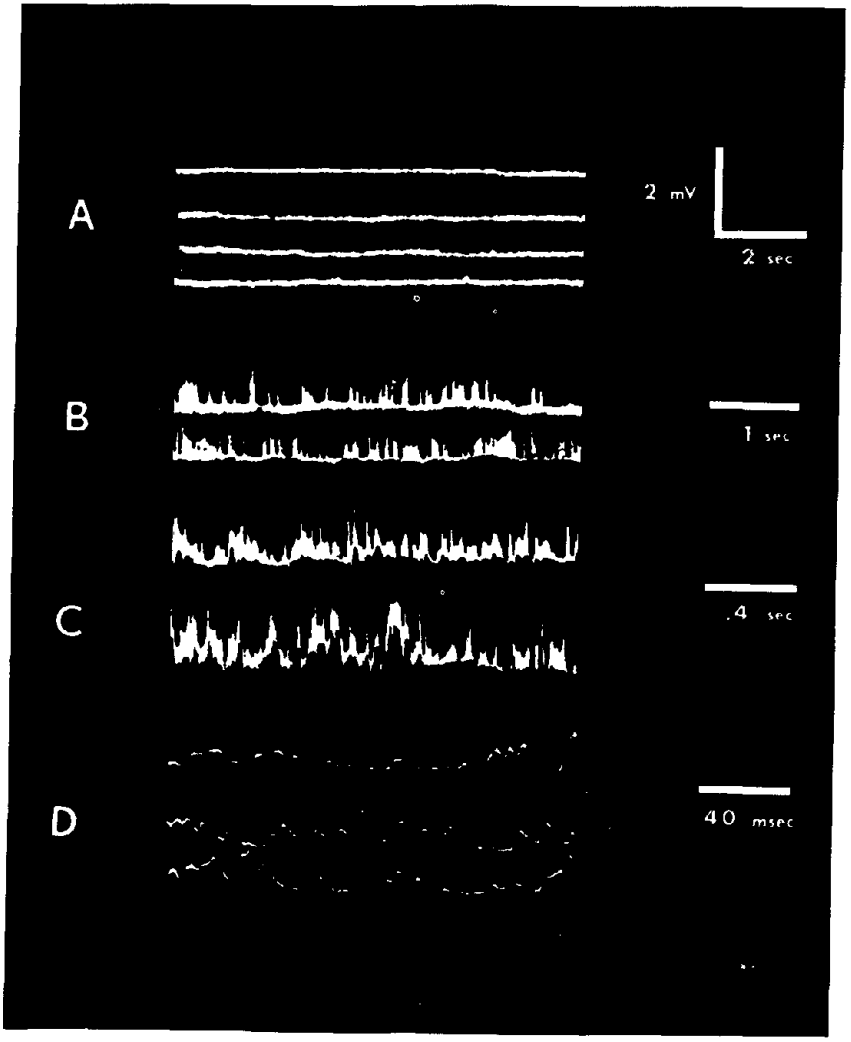

FIGURE 2. Spontaneous miniature end-plate potentials recorded intracellularly from single muscle fiber. Trace A was recorded before application of methyl mercury, and traces B, C, D at 13, 16, and 18 minutes respectively after application of 10 ppm methyl mercury to the solution surrounding the muscle, at 10°C. Note the faster time scale of the later traces.

muscle contraction. At this stage no synaptic potential was recorded in the muscle fibers in response to nerve stimulation, but the frequency of spontaneous miniature end-plate potentials (mepps) was increased 100 to 1000 times above control levels (Fig. 2).

After methyl mercury treatment, the resting potential of the frog muscle fibers was usually depolarized 10 to 50 mV from the normal value of −90 mV.[10] An hour's exposure at room temperature to methyl mercury concentrations greater than 10 ppm completely abolished the resting potential.

Low Ca Ringer

By reducing the bath Ca to 0.8 mM and raising bath Mg to 5.0 mM, the amplitude of the nerve-evoked end-plate potential was reduced to a value (1 to 5 mV at 20° C) too small to evoke a muscle action potential and the resulting contraction. This method of avoiding contraction allowed continuous monitoring of the nerve-evoked synaptic potential during methyl mercury treatment. The end-plate potential declined slightly with time in solutions containing methyl mercury, until suddenly no end-plate potential was seen in response to nerve stimulation. An increase in the frequency of spontaneous miniature end-plate potentials was observed, starting before block of the evoked end-plate potential.

Disappearance of the presynaptic nerve action potential

The presynaptic nerve action potential was monitored using glass micro-electrodes (tip diameter $< 0.5\ \mu$) placed over the fine nerve branches. Such extracellular recordings give potentials reflecting the currents that flow through the nerve membrane during a nerve action potential[11]. Fig. 3 illustrates extracellular recordings from a nerve fiber in a bath containing low Ca (to avoid muscle contraction) and 10 ppm methyl mercury. The extracellular action potential failed at about the same time that the intracellularly recorded end-plate potential had failed in similar experiments. No presynaptic nerve action potentials could be recorded after block of the nerve-evoked muscle contraction, even in solutions containing normal Ca.

DISCUSSION

Mechanism of action of methyl mercury

Methyl mercury may damage nerve and muscle cells in many different ways, especially at high concentrations. This study attempts to define the damage produced in an isolated neuromuscular preparation by acute exposure to low levels of methyl mercury chloride.

Under these experimental conditions it appears that methyl mercury first blocks neuromuscular transmission by abolishing conduc-

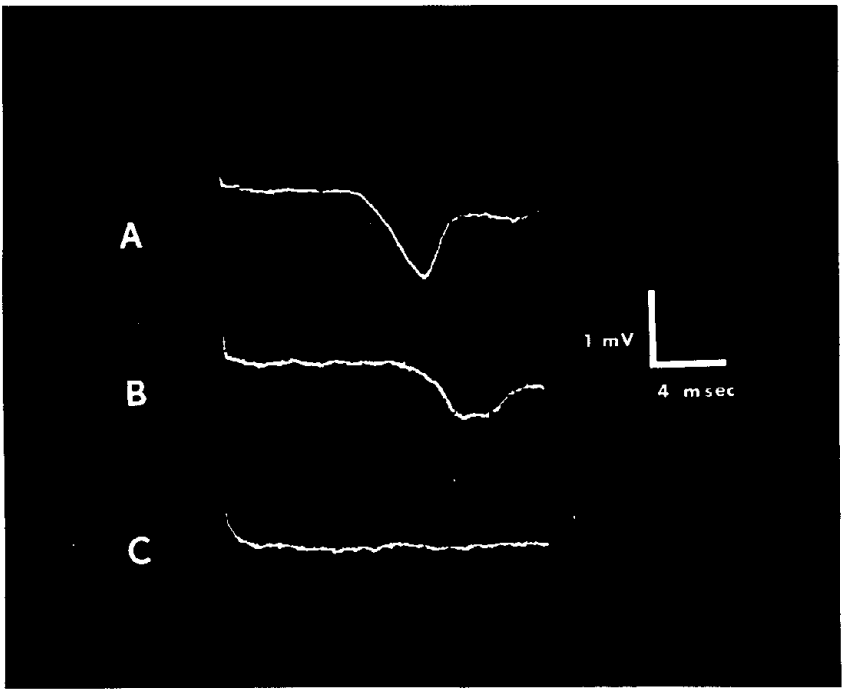

FIGURE 3. Extracellular recordings of compound nerve action potential, evoked by an electrical stimulus (4V, 0.1 msec duration) applied approximately 1 cm from the recording site. Stimulus artifact occurs at the beginning of the trace. The downward voltage deflection in trace A is the compound nerve action potential before application of methyl mercury. Trace B was recorded 20 minutes after addition of 20 ppm methyl mercury at a bath temperature of 2°C. 100 minutes after the addition of methyl mercury the nerve action potential failed completely as shown in trace C. At the same time the muscle ceased to contract in response to nerve stimulation.

tion of the action potential in the presynaptic nerve. Nerve action potentials were never recorded in a preparation after synaptic transmission failed. The mechanism of this nerve block is not certain, but it seems likely that methyl mercury depolarizes the nerve terminal enough to inactivate the early sodium conductance channels that normally initiate action potentials. Depolarization of nerve terminals (by excess potassium or by focal or electrotonic polarization) is known to increase the frequency of spontaneous miniature end-plate potentials[12, 13], an effect observed following application of methyl mercury. Also, slightly higher concentrations of this compound (10 ppm or more) produce an irreversible depolarization of muscle fibers. However, some agents (e.g., alcohol[14] and increased osmotic pressure[15]), can increase the frequency of spontaneous poten-

tials without greatly depolarizing the nerve, so there is as yet no direct evidence that methyl mercury initially blocks the nerve by depolarization.

The measurable muscle fiber depolarization occurring at high concentrations and/or long exposure times may be due to changes in the ionic permeability of the muscle membrane or to inhibition of the active transport of ions across the membrane. Also methyl mercury could interfere with cellular energy supplies by damaging mitochondria, as may lead ions[16].

The persistence of contraction in response to direct muscle stimulation in low (1-3 ppm) methyl mercury solutions indicates that the muscle action potential and contractile mechanisms remain intact for some time after transmission blocks. Similarly, the persistence of spontaneous miniature end-plate potentials of approximately normal amplitude means that the nerve can still release normal transmitter packets and that the postjunctional acetylcholine receptors continue to function.

Comparison to *in vivo* effects of methyl mercury

We found that a dose of 10^{-5} grams methyl mercury per gram body weight (10 ppm) did not produce muscular paralysis when injected into the lymphatic system of live frogs. Paralysis is produced by whole body dose levels above 200 ppm. This result contrasts with the *in vitro* neuromuscular block produced by concentrations as low as 1 ppm (10^{-6} grams per gram of solution). A likely explanation for this discrepancy is that most of the methyl mercury injected into the whole frog becomes bound to sites other than those leading to nerve damage. Ninety per cent of the methyl mercury in blood is bound to red blood cells[17], and much of the methyl mercury remaining in the plasma is bound to plasma proteins[18].

Thus while we observed inhibition of nerve action potentials by concentrations of methyl mercury as low as 1 ppm *in vitro*, it is likely that much higher total dosages would be required to duplicate the same effect *in vivo*.

SUMMARY

Low concentrations of free methyl mercury (1-3 parts per million) irreversibly block synaptic transmission in an isolated frog neuromuscular preparation. The time to block decreases with increasing methyl mercury concentration and with increasing temperature. This block of transmission is due to failure of the presynaptic nerve action potential to propagate to the nerve terminals. The spontaneous release of acetylcholine from nerve terminals (detected as miniature end-plate potentials in the muscle) is greatly accelerated by methyl mercury, suggesting that methyl mercury depolarizes the presynaptic nerve terminals. Higher doses of methyl

mercury (10 ppm) significantly reduce the recorded resting potential of muscle fibers.

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WORKER EXPOSURE
TO
TOXIC GASES, IRRITANTS, ODORS, ALCOHOL
AND
EFFECTS OF DRUG INTERACTIONS

SESSION V

Chairman and Keynote Speaker

Professor Ido de Groot
University of Cincinnati

BEHAVIORAL TOXICOLOGY OF ODORANTS IN WORK ENVIRONMENTS

Ido de Groot, M.P.H., John A. Morrison, Kathe Ann Kelly

This afternoon our attention will be directed to the toxic gases, irritants, alcohol, drugs, and odors, viewed singularly and together, in regard to their synergistic, potentiating or other combinatorial effects. Special emphasis will be on the occupational hazard of these agents in terms of their potential to threaten the health or alter the behavioral activities and patterns of exposed workers.

These classes of exposures appear different and disparate, but their interrelationship and indeed mutual interdependence and thus their classificatory integrity is apparent, albeit that we deal with them as separate and distinct entities. For example, CS_2 is an irritant, a gas, a drug, but also an odor.

Consequently, it is in terms of the reactions or responses in the human receptor that an agent becomes termed a "hazard," while the social circumstances surrounding the event of exposure cause the determination that such an exposure is "occupational" in nature. Thus, "occupational hazards," in this view, fall into such a category without regard, necessarily, to severity, clinical importance, or relative incidence or prevalence.

Odorants, and the odor environment, appear to have little importance as agents of disease, disability, dysfunction or with complaints aired by workers. The exposures but rarely result in palpable pathology, dramatic death, or clear-cut therapeutic regimes of prophylaxis, therapy and maintenance. When clearcut pathology is observed, we generally are dealing with accidental and thus relatively rare circumstances, that are relatively easily remedied. It is our experience, however, that when the agent of disease is not clearly understood, in part due to the complexity and variety of human responses that may ensue from exposures, the emphasis of investigation often shifts from relatively more clinical approaches to more epidemiological assessments for the initial elucidation of the problems at hand. The proper epidemiological assessment of the relationship of odors, the odor environment, and man's behavior demands the collation of data and knowledge from the basic clinical sciences, medicine and the social sciences, a process which is reflective of the epidemiologic method. Simultaneously, however, this process reflects the weakness of this approach, especially when we

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are dealing with ill-defined, poorly measurable agents or stimuli affecting man.

As an apology for the involvement of the epidemiologist in the field of odors per se, we can note that few of us have been able to escape the assessment of the effects of air pollution or tobacco-smoking on human health, in which case odors, be they displeasing or sought-out ones, certainly play an important role. Certainly, the incidence and prevalence of heart disease are important outcome-measures in these relationships. The observations for example by Brieger and Teisinger concerning the increased prevalence of atherosclerosis among workers in the viscose rayon industry following long-term exposures to CS_2 thus not only justifies, but also elucidates the interest in the problem of odors by epidemiologists[1].

The concept of "behavioral toxicology," especially as it is being utilized at this conference, reflects and comprehends all the operational and theoretical constructs and interests familiar to the epidemiologist.

The serious consideration and investigation of the odor environment of worker and non-worker alike is far from frivolous or even esoteric and certainly is not oriented solely to making things "nicer" or "more pleasant" for the worker. Although the improvement of life's circumstances is a worthwhile and needed cause for our interest, it is by no means a prime one. As we have learned in our earlier studies of odors especially in community environments, all levels of physiological, biological, personal and social functioning are involved and ultimately economic and political considerations become superimposed, as well[2, 3]. The apparent frivolity of being concerned with odors readily disappears especially when the worker and the work-environment are being considered (characterized by fixity, if not captivity).

In addition to the widespread response-capability of the human to odor exposures, such responses can occur without regard to the hedonic quality of the odorant, such as when amyl acetate and thiophan are compared for their effect on heart rates in exposed subjects[3]. This indicates that the effects studied should not be limited to the presumed or reported undesirable or deleterious end of the spectrum.

Indeed, we must consider the possibility for example that biologically undesirable odor exposures may have desirable psychological or sociological consequences and effects. For example, the exposure to foul odors among workers may well cause them to form close-knit friendship patterns[4, 5]. Similarly, the removal of odors from garbage may remove the signal to careful, sanitary handling of the material. Such social-psychological antecedents and associa-

tions to health and disease behavior are not well described in the literature.

The rather general lack of interest in the moods, or intra-psychic operations of people, and the worker specifically, is dramatic when the literature on odors is reviewed, as is apparent in the recent bibliography on odors and air pollution published by the U.S. Environmental Protection Agency[6].

Most of what we know about odors outside the purely basic scientific arena appears to derive from community studies. In part, this is so because odors cause more complaints from members of the community than any other form of air pollution[7]. This may lead us to the conclusion that workers are less aware, concerned or affected than all other people, or less involved with problems associated with odors either within or without the workplace, but there is no evidence to warrant this conclusion. Rather, part of our sociology is simply that "By the sweat of thy face shalt thou eat bread." Thus, worker acceptance of odiferous work environments would appear to reflect different attitudes and expectations to the work environment, and need not reflect differences between workers when compared to other groups in the population.

Indeed, the work place ought not be considered a secondary site of study, awaiting the sophisticated and technically valid findings of laboratory and community studies. On the contrary, the work-place appears to be a site of choice to investigate some of the behavioral effects of differential exposures to odors, pleasant or unpleasant, deleterious or harmless.

First, the situation allows for comparisons of relatively homogenous groups of people, according to class, income, job, education, life out-look, etc., all such variables generating problems in community studies. Second, the odorants in all their complexity in which they occur in "natural settings" are identified far more realistically, than in the community, while experimental chambers are limited in capability. Third, variables that affect the behavior of odorants themselves, such as temperature, wind-velocity, electro-magnetism, can be studied more readily. Fourth, the odorants and olfactory response-sets can be studied without regard to their hedonic quality, as we perceive them in *other* situations or role-sets.

The effects of undue exposure to odors on mood, headache, gustatory responses, etc., have been increasingly examined[8,9]. Olfactory problems, however, are not necessarily indicative of mood *disorders* or even more severe diseases, though such problems often do constitute significant components of a variety of disorders[10]. Any effect of odors on headaches, dietary preference, or on general mood should direct our attention immediately to possible effects on vigilance, cooperation, safety-consciousness, etc., among workers and in the

work place[11]. Thus, for example, lysol is not likely to be perceived as pleasant in a restaurant, but appears to generate confidence in the cleanliness of a hospital (to the point of using concentrations which have no further medicinal value).

Similarly, the signal quality of odors can be studied, that is, which odors signal danger (the *abatement* of which could possibly generate accidents, uncertainty, ambivalence). How do other workers utilize odors to determine, for example, whether work has been accomplished by their associates (for example, the role of the smell of kerosene in clean-up crews)? In other words, odors become a definition of the situation, a definition the alteration of which may be *more* deleterious than the odorant initially, such as via the use of the drug, heroin by the negatively affected individual, or the introduction of masking odors.

What this proposes, is that the work-place is not only a unique social situation, but profitably can be viewed as a place to study all of society. Such studies might provide the insights into the dynamic operations of society at large we so sorely need. This approach would also help delineate the true difference between work and non-work (work-place versus home, for example), which appears to be a large component in the mood shifts among workers.

Such workplaces as telephone-switchboard rooms readily come to mind for observation and study of all variables we commonly desire information about. For example, the effects of such variables as diurnal cycles on olfactory responses can be studied by comparing shifts[12]. Also one can introduce selected odors in any desired concentration or combination and study the effect on dialing digit-error, the need for supervisor intervention, illness and absenteeism rates, aggregate menstrual cycling, breathing rates, frequency of customer complaints, use of deodorants and perfumes, use of tobacco, and finally, such important antecedents and precursor behaviors as are involved in deleterious changes in diet.

In conclusion, since toxicologists are normally concerned with the work-place, we propose to you that such studies of behavior in working populations are not esoteric, but in fact will render extremely potent data on the differential distribution between normal, desired versus deviant or undesired human functioning. Thus, the study of workers can help in furthering and maximizing man's functioning outside the workplace as well.

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CARBON MONOXIDE AND HUMAN FUNCTIONS

Netta W. Grandstaff, Ph.D.

Evidence of impairment of human functions during exposure to low dosages of carbon monoxide has continued to accumulate over the past year and it is these data with which we will be concerned. There have been two basic lines of evidence in this regard, one related to the central nervous system and the other to the cardiovascular system. It has been well established that the brain and heart are the most susceptible organs in the body to an oxygen deficit. A brief look at their use of oxygen makes this apparent. The metabolic demand for oxygen by the brain is so great that approximately 20% of the basal oxygen requirement of the entire body is necessary to meet its needs, although the brain only represents from 2 to 2.5% of the total body weight.

The heart muscle, even under resting conditions, extracts nearly all of the available oxygen from the coronary blood flow. The remainder of the body, at rest, extracts only about 25% of the oxygen available from arterial blood. Whereas increased activity of skeletal muscles can be partially met by extraction of more oxygen from the blood, the heart muscle has no such reserve. An increased oxygen demand can be met only by an increased coronary blood flow. Coronary artery disease may limit the capability of such an increase, readily leading to tissue hypoxia.

The response of the myocardium to diminished oxygen transport induced by CO uptake has been studied by several investigators using both normal and coronary heart disease subjects.

Before reviewing some of the more recent studies in this area, I would like to digress a moment and look at some basic aspects of CO uptake.

Carbon monoxide enters the bloodstream through inspired air via the lungs. Once in the bloodstream it binds to the hemoglobin of the red blood cells and becomes carboxyhemoglobin (COHb). There are several primary factors which determine the uptake of exogenous CO: (1) the CO concentration; (2) the length of exposure, and (3) the ventilating rate of the individual. A fairly constant rate of CO uptake occurs until approximately one-third of the equilibrium value is reached and then the uptake becomes progressively slower. The COHb concentration at equilibrium is about 0.16 times that level of CO in ppm to which the individual has been exposed. Under "normal" circumstances, almost all hemoglobin is saturated with oxygen, with only about 0.5 to 1.5% being bound with endo-

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genously produced CO. Since the chemical affinity of CO to hemoglobin is 210 times greater than that of oxygen, "a little CO goes a long way!"; thus, the main effect of CO is its direct reduction of the oxygen-carrying capacity of the blood. The presence of COHb in the bloodstream also shifts the oxyhemoglobin dissociation curve to the left. This means that at the tissue level of circulation there is less oxygen available to the cells due to a decrease in oxygen tension (Po_2), i.e., the oxygen that is in the blood, as COHb increases, becomes less readily available at the cellular level.

Permutt and Farhi[1] discussed the theoretical implications of this relationship, and showed that with small doses of CO, tissue hypoxia might occur in such high oxygen demand organs as the heart and brain. Ayres[2] showed that inhibition of oxygenation of cardiac muscle by small doses of CO could be readily induced experimentally.

A very relevant study showing cardiovascular system impairment was done this past year, 1972, by Aronow[3] and his group in Los Angeles. Ten patients with angina had cardiopulmonary tests performed under three conditions: (1) in the control state, i.e., freeway driving breathing compressed air; (2) after being driven for 90 minutes during heavy morning freeway traffic; and (3) two hours after traffic exposure.

The primary findings after breathing freeway air were (1) that there was a decrease in time during exercise performance until the onset of angina pain; (2) a decrease in systolic blood pressure at angina; and (3) in heart rate at angina. Three of the ten patients also had ischemic S-T segment depression while breathing freeway air. These findings were attributed to an increase in COHb levels from CO exposure in heavy traffic.

There have been several justifiable criticisms of Aronow's study. However, it should be pointed out that another research group, that of Knelson and Anderson, et al.[4,5], have reported somewhat similar findings under highly controlled laboratory conditions.

Knelson, Anderson and associates[4,5] studied the possible contribution of CO to myocardial ischemia on a group of adult males with stable angina pectoris and on both young (25-36 years) and middle-aged (41-60 years) clinically normal men.

The ten angina subjects (average age 49.9 years) were exposed to 0 ppm, 50 ppm and 100 ppm CO on five successive days. Mean COHb levels were 1.3% after 0 ppm exposure, 2.9% after 50 ppm, and 4.5% after 100 ppm CO. Following each 4-hour CO exposure, each subject was tested on a standard exercise treadmill while monitoring the ECG.

The mean duration of exercise before the onset of pain was significantly shortened after both 50 and 100 ppm exposures when compared with 0 ppm data. The duration of induced pain was also

significantly prolonged after the 100 ppm exposure, but not after the 50 ppm.

ECG's showed worsening of the S-T segment changes and an earlier onset and longer duration of S-T segment depression both during and after exercise.

Their conclusion was that "low level exposures to CO can cause decreased exercise tolerance and worsening of myocardial ischemia in angina patients.

The normal subjects were exposed to 100 ppm CO for 4 hours and had COHb levels which varied from 5 to 9%, with uptake being higher in the younger subjects. Both pre- and post-CO exposure electrocardiograms (ECG) were monitored as each subject exercised on a standard treadmill unit to submaximal treadmill exercise. Both the younger and middle-aged men attained target heart rates at a lower level of exercise after CO exposure. There were no changes in the exercise ECG for the younger men. Post-exercise systolic time intervals changed in the middle-aged group, accompanying a depression of the S-T segment of the ECG. It should be noted that all seven of the older subjects with abnormal ECG's were either former or current smokers. The conclusion was that normal middle-aged men are definitely susceptible to the hypoxemic effects of CO, and that it "may augment the production of exercise-induced myocardial ischemia in pre-existing subclinical heart disease, contribute to the development of myocardial dysfunction, and may lead to an increased incidence of arrhythmias in such persons".

A very recent survey study by Stewart's group[6], gives a breakdown of the "normal" COHb levels among blood donors by locale in the United States. Data were collected in 18 major cities on a total of 20,000 adults at arbitrarily chosen blood bank collection sites. The venous blood samples were analyzed, using the CO-Oximeter and a gas chromatographic procedure. The high point of the study was the observation that 45% of all the non-smoking blood donors sampled had COHb saturations in excess of 1.5%, and that in areas with high automobile densities, such as Los Angeles and Denver, the proportion of non-smokers with COHb levels above 1.5% rose to an alarming level of 76% of the total donors sampled in each geographical location. Chicago was close behind with 74% of the non-smoking donors with COHb levels above the "normal" baseline of 1.5%. Smokers, of course, exhibited considerably higher mean COHb levels, with Los Angeles having a mean of 6.2% (range 2.0 to 10.3%), for example. The conclusion was that a significant percentage of the population is chronically exposed to CO concentrations exceeding those permitted by Air Quality Standards.

Dr. Barry Johnson and associates[7] at NIOSH have just completed a study in Kentucky on the effects of CO (and other possible

air pollutants) on toll-booth operators. The subjects were tested twice daily over a two-week period. Each subject was tested just before and at the end of an eight-hour shift (2-10 pm). Analysis of data from a battery of seven performance tests has not been completed yet. Included in the tests were reaction time, arithmetic tests, time estimation, "time sharing" or dual task (red/green choice and tapping).

Ambient CO levels were monitored and blood COHb estimations were made. Mean pre-COHb levels ranged from 0.8-1.0%, and after an eight-hour work shift the range was from 2.5 to as high as 12%. Since five out of six of the employees presently being hired to be toll-booth operators are young women, the question of CO on pregnancy or during pregnancy arose. So in addition to the usual health and performance risks, this added possibility of risk to the fetus must be considered in evaluating the effects of CO exposure on this special population.

Of grave importance is the question, What effect does this kind of chronic CO exposure have on the general worker population in this country? A few more relevant facts may be in order. An astounding result which came out of the Korean War autopsies, performed on young soldiers, was that 77.3% had gross pathological evidence of coronary heart disease at the mean age of 22 years. Brest[8] has gone so far as to state that after twenty it would be impossible to delineate a control group "without atherosclerosis" in the U.S. in the white male population. Brest reported that it has been estimated that each year (in the U.S.) more than five hundred thousand persons sustain silent myocardial infarctions.

A study of the autopsies in a stable community of thirty thousand has shown that coronary heart disease was the cause of death in 40% of the males. It is a well established fact that more persons in the U.S. die from coronary heart disease than from any other disease.

It is clearly evident from the foregoing information that the general work force in the U.S. is composed of a significant number of individuals who have asymptomatic coronary heart disease. It is supposedly virtually impossible to identify these persons in the absence of any overt clinical symptoms. Unfortunately, the first clinical symptom is sudden death in one-sixth of the cases of coronary heart disease.

Central Nervous System impairment to low dosage exposures of CO has been shown to occur for a number of psychological, perceptual, and psychomotor functions.

Early work in this area was done by McFarland, et al.[9,10], and by Halperin, et al.[11,12], who showed a deficit in visual functions with CO exposure. Schulte[13] was the first to demonstrate an adverse

effect of CO on several psychological functions.

In our laboratory, an early study by Beard and Wertheim[14] showed that CO at low dosages caused a decrement in the ability to perform a temporal discrimination task. We also reported a deficit in visual acuity and relative brightness thresholds during CO exposure[15].

Groll-Knapp and Haider, et al.[16], showed that CO adversely affects performance on an auditory vigilance task. Visual vigilance performance has also been shown to be affected by low levels of CO, both in our laboratory[17] and by Horvath, et al.[18].

Recently, Bender, et al.[19], have shown a CO-induced impairment in manual dexterity, visual perception, and short-term memory, as well as a performance decrement in a highly cognitive task relative to abstraction.

All research groups have not been unanimous in finding low-level CO effects on central nervous system function. In fact, Stewart's group[20] has reported that no behavioral effects were evident for COHb levels of 17% for a number of performance tasks.

In our own work, several tests have failed to show a reliable decrement during CO exposure, including (1) a simple short memory task; (2) a very complex memory task, and (3) a spatial perception task. However, a time estimation task with a spatial factor did show that CO affected this particular perceptual ability.

PERIPHERAL VISION

Peripheral vision is often critical for efficient and safe performance of numerous tasks. Location and detection as well as reaction time or response latency are all relevant variables to many on-the-job performance requirements.

Peripheral visual fields have been shown to be affected by a number of stressors, including heat[21], anoxia[22], loud noise[23], and hypoxia[24].

Since peripheral vision plays such an important role in detecting visual stimuli in the useful field of vision, determination of the effects of CO on this aspect of vision seemed most germane in evaluating CO on cerebral functions. The past year has been spent attempting to do this.

METHODOLOGY

Testing has been completed on 15 young adult subjects (18 to 25 years of age) who were non-smokers.

Prior to participation in the study, each subject was given a visual field examination in the clinical ophthalmology laboratory, Stanford Medical School, using the Goldman Perimeter. Only individuals with normal visual fields were tested in the experiment. Other clinical tests given were baseline ECG and EEG, in addition

to an extensive medical history and a physical examination when there was any question regarding the health of the individual.

All subjects were tested in a continuously ventilated, sound attenuated, single-person audiometric chamber to atmospheres of 0, 50, 175 or 250 parts per million (ppm) CO. The CO concentration was continuously monitored by a nondispersive infrared analyzer (Beckman Model 315B) and maintained at the predetermined level by manual adjustment of the flow of CO into the ventilator inlet.

The presentation of stimuli and recording and analysis of behavioral responses were controlled by a PDP-8 computer and related peripheral equipment. The analysis of variance data were processed on an IBM 360-65. Electrophysiological recordings included EEG (evoked potentials), ECG, and eye movements. Results from these data will be reported at a later date.

Breath samples and venous blood samples were used to estimate carboxyhemoglobin (COHb) levels. COHb etimates from breath samples were calculated using a nondispersive infrared analysis and the Goldsmith[25] formula (ppm x 0.16). The venous blood samples, collected in a hypodermic syringe with a small amount of heparin, were analyzed using an automated spectrophotometric procedure (CO-Oximeter).

The apparatus for testing peripheral vision was fabricated in our laboratory. It is a hemisphere having 8 radial axes dispersed around a central fixation light with each axis separated by a standard angular distance of 45°. Four stimulus lights (visual angle $-0.256°$) are located at 20°, 40°, 60°, and 80° from the center fixation point along each of the 8 axes, making a total of 32 different stimuli. The diameter of the hemisphere is 31 inches. Each light is approximately 15 inches from the mid-point between the eyes. The stimulus lights are viewed against an off-white background with an ambient illumination of approximately 4 ft. L. The small size (0.256°) of the stimulus lights has made exact measurement of luminances impossible with available equipment. The stimulus intensity was established empirically by pilot studies in which the light intensity was increased until a specified performance criterion was met. This criterion required the detection of a minimum of 5 lights out of the total 6 at each stimulus location during each block of trials for all stimuli in the functional field of vision. Subjects in all age groups were tested in the pilot study (18-65). A chin-rest was used to keep the subject's head in a constant position during testing. The entire apparatus was adjustable in order to accommodate the various heights of different individuals so the eyes were always aligned with the horizontal meridian. Binocular vision was used for all testing.

The task required the subject to fixate on a small blue light in

the center of the hemisphere and to press a hand-held button as soon as possible after seeing a light at any position. In each block of trials, 6 successive series of the 32 stimulus lights were presented (in random order) with the restriction that no light position be repeated until all 32 positions had been presented. Since there were 4 blocks of trials or runs (of 192 trials each) per session, the total number of trials was 768 for each subject at each CO concentration.

The stimulus-response paradigm was as follows: the duration of the stimulus light was 0.5 second. The subject could respond at any time after the onset of the stimulus and for a post-stimulus period of 1.5 seconds. All other responses made during the inter-stimulus interval were classified as false responses. There were 8 inter-stimulus intervals which varied between 5-12 seconds and which were presented in random order.

Each subject received 2 orientation sessions in order to help eliminate possible practice or learning effects. The four conditions of 0, 50, 175, and 250 ppm were presented in different random orders for each subject on the four subsequent test sessions. All testing was under double-blind conditions. Each subject's 2-hour and 40-minute test session consisted of a 10-minute pre-test period in the chamber to adapt to the ambient light level. This was followed by the presentation of four 30-minute trial blocks, each separated by a 10-minute rest period. A flashing red light served as a warning signal for the onset of each block of trials.

Data used for the analyses were the 2nd, 3rd and 4th blocks of trials. (The first block appeared to still show some "warm-up" effect, and therefore was not used.) That is, data used were the trial blocks after CO was present in the chamber or the COHb was still elevated (after the CO was off) and the identical trials for the 0 ppm condition. Trials on which there was no response were automatically given a latency value of 2.0 seconds (i.e., the maximum response time-out).

RESULTS AND DISCUSSION

The two behavioral measures used in evaluating the effects of CO on visual fields were (1) the response latency at each light position, and (2) the corresponding number of stimuli detected. Data were analyzed using the subject as his own control and combining data over all subjects. An analysis of variance was used to determine the effects of CO on these performance measures. In looking at the group data first, the results showed that a statistically significant increase in the response latency occurred during exposure to CO ($p < 0.005$).

Figure 1 shows the group mean response latency for each light position at 0 ppm CO. Kobrick and Dusek[24] have shown that

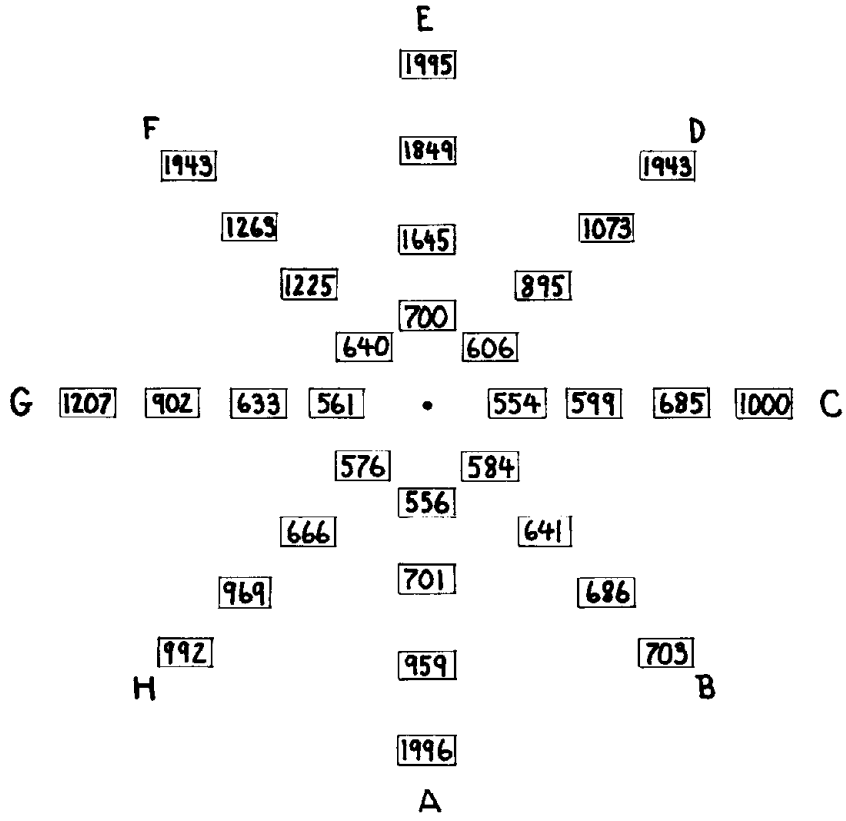

FIGURE 1. The group mean response latency (msec) for each light position throughout the visual field at 0 ppm CO concentration.

response latencies increase with stimulus displacement toward the visual periphery and that these increases become greater with severity of hypoxia. Our baseline (0 ppm CO) observations also showed a definite increase in response time with displacement toward the periphery and a trend for CO exposure to cause a small but greater increase in response time throughout the visual field. A comparison of 0 ppm data with data from each of the CO levels indicated that at 50 ppm the mean response latencies increased at 28 out of the 32 light positions; at 175 ppm CO, 22 out of 32 increased, and at 250 ppm again 28 of the 32 positions showed a lengthening in the response time.

It is obvious that a specific dose-response relationship was not observed; there was no evidence of a gradient effect as a function of CO level. The increases in mean response latencies ranged from a maximum of approximately 200 msec down to a change in the opposite direction in the few cases mentioned above.

One other finding bears mention, although we can only speculate about its meaning. The latencies in the left visual field were predominantly longer than the corresponding position in the right, and the latencies on the superior vertical axis were noticeably longer than those on the inferior vertical axis. (We should mention that lights at 80° on Axes A, D, E, and F were essentially outside of the functional field of vision for most subjects, as eyebrows and/or chin obstructed these lights.) Such an observation suggests that in the dynamic visual field testing situation we have here, there appears to be a difference in the processing time as a function of which cerebral hemisphere is stimulated.

This possible asymmetry of function may become clearer if we look at the visual system and its relationship to the functional field of vision. Visual pathways are crossed so that when the eyes are fixated on a point, all of the field to the left of the fixation point excites the visual cortex in the right hemisphere and stimuli from the right visual field excite the left visual cortex. Communication between the two visual cortices occurs via the corpus callosum, which is a large bundle of fibers connecting the two cortices.

To further complicate the matter, the sensory-motor cortex, involved with pressing the button on such tasks, is connected to the contralateral hand, i.e., the left cortex is connected with the right hand and the right cortex with the left. Consequently, visual information projected from the left visual field to the right visual cortex must then cross over to the left motor cortex to initiate the response of the right hand. Thus our conclusion is that the longer response latencies observed in the left visual field are due to simply a longer processing time related to handedness.

To further evaluate the effects of CO on response latency, each subject was used as his own control. The results showed that 12 of the 15 individual subjects had a statistically significant ($p < 0.005$) increase in response latency with exposure to CO.

The other behavioral measure, the number of lights detected, indicated that for group data, a significant decrement occurred in the mean number of lights detected during CO exposure ($p < 0.005$, analysis of variance). There was a very close correspondence between the number of missed lights at any given position and the mean response latency, with the positions having longer latencies definitely showing the greatest number of missed lights.

Again, the subject was used as his own control to further evaluate the number of stimuli detected by individual subjects. Ten of the 15 subjects showed a highly significant decrease in the number of lights detected during CO exposure ($p < 0.005$).

The number of false reports, i.e., pressing the button when, in fact, there was no stimulus, tended to decrease with time and was

not correlated with CO level. A decrement with time is the typical response pattern reported for vigilance data in numerous studies[26,27].

A word regarding the subjects who did not appear to show a performance decrement during CO exposure. Such data should always be viewed with some degree of caution. Obviously, all individuals are not equally sensitive to a toxic agent or drug. This has been especially well documented in the area of research on anesthetics. And the assumption may be quite valid that this is the case for those subjects who do not show an effect with CO. However, the likelihood that a subject is affected, but that the effect is obscured by a very unstable or variable performance is just as possible. It has been our experience in evaluating human performance that intrasubject variability is a critical factor to be considered in interpreting behavioral data.

Figure 2 shows the mean percent COHb as estimated from alveolar breath samples at 0, 50, 175, and 250 ppm CO concentration. Data include all 15 subjects. There were four breath samples ob-

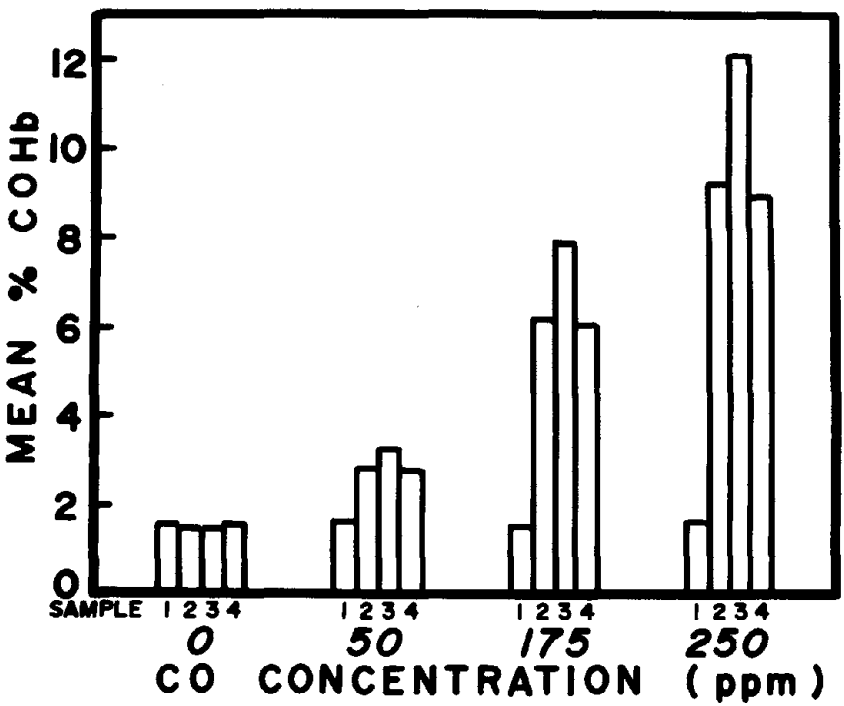

FIGURE 2. The mean percent COHb as estimated from alveolar breath samples at 0, 50, 175 and 250 ppm CO concentration.

tained at each CO level for each subject. Sample one was a pre-test sample, and therefore was always at baseline (1.5% COHb) regardless of CO concentration. Sample two was taken after 40 minutes of CO exposure (at the end of the second block of trials) and sample three was taken 80 minutes after onset of CO (at the end of the third block of trials). The CO was turned off at this point in testing. The fourth breath sample was collected after the fourth and last block of trials, at the end of the test session (approximately 40 minutes after CO was terminated). The maximum for each level was approximately 1.5% at 0 ppm, about 3.25% at 50 ppm, almost 8% at 175 ppm and 12% for 250 ppm CO. The values are comparable to COHb data from other studies in our laboratory.

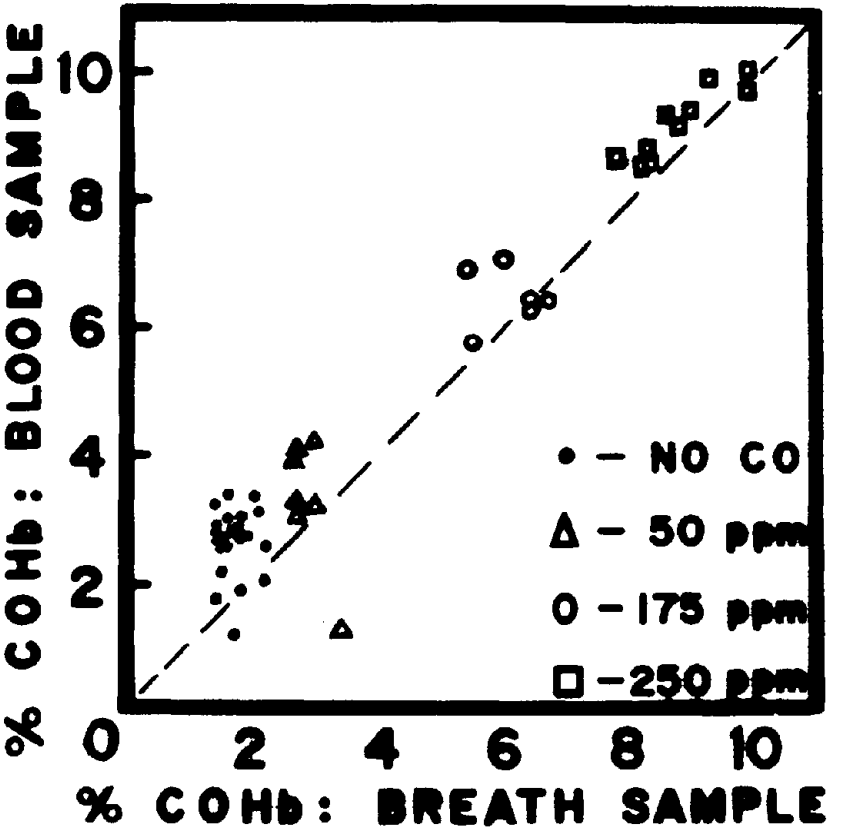

FIGURE 3. A comparison of COHb estimates from alveolar breath samples with venous blood samples at 0, 50, 175 and 250 ppm CO.

A comparison of COHb estimates from alveolar breath samples with venous blood samples is shown in Fig. 3. Both types of samples were collected concurrently. There were 49 determinations on 13

subjects. Venous blood samples were collected only for pre-test and post-test samples. The over-all correlation of blood samples with breath samples was very good with a correlation coefficient of 0.97 (slope = 1.04; intercept = 0.91, standard error = 0.008). However, if pre-test data (0 ppm data, N = 22) are analyzed separately, the correlation coefficient is −0.11 (slope = 0.08; intercept = 1.86; standard error = 0.22). Post-test data analyzed alone (N = 27) had a correlation coefficient of 0.96 (slope = 0.99; intercept − 0.40; standard error = 0.02). The point is, that for low COHb saturations (below 3%) there is a very poor correspondence between alveolar breath samples and venous blood samples, but with higher COHb saturations, the correlation is very good.

Our investigation into the reliability (or perhaps lack of reliability) of the CO-Oximeter as an instrument for estimating the COHb saturations from venous blood samples has helped to clarify at least one of the problems. The instrument has a calibration bias (1.5%) which in turn introduces a constant error of approximately 1.0% in low COHb (3% and less) levels. When the calibration bias was adjusted or eliminated, i.e., set at 0 (instead of 1.5 as suggested by the manufacturer), replication of COHb saturation estimates on blood samples with a constant and known value proved impossible. The instrument bias appears to be absolutely necessary for the operation of the machine, but users should be aware of this source of error for calculation of low COHb saturations.

These behavioral observations support the prediction of a possible decrement in the processing of information in the visual field as a function of low level CO exposure. The deficit occurs throughout the visual field, but there appears to be a definite trend for the superior vertical portion of the field to be slightly more affected. The implications of this are that not only is there a decrease in the general arousal level which reflects a decrement in the sensitivity of the brain itself, but the peripheral receptors in the retina appear to be differentially affected. CO has always been considered a central nervous system depressant, and this is the first report to implicate the peripheral receptors as a site of action for CO.

The focus at this point in our knowledge concerning CO should perhaps be directed more toward a clearer identification of those specific groups of individuals or populations who are definitely at risk during such low-level CO exposures. Further elucidation of the extent of impairment of psychological and physiological processes in normal subjects continues to be important in and of itself, as well as affording baseline data for comparative studies with independent populations. Essential to the identification of populations at risk is, of course, a good operational definition of the term, *at risk*, for

example, how does one establish a critical threshold for CO exposure for those individuals with subclinical cardiovascular disease?

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FIELD EVALUATION OF CARBON MONOXIDE EXPOSED TOLL COLLECTORS

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INTRODUCTION

The effects of carbon monoxide (CO) on behavioral performance have been summarized in two reviews by the U.S. Public Health Service [1,2] and, more recently, further elaborated by Grandstaff[3]. A review of these and other related articles indicates that few studies have been conducted concerning the effects of CO on the behavioral performance capacities of worker populations found in a non-laboratory setting. Indeed, most of the CO behavioral toxicology studies have utilized college students with the studies being conducted within university laboratories. The question of whether or not the findings from such laboratory studies can be replicated in worker populations in actual industrial settings has not been answered.

METHODS

The Study Group

The group chosen for study in this investigation consisted of six workers employed as fare collectors at a toll highway (Interstate 65) south of Louisville, Kentucky. This particular work group was chosen for study because of known exposure to CO from vehicle exhaust and due to workers' subjective complaints that indicated a possible excessive exposure to CO. The study group consisted of 1 male and 5 female workers. The subjects ranged in age from 19 to 49 with a mean age of 32.1 years. All subjects were nonsmokers in order to maximize the difference between the resting blood CO (carboxyhemoglobin, COHb) level and the equilibrium COHb level. Also, it was considered important to ascertain the degree of exposure to ambient air CO without the confounding influence of COHb accumulation due to smoking. Prior to acceptance as subjects in this study, all six volunteers were given a general medical examination to ascertain their suitability for inclusion in the study.

The workers collect the vehicle fares while situated in a semi-enclosed booth as illustrated in Figure 1. The collection plaza con-

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sisted of 6 collection booths arranged as shown in Figure 2. The toll collectors rotate booth assignments each day, but do not change booths during the course of an eight-hour workshift. All work breaks were taken in the central office area (Figure 2), which also contains two collection booths. As can be discerned from Figure 3, the entire toll plaza was situated under a canopy formed by a bridge overpass, a situation which contributes to accumulation of vehicle exhaust fumes in the toll collection booths.

FIGURE 1. Toll collection booth.

Performance Tests

Each day the subjects were administered a battery of behavioral performance tests. The various tests measured performance functions demonstrated by laboratory studies[4, 5, 6, 8] to be affected by CO or tapped those functions believed of importance to worker safety. The task battery consisted of measures of eye-hand coordination, time estimation, visual perception, complex arithmetic, choice reaction time, critical flicker frequency, and task time sharing. These individual tests are described in the following sections.

Time Estimation. The time estimation task was chosen based on a report by Beard[4], that subjects' ability to estimate, without cues, the passage of 30-second intervals of time was impaired by a 50 parts per million (ppm) CO exposure given for 80 minutes. The time judgment task utilized in the present study consisted of manually presenting a 1000 Hz tone to the subjects via headphones. The

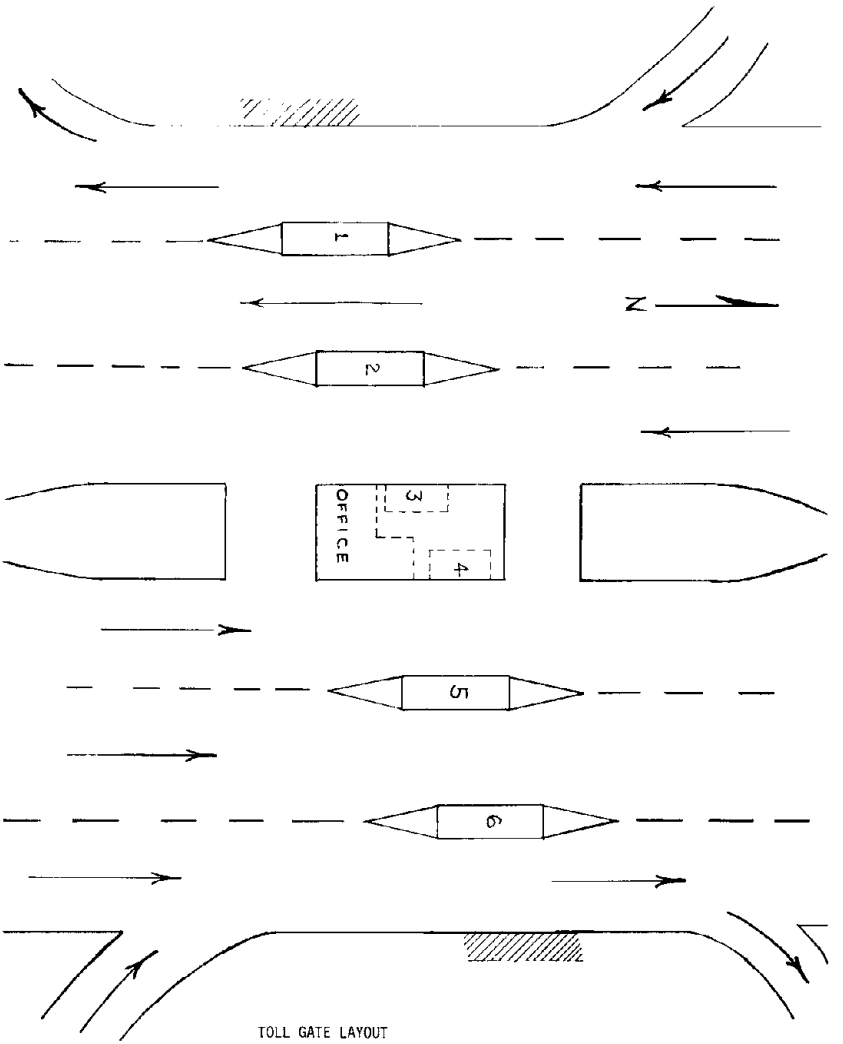

FIGURE 2. Arrangement of toll collection booths and office.

duration of the tone was controlled by the experimenter and varied between 1 and 30 seconds in duration. Each session consisted of a randomized set of 27 such tones. The subjects' task was to listen to each tone, then press a hand-held switch for an amount of time equal to the duration of the stimulus. Both the stimuli and the responses were recorded as pulses on a FM analog magnetic tape recorder (Honeywell, Model 5600)* for subsequent data analysis. The response data were characterized in terms of percent estimation error for each stimulus. For example, if the stimulus persisted for

FIGURE 3. Toll collection booth and plaza canopy.

10 seconds, and the subject's estimation was that 8 seconds had elapsed; the percent estimation error would be −20 percent.

Complex Arithmetic. The complex arithmetic task was included in view of Schulte's report[5] that low level CO exposure affected subjects' ability to perform arithmetic calculations. The arithmetic task utilized in the present study consisted of adding sets of five five-digit numbers arranged in columns. Each subject was limited in time to 6 minutes and had been instructed to complete as many addition problems as possible and as accurately as possible. The number of problems completed within the alloted time and the number of errors constituted the performance measures for this task.

Reaction Time. Although a review of the CO literature suggests no effect of CO on choice reaction time, an eight-choice reaction time test was utilized in this study due to the recognition that safe performance of the toll collectors' job required the ability to react on occasion quickly to potentially hazardous situations. The reaction time apparatus consisted of eight red pushbutton switches (A-H #83503) arranged equally spaced around a semicircle of 4 inch radius. Within each pushbutton switch was a miniature lamp (Chicago #334). Situated on the same panel 6 inches directly above the center of the semicircle of pushbutton switches was a green cue lamp. At the center of the semicircle was a pushbutton switch

*Mention of company or product does not constitute endorsement by the Department of Health, Education, and Welfare.

(A-H #83503). Through use of commercial, programmable solid state logic (Lehigh Valley Electronics), a randomized series of 24 stimuli was presented in the following manner. When the green cue lamp was enabled, the subject was required to depress the central "hold" button until one of the eight red lamps was lighted. When this occurred, the subject was required to extinguish the red lamp by depressing the corresponding response button as quickly as possible. By use of electronic clock counters (Hunter, Model 220C), the subject's reaction time was measured and divided into two components, movement time and detection time. Detection time was measured as the elapsed time from time of onset of a red stimulus lamp to the time the subject released the hold button. By subtracting this time, detection time, from the total reaction time (i.e., the elapsed time from the onset of a red lamp stimulus to the time the red lamp was extinguished), subject's movement time from hold button to response button was calculated.

Eye-Hand Coordination. Bender, et al[6]., reported from laboratory studies that a COHb level of 7.3 percent saturation impaired subjects' performance on tests of eye-hand coordination and visual perception. In the present study eye-hand coordination was tested through use of a modification of a test developed by Poock[7] at the University of Michigan. The test required the subject to insert a metal stylus with his preferred hand into a series of 119 holes drilled into a plastic plate. The holes were connected by lines drawn from hole to hole in such a fashion as to indicate a maze that possessed start and end points. The subjects, in effect, traced out this maze by moving the stylus pen from hole to hole. The subjects performed this task while seated at a table and while maintaining the arm holding the stylus parallel to the table. Each time the stylus was placed into a hole, a metal plate beneath the plastic cover plate was contacted, a buzzer sounded, and an electrical pulse was generated and recorded on FM analog tape. The set of 118 interhole movement times constituted the data for this test.

Visual Perception. Bender, et al[6]., found that subjects' visual perception was impaired at a COHb level of 7.3 percent. Our test, which was similar to Benders' test, consisted of presenting via a tachistoscope (Lafayette, Model U-1) a series of 20 slides, each of which contained 5 letters arranged on a horizontal line. The sequence of letters on each slide and the order of the slides were different for each experimental session. The letters on the slides each had the following dimensions: 0.5 inch tall, 0.25 inch wide, 0.5 inch between adjacent letters, and a 0.125 inch width for the lines comprising the letters. The stimulus duration was 8 milliseconds for each stimulus. The subjects were required to view the stimulus and write on a data sheet the exact sequence of

letters that was observed. The number of errors and the position of the errors made in reporting each set of 5 letters were subsequently evaluated.

Critical Flicker Frequency. The effect of CO on the estimation of critical flicker frequency (CFF) was determined through use of a CFF instrument (Lafayette, Model 1202-A). The CFF was determined binocularly at an on/off ratio of 50/50 percent and constant luminosity. Fodor's[8] finding that CFF is most sensitive to chemical effects if determined by the descending method, i.e., by adjusting the frequency of the light source downward from a point of perceived light fusion to a point where flicker is just apparent, was utilized. Accordingly, each subject was given five trials for which initial light frequency was self-adjusted from 70 cycles per second downward to the CFF. The mean and standard deviation of the 5 CFF determinations were used to characterize the data for each subject's session.

Task Time Sharing. In order to investigate the effects of CO on a subject's ability to perform two tasks concurrently, a time-sharing test consisting of two separate tasks was devised. The primary task was a modification of a binary choice task described by Ettema[9] and in our case consisted of a visual task involving two vertically oriented red and green lights. Through use of Lehigh Valley electronic control logic, the two lights were turned on and off in a random order and at a rate controlled by the experimenter. The subjects were required to respond to each occurrence of the red light by pressing a pushbutton switch held in the right hand and to each green light by pressing a switch held in the left hand. The number of response errors and omissions were determined by use of electronic counters.

The secondary task utilized in the test of task time-sharing performance was an interval production task originally described by Michon.[10] The task utilized in this study required each subject to establish and then maintain a regular interval of tapping with the preferred foot. The emphasis given to the subjects was to maintain as constant a rate of tapping as possible. To record the tapping performance, a contact switch was placed beneath the sole of the foot. Each closure of the switch caused the generation of an electric pulse, which was recorded on FM analog tape.

The time-sharing task was administered at each session in the following manner. First, the subject performed one minute of basic tapping without the presence of the binary choice task. Then followed two periods (3 minutes each) of tapping plus concurrent performance on the binary choice task given at two different rates of visual stimulus presentation. The 'moderate' presentation rate was 50 stimuli per minute, while the 'fast' rate was 60 stimuli per

minute. Each stimulus remained on for 500 msec. The ability of the subject to perform both tasks simultaneously was measured by the irregularity induced in the tapping task by the presence of the primary task. The subjects' performance on the tapping task was calculated in terms of Michon's[10] perceptual motor load (PML) and Drury's[11] correlation index for all three perceptual load conditions: no load, moderate load, and fast load.

CO Determinations

Since carbon monoxide was considered to be the primary constituent of vehicle exhaust thought to be responsible for such workers' subjective complaints as headaches, mild dizziness, and nausea; two biological methods for assessing the degree of CO exposure were utilized. First, in order to ascertain directly the level of carboxyhemoglobin, two blood samples were obtained from each subject on each day of testing. The blood samples were obtained prior to the start of the subjects' eight-hour workshift and then following completion of the workshift. All blood samples were obtained through use of a finger stick procedure. In this procedure a sterile lance was used to puncture a subject's finger near the tip. The first drop of blood was wiped away with a sterile gauze pad, then two heparanized capillary tubes were filled with blood and sealed with capillary tube sealant. The blood samples were refrigerated at 4°C and analyzed for COHb content within 3 days from the time of blood collection. The spectrophotometric method of Commins and Lawther[12] was used to measure COHb content.

In order to measure the rate and extent of CO uptake of the subjects during the course of their workshift, breath samples were collected prior to the workshift, every two hours at the worksite, and following completion of the workshift. These samples were obtained using the twenty-second breathholding technique described originally by Jones, et al.[13] This procedure requires the subject to expire maximally into room air, inhale maximally and hold the breath for a minimum of 20 seconds, following which about one-fourth the breath sample is released into room air. The remaining breath sample is collected in a closed plastic bag. The samples collected in this manner represented alveolar air and were analyzed for CO content through use of a calibrated (0-100 ppm CO) infrared gas analyzer (Beckman, Model 315BL). The CO analyzer contained a desiccant (Ascarite) to remove the interference of H_2O vapor and CO_2. Breath samples were typically analyzed within 15 minutes from the time of collection.

Worksite Measurements

In addition to the previously described breath and blood analyses for CO, ambient air monitoring was performed at the toll collection

plaza. Portable, direct-reading, CO analyzers (M.S.A., Model D) were used by industrial hygienists from the Kentucky Department of Health to measure ambient CO levels at the 6 collection booths. Each subject was also required to wear a personal sampling pump (M.S.A., Model G) which drew ambient air (1.5 liters per minute) through a millipore filter pad which was subsequently analyzed by atomic absorption spectrophotometry for lead and manganese content.

Since noise levels at the worksite were considered a potential source of worker discomfort and fatigue, measurements of ambient noise levels were obtained using a sound level meter (B&K, Model 2205). Noise level measurements were normally obtained every two hours at different locations within the toll plaza.

In order to correlate the degree of ambient air pollution with vehicular traffic patterns through the collection plaza, printouts from the automatic traffic logging system were obtained from the Kentucky Department of Highways. Also, since the degree of vehicle exhaust accumulation was affected by weather conditions; temperature, relative humidity, and wind direction and velocity were obtained for each day of testing from the local office of the U. S. Weather Service.

TESTING PROCEDURE

The six subjects comprising this study were tested three per day on alternating days over a twelve day period from April 2 through April 14, 1973. Preceding the start of the study, each participant was given two training sessions of 90 minutes duration each. The testing facility consisted of a room at a motel adjacent to the toll collection plaza. The test room measured approximately 12 by 15 feet and was functionally divided into three distinct performance testing areas. Each of these three areas contained two or more of the seven performance tests comprising the performance test battery.

A room adjacent to the performance testing room was used to collect subjects' blood and breath samples and complete a questionnaire given to ascertain subjective feelings of fatigue and discomfort. Each subject was asked at each session to provide an indication of the degree of subjective fatigue on a scale ranging from 1 (no fatigue) to 10 ("bone-tired"). Also, the questionnaire requested each subject to report by 'yes' or 'no' answer whether or not they believed they had been exposed to a lot of CO during their workshift.

The protocol for each day of testing was as follows. One hour prior to the start of their workshift (2 p.m.-10 p.m.), three of the participants reported to the test site. Blood and breath samples were obtained from each as well as completing the subjective feelings

questionnaire. All subjects were then administered the performance test battery. Following the pre-workshift testing, the subjects reported to the toll collection plaza and worked their normal 8-hour shift. Every two hours alveolar breath samples were collected at the worksite. Noise levels sampled throughout each workshift were from points within the collection booths and immediately adjacent to the booths. Following completion of the workshift, the three workers being tested reported to the testing station for a repeat of the pre-shift protocol.

RESULTS

The findings from this investigation are given in Tables I through IX and will be discussed in terms of subjects' ambient exposure to vehicle exhaust, results of biological measures of CO exposure, behavioral performance test results, and findings from subjects' subjective feelings questionnaire.

Ambient Exposure Data

The summary results of various measures of the subjects' exposure to airborne lead and CO, together with mean hourly vehicle counts, are given in Tables I-IV. Table I contains the mean hourly vehicle counts for each day of the investigation. The mean hourly number of vehicles processed during all 12 days of the study was 249 and the hourly traffic count ranged from 40 to 1035 vehicles. Two patterns are evident from examination of Table I. First, for both weeks of the investigation the number of vehicles processed through the toll plaza remained relatively constant from Monday through Wednesday, then began increasing on Thursday. The greatest amount of traffic was found to occur on Friday and Sunday, a situation attributed in large measure to the large number of factory workers proceeding south on Fridays to homes in southern Kentucky and northern Tennessee and then returning on Sundays. The second pattern evident in Table I is that the collection booths in the interior of the plaza, i.e., booths 3 and 4, process more vehicles per hour than do the exterior booths (booths 1 and 6). However, as will be subsequently described, this difference in traffic was not reflected as pronounced differences in ambient lead and CO concentrations.

The results of ambient air sampling for CO content are given in Table II. These data represent an 8-hour time weighted average (TWA) for CO concentrations measured in and around collection booths 1, 4, and 6. Examination of these data indicates that the mean 8-hour TWA CO level for the 12 days of the investigation was 22.8 ppm. On three of the days of the study the 8-hour TWA CO level exceeded the NIOSH recommendation of 35 ppm, and if CO data from collection lane #2 are included with those shown for Thursday, April 5th, then the TWA CO level for this day also ex-

Table I. MEAN VEHICLE COUNT FOR 2-10 P.M. WORKSHIFT IN VEHICLES PER HOUR

Collection booth number	Day													Booth Mean and (S.D.)
	M	T	W	Th	F	S	Sn*	M	T	W	Th	F	S	
1	201	203	204	216	351	175	249	192	183	225	249	367	263	236 (63), (N=12)
4	275	259	257	303	401	389	471	245	255	269	295	412	414	315 (68)
6	164	158	165	181	231	220	320	146	148	171	177	304	292	196 (54)
Mean of Day Means and (S.D.), (N=3)	213 (57)	207 (51)	209 (46)	233 (63)	328 (87)	261 (113)	347 (113)	194 (50)	195 (55)	222 (49)	240 (59)	361 (54)	323 (80)	249 (78), (N=36)

*Vehicle counts for this day not included in booth or grand means.

Table II. 8-HOUR CARBON MONOXIDE TIME WEIGHTED AVERAGES IN PARTS PER MILLION

Collection booth number	Day													Booth Mean and (S.D.)
	M	T	W	Th	F	S	Sn	M	T	W	Th	F	S	
1	16	19	7	24	31	18		13	22	12	21	57	40	23.3 (13.7), (N=12)
4	23	14	26	32	41	21		13	8	8	13	28	30	21.4 (10.4)
6	12	18	9	44	38	17		32	16	25	13	25	36	23.8 (11.4)
Daily Mean and (S.D.), (N=3)	17.0 (5.6)	17.0 (2.6)	14.0 (10.4)	33.3 (10.0)	36.7 (5.1)	18.7 (2.1)		19.3 (11.0)	15.3 (7.0)	15.0 (8.9)	15.7 (4.6)	36.7 (17.7)	35.3 (5.0)	22.8 (11.7), (N=36)

Table III. AMBIENT AIR CONCENTRATIONS OF LEAD IN MICROGRAMS PER CUBIC METER

Collection booth number	Day													Booth Mean and (S.D.)
	M	T	W	Th	F	S	Sn	M	T	W	Th	F	S	
1	46	16	6	16	87	21		12	6	14	28	74	57	31.9 (27.5), (N=12)
4	36	15	8	26	5	31		15	21	34	33	58	35	26.3 (14.6)
6	16	15	16	12	50	33		5	9	19	24	52	34	23.8 (15.4)
Means by Day and (S.D.), (N=3)	32.7 (15.2)	15.3 (0.6)	10.0 (5.3)	18.0 (7.2)	47.3 (41.1)	28.3 (6.4)		10.7 (5.1)	12.0 (7.9)	22.0 (9.8)	28.3 (4.5)	61.3 (11.3)	42.0 (13.0)	27.4 (19.8) (N=36)

ceeded the NIOSH recommended exposure level. Also noted from the data of Table II is the rather uniform degree of CO exposure as a function of collection booth location. Due apparently to dispersion phenomena, CO levels for the 12 days were approximately equal throughout the toll plaza area. In general, wind conditions in the plaza area were not a consideration throughout the 2-week investigation.

The ambient air concentrations of lead are given in Table III. The mean lead concentration for the 12 days was found to be 27.4 $\mu g/m^3$ with a range from 5 to 87 $\mu g/m^3$. As with the ambient CO concentrations, the mean ambient lead levels were approximately the same throughout the toll plaza area. The filter pads from the personal sampling pumps worn by the subjects which were analyzed for lead content, were also analyzed for manganese content. All ambient manganese levels were found to be less than 1 $\mu g/m^3$.

Background noise levels were found to average 78 dbA while automobile traffic was being processed, but would sometimes reach peak levels as high as 120 dbA while large diesel-powered trucks were being processed through a collection lane. The duration of such individual truck noise levels was 0.5-3 minutes, depending on the type and amount of truck fare being collected. For example, truck drivers utilizing credit card payment required a greater amount of time to process through a collection lane than did cash customers.

Table IV. EXPOSURE DATA AS CORRELATED WITH MEAN HOURLY TRAFFIC COUNT

Vehicle exhaust exposure measure	Linear correlation coefficient	Number of data pairs
1. Ambient lead (μg Pb/m^3)	+0.591*	36
2. 8-hour TWA ambient CO (ppm)	+0.445*	36
3. Post-shift alveolar CO (ppm)	+0.805*	36
4. Post-shift COHb (% sat.)	+0.795*	36
5. COHb differential (% sat.)	+0.744*	36

*($p < 0.01$)

Table IV lists the results of correlation analyses of the mean hourly traffic count over 8 hours versus five indicators of subjects' exposure to vehicle exhaust. As can be noted from Table IV, all correlations were statistically significant ($p < 0.01$), and the linear correlation coefficients ranged from a low of +0.445 for ambient CO to a high of +0.805 for post-shift alveolar CO. The relationship

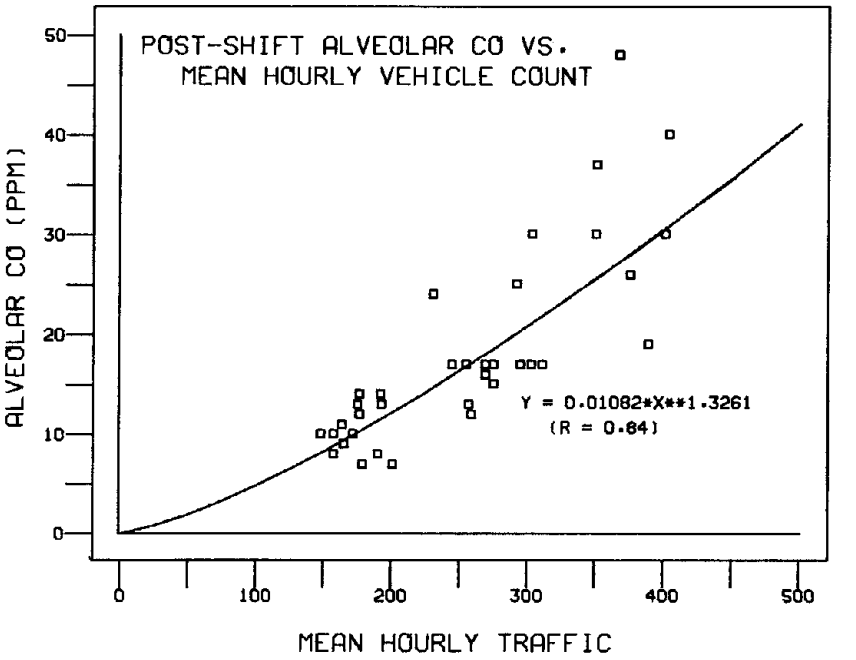

FIGURE 4. Relationship of subjects' alveolar CO concentration to mean hourly traffic.

between post-shift alveolar CO and mean hourly traffic count is illustrated in Figure 4. The relationship was found to best (in a least squares sense) obey a power function description given in Figure 4. The correlation coefficient for this power function was 0.84. The other measure of body burden accumulation of CO, carboxyhemoglobin, also correlated well with mean hourly traffic count. The correlations of post-shift COHb and differential (post-shift minus pre-shift) COHb with mean hourly traffic were found to have correlation coefficients of +0.795 and +0.744, respectively. The slight difference in correlation coefficients is attributed to greater measurement error inherent in COHb determinations at COHb levels less than 3 percent saturation than for values greater than 3 percent.

Results from alveolar CO samples obtained every two hours at the subjects' worksite were analyzed to give an indication of the rate of CO accumulation by the subjects during the course of their 8-hour workshift. The alveolar CO data for Subject #6 for two Fridays (4/6/73 and 4/13/73) are plotted in Figure 5. The data depicted in Figure 5 indicate a gradual increase in CO accumulation by the subject for 6 hours as a consequence of 5 hours of approximately equal hourly traffic. As illustrated, during the last two hours of work the subject experienced a decrease in alveolar CO as a con-

Table V. POST-SHIFT COHb LEVELS (% saturation)

Collection booth number	Day													Booth Mean and (S.D.)
	M	T	W	Th	F	S	Sn	M	T	W	Th	F	S	
1	1.6	2.8	3.8	3.5	6.8	3.0		3.0	2.1	2.8	2.0	8.8	6.3	3.9 (2.2) N=12)
4	1.8	4.6	2.8	3.2	6.4	4.3		2.8	2.0	2.5	2.4	7.0	11.7	4.3 (2.9)
6	2.0	2.8	3.1	3.1	5.0	3.4		1.5	1.8	3.0	1.8	5.7	7.8	3.4 (1.9)
Day Means and (S.D.), N=3	1.8 (0.2)	3.4 (1.0)	3.2 (0.5)	3.3 (0.2)	6.1 (0.9)	3.6 (0.7)		2.4 (0.8)	2.0 (0.2)	2.8 (0.3)	2.1 (0.3)	7.2 (1.6)	8.6 (2.8)	3.9 (2.3) (N=36)

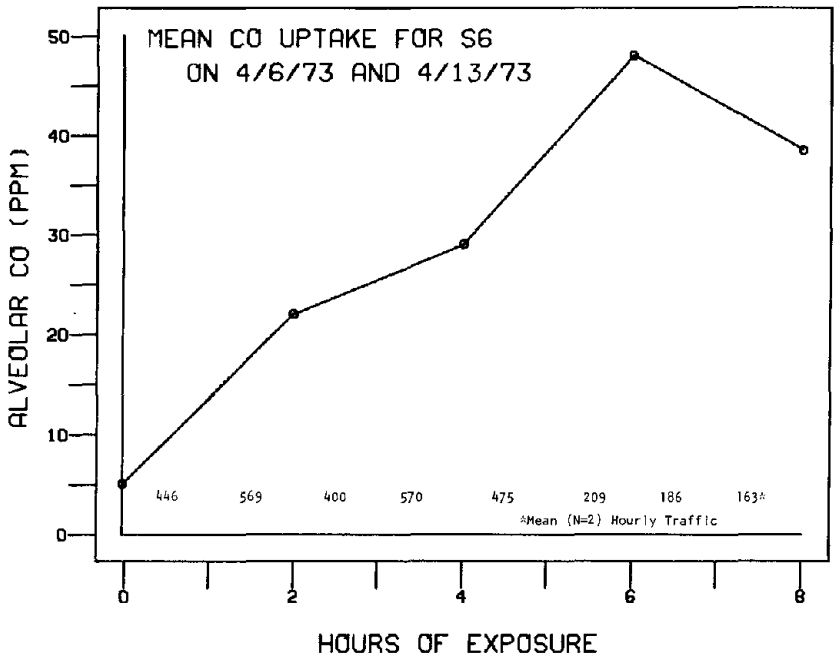

FIGURE 5. Rate of mean CO uptake by subject #6 for 4/6/73 and 4/13/73.

sequence of diminished traffic. Although the data in Figure 5 are specific for one subject, the peak in alveolar CO after 6 hours of exposure was found to be characteristic of all subjects. This situation is attributed to a decrease in traffic that normally commences after 7 p.m.

Biological Exposure Data

The extent of subjects' exposure to CO, as measured by COHb levels, is given in Table V, which gives the post-shift COHb levels. The pre-shift COHb values were typically 0.8 to 1.5 percent saturation. The maximum COHb value found in the 12-day study was 11.7 percent, a value which is more than double the NIOSH recommendation of 5.0 percent. Comparison of the COHb data of Table V with the mean hourly traffic count data of Table I shows that the NIOSH COHb recommendation was exceeded whenever the mean (over 8 hours) hourly traffic count exceeded 300 vehicles.

The correlation of alveolar CO with COHb levels is given in Table VI. The linear correlation coefficient was +0.893 and was highly significant ($p < 0.01$). This relationship is plotted in Figure 6, which contains the linear regression equation for COHb as a function of alveolar CO. The slope of the regression line in Figure 6

Table VI. CORRELATIONS OF COHb AND LEAD LEVELS WITH POST-SHIFT ALVEOLAR CO

Exposure measure	Linear correlation coefficient	Number of data pairs
1. Ambient lead levels	+0.781*	36
2. Carboxyhemoglobin levels	+0.893*	71

*($p < 0.01$)

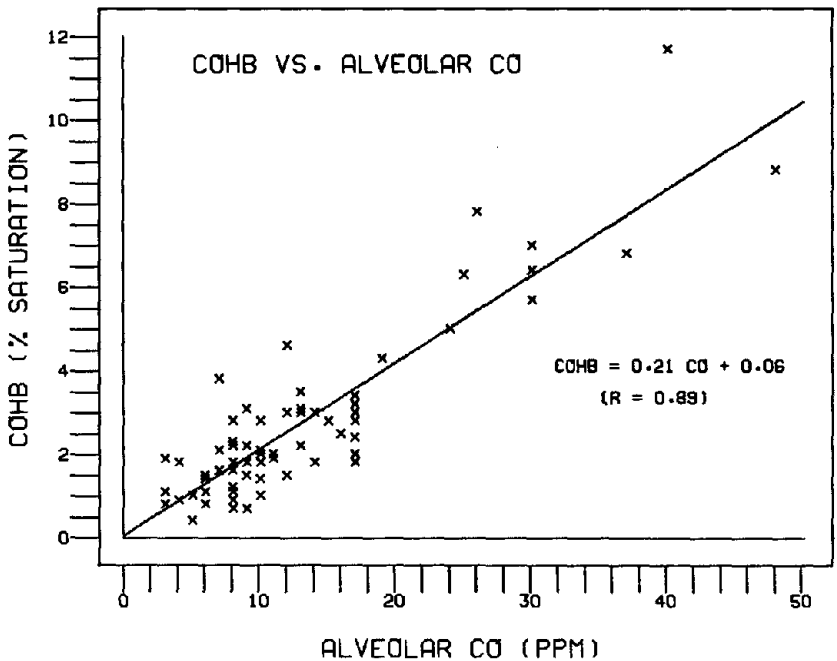

FIGURE 6. Relationship of subject's alveolar CO to carboxyhemoglobin levels.

is +0.207, which is in good agreement with Stewart's[14] value of +0.217 and Ringold's[15] slope of +0.20. The intercept of our regression equation was found to be +0.06, a value not in good agreement with either Stewart's or Ringold's values, which both approximated +0.50. It is felt that the greater measurement error inherent in the spectrophotometric method employed in this study for determination of COHb for COHb levels below 2 percent was responsible for the difference in intercept values.

Performance Data

The results obtained from the seven behavioral performance tests are given in Table VII. Since considerable inter-subject variability existed among the six participants, each subject's data were

characterized in terms of percent change between pre-shift and post-shift values. Table VII lists the results of linear correlation analyses relating percent change in performance with post-shift COHb. A positive correlation coefficient indicates that the performance measure in question increased in value as post-shift COHb increased. Examination of Table VII indicates only five performance measures were significantly ($p < 0.05$) correlated with post-shift COHb: eye-hand coordination mean interhole movement time, 'heavy' load (60 visual stimuli per minute) mean intertap interval, standard deviation of the basic load (zero visual stimuli) intertap interval, standard deviation of the 'moderate' load intertap interval, and the perceptual motor load measured between the basic and moderate information load conditions. Consideration of these behavioral performance results suggests slowed performance on the concurrently. It must be pointed out that due to the large number of performance measures that were used in the correlation test, some of the significant correlations may be due to chance.

Although no behavioral performance measures other than the five previously described were significantly correlated with post-shift COHb, two other trends in the results given in Table VII are worthy of comment. First, although no measure of ability to perform the time estimation task was significantly correlated with post-shift COHb, it can be noted from Table VII that mean estimation errors for long duration stimuli (i.e., 15-30 sec.) were better correlated with CO exposure than were the mean estimation errors for the shorter duration stimuli. This trend is in agreement with Beard's[4] report that subjects' estimation of 30-second intervals of time was impaired by exposure to CO, whereas estimation of 10-seconds intervals was not.

A second observation that can be made from the data of Table VII is that of the two performance measures concerning choice reaction time; detection time, not movement time, was correlated in a positive sense with increased CO levels. This suggests that the subjects' responses in selecting the correct stimulus lamp were slowed, but not the ability to quickly move to extinguish the stimulus lamp. Also, since movement time as measured in the reaction time task did not correlate with CO exposure, whereas interhole movement time in the eye-hand coordination task correlated with increased COHb, the implication is that CO is more likely to affect tasks involving fine motor control than those requiring gross motor responses. The effect of CO on eye-hand coordination suggested here is in agreement with the report by Bender, et al.[6] that COHb levels averaging 7.3 percent were sufficient to impair performance on another test of eye-hand coordination, the Purdue pegboard test.

As described previously, each subject was asked to complete a

Table VII. CORRELATION OF PERCENT CHANGE IN PERFORMANCE DATA WITH POST-SHIFT COHb LEVELS

Performance measure	Linear correlation coefficient	Number of data pairs
1. Eye-hand coordination:		
a. Interhole movement time (mean)	+0.317*	36
b. Interhole movement time (S.D.)	+0.201	36
c. Interhole movement time (Skewness)	−0.069	36
2. Mathematics test:		
a. Number of problems attempted	−0.133	36
b. Number of errors	−0.263	36
3. Time estimation:		
a. Estimation error mean (1-30 sec.)	−0.118	36
b. Estimation error mean (5-15 sec.)	+0.114	36
c. Estimation error mean (15-30 sec.)	+0.261	36
d. Estimation error S.D. (1-30 sec.)	−0.183	36
e. Estimation error S.D. (5-15 sec.)	−0.240	36
f. Estimation error S.D. (15-30 sec.)	−0.011	36
4. Choice reaction time:		
a. Detection time (mean)	+0.240	36
b. Movement time (median)	−0.119	36
c. Decision-detection time (S.D.)	−0.115	36
d. Movement time (S.D.)	−0.139	36
5. Critical flicker frequency		
a. CFF (mean)	+0.126	36
b. CFF (S.D.)	+0.161	36
6. Visual perception:		
a. Errors, irrespective of position	+0.083	36
b. Errors in choice and position	+0.207	36
7. Time-sharing task:		
a. Basic load intertap interval (mean)	−0.137	33
b. Moderate load intertap interval (mean)	+0.227	33
c. Heavy load intertap interval (mean)	+0.379*	33
d. Basic load intertap interval (S.D.)	+0.595*	33
e. Moderate load intertap interval (S.D.)	+0.427*	33
f. Heavy load intertap interval (S.D.)	+0.309	33
g. Perceptual motor load (basic-moderate)	−0.393*	33
h. Perceptual motor load (basic-heavy)	+0.014	33
i. Correlation index (basic load)	+0.123	33
j. Correlation index (moderate load)	−0.086	33
k. Correlation index (heavy load)	−0.145	33

* ($p < 0.05$)

Table VIII. SUMMARY OF SUBJECTIVE FEELINGS

Complaint	Day												Totals by category
	M	T	W	T	F	S	M	T	W	T	F	S	
Backache	1	..	1	..	..	..	..	..	1	..	1	2	6
Upset stomach	..	..	..	..	..	..	..	..	1	..	..	..	1
Headache	..	1	..	..	..	1	1	..	..	..	2	2	7
Sore feet	..	..	..	..	..	..	..	..	1	..	1	..	2
Dizziness	..	..	..	..	..	..	..	..	..	..	1	..	1
Irritability	..	1	..	..	1	1	..	..	1	1	2	..	7
Muscle cramps	..	..	..	..	..	..	..	..	..	..	..	..	0
Blurred vision	..	..	..	..	..	..	..	..	..	..	1	..	1
Nausea	..	..	..	..	..	..	..	..	..	..	..	..	0
Nervousness	2	..	1	..	3	1	..	..	1	..	1	1	10
Sluggishness	1	..	..	..	..	..	..	..	1	1	1	1	5
Total complaints by days	4	2	2	0	4	3	1	0	6	2	10	6	40
Mean fatigue (max), (N=3)	8.0	4.7	6.3	6.0	6.7	6.3	4.3	6.3	7.3	6.7	10.0	9.0	
Mean fatigue (diff.), (N=3)	4.3	2.0	4.3	2.3	2.7	3.0	1.7	3.0	4.0	3.7	6.0	4.3	
Lots of CO? (Yes answers)	1	0	2	2	1	0	1	1	1	1	3	3	

subjective feelings questionnaire prior to the start of each session's performance testing. The results from these questionnaires are summarized in Table VIII. As noted in this table, the most frequent complaints were those of nervousness (10 responses), irritability (7), headache (7), and backache (6). In general, it appears that the number and type of complaints were not correlated with either traffic load or degree of exposure to vehicle exhaust. The exception to this statement were the 10 subjective complaints indicated on Friday, April 13th, the day of greatest traffic during the 12-day study. However, the next greatest number of complaints (6) were received on Wednesday, April 11th, which was a day when traffic was rather low in comparison to the weekend traffic.

Also given in Table VIII are data concerning subjects' rating of their state of fatigue. The correlations of subjects' fatigue ratings (differential fatigue, post-shift minus pre-shift) with mean hourly traffic counts and differential COHb levels are given in Table IX.

Table IX. CORRELATIONS OF COHb AND TRAFFIC COUNTS WITH SUBJECTIVE FATIGUE RATINGS

Measure	Linear correlation coefficient	Number of data pairs
1. COHb differential	+0.250	36
2. Mean hourly traffic count	+0.461*	36

*($p < 0.01$)

It can be noted that fatigue and traffic counts were significantly correlated ($p < 0.01$), but fatigue and COHb levels were uncorrelated. In an attempt to assess the contribution of fatigue on the behavioral performance measures previously discussed, the five performance measures found to be correlated with alveolar CO were subjected to a corresponding analysis with subjects' differential fatigue ratings replacing post-shift COHb in the analysis. All five correlation coefficients resulting from this analysis were found to be nonsignificant, indicating that fatigue as rated in this study was not a major factor in subjects' impaired behavioral performance.

DISCUSSION AND SUMMARY

Consideration of the previously described results indicates that the subjects comprising this investigation were found to be exposed to excessive (by NIOSH recommendation[1]) amounts of carbon monoxide on 25-33 percent of the days covered by this study. It appears from the data that this situation occurs whenever the mean (over 8 hours) hourly vehicle count exceeds 300 vehicles. This investiga-

tion also indicates that the ambient CO concentrations are approximately uniform across the toll plaza area if wind conditions are not a factor. This means that workers who use the central office for lunch and work breaks will be exposed to CO levels approximating those found in the toll collection booths and that office personnel stationed within the office are exposed to CO concentration equal to those within the collection booths.

Mean ambient lead levels for the 12 days of the investigation were found to range from 23 to 32 $\mu g/m^3$ within the collection booth lanes. These values can be compared with findings from an earlier study by Hofreuter, et al.[16], who reported that the average particulate lead concentration within a municipal vehicle inspection lane was 14.8 $\mu g/m^3$. The ambient lead concentrations observed in this investigation were all less than the current NIOSH recommended[17] level of 150 $\mu g/m^3$. Ambient manganese levels were all less than 1 $\mu g/m^3$, which is considerably below the TLV[18] level of 5 mg/m^3.

Two correlations of CO with impaired behavioral performance were indicated by results from this study: First, slowed performance on an eye-hand coordination task requiring fine motor control, and second, a disruption of subjects' performance on two concurrent tasks. The implication these performance findings may hold for worker safety remain to be delineated, and should be verified in other studies, but the results do suggest the picture of a worker less able to respond quickly and accurately to demands of the job.

ACKNOWLEDGMENT

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EYE IRRITATION: A LITERATURE REVIEW

Lowell G. Wayne, Ph.D.

My original interest in eye irritation arose from my involvement in the chemistry of photochemical smog. From that perspective, one wishes to assess eye irritation as a possible indicator of air quality. Thus the emphasis is on the atmosphere, rather than the population, and on the behavior of air, rather than that of subjects. As a chemist, if I should learn that a particular atmosphere irritated people's eyes, I would want to find out what chemicals might be in it which would cause that. Let me review that aspect briefly.

In Los Angeles, and other places where there are both automobiles and sunshine, photochemical reactions in the air generate irritants, as we discovered about twenty-five years ago. They also generate ozone - but ozone appears not to be an eye irritant. Twenty-five years of study have yielded a surprisingly small harvest of chemical culprits apparently responsible for the irritation; namely, formaldehyde, acrolein, peroxyacetyl nitrate, and (more recently), peroxybenzoyl nitrate[1]. These products are formed by the partial oxidation and nitration of vapors of unburned gasoline and partially burned gasoline. The most recent progress in the study of eye irritation in smog has brought us close to accounting for the eye irritation potency of various gasoline constituents as smog precursors - that is, close to understanding why it is that the most reactive constituents, chemically, do not (in the laboratory) yield the most irritating products. A very promising model for this reaction system has recently been published by Yeung and Phillips[2] of the University of Toronto.

So, there has been progress in studying the air; but what about the people? Their behavior, apparently, is to rub their eyes and cry. Just about all we have found out, so far, is that when they cry, there is less lysozyme activity in their tears if their eyes are irritated by smog at the time; at least, so say Sapse[3] and his colleagues in Los Angeles, who have studied the tears. One other possibility: if people are like rabbits, perhaps their hearts slow down when their eyes are irritated. That's what happened to some rabbits in Toronto, where it was shown by Basu et al[4]., that acrolein can produce bradycardia upon application to the eye . . . an example of the *oculocardiac reflex.* Blocking of this response by atropine indicated to the authors that it was mediated through the parasympathetic system.

I have also reviewed some of the occupational aspects of eye

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irritants, as reflected in the toxicology literature; again, the findings were rather meager.

One gains the definite impression that there is little continuity between the environmental and the occupational interest in this subject. Occupationally, people seem to be more concerned about the possibility of injury from splashes than from exposure to vapors. Thus, the FDA standard method for testing the irritant potential of suspect material is to apply it (in the liquid phase) to the eyes of rabbits, then examine those eyes later. There is no standard method for assessing the irritant potential of gases or vapors, as far as I know. But perhaps that is just as well, in view of the results of a study by Weil and Scala[5], who investigated the reproducibility of the scores obtained by using the standard method. They found that the degree of variation in the scores, as between laboratories, was so great as to render the procedure very unreliable, and they recommend that it not be included in any new regulations. Later, Burton[6] found that optical measurement of the thickness of the corneas of rabbits can be closely correlated with the subjectively assessed conjunctival reaction, as well as with various partial scores from the standard method, and he suggests this as a more objective procedure.

But, for behavioral toxicology, eye irritation as an occupational hazard must be a more interesting subject. A recent review[7] cites an estimate of more than 500 substances known to have some potential to affect the eyes. Occupationally, the most important are said to include: benzoquinone, hydroquinone and related compounds; aromatic nitro and amido compounds, especially choline (encountered in fish processing); vapors of various solvents, for example, higher alcohols, xylene, toluene, esters, ethylene glycol; hydrogen sulfide; carbon disulfide; hydrogen cyanide, carbon monoxide, methanol; lead, mercury; and other metals. Some of these, of course, are not irritants but systemic poisons, and affect the eyes through metabolic mechanisms or effects on the central nervous system. Noteworthy in this light are some of the substances which have been discussed at this workshop.

The recent literature also provides some interesting and more specific examples of hazards in particular industries. In a *synthetic rubber* plant, Groves and Smail[8] reported an outbreak of superficial keratitis resulting from exposure to ethyl isothiocyanate, which was released as a vapor by thermal decomposition of an accelerator, diethylthiourea, used in a vulcanizing process. Thirty-two workers reported to the hospital complaining of pain, photophobia, and blurring of vision. All lesions healed without corneal scarring or residual damage, but various therapeutic agents failed to accelerate recovery.

Latex used in the production of *natural rubber* can also be a hazard to eyes unprotected from splashes, as reported by Sofat[9] in a review of three cases.

In the manufacture of *foam plastic*, various amines are sometimes released which make the visual field look blue or grey. Jones and Kipling[10] studied this phenomenon, which they call "glaucopsia". They observed mild conjunctival injection in some cases, but no gross irritation, no epiphora, no alteration in visual acuity, and no permanent sequelae. Similar conditions from a similar cause were also observed in a cold curing process in *a foundry*.

Gemke et al[11]., surveyed the status of the eyes of 891 workers in a *zinc plant*, many of whom had possible exposures to fumes of zinc, lead, and arsenic as well as oxides of sulfur. The incidence of conjunctivitis was reported to increase with length of service, up to 16 percent in the most senior cohort; some punctate cataracts were seen. Lesions of retinal vessels occurred in 25 to 44 percent, and increased intraocular pressure in 47 to 58 percent, of those examined.

Solvent usage may be a significant cause of conjunctivitis in some industries. Valvo *et al*[12]., reported on 30 cases of acute and chronic irritation of the conjunctiva due to vapors of amyl and ethyl acetate at concentrations up to 80 mg/l, in such operations as *spray-painting* and *film-checking*. Raleigh and McGee[13] commented that acetone at 500 ppm can cause eye irritation in subjects "unaccustomed to the exposure".

Finally, there may be an interesting clue to possible physiological interaction of sensory stimuli in the following abstract: "Hygienic Significance of Methaphose as an Atmospheric Pollutant. K. Akhmedov. Gig. i Sanit, (1968 Oct) p. 10-15. Author determined threshold values of smell that alter the light sensitivity of eyes and the electric cerebral activity in short-term action of methaphose."

I know that this review has been less than complete, and certainly discursive; I do hope, however, that it may serve to stimulate some new consideration of an insufficiently considered field.

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DRUG EFFECTS IN RELATION TO INDUSTRIAL SAFETY

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Many of the substances which are potential work hazards are encountered at the employment site, but others are brought to the work situation by the employee. These include a broad classification of drugs, ranging from prescribed anti-histamines, tranquilizers, and barbiturates to self-selected stimulants, hallucinogens, marihuana, and alcohol. These substances, which may not be intrinsically dangerous, potentially can become so as they affect task performance or interact with other substances in the work environment. Since Dr. Leblanc will be discussing the interactions of various drugs, this paper will be restricted to those health hazards which exist when a worker attempts to perform certain tasks while under the influence of alcohol or marihuana.

ALCOHOL

Alcohol has been listed as a causal factor in 6% of all driving accidents, in 30% of serious driving accidents, and in 50% of traffic fatalities. The statistics for alcohol-related accidents in industry are less reliable, but it has been estimated that alcohol may be a causal factor in 10% to 30% of industrial accidents. There is little available data to describe the distribution as a function of severity. Unfortunately, we have no epidemiological data for marihuana, a behavior altering drug which has been used by more than 20 million Americans.

There are few drugs which do not alter behavior to some extent. In some instances, there are side effects which may be minor or which may themselves constitute health hazards. In other cases, as with alcohol and marihuana, the drugs are taken specifically for the behavior and mood altering effects. The studies to be described were undertaken to determine the nature of impairments produced by these drugs.

The nature of the impairment which underlies behavioral changes must be understood in order to determine when the drug can be used safely. For example, the hazard created by imbibing alcohol during a sales luncheon is relatively small, but the consequences for forklift operators could be serious. It is the relationship between the drug-induced behavioral changes and the behavior required for a particular employment function that establishes the hazard.

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It has become evident in recent years as task demands have grown increasingly complex that the prime limitation upon skilled performance is not in physical strength but in attention, perception and memory. Accidents are most likely to occur at times and places where the amount of information to be processed is beyond the worker's capacity. The most obvious example of hazards resulting from information overload is the driving situation, but I am sure many of the readers are aware of situations in industry where an individual must control potentially dangerous functions. Accidents occur not because the worker lacks strength but because he does not notice crucial cues or incorrectly manipulates a control device.

The relevance of this discussion is that one cannot test a drug for its effect upon simple sensory or motor skills and assume thereby that one has tapped all the skills involved in safe performance in the modern factory. Moreover, drugs are more selective in their actions than commonly thought. A drug can leave one behavioral function completely unaffected while seriously impairing others. It is important to identify the demands of a task and to test the specific abilities required by the task under the drug. These concepts will be discussed for alcohol, the drug most frequently used in industry.

Alcohol serves to illustrate earlier comments, because it has little effect upon simple sensory and motor control. It must be ingested in large quantity before there is a significant effect on vision or hearing. The ataxia or motor control loss that characterizes the movies' version of intoxication occurs at blood alcohol levels (BAL) above .15%. At this level driving accident probabilities are increased by a factor of 20. Although the probability of a driving accident is 12 times greater (1200% increase) at .10% BAL than at zero BAL, it may not be apparent that a person at this level is intoxicated.

Visual tests performed at .075% BAL (300% increase in driving accident probability) have found no effects upon visual acuity, dark adaptation, binocular fusion or convergence. Yet many drivers involved in accidents while under the influence of alcohol report a failure to see or a misinterpretation of visual input. The following experiment may help to explain this paradox.

Moskowitz and Sharma (In press) tested the ability of subjects to perceive signals in their peripheral field of vision while fixating upon a central light. Three levels of alcohol treatment were used: 0, .414, and .828 grams alcohol/Kg bodyweight. The test involved perception of a light presented for one second at one of 32 possible positions in a horizontal array on both sides of the central fixation light, at angles ranging from 12 to 102 degrees. There were three

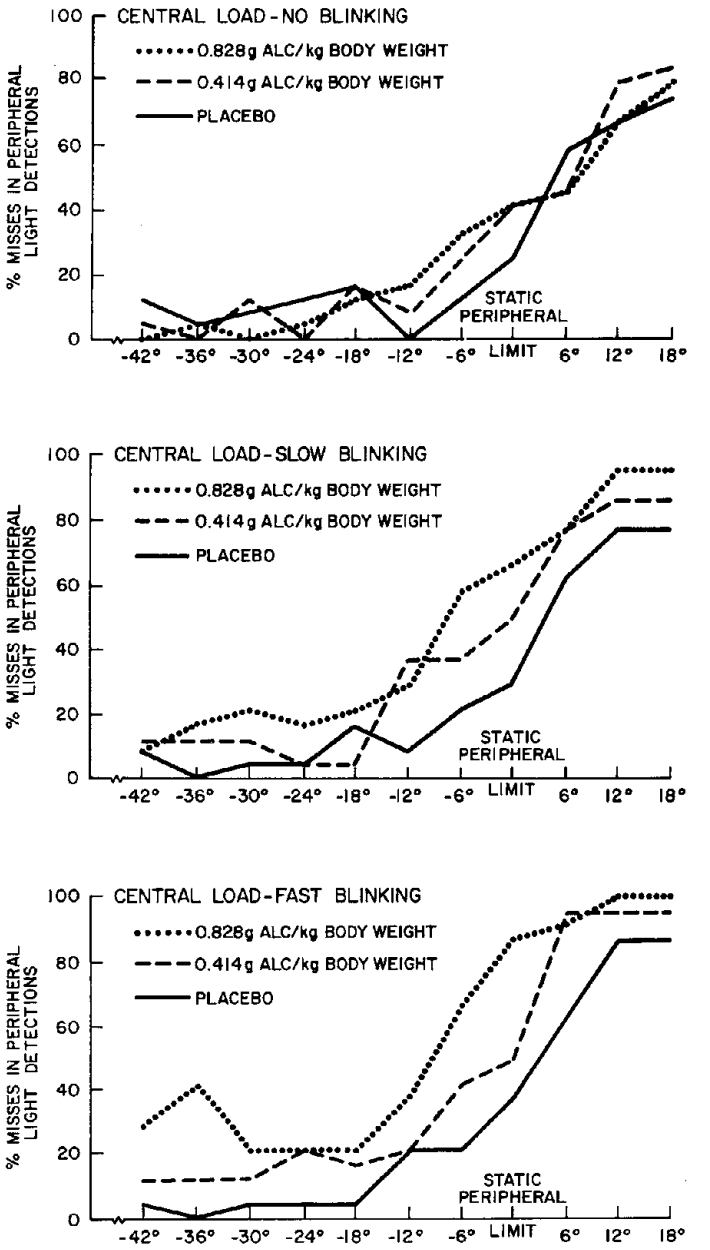

FIGURE 1. Missed peripheral lights as a function of peripheral visual angle, alcohol dose, and information processing demands from central vision.

information load test conditions. In the first condition, the central fixation light was steady, but in the second and third conditions the light blinked at an average rate of 0.4 and 0.8 blinks per second, respectively. In the latter two conditions, subjects were required to count the blinks, reporting the number at the end of each 20 second trial.

Figure 1 illustrates the results in terms of missed peripheral lights as a function of peripheral visual angle, alcohol dose, and information processing demands from central vision. Note that the figure is graphed in reference to the "static peripheral field limit" which is the average temporal field limit of vision measured under standard psychophysical techniques. Minus signs indicate angles from the temporal limit towards the center of the visual field and positive angles indicate lights beyond the temporal limit.

Under the condition where central vision was fixated upon an unblinking light, neither of the two active alcohol treatments produced any change in peripheral light detection. This finding agrees with the several optometric studies of peripheral vision, which have failed to find any peripheral vision impairment by alcohol (Colson, 1940; King, 1943; Newman and Fletcher, 1941). However, when subjects were required to process information from the central light (the conditions requiring counting of light blinks), there was a very large decrement in the proportion of peripheral light signals perceived. Note that the performance decrement under alcohol occurred only when subjects were required to divide attention.

Again, alcohol did not impair a sensory function, in this case visual, when examined by itself. The apparent perceptual failure is a function of the information processing demands on central processing mechanisms, appearing when several sources of information must be monitored simultaneously.

The results of this study are offered as evidence that it is higher brain functions which are affected by low BAL's in complex information processing, rather than the simpler functions which compose the complex demands. Several investigators now have demonstrated a loss in peripheral vision under conditions of complex information processing in a divided attention task (Huntley, 1970; Hamilton and Copeman, 1970; Von Wright and Mikkonen, 1970).

Since the effect of alcohol was not simply upon the ability to handle visual information, it is clear that examination of that system under alcohol would not reveal the deficit. Note that these alcohol-induced deficits are critical, because contemporary society is demanding highly complex skills of its citizens.

Another relevant experiment (Moskowitz and DePry, 1968)

examined the effect of alcohol upon the detection of an auditory signal under conditions of concentrated and divided attention. Subjects were required to perform two simultaneous auditory tasks, one presented to each ear by binaural earphones. One task required the detection of a 1,000 Hertz tone embedded in a 3 sec. burst of random noise; the second task required recall of six random digits presented to the other ear concurrent with the three second noise burst. Concentrated attention required subjects to ignore the input to one ear and focus attention solely on the task presented to the other ear. Divided attention required subjects to perform both tasks; i.e., detect presence of a tone within a burst of noise presented to one ear, and recall six digits simultaneously presented to the other ear.

An alcohol treatment of .52 grams alcohol/Kg. bodyweight produced no significant impairment of either of the two tasks when each was performed under concentrated attention conditions. However, a large performance decrement under alcohol appeared when division of attention between the two tasks was required. The result demonstrates that the primary influence of alcohol was not upon the separate tasks, but upon the brain processes involved in handling two or more inputs simultaneously.

A replication of this experiment was undertaken, using alcohol doses ranging from .21 to .83 grams alcohol/Kg. bodyweight (Moskowitz and Shea, unpublished study). For this replication, the concentrated attention task required signal detection alone. For divided attention both signal detection and recall of the simultaneously presented digits were required.

Figure 2 presents the results of the signal detection task when it was the sole task requiring attention. The figure also clearly illustrates the significant and increasing impairment of the ability to divide attention with increasing alcohol doses. The divided attention test was performed almost one hour after completion of drinking at which time the average Breathalizer reading for the lowest dose was below .015%.

These results indicate that one of the abilities required to perform complex man-machine interactions, i.e., division of attention, is impaired at any measurable departure from zero BAL. The importance here is that again this is evidence that central processing of information is impaired, not the sensory system. If a worker's activity were confined to a single monitoring task or physical activity, the impairing effect of alcohol would be of less importance. It is precisely because the jobs in modern factories demand attention to many concurrent activities that alcohol is likely to lead to an accident.

Unfortunately, the large performance deficit often is not ap-

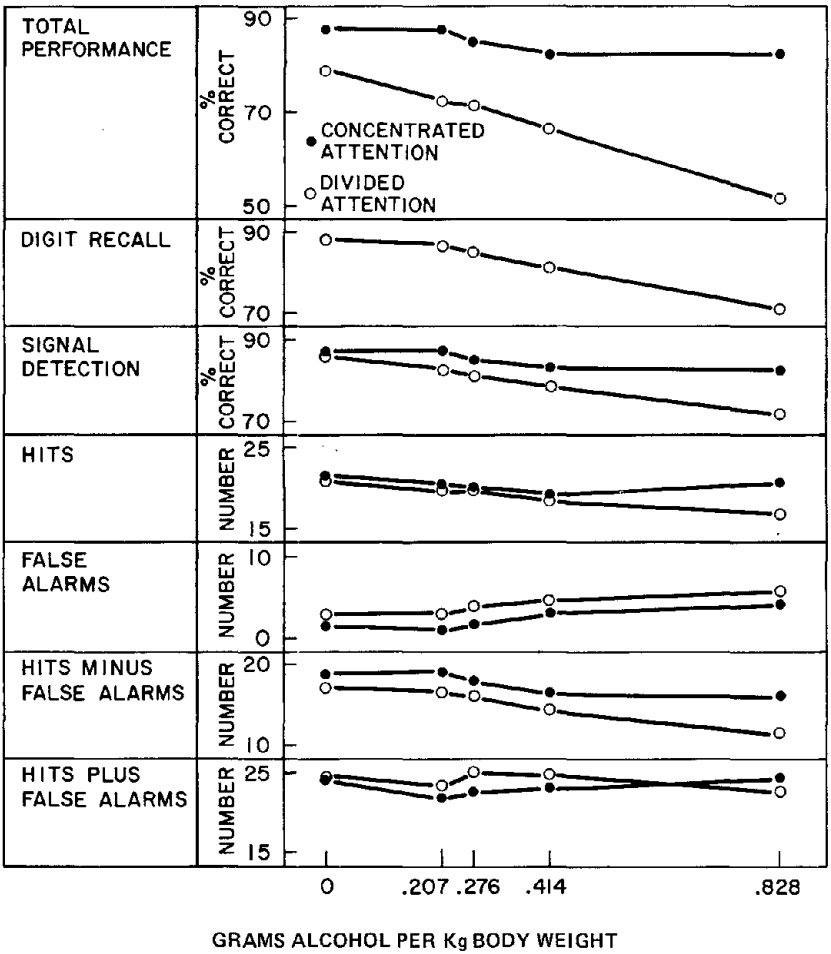

FIGURE 2. Effect of alcohol on the detection of an auditory signal under conditions of concentrated and divided attention.

parent to the individual who is experiencing it. Under the stress of alcohol the capability of surveying the environment for important signals is reduced and must be allocated to those deemed most important. If this emphasized area does not constitute an overload itself, then performance may be maintained close to normal there, but with failure in unattended areas. The individual subjectively is unaware of the inputs which receive no attention and does not recognize the hazards which this represents for himself and fellow workers.

These experiments may seem to be rarefied laboratory findings, but they have been substantiated with driving simulators and

with flying while under the influence of alcohol. A study at UCLA (Moskowitz, 1971) has compared the action of alcohol on both concentrated and divided attention in driving.

A rather complex simulator was used with an actual car mounted on a chassis dynamometer, facing a 150 degree screen on which is projected a filmed ride covering some 31 miles of road. The speed of the car motor controls the projector speed, so that the subject can progress at his own desired rate. The subject must manipulate the steering wheel appropriately as the projected scene moves laterally to follow the contours of the road.

Since the scenes were filmed from an actual car traveling over roads in a variety of traffiic situations, the rate at which information is presented and the tracking demands required are similar to those of actual driving in contrast to simulators which have artificially difficult tracking demands. Two experiments were performed in this study. In the first experiment nearly all the information processing demand came from the single source of the front screen. Although there was a rearward projection of the retreating scene, this rarely was sampled by the drivers.

The experiments compared drivers with and without alcohol treatments on the 31-mile drive which most subjects completed in about an hour. In both experiments, peak BAL's were close to .085% for the alcohol condition.

The first experiment failed to find any significant differences due to alcohol on the mean scores of the 25 performance measures of speed, steering wheel and pedal usage, and tracking. The sole apparent effect of alcohol was an increased variability in speed between subjects.

The second experiment introduced a subsidiary task to bring the simulator into greater conformity with the divided attention nature of information processing in actual driving. The subsidiary task consisted of lights mounted near the car visors, within the peripheral visual field. There were four possible lights, and an appropriate lever actuation was required whenever the corresponding light appeared, which was about once a minute.

In this second experiment, the presence of alcohol produced significant performance changes. Even though the drivers concentrated primarily upon the tracking aspect of their dual task as presented on the front screen, nevertheless there was now a small but consistent drop in the mean performance scores. More important, however, was that 12 of the 25 scores showed large and statistically significant increases in between-subject variability and a lesser but consistent rise in within-subject variability. And as Lewis (1956) has demonstrated, constancy is the hallmark of the skilled and experienced driver. Variability is a greater indication

of lack of control than mere increase or decrease in mean performance.

Thus, under information-processing requirements closer to actual driving, alcohol produced a significant decrement in tracking performance. An even greater impairment was produced in the subsidiary task which represents the driver's sensitivity to the non-tracking aspects of the driving function, and there was a 30% increase in average reaction time to the peripheral lights. Between-subject variability increased 121% and within-subject variability increased 67%. Finally, there was an increase in total omissions of any response, indicating complete failure to see.

For the environment surveillance aspect of driving as well as for the tracking aspect, increased variability represents a greater driving hazard than would a uniformly increased reaction time. For the simulator as for laboratory studies, maximum impairment by alcohol occurs for information processing in a divided-attention situation.

Another illustration can be found in a recent study of flying under the influence of alcohol (Billings, Wick, Gerke & Chase, 1972). Sixteen subjects took off, instrument flew and landed a plane under four alcohol treatments which produced 0, .04, .08 and .12% BAL. Eight subjects were highly experienced professional pilots and 8 were non-professional pilots. Flights took place with a safety co-pilot and also a physician behind the pilot to incapacitate him, if necessary. Although the tracking demands of flying are far more difficult than those of driving, the experienced pilots suffered no significant decrement in their tracking ability, even at the highest dosage. However, even at the lowest dosages they committed procedural errors which were a hazard to flight. At the highest dose level, the safety co-pilot had to take command of the plane eleven times to prevent an imminent accident. The inexperienced pilots exhibited impairment in tracking skills and accumulated far more procedural errors than the experienced pilots. Major procedural errors included taking off with full flaps, flying without lights, taking off with carburetor heat on, turning the wrong way in response to instructions, and flying a landing approach tuned to the wrong frequency. Catastrophic procedural errors included loss of control in flight, turns towards oncoming traffic and landing errors which would involve striking the ground.

The authors comment, "If we assume that instrument-rated pilots, flying ILS approaches, consider the job of guiding their aircraft to a position from which a visual landing can safely be made as their primary task, then it follows that the other, discrete, procedures involved, while no less essential to safe operations, are relegated to a secondary role. The evidence is clear this is in

fact the hierarchy which exists. It is equally clear that as pilots are progressively affected by alcohol, they become progressively less able to cope with the various facets of their task, and it is the secondary tasks which suffer first and most." The prime alcohol deficit does not impinge on the tracking task, because it receives more attention than the search-and-recognition task.

We have continued experimentation in an effort to understand why divided attention is particularly vulnerable to alcohol effects. Our conclusion is that alcohol slows the *rate* at which the brain can process information. Deficits are readily apparent in the divided attention situation with more than one source to be monitored, but this is not to suggest that alcohol does not impair performance otherwise. Indeed, the input from one very high-information source may not be processed efficiently under alcohol, in which case response times increase the likelihood of an accident.

Experimental work with visual masking supports the slowed processing rate hypothesis. The following reports a typical experiment.

Moskowitz and Murray (unpublished) examined the effect of alcohol on the speed of processing visual information using a technique in which the effective duration of visual information is controlled by an interrupting visual mask.

The procedure involves the presentation for 15 msec. of a card with a random set of four letters. At some subsequent inter-stimulus interval (ISI) a card with a random distribution of letters and letter fragments is presented and serves as a masking stimulus. Were this masking stimulus not used, the 15 msec. physical duration would be sufficient for all four letters to be processed because of the existence of a persisting visual image, i.e., the short-term sensory store. This short-term sensory storage greatly increases the effective duration or availability of the 15 msec. presentation. However, if the masking card is presented at some ISI less than approximately 100 msec., the subject is unable to process all the information which was presented on the stimulus card.

In essence, the presentation of the masking card controls the effective duration of the letter card by obliterating the later portion of the very short-term sensory store. By presenting the mask at various intervals after the stimulus, it has been determined that extraction of information from the sensory store is complete within 100 msec.

With the normal time course under placebo treatment, subjects were able to extract approximately 56% of the information by 45 msec. The rate of information extraction was slowed significantly by the two alcohol doses which produce BAL's of approximately .05% and .10%. At 45 msec. only 46% and 26% of the information

had been extracted under lower and higher doses, respectively. There was *no* alcohol interference with the ability to extract data from the 15 msec. presentation of the stimulus card and place it in the very short-term sensory store, as was evident by performance when no mask was presented.

MARIHUANA

Another drug which has gained widespread popularity is marihuana. Currently, use appears to be spreading from the under and early 20's population to a much wider age range. Importantly, although it is used for subjective effects on mood and feelings, as is alcohol, experimental results indicate that it has completely different effects on performance. Our interest here is in potential hazards associated with its use.

Among the studies we have conducted with marihuana is an examination of the effect of a 200μg delta-9 Tetrahydrocannabinol (THC)/Kg. bodyweight dose in the form of smoked marihuana upon auditory concentrated and divided attention tasks. The tasks previously discussed in the Moskowitz and Shea experiments were used. The results showed no significant effect of marihuana upon digit recall when attention was concentrated upon that task. However, when signal detection was the concentrated attention task, there was a 12 percent performance decrement as compared to placebo treatment. Under the divided attention condition performance dropped 28 percent as compared to placebo.

A drug dose replication of this experiment was performed (Figure 3) using doses of 0, 50, 100 and 200μg delta-9 THC/Kg. bodyweight in the form of smoked marihuana. (Moskowitz, McGlothin, Hulbert, 1973). Again, the results differed from those of alcohol effects in that a performance decrement occurred under the concentrated attention condition as well as under the divided attention condition (recall that alcohol had no significant effect on concentrated attention). The decrements were linearly related to the drug doses. A signal detection theory analysis of the nature of the errors confirmed that under both concentrated and divided attention, the prime deficit was under the detectability of the signal; that is, upon subjects' sensitivity to the signal as measured by d'. There was no significant effect upon subjects' criterion as measured by beta.

The data indicate marihuana produced a greatly increased number of false alarms (a statement that a signal was present when in fact it was not). Also, there was a decrease in hits (a hit being detection of a signal acutally present) but this phenomenon was not as large as the change in false alarms.

The effect of marihuana upon signal detection performance

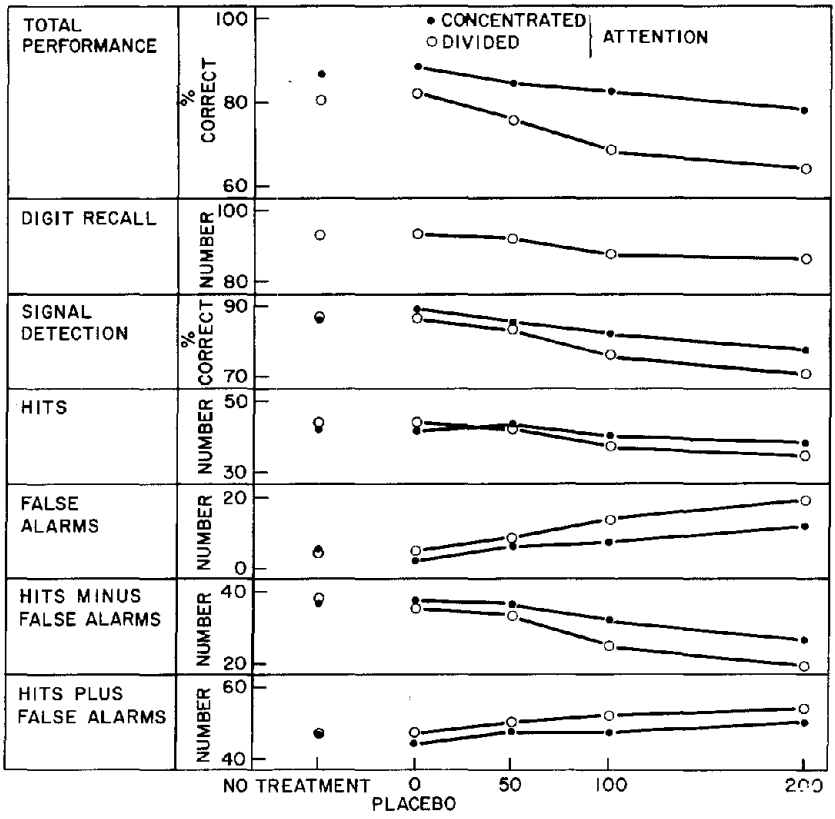

FIGURE 3. Effect of marihuana on auditory concentrated and divided attention tasks.

in the visual modality was investigated using the technique of Moskowitz and Sharma (in press), as previously discussed. This experiment (Moskowitz, Sharma and McGlothin, 1972) again used doses of 0, 50, 100 and 200μg delta-9 THC/Kg. bodyweight in the form of smoked marihuana.

Figure 4 presents the results. Statistical analyses verify the impression that marihuana produced a large and significant decrease in the number of peripheral signals detected. However, in contrast to the alcohol experiment, the decrement is not a function of demands on central vision. There was almost as great a deficit under the condition where the central light was not blinking as when the subjects were required to count the blinks of the fixation light.

Thus, whatever the nature of the marihuana-induced deficit in visual perception, it is not dependent, as it was in the case of

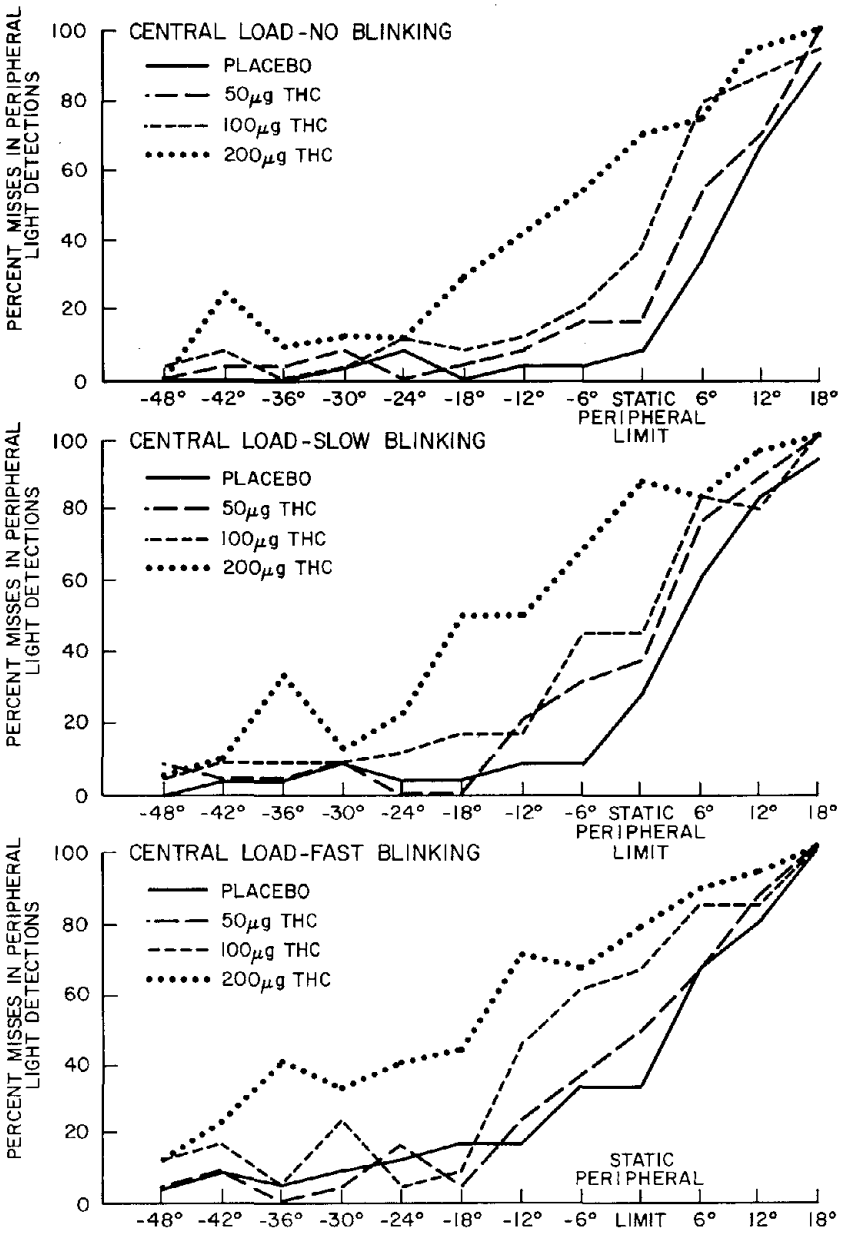

FIGURE 4. Effect of marihuana on signal detection performance in the visual modality.

alcohol, upon information overload imposed by demands for division of attention. It is clear from these figures and from the statistical analysis that marihuana had no effect on the reaction times to the perceived signals. This differs slightly from the results in the alcohol dose experiment where there was a statistically significant increase (of very small size) in reaction time.

How can we account for marihuana-related performance deficits? A tentative hypothesis is that it affects the ability to sustain concentrated attention. Recall that there is no evidence that alcohol affects concentrated attention unless large amounts of information must be handled. For example, if a person under alcohol is required to monitor a radar screen for an infrequently occurring signal, the performance decrement is no greater than that exhibited by anyone facing this boring task. As is clear from the following experiment, marihuana has quite a different effect.

This study tested the effects of marihuana on a sustained attention task using a modified Mackworth clock technique (Mackworth, 1961). Overall differences between a placebo treatment and three levels of marihuana, and time-related changes in performance were examined.

Thirty-three neon bulbs were distributed evenly around the edge of a 12 in. diameter black-clock display. The lights were lit sequentially, moving clockwise, at 500 msec. intervals. Subjects monitored the display for two types of target signals: A Double Jump (DJ), meaning one light position was skipped, and a Repeat (R), meaning the same bulb was lit twice. Both types occurred five times in each of four 15 min. periods, and there was a total of 7200 light presentations at each hour session. Subject pressed a telegraph key under his left hand in response to a DJ and a telegraph key under his right hand in response to an R.

Treatments were administered as cigarettes of active and detoxified marihuana which yielded doses of 0, 50, 100, or 200μg delta-9 Tetrahydrocannabinol (THC)/Kg. bodyweight. Treatment order was determined by a 4 x 4 Latin square design.

Results are in terms of two error categories, failures to detect a signal and responses to non-existent signals. The latter, false alarms, was not affected by marihuana, but 100μg and 200μg doses significantly impaired the correct detection of signals. This finding is consistent with data from Moskowitz, Sharma and Schapero (1970) which shows a perceptual performance decrement under marihuana.

Marihuana also significantly impaired vigilance performance. Placebo and 50μg THC treatment conditions show only a first half-hour decline followed by a performance plateau as is typical of vigilance studies. Performance under 100μg and 200μg doses,

however, continues to decline over the hour session. Since performance did not stabilize, it is not known over what time period the decline will extend.

These data suggest that marihuana use represents a potential hazard for tasks which require sustained attention. It is unclear what mechanisms underlie the impairment, and further experimental effort is needed.

In summary, testing for drug-related hazards in the conditions of modern industrial environments requires an examination of the effects of a drug on perceptual and information processing abilities. Assessing more elementary sensory or motor deficits is an insufficient, though perhaps necessary, step in determining the hazards associated with the drugs. It is essential to understand the drug effects in the context of the requirements of the industrial activity.

It is unfortunate that little research effort has focused on the complex human functions demanded by many new industrial activities. Some situations are so engineered that they push workers to the extreme of their perception and attention abilities. Then any stress, whether it be drug, noise, fatigue, or some other, will lead to increased accident probabilities.

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DRUG INTERACTIONS — SOME FIRST PRINCIPLES

A. E. LeBlanc, Ph.D.

Drug interactions have received increasing attention in recent years. This is evidenced by several recent bibliographies[1, 2, 3] as well as a new literature alert system. This interest has, however, not been matched with the development of an organized body of information. This paper will attempt to indicate some of the difficulties, especially in the area of vocabulary.

The probability of drug interactions has dramatically increased. Among the reasons are (a) drugs prescribed in combination, (b) people going to more than one physician, (c) non-prescription drugs taken concurrently with prescription drugs, and (d) drug abuse[4].

All of the above are further compounded by the potential combination of these drugs with chemicals at the industrial site[5]. With increased complexity of chemical processes, the likelihood of a worker being exposed to two chemicals is quite high.

LOGICAL DEVELOPMENT OF KNOWLEDGE

Identification of Interaction

Since even a few hundred chemicals/drugs taken in pairs will yield a very large number of pairs, no procedure based on random pairs will be very productive.

In experimental studies, a choice is made because of a dramatic interaction such as that which led to the discovery of the Antabuse reaction. Industrial workers who were exposed to some sulfur-containing substances developed an unusual sensitivity to alcohol consumption.

Other choices of drug pairs to study have come from epidemiological studies in which factor analyses identify probably important interactions.

Other interactions are pursued because of (a) probability of there being an interaction based on knowledge of metabolism, etc., for example the interaction of some anticoagulants and sedatives, (b) probability of there being an interaction based on similarity of actions; e.g. alcohol and solvents or sedatives.

Characterization of Drug Interactions

Having identified the interaction that is of interest, the next logical step should be to characterize the interaction with respect to dose ranges, effects and direction of change.

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Here, care must be taken to deal with appropriate tests and dose ranges. To look at lethality for drugs which are not consumed at these high levels is of academic interest but of less practical value. The use of anaesthesia to do interactions of tranquilizers is similarly inappropriate.

In addition one should be alert to the possibility of the production of an unusual interaction not obviously predictable from the primary effects. For example, the simultaneous administration of a major tranquilizer, an opiate and a local anaesthetic may cause death even though at these doses one would look for alterations in analgesia or tranquilization.

Although this area is a very important step, it is usually neglected. Instead there is a rush to examine the more fashionable mechanistic explanations. Before this happens there is usually a pause to use some of the very confused vocabulary of drug interactions.

Vocabulary of Drug Interactions

Many vocabulary terms will be introduced below with no attempt being made to be definitive, since the author believes these terms to be irretrievably distorted in their meaning. We face the situation that the workers in the field of drug misuse faced with the term *addiction*. After many attempts at redefining the term they used a new term *drug dependence* which had a clear definition.

Loewe[6] said that though these terms "have no definable place in the treatment of combinations problems and should be eliminated from the field because of the menace of confusion, the greater probability is that they will live on as so many other undefined and undefinable 'terms'."

Synergism

This term has two major meanings. For some workers the word means all drug interactions generally[7]. That is to say, synergism is synonymous with interaction. A second use of the term has it meaning the same as potentiation[8].

Potentiation

This term means the resultant of two drug effects that is bigger than that which is expected. The problem with this is the definition of the term *expected*[9].

Addition

This term means that the effect is as big as one would expect from the combination of two drug effects. A further qualification

is made to separate this from *summation*[10, 11, 12]. Addition is said to occur when the mechanism of the two drugs is the same. This implies that the maximum effect of a combination cannot be bigger than the maximum of either drug.

Summation

This term is used when two drugs together produce an effect equal to that predicted by the two drugs separately. This term makes no assumption about similarity of mechanism. Thus the maximum summated effect can be as large as the algebraic sum of the two maximal responses produced by the two drugs.

Antagonism

This term is used when the sum of two drug effects is less than that predicted by their individual effects. It is possible for antagonism to be so large as to produce negative values. For example, if one studies a bronchodilator and a bronchoconstrictor on a normal bronchi, the crest change may be either positive or negative.

Hetergic

This term is used to describe an interaction when there is no common action between the drugs.

Homergic

This term is used to describe an interaction when both compounds produce effects on the same test situation.

Mechanistic Explanations of Drug Interactions

This area of research has been very active[4, 13]. It in large part reflects the scientific culture which favors reductionist approaches to problems.

There is a very large complex of potential interaction mechanisms. Some of the more common ones are given in Table I. The Table indicates the two broad kinds of interactions. Pharmacokinetic interactions are based on an altered handling of the drug by the body. These processes are more easily studied than pharmacodynamic interactions which are based on some knowledge of the mechanism of drug action.

Pharmacodynamic interactions are invoked when the mechanism is known or when the other mechanisms are ruled out. This is usually done by using appropriate target tissue drug levels rather than doses.

These mechanisms are usually studied separately. A recent report[14, 15] exemplifies the difficulties of looking at more than one process.

Table I. POSSIBLE MECHANISMS OF DRUG INTERACTIONS

Pharmacokinetic (drug handling)	
Absorption	
	alteration in pH
	alteration in blood flow
	complex formation
	alteration of gastrointestinal motility
Distribution	
	alteration in blood flow
	competition for binding sites
Metabolism	
	induction of drug metabolism
	inhibition of drug metabolism
Excretion	
	alteration in urinary pH
	interference with excretion
Pharmacodynamic (drug actions)	
	interactions with known physiological processes
	interaction with drug receptors

Factors Affecting Drug Interaction

At any point along the above continuum it is necessary to look at such factors as age[16], sex[17], race[18, 19], nutritional and pathological state. Several of these have clearly been shown to be involved in drug interaction. All have been shown to be influential in drug effects.

A "NEO-CLASSICAL" APPROACH TO DRUG INTERACTION

The Self Potentiating Paradox

Pharmacologists routinely expect to see dose-response curves for drug interactions. If this procedure was appreciated, some of the problems with vocabulary would be eliminated.

This need can be clarified with the following imaginary experiment. If a dose of drug A were given on a certain test and a dose of drug B were given separately, each of which produced a significant effect, then the sum of these two drug doses will then produce an effect. If this combined effect equals the sum of the two separate effects, many experimenters would take that to be evidence of summation or addition. If it were larger than this sum, synergism or potentiation would be demonstrated, and if less, antagonism.

This proposition will be true only if the dose response curve for the test is linear and passes through zero. This almost never happens. Most drug effects have a threshold and a sigmoidal or some other relationship between dose and effect.

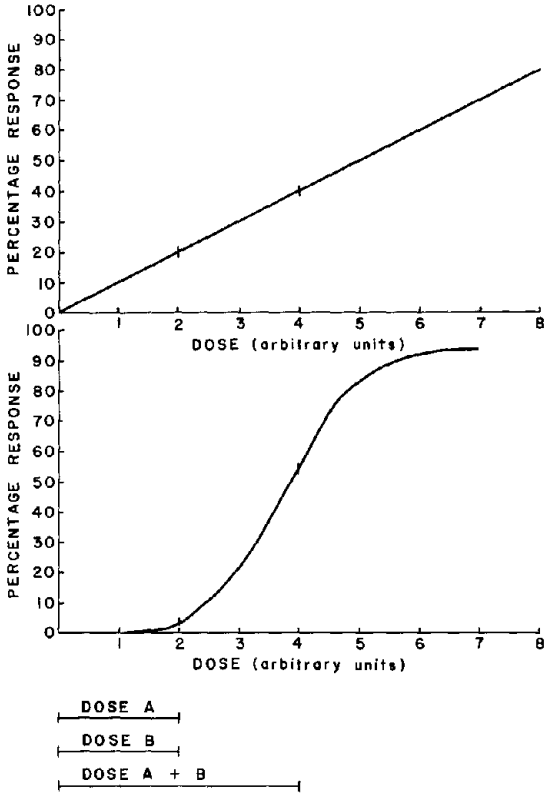

FIGURE 1. Theoretical dose response curves. Doses A and B are the same and equal to 2 units.

If you examine the curves in Figure 1, you can see the sense of the above discussion. The reader is encouraged to try the exercise with dose response curves of different shapes and intercepts.

To really appreciate the necessity of at least this type of an analysis is to consider the general experience of alcohol consumption. Two drinks consumed by a normal individual will have little effect. If at a separate time two equal but different drinks are consumed, this experience will be repeated. If both doses are consumed together, the effect is much larger than that predicted by the sum of the separate effects. If the two doses had been labelled drug A and drug B, many would claim this as evidence of potentiation. In other words, we would conclude that a drug potentiated itself — obviously a peculiar concept.

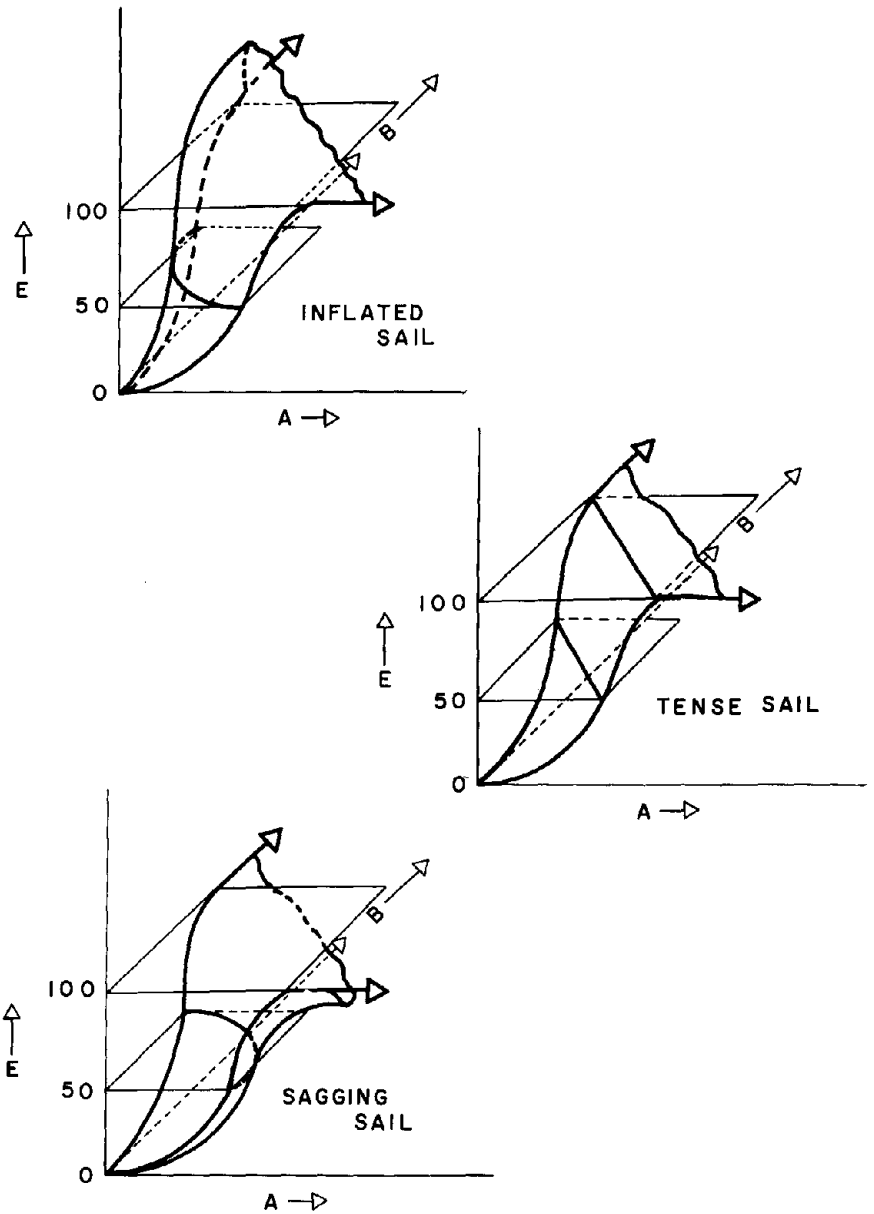

FIGURE 2. Examples of the possible dose/effect relationships of two drugs in combination. Doses of A and B are shown on the abscissae delineating the horizontal plane. The ordinate is the intensity of effect in the range between zero and maximum possible effect (=100). The S shaped curves in the vertical A/E and B/E planes are the respective dose effect curves of A and B separately. The surface between these lines represents the dose-effect surface of the combination. The horizontal plane at E=50 transects the drug effect surface at lines of equi-effectiveness. These Figures are derived from Loewe (6).

Drug Surfaces

A pharmacologist would say that a single drug requires a dose response curve to adequately describe its effects. Two drugs concurrently administered require a dose response surface.

Twenty years ago, Loewe[6] described in considerable detail just such an approach to drug interaction. He described a "sail" produced by a three dimensional array of dose combination effects. These sails are portrayed in Figure 2.

In these figures the term addition would apply to the "tense sail", infra-addition to the "sagging sail", and supra-addition to the "inflated sail".

These terms do not imply mechanism any more than a dose response curve does. If the dose axes are replaced by blood levels, many of the pharmacokinetic explanations of drug interactions can be ruled out. For example, alterations in absorption are irrelevant if equivalent blood levels are examined.

It should be clear that these "sails" can be mixed such that no single term will describe an interaction at all dose ranges. Thus one can conceive of an interaction being hetero-additive.

The analysis shown here applies to homergic drug interactions. This means the dose response curves for the two drugs are similar. In the case of hetergic interactions, the surfaces are described as a minimum surface area defined by the two dose response curves. Supra-addition and infra-addition are indicated by deviation from this minimum surface. The deviations are upward and downward respectively as in the homergic situation.

Alcohol-Chlordiazepoxide Interaction

The data in Figure 3 are derived from a study of alcohol-chlordiazepoxide (Librium) interaction which attempted to use Loewe's approach. This study used the moving belt test which measures moderate degrees of motor impairment. If a rat trained to walk a moving belt steps off the belt on to the surrounding grid, the animal is shocked and a timer is activated. The time off the belt is the measure of impairment[20].

Thirty dose pairs in the range 0-5.0 mg/kg of chlordiazepoxide and 1.2-2.2 g/kg of ethanol were administered to thirty trained rats. After a wait of at least 3 days the animals were randomly reassigned to dose pairs and rerun.

Blood samples were taken after behavioral measures to determine blood alcohol by the internal standard method of gas chromatography[20] and the chlordiazepoxide by a radioactive-combustion method[21]. The results are shown in Figure 3.

Statistical analysis soon showed that for this density of data points only a family of dose response curves could be reliably

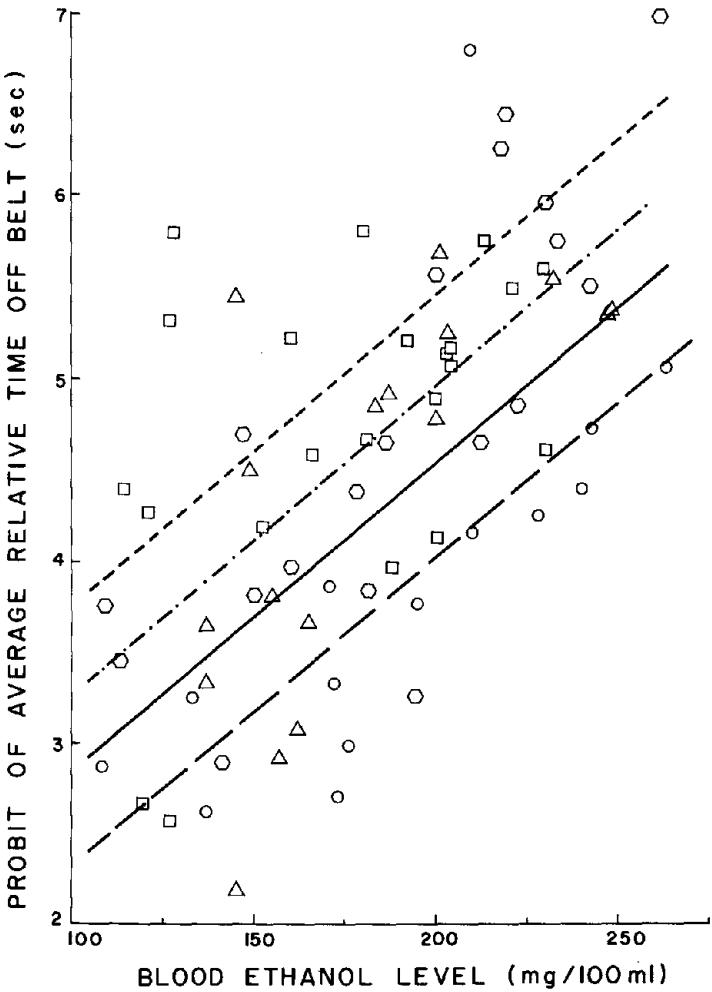

FIGURE 3. Effects on motor impairment of increasing blood levels of chlordiazepoxide (CDE) combined with increasing blood levels of alcohol

KEY: ○— 0-48 ng/ml CDE — — —
△ 50-98 ng/ml CDE ————
⬡ 100-198 ng/ml CDE — · — · —
□ 200-400 ng/ml CDE - - - - - - -

The probit values for the average relative time off belt were plotted against ethanol concentration.

calculated and not a surface. What the data do permit one to say and only to say, is that chlordiazepoxide, at doses which do not themselves cause motor impairment, monotonically increase the impairment produced by ethanol. Moreover, one can say that this is most probably a pharmacodynamic interaction, i.e., occurring in the target tissue, the brain.

It is emphasized that these limited conclusions required thirty dose pairings. Drug interaction conclusions often outstrip much more limited data.

CONCLUSIONS

There is no question that drug interaction will continue to be an important field of study. There is, however, an inefficiency in the work of the field, in part because of concept confusion and in part by premature reductionism. There are now trends hopefully continuing which will facilitate development of this important area. If we are unable to cope with a two drug situation, we will be poorly prepared to cope with the multi-drug problems now appearing.

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BEHAVIORAL TOXICOLOGY WORKSHOP DEMONSTRATIONS

SESSION VI

BEHAVIORAL TOXICOLOGY WORKSHOP DEMONSTRATION

Measurement of Physiological and Behavioral Responses in a Controlled-Environment Chamber

Medical College of Wisconsin

The Department of Environmental Medicine operates two controlled-environment chambers for the purpose of conducting human-inhalation toxicology investigations. These chambers in which the environmental factors can be controlled with precision provide an ideal setting for the study of the behavioral and physiological responses of human volunteers to a chemical agent (Figure 1).

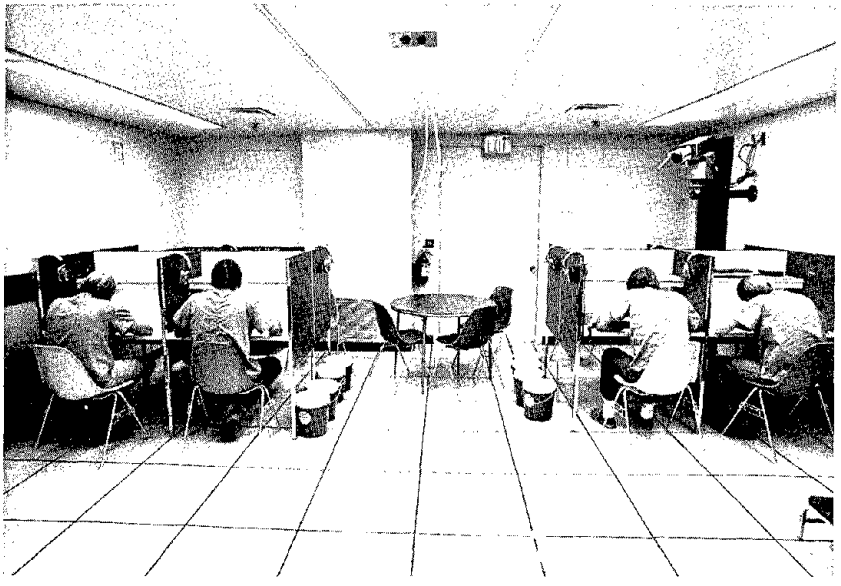

FIGURE 1. Controlled-Environment Chamber, 3,200 cubic feet. All activities are monitored by closed circuit television (upper right). The two air monitoring probes are in the upper center of the photograph. Containers for 24-hour urine collection line the corridor separating the two, 4-person carrels.

Each controlled-environment chamber in which the exposures to gases or solvent vapors are carried out is a room measuring 6.1 x 6.1 x 2.4 meters (20 ft x 20 ft x 8 ft). Each is equipped with an independent ventilation system permitting control of temperature and relative humidity. The chambers feature pleasant

This workshop material was presented as a slide presentation with prerecorded narration by Dr. Richard D. Stewart. Other staff members who participated in this demonstration included Jack E. Peterson, Ph.D., Carl L. Hake, Ph.D., Andre J. Lebrum, M.D., D.P.H., and Paul E. Newton, M.S.

lighting, comfortable chairs, individual testing cubicles, a rest-room facility, and an audiometric booth.

The experiments with human subjects are conducted in strict accordance with the ethical and technical requirements for human experimentation previously set forth[1]. While in the chamber, the subjects are under continuous visual surveillance by medical per-

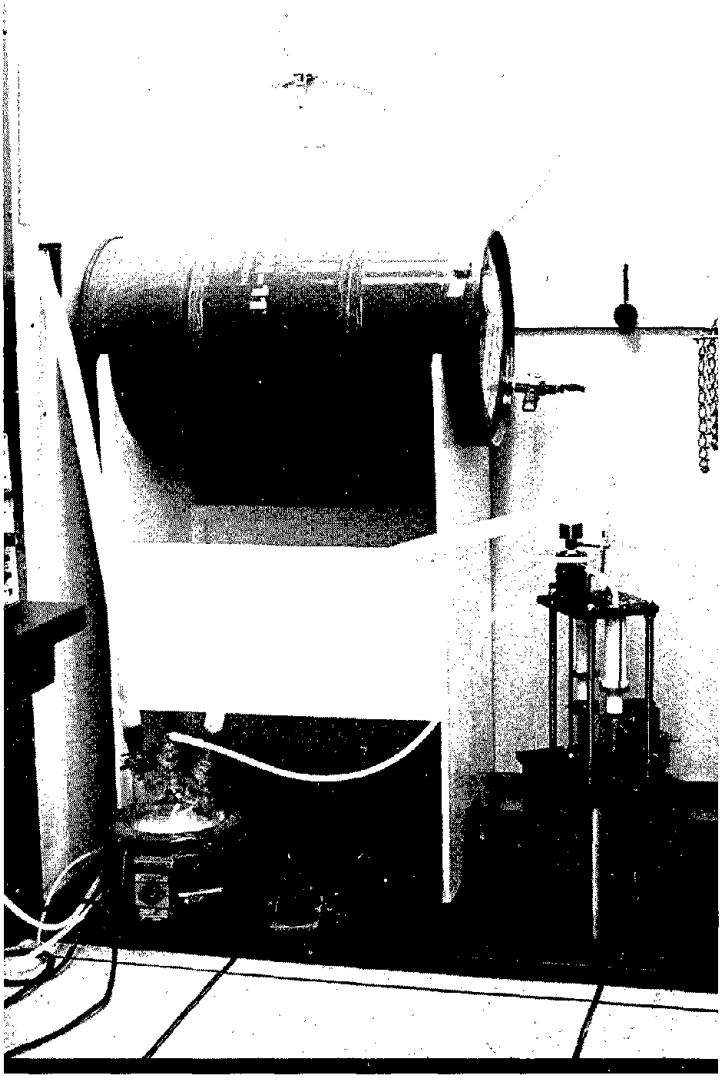

FIGURE 2. The contaminant to be introduced into the Controlled-Environment Chamber is contained in the walk-in hood adjacent to the chamber's air supply ducts. Here fluorocarbon-11 is being metered by a dual syringe pump into an evaporating flask, whose contents are being swept into the air supply duct.

sonnel and, in addition, all activities are monitored by closed-circuit television and are periodically video-taped for future review since the majority of experiments are conducted in a double-blind mode.

In the command laboratory, immediately adjacent to the environmental chamber, is a walk-in hood. The compound to be studied is introduced into the chamber's air supply from this hood (Figure 2). The contaminated air enters the chamber through four diffusers in its ceiling. This air is drawn through the chamber at approximately 14 cubic meters per minute (500 cfm), creating a slight negative pressure within the chamber. The air may be recirculated in part or discarded after one pass.

Each time human subjects are deliberately exposed to a toxic agent two independent monitoring systems are used to determine the concentration of the contaminant in the chamber air. In Figure 3 a gas chromatograph equipped with a sequential sampler and an infrared spectrometer equipped with a 20-meter path-length gas cell are monitoring air continuously drawn from the chamber through 0.635 cm (¼-inch) diameter polyethylene tubing.

The analytical information from the infrared spectrometer is continuously monitored by a PDP-12 mini-computer. Every 30 seconds the mean concentration of the contaminant in the chamber for the previous 30 seconds is displayed on the instrument screen, along with the cumulative concentration over the period of time that the chamber has been in operation. Using computer vigilance of the infrared monitoring system makes possible careful control of a gas or solvent vapor concentration in the chamber air.

The design of the air flow dynamics within the controlled environment chamber is such that no gas or vapor concentration gradient exists except in the zone immediately within the entrance door, this due to the influx of uncontaminated air from the command laboratory. In the rest of the chamber the concentration of the gas or vapor from wall-to-wall or floor-to-ceiling is uniform.

Gas and vapor standard preparation is critical for accurate analytical results. In our laboratory we use the following technique: clean air is metered into 30- and 70-liter saran bags, passing first through absorbents to remove water vapor and any trace contaminants. To the known volume of clean air a known volume of gas or vapor is introduced from a gas sampling syringe through the wall of the saran bag. After the introduction of the contaminant the small needle hole in the saran bag is sealed with plastic tape. The bag is then agitated until mixing is complete. It has been our policy to prepare a series of standards, spanning the concentration of the gas or vapor we wish to monitor. Aliquots from this series of standards are drawn through the air sampling probes from within the controlled-environment chamber to the

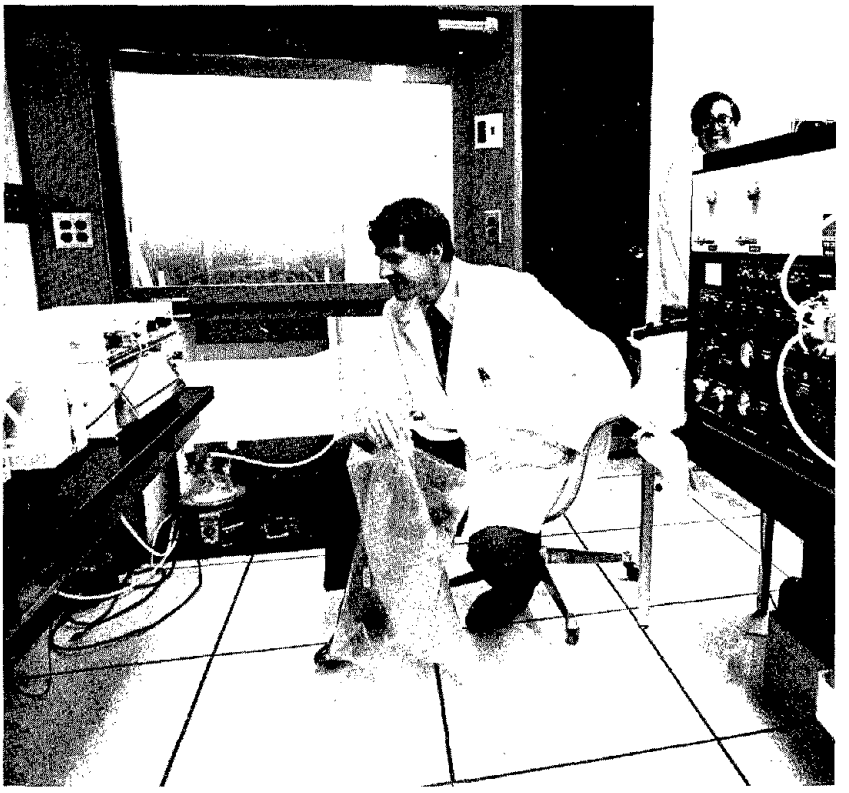

FIGURE 3. Two independent analytical methods are used to monitor the concentration of the contaminant in the Controlled-Environment Chamber. On the left is an infrared spectrometer equipped with a 20-meter variable path-length gas cell. On the right is a gas chromatograph equipped with an automatic sequential sampling valve. Analytical data are monitored by a PDP-12 computer, providing finger tip control of the concentration of the contaminant in the chamber.

analytical instruments for measurement. This is done pre-exposure, hourly during exposure, and following each exposure. The computer program used in monitoring the chamber is so written that information from the hourly standard analyses is instantaneously used to refine and adjust the calibration curve.

FIGURE 4. A team of experts representing several scientific disciplines is required to conduct controlled-environment chamber experiments with humans. Members of the team are shown in the laboratory which surrounds the chamber. The mini-computers on the left provide for simultaneous collection of physiological and behavioral test data from multiple subjects.

Figure 4 is a view of the exterior of the controlled-environment chamber taken when volunteer subjects were being exposed to a toxic gas. Note that the large observation windows are fitted with draperies to reduce the "fish bowl" effect upon the subjects. This is especially critical when the primary object of the investigation is

to study behavioral effects of an exposure to a toxic agent. To the left, immediately in front of the main chamber entrance, stands a physician well qualified in emergency resuscitation techniques. No human experiments are ever conducted in the absence of a physician so qualified.

All of the human experimentation conducted in our laboratory is performed in such a manner as to entail "zero" on negligible risk to the volunteer subjects. However, there is always the possibility that a catastrophic illness could occur, coincidentally or accidentally. To deal effectively with such an improbable event, all personnel are trained to function as a team so that the optimum emergency medical care can be immediately available. Emergency equipment includes a portable defibrillator, the paddles of which can be used to monitor the electrical activity of the heart, and a laryngoscope with a set of endotracheal tubes.

The principal investigator may oversee the entire experimental procedure from the "citadel", a satellite office with large observation windows. From this vantage point he is in a position to make the appropriate decisions, should difficulties arise, and yet he is remote enough not to interfere with the double-blind experimentation (Figure 5).

Over a period of 15 years, our laboratory group has investigated

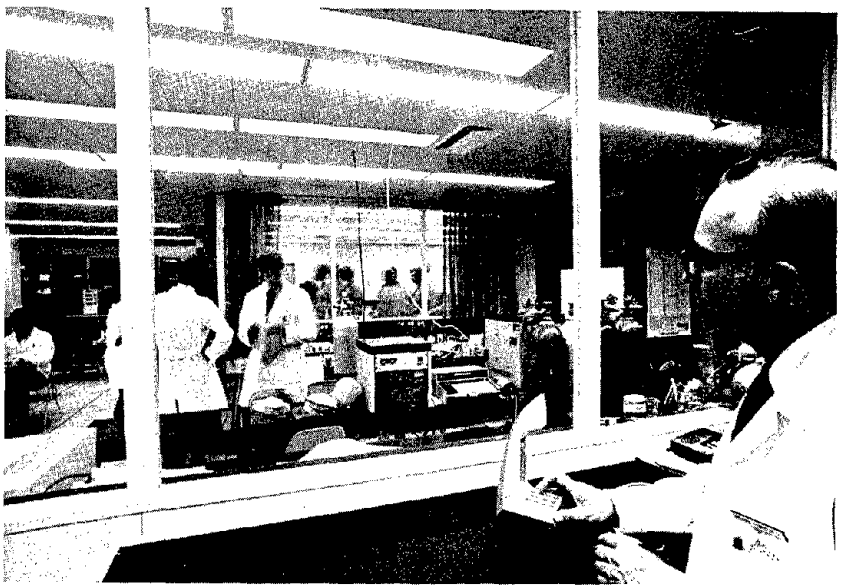

FIGURE 5. Adjacent to the chamber laboratory is the "citadel" from which the principal investigator can oversee the entire operation. In the background the chamber subjects can be seen in the Controlled-Environment Chamber.

the absorption, the metabolism and the physiological effects of approximately two dozen volatile compounds and half-a-dozen gases upon man. In the majority of instances we have used paid volunteers. In every instance the volunteers have been completely informed as to the purpose of the experiment, the manner in which the investigation will be conducted, and the potential risks to themselves. The subjects are asked to sign a document which indicates that they have been so informed and that they are freely volunteering to participate. All subjects are, of course, free to withdraw from the investigation at any time.

While we have employed subjects from all walks of life, our best success has been with university graduate students, whom we have found to be emotionally stable and highly reliable, especially in the critical post-exposure collection of breath and urine samples.

Prior to being accepted for any research program, each subject is given a comprehensive history and physical examination with the appropriate clinical laboratory tests so that his health status may be well defined. During exposure experiments the minimum medical surveillance program we employ includes a 12-lead electrocardiogram prior to exposure, a repeat physical examination each day prior to exposure, and the following clinical laboratory studies performed prior to, during, and at appropriate intervals following exposure: complete blood count, complete urinalysis, and SMA-12 clinical chemistry panel.

Lead II of the EKG is routinely monitored by telemetry. In the majority of instances it could be argued that neither a pre-exposure 12-lead electrocardiogram nor continuous surveillance of the electrical activity of the heart during exposure is a necessary precaution. However, since many of the gases and volatile agents studied in our laboratory do possess the potential for inducing cardiac arrhythmias were an "accidental" over-exposure to occur, these safety precautions are desirable. Ten years ago a 50-year-old volunteer for an eye irritation experiment, who had had a normal exercise EKG one week previously, reported for the experiment complaining of mild nausea and indigestion. A 12-lead EKG revealed that the subject was in the early throes of an acute myocardial infarction and he was quietly ushered to a hospital. Since coronary artery disease is endemic in our male population and since precious little experimental animal data are available to indicate the influence of toxic agents upon the natural course of a developing myocardial infarction, we insist upon the cardiac surveillance described above.

As an additional check on the magnitude of the exposure, blood samples are obtained periodically from each of the chamber subjects so that the exact amount of the contaminant which has been

absorbed can be determined and correlated with the physiological responses and behavioral testing. This correlation is essential in behavioral testing because of the biologic variation which exists between people in the absorption and metabolism of chemicals. For example, in a series of carbon monoxide exposures females were found to absorb the gas more rapidly than did males. Hence, it was critical that accurate carboxyhemoglobin determinations be made so that the precise correlation with test performance would be possible. Venous blood samples are obtained easily while the subject is being exposed by having him hold his arm through an armport in the chamber wall. The venipuncture is then performed in the adjacent laboratory in an uncontaminated atmosphere without interrupting the subject's exposure.

In addition to determining the exact magnitude of the exposure during behavioral testing, monitoring the function of all major organ systems is essential, with special attention to those known to be most sensitive to the chemical agent under investigation. This is required to safeguard against unintentional organ injury. When studying the effects of an agent capable of depressing the central nervous system, we periodically monitor the spontaneous EEG. This is accomplished during the exposure with the subject seated in a grounded audiometric booth whose air supply comes from the controlled environment chamber (Figure 6). In this

FIGURE 6. The spontaneous EEG and visual evoked response are obtained in the audiometric booth attached to the Controlled-Environment Chamber.

setting the visual evoked response can be serially recorded[2]. For repeatedly exposed persons the scalp is marked by trimming away a small patch of hair which permits the electrodes to be placed in identical locations day after day.

The technique for obtaining the visual evoked response has been described in detail[2]. Many gases and solvent vapors capable of producing central nervous system depression in man alter the visual evoked response at doses lower than those which result in decrements in other behavioral tests. For example, alterations in the visual evoked response have been observed following exposure to trichloroethylene 100 ppm for eight hours and to methylene chloride, 1000 ppm for one hour.

Our primary endeavor over 15 years of experimental work in the controlled-environment chamber has been to study the absorption and metabolic fate of various compounds. We feel that this technique has great application in the field of behavioral toxicology. Eliciting a sensitive behavioral change is meaningless if the exact magnitude of the exposure responsible for eliciting that change is unknown. Several examples of the usefulness of the chamber for this type of research have been reported[3-11]. The most recent work with trichloroethylene is the subject of another paper of this book. Figure 7 is an example of the precision with which the

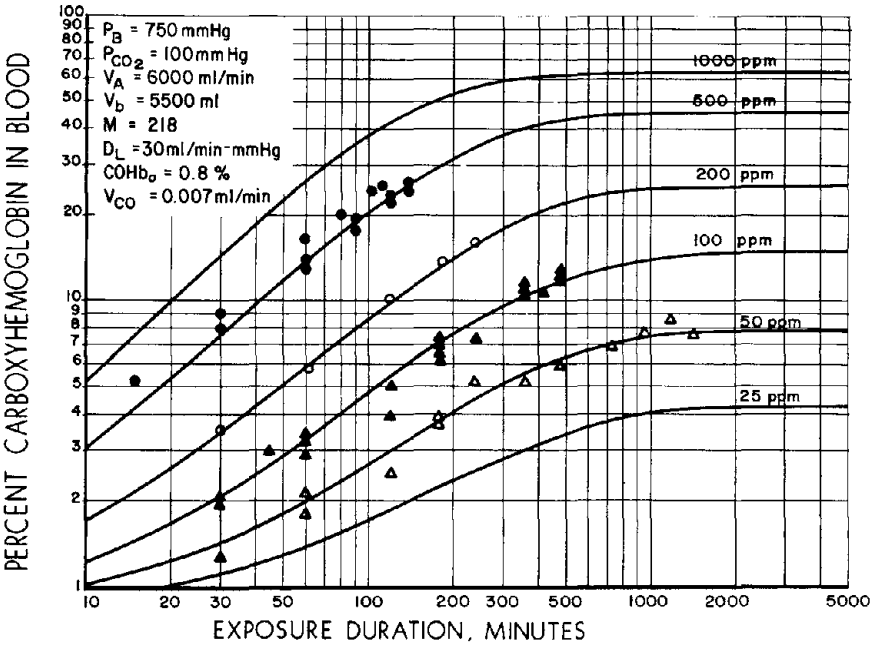

FIGURE 7. The absorption of carbon monoxide as reflected in the increase in carboxyhemoglobin of human volunteers. (Reprinted with permission from the **Archives of Environmental Health**, Vol. 21, pp. 154-164, August, 1970.)

absorption and excretion of carbon monoxide were controlled during behavioral toxicology experiments[5].

Breath analysis has proved of extreme value in estimating the magnitude of an exposure to a toxic gas or volatile compound[12]. The rational for monitoring the breath concentration of a compound

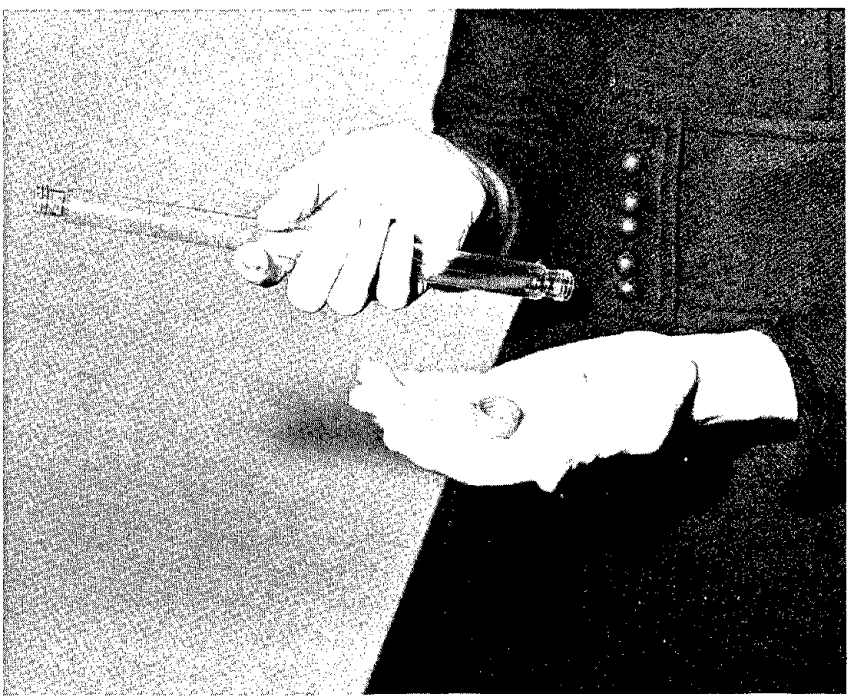

FIGURE 8. Glass pipette (50 ml) is used for collecting breath samples. One cap has a pre-drilled hole for gas sampling. Both caps have saran liners which seal the pipette chamber, permitting storage of the breath sample for prolonged intervals. (Reprinted with permission from **Essays in Toxicology,** "The Use of Breath Analysis in Clinical Toxicology," Academic Press, 1973.)

is that each gas or volatile compound which is absorbed and then circulates in the blood stream is excreted in a predictable concentration following cessation of the exposure. From an analytical standpoint, breath analysis is a rapid and economically feasible technique. From the subject's standpoint this is the preferred monitoring technique as it obviates the pain of venipuncture or the nuisance of urine collection. Figures 8 through 11 illustrate the collection technique and Figure 12 is an example of one of the breath decay curves which has been obtained[10].

FIGURE 9. The breath sample is "collected" by sealing one end of the pipette with the lips, flushing the chamber with three exhaled breaths and then, after holding the fourth breath for 30 seconds, exhaling through the pipette chamber so that the end-tidal portion can be collected.

In conducting behavioral toxicology testing in the controlled-environment chamber, subjects may be exposed and tested individually or in a group setting. The advantages and disadvantages of each have been discussed in a recent publication involving the effect of carbon monoxide on time perception[13]. When small groups of subjects are tested in the chamber, group interaction commonly occurs and the experimental design must be such that the influence of this variable can be assessed accurately. Figure 1 shows eight

FIGURE 10. The distal cap is secured while the subject is still expiring through the pipette chamber. After the distal cap is secured, the proximal end of the pipette is removed from the mouth and quickly sealed with the finger tip.

subjects in a group setting where individual testing cubicles provide adequate isolation for many cognitive and behavioral tests.

The tests which have proved to be of most value in our testing include the Flanagan coordination test, the modified Flanagan arithmetic test, the random number inspection test, the Crawford collar and pin test, the Crawford screw test, the Marquette time estimation test which includes a measurement of complex reaction time, the General Motors physiological readiness-to-drive tester, the Marquette tracking test, the 10- and 30-second time estimation tests, and as described above, the visual evoked response.

In summary, the role of the controlled-environment chamber in behavioral toxicology has been discussed. Scientists investigating behavioral toxicological changes in human subjects must measure accurately the magnitude of the exposure to which the volunteers are subjected. For toxic gas or vapor investigations, the controlled-environment chamber currently affords one of the best facilities in which this goal can be achieved.

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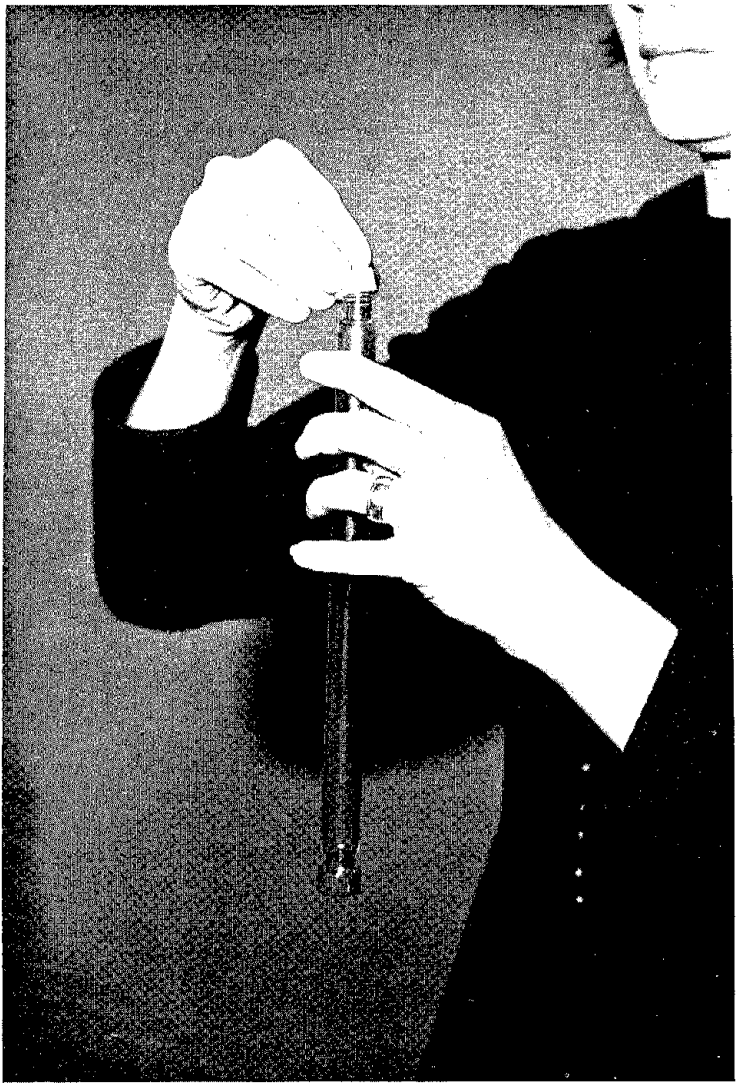

FIGURE 11. The breath sampling is completed by securing the proximal cap.

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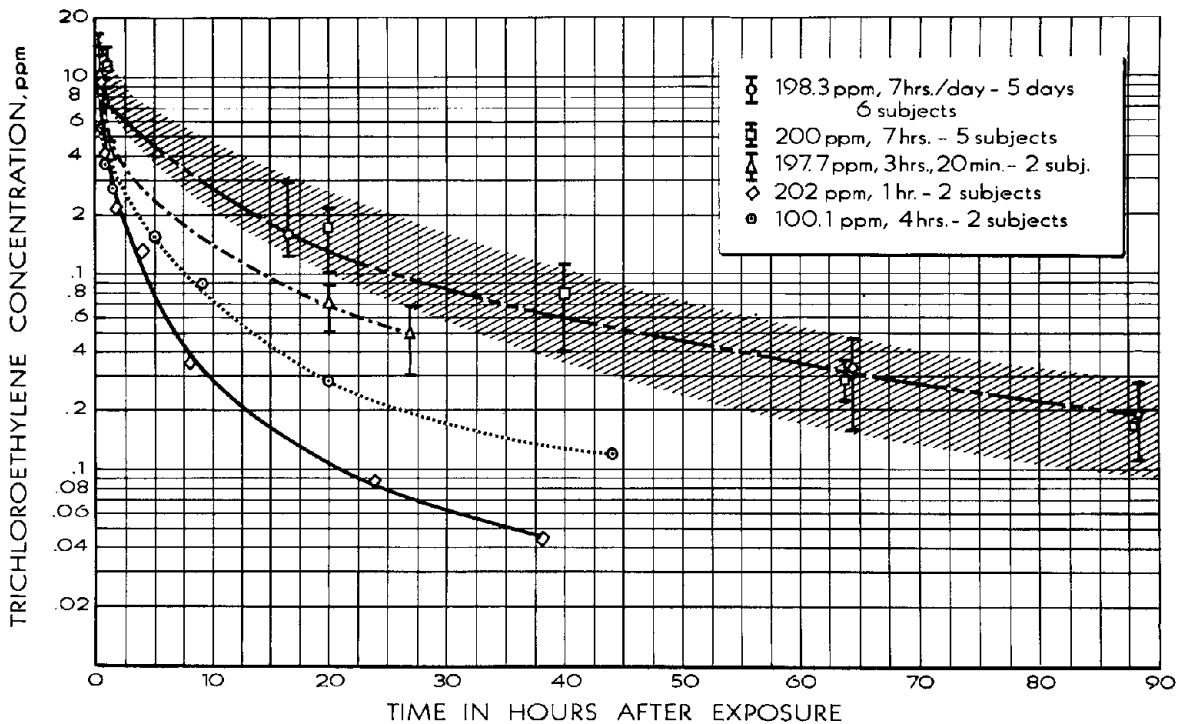

FIGURE 12. The concentration of trichloroethylene in the expired breath is related to both duration of exposure and concentration of solvent during exposure. These breath decay curves are reprinted with permission from the **Archives of Environmental Health,** Vol. 20, pp. 69, January, 1970.

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BEHAVIORAL TOXICOLOGY WORKSHOP DEMONSTRATION

Behavioral Patterns of Workers and Accidents in High Risk Jobs in Construction Work

Theodore Barry and Associates

This study is an initial investigation by NIOSH of safety and accident causation in the construction industry and is intended to look at accident experience of workers and companies in the construction industry from three perspectives:

A. An industrial engineering oriented analysis of the work directed toward identification of work practices and procedures associated with high accident experience. This would include characteristics of the design or lay out of the job and conditions that may increase the hazards or otherwise be associated with high accident incidence. In addition, this analysis will involve the personal and mechanical observation of the behavior patterns of construction workers who have been identified as having had significantly high and significantly low accident experience. This observation will be done under real work conditions, with the purpose being to attempt to isolate or identify any behavioral or habit patterns of the workers themselves that may be associated with high and low accident experience.

B. A psychological evaluation of characteristics of personality, attitude and adjustment of construction workers identified as having histories of significantly high or low accident experience. In part, what we shall consider here is the existence or nonexistence of "the accident prone worker".

C. The measurement and analysis of certain behavioral or functional performance capacities that may differentiate between construction workers with high and low accident experience histories. Such behavioral capacities as vision, reaction time, coordination and aspects of decision making will be considered.

The investigation involves the following five key steps:

Task I. Determination of high risk jobs in construction work.

Task II. Selection of companies and workers.

Task III. Development and administration of the psychological and performance capacity test batteries.

This study is supported by a NIOSH Contract to Theodore Barry and Associates, Los Angeles, California. Kirk Prather, Dr. Robert Crisera and Dr. Sandy Fidell are research investigators on this contract.

Task IV. Job sample analysis of the jobs at the work site with observation of behavioral patterns of the subjects under real work conditions.

Task V. Analysis of differences and interaction effects between workers in the high and low accident groups in terms of measured psychological characteristics, performance capacities, and behavioral patterns on the job.

Construction is a broad and diverse industry that encompasses many different operations each with differing degrees of associated danger or hazard. Over-all, however, construction ranks 4th nationally in terms of accident severity and 6th in terms of accident frequency against other industry categories according to the Bureau of Labor Statistics. The roofing phase of construction was chosen for study. The industry employs about 100,000 workers nationally and 10,000 in Southern California.

Tasks I and II are largely a matter of gathering statistics from such agencies as the Bureau of Labor Statistics, State Workman's Compensation Commission, Insurance Companies and obtaining accident experience information from the roofing companies and the union.

The critical or important step in Task III, the development of the test batteries, is the identification of the characteristics to be measured. What characteristics of personality and performance capacity are important or appear to be important to safe performance on the job? The process by which these characteristics were identified involved: (a) a review of the relevant literature, (b) job analysis of roofing work and the environment in which it is performed.

Observations were made of roofers, their foremen, members of management of roofing companies and union leaders. Based on this analysis and the conclusions derived from the literature review the following general areas were selected for study.

A - Sensory motor behavior or capacity.
B - Cognitive process.
C - Personality
D - Attitudes
G - General Life Adjustment

Each of these general areas is broken down into specific characteristics selected for investigation in the succeeding paragraphs.

Sensory Motor Behavior or Capacity

A. Balance

A study of job related injuries in roofing and sheet metal work by O'Grady for the California Department of Industrial Relations

that was published in 1972 indicated that falls and slips are "responsible for more injuries suffered by workers in the roofing industry than any other single type of accident". This study further observed that loss of balance is a critical factor in disabling accidents in the roofing industry due to the roofers' frequent use of ladders and temporary scaffolding, not to mention the elevated surfaces on which they habitually work. Our own field observations corroborate the importance of balance in roofers' work.

B. Visual Acuity

This visual task involved in roofing is admittedly not very demanding, since it is performed under ample illumination and requires little or no training or specialized visual skills, but it is critical to safe performance on the job.

C. Auditory Sensitivity

Eighteen per cent of roofing accidents as summarized by O'Grady (1972) are attributed to workers being struck by or struck against some object. A relatively large proportion of these accidents involve workers being struck by falling objects, such as dropped tools, discarded roofing materials, etc. To the extent that some of these "struck by" accidents are preceded by shouted warnings, a roofers' auditory sensitivity may influence his accident rate. Older workers in particular may suffer from undiagnosed hearing impairment. At least one foreman interviewed in the field mentioned a worker's inability to hear shouted instructions over a reasonable distance as a factor that could influence his job performance and safety.

D. Eye Finger Coordination

O'Grady implicates the use of hand tools (saws, axes, hatchets, knives, etc.) in a large proportion of roofing accidents. Our own field observations revealed the hazard associated with the razor blade knives used by roofers to trim tarpaper sheets to fit irregular roof openings. Injuries to fingers in particular were cited by workers we interviewed as attributable to reaching for or using trimming knives usually worn in holster belts, or pockets. The use of such tools requires fairly accurate eye-finger and eye-hand coordination, particularly when careful trimming must be done in proximity to fingers that are holding a piece of roofing material in place.

E. Choice Reaction Time

A roofer may be able to prevent an accident if he is able to respond rapidly and correctly to an imminent hazard. If a bucket of hot asphalt is seen falling, for example, a roofer may be able to

avoid scalding if he can decide how to move himself from its path by one of a number of safe routes. This ability to avoid an accident depends not only upon gross motor capacities, but also upon decision making capabilities. There is also a modicum of evidence (e.g. Laurer et al., 1952) that choice reaction time may be related to error rates in certain industrial tasks.

F. Performance Speed vs. Accuracy

Profitability for roofing companies, particularly small ones, depends principally upon minimizing labor costs. Strong pressures are therefore exerted on foremen to encourage workers to work as rapidly as union work rules will permit. It is commonly observed that accuracy of skilled performance is inversely related to speed of performance. If inaccuracy in the manual skills involved in roofing is associated with accidents, as seems intuitively reasonable, a test of workers' inclination to be careless to achieve speed may offer some predictability of accident rates.

Cognitive Performance

A. Risk Preference

Much roofing work is repetitive and tedious. A busy apprentice servicing a number of journeymen may find himself climbing a ladder with a hot mop or a tar bucket a dozen times in the space of a few minutes. A temptation might exist to take advantage of a number of hazardous shortcuts such as trying to carry too heavy a load, or attempting to carry too many tools or materials. The roofer must therefore balance the risk associated with the hazardous shortcut against the payoff in reduced workload.

B. Transfer of Training and Reversal Learning

Roofers typically work for short periods of time (measured in days or weeks at most) at a given site, using different materials at different job sites, and performing different tasks under different circumstances. Thus, both the jobs the workers perform and the materials and methods of application that they employ are continually changing. Controls on the machinery that they use are not well standardized. For example, the gas valves may be dissimilar on a number of frequently used tar kettles. Even experienced workers must therefore be able to switch frequently from one set of skills to another. To the extent that confusion over similar skills associated with different materials or tasks can be implicated in accident causation, tests for reversal learning and transfer of training may be important in discriminating between low and high accident rate workers.

C. Short Term Memory & Perceptual Span

A roofer working at a particular task may be interrupted frequently for any of a number of reasons. For example, he may run out of hot material, he may be called to help another worker, or the foreman may stop work to issue warnings or other instructions. After each interruption the roofer must resume work where he left off. Safe resumption of work may require the roofer to recall how hot a piece of material was, estimate how rapidly it may have cooled, remember where knives or other hand tools have been placed and so forth. These requirements depend in part on the adequacy of the roofers' short term memory.

D. Intelligence

Few significant relationships between industrial accident experience and intelligence have been found, and those that have been identified have involved complex tasks such as flying an airplane. Nevertheless, it is a factor that must be controlled or accounted for in any study of group or individual differences in the performance of complex behavior.

Characteristics of Personality

The studies of personality correlates of high accident experience and industrial populations have not produced clear cut results. In many cases, conflicting results and conclusions have been found, and many of the studies have been fraught with methodological problems. Based on our analysis of the job and the relevant literature we have identified or hypothesized 6 dimensions of personality that we believe may be important in roofing accident experience.

A. Reflectivity vs. Impulsivity

Another way to describe this dimension is in terms of personal discipline and control. We believe that there may be a difference between the high and low accident groups in the degree to which they interpose thought between stimulus and action. In other words, the degree to which workers think of the consequences of their actions as opposed to responding impulsively may directly affect their accident experience. This conclusion is based on empirical observations and has not been investigated in the literature.

B. General Activity Level

Again, based on observation and discussion with workers, extreme hyper or hypoactivity would appear to be a source of accidents. Hyper and hypoactives tend to be out of pace with their

co-workers and this could be significant as roofing is largely a coordinated group function. Hyperactives tend also to over react to potential danger situations and can thereby cause another accident in the avoidance process.

C. Emotional Stability

Tension, anxiety, emotional stress from any source can be and frequently is a source of distraction, or a barrier to concentration on the task at hand. This dimension is one of the few that has found support in the literature as an accident correlate. (Smiley, 1955; Smart & Schmidt, 1960; Tiffin & McCormick, 1962; Whitlock & Crannell, 1949).

D. Introversion - Extroversion

Again, in a group or team task, ability or inclination to relate to people has significant influence on the way in which one is perceived by his coworkers as well as their attitudes toward him. We are particularly interested in the extremes of extroversion with its connotation of exhibitionism or "show off behavior" and the general lack of control and inhibition it implies. This dimension is another that has found some support in the literature as a correlate of accidents. (Fine, 1963; Mackay et al., 1969; Powell, 1971).

E. Ascendency vs. Submissiveness

The ascendency extreme of this dimension is associated with extreme independence and resistance to authority, a potential source of conflict in the group and therefore a source of distraction. It may also be reflected in a refusal to follow procedures and policy, for example a refusal to wear safety equipment. The submissiveness end of this dimension may be associated with a willingness to perform unsafe acts as directed by superiors rather than refusing or protesting.

F. Need for Achievement

This is a factor of current interest to researchers in the area of safety and accident prevention, and it was included in this study for that reason. It is assumed that people driven by an extreme or intense need for achievement may be more likely to be careless than their co-workers who are not so motivated.

Attitudes

Attitudes toward certain aspects of the work and work environment and job satisfaction in general have been found to relate to accident experience. Examples of work related attitudes that

have been found to relate in some way to accident experience include those toward risk taking, co-workers, work itself and the perceived conflict between high productivity and safety.

General Life Adjustment

Factors that reflect general adjustment in life such as marital discord, irregular work history, conflict with the law, etc. have been found significantly more common among accident repeaters than among low accident experience workers. (Wong & Hobbs, 1970)

SELECTION OF TEST BATTERIES

The objective of the testing program is to determine if there are in fact measurable differences between the high and low accident experience workers on the characteristics that have been hypothesized as critical in accident causation based on the literature and job analysis. In selecting tests the following principles were emphasized:

1. High reliability in terms of test re-test repeatability.
2. Validity in terms of applicability to the identified skills and characteristics.
3. Standardization in terms of availability of performance information on reference populations.
4. Economy of administration in terms of both time and equipment, for ease of testing under field conditions.

Sensory Motor Performance Battery

A. Equilibrium Duration Test

Subjects will be asked to stand erect with one leg bent at the knee, both eyes shut, and arms outstretched. A stopwatch will be used to record the duration of the period that subjects are able to maintain the stance before returning the second foot to the ground. The test will be repeated with the alternate leg after a short rest. An equilibrium duration test was selected from a number of potential measures of gross balancing ability primarily for pragmatic reasons. First of all, it requires no special apparatus to be transported from site to site. Second, it is easily scored and requires no extensive instructions to workers. Third, it can be quickly administered and it is not sensitive to experiential or learning artifacts as are certain balance tests that utilize specialized apparatus.

B. Visual Acuity Test

A standard Landolt Ring Test, utilizing broken circles with slits oriented in random directions, will be administered to workers

to assess gross visual acuity. The reader is referred to Geldard (1972 The Human Senses) for a discussion of the superiority of this technique over the more familiar Snellen Letter Charts. Administration of the Landolt Ring Test requires only a readily transported chart and tape measure to insure the targets subtend a 5′ angle at the subject's eye.

C. Auditory Sensitivity Test

Standard pure tone audiometry will be accomplished at speech interference frequencies utilizing a field operable portable clinical audiometer. Efforts will be made to find a sufficiently quiet environment in which to conduct the tests.

D. Eye Finger Coordination Test

Using specially designed testing apparatus, subjects will be instructed to press the response buttons of each hand corresponding to the illuminated lights immediately above them. The number of lights that will be illuminated on each trial at random may vary from one to four, depending on experimental conditions. Two minutes' practice with a standard display condition will be required before data are taken. It is estimated that 25 trials will be administered in each of two display conditions. The measure of performance will be time (to the nearest millisecond) from the onset of a display to depression of the correct combination of response buttons.

E. Choice Reaction Time Test

Subjects will be first instructed to press a response button immediately upon seeing a display light flash. Fifty trials will be administered to a fixed pace of approximately one every two seconds. The measure of performance will be average elapsed time or correct trials. As a second test condition, subjects will be required to make the identical response contingent upon the presence of a second display light. False alarms (responses made in advance of signal) and misses (responses made in the absence of the confirming signal) will be tabulated. The two measures of performance under the second condition will be number of correct trials and average elapsed time on correct trials. The above procedure permits rough estimation of the motor and decision time components of reaction time. The average choice reaction time minus the average simple reaction time gives some indication of decision time.

F. Speed Accuracy Trade Off Test

Subjects will be instructed to alternately depress response buttons beneath each index finger. The number of depressions with

the same finger before alternation will be determined by the number of lights illuminated. Thus, if one display light is illuminated, subjects are to tap the two buttons in immediate succession. If two display lights are illuminated, two depressions of one button will be required before the alternate button is to be pushed twice. The task will be structured such that increasing numbers of successive repetitions of response sequences will be required before the next higher number of display lights is illuminated. The measure of performance will be elapsed time from the start of an alternating sequence to its conclusion.

Cognitive Performance Tests

A. Short Term Memory & Perceptual Span Test

Subjects will be instructed to press buttons corresponding to illuminated lights in a delayed paradigm. Lights will be illuminated in a random pattern for a brief period (a few msec), immediately after which subjects will be required to reproduce the pattern of lights with their response buttons. The measure of performance will be the total of correct trials out of a sequence of 25. As a second test condition, subjects will be exposed to a similar display, but will not be permitted to respond until a ten second waiting period has elapsed. During the waiting period subjects will be required to sit with fingers outspread to preclude overt motor response reversal. The measure of performance will be total number of correct trials out of a sequence of 25.

B. Risk Preference Test

Subjects will be instructed to depress a response switch repeatedly to illuminate display lights sequentially. The number of button pushes required to illuminate each light will be contingent upon depression of three other buttons. If no other button is depressed, 16 pushes will be the requirement to illuminate a single display light. If one other button is held down, eight pushes will be required to illuminate the light with probability 0.50, while a light will fail to be illuminated with probability 0.50. If another button is held down while pushing, four pushes will be required to illuminate a light with probability 0.25, while a light will fail to be illuminated with probability 0.75. If a third button is held down while pushing, only two pushes will be required to illuminate a light with probability 0.125, while a light will fail to be illuminated with probability 0.875. The measure of performance will be the number of trials on which each of the accessory buttons is depressed.

C. Transfer of Training & Reversal Learning Test

The general conditions of this test will be similar to those of the short term memory test. The two major differences are that subjects will be instructed to depress response buttons corresponding to non-illuminated lights, and that the assignment of columns of lights to hands will be reversed. No practice trials will be permitted.

Personality Test

A. The Guilford Zimmerman Temperament Study

After reviewing the various personality inventories available on the market in terms of the applicability of their scales to the characteristics under consideration and their technical soundness, the Guilford Zimmerman was selected for use in this study. The instrument is composed of 300 items descriptive of behavior, feelings, and motivation to which the subject agrees or disagrees as they apply to him. The test yields scores on 10 factors or dimensions of personality. These factors are listed below. Their similarity to the 6 personality characteristics isolated for study in this investigation is evident in the descriptive titles.

Guilford Zimmerman Temperament Survey
Test Dimensions

High general activity and energy vs. inactivity and slowness
Restraint, seriousness vs. impulsiveness
Ascendance and social boldness vs. seclusiveness
Social interest, sociability vs. shyness, seclusiveness
Emotional stability vs. emotional instability
Objectivity vs. subjectivity, hypersensitiveness
Friendliness, agreeableness vs. unreflectiveness
Cooperativeness vs. intolerance, criticalness
Masculinity vs. femininity

Attitudes

A. Interview Questionnaire

The workers' attitudes toward and opinions about the various elements in their work environment which have been identified as potentially important correlates of accident experience in roofing will be studied through an interview questionnaire. This questionnaire was designed specifically for this study to elicit the workers' opinions, feelings, and attitudes toward the critical elements in their job and work environment.

B. Projective Tests

The workers will be shown pictures or sketches depicting cer-

tain of the most common roofing accident situations. They will be asked to describe their reaction to the pictures, and to assess the likely consequences of the accident situations. The workers' responses should reveal information about their perception of and experience with work related hazards, and their awareness of the consequences of accidents.

General Lifestyle or Adjustment

A. Interview Questionnaire

Differences that may exist between the high and low accident experience groups in general life adjustment as reflected in such factors as marital and work stability will be investigated through the Interview Questionnaire and the personnel files of the participating companies. The biographical questions relevant to this dimension are included among the first questions of the Interview Questionnaire.

WORK SAMPLE ANALYSIS AND BEHAVIORAL OBSERVATIONS

The third major thrust of this study involves the observation of the work behavior of the high and low accident workers on the job sites. Using live visual observation and photographic recordings, efforts will be made to identify practices and behavioral characteristics that differentiate between the two groups. Any such practices and behavioral patterns that are found to be consistently associated with high and low accident experience would have implications for improved worker training as well as the establishment or modification of company and union policy regarding safety.

PRELIMINARY RESULTS

Preliminary Accident Data

The following are some key excerpts from our analysis of California accident data. We have not found any detailed accident data. The State of California Division of Labor Statistics and Research has published a report on 1970 roofing accident data. We obtained a computer tape of this data and attempted to analyze it.

1. *California Apprentices vs. Journeymen Accident Rates 1970*

	Reported 1970 Accidents	Est. 1970 Population	Estimated Accident Rate per 1000 Men***
Journeymen roofers and foremen	778	9000*	86
Apprentice roofers	89	400**	228
	867	9400	92

* 1970 seasonal employment varied from 9000 to 10,300. This number includes some other work classifications such as sheet metal workers, helpers, loaders, and truck drivers. If anything, 9000 is too high a number for journeymen roofers only.

** Don Banks, Director of Southern California Roofing Apprenticeship Program developed this estimate of the number of roofing apprentices after comparing notes with the other directors in California.

*** Divide by two for rough estimate of accident frequency per million man-hour, assuming 2,000 hours per year.

NOTE: 92 accidents per thousand workers would be 46 accidents per million man-hours, if a man-year of employment equals 2,000 hours. Some data within the contractor associations suggests the rate may often exceed 50 accidents per million man-hours, thus exceeding underground coal mining accident frequency rates which have long been considered the highest occupational accident rate for work populations in excess of 50,000 men.

2. *Frequency of Accident Type*

A special analysis by California Division of Labor Statistics and Research indicates the following in terms of 1132 reported lost-time accidents analyzed from 1970:

		Number	Percent
a. *Fall or Slip*		329	29.1%
From elevation	200		
On roof	55		
Other	74		
b. *Burns*		256	22.6%
Hot asphalt	253		
Other	3		
c. *Strain, Overexertion*		219	19.3%
Lifting	145		
Pushing/pulling	23		
Using hand tool	18		
Other	22		
d. *Struck by, striking against*		202	17.8%
Falling object	69		
Hand tool	51		
Sharp object	34		
Other	48		
e. Other, misc.		126	11.2%
		1132	100.0%

Burns represent the highest frequency category of lost-time accidents. Eighty percent of the burns are to the hand and forearm. Many men indicate they incur numerous small unreported burns, and we rarely visit a crew on site where at least one man does not show evidence of having recently incurred a small splash burn on his hand or forearm.

3. *Severity of Accidents*

Death or total disability is usually associated with bad falls from elevation. Consequently, falls and back problems usually related to strain are the two problem areas that result in the large claim expenditures associated with total or partial disability cases. These costs cause roofing to have one of the highest workmen's compensation insurance rates, now approximating $15 per $100 of payroll.

Preliminary Information From Test Battery Interviews

The following information is based on reading approximately 30 interviews. No effort has been made to distinguish between workers in the high and low accident categories. The following information is not to be regarded as conclusive but is indicative of some themes that will be pursued more carefully in the analysis of the interviews.

Interview Theme I

The roofers cite almost unanimously that excessive work pace is the most important cause of roofing accidents. The roofers feel that accidents occur when workers are being "pushed too hard."

Comment:

The examples and anecdotes describing how excessive pace creates accidents are often related to apartment building construction. Smaller low-rise apartment construction work has a reputation with insurance underwriters as being a higher risk type of construction than either residential or commercial buildings. The competition is very fierce among small contractors for this kind of work, and large construction companies are usually not involved in this kind of roofing work. The complaint about work pace is also consistent with our interviews with numerous roofing contractors and insurance personnel who indicate that smaller non-union roofing shops are greater risks for industrial accidents.

Since a large number of our initial interviews were with union workers, it will be interesting to see if this theme is also as strong with non-union workers. It would be interesting to

verify these observations on work pace by a carefully constructed field survey utilizing work sampling procedures.

The safety literature we have examined does not really offer very many conclusions in regard to the relationship between work pace and accidents. Most researchers who have briefly explored the question have noted that accident rates are roughly proportional to work pace or productivity. However, Hale, in a study of errors, found that the relationship between work done and errors was roughly proportional except at the two extremes of workload. Some of the descriptions by the roofers describing excessive work pace include such examples as running across a roof with two buckets of hot asphalt, or running backwards pulling a felt machine.

Ironically, the work crews that exhibit this frantic work behavior very likely don't get much more work done in the long run than an experienced crew that exhibits a steady coordinated work pace. Many experienced roofers have made this point which is consistent with our own industrial engineering observations. Frantic work behavior rarely provides that much of a gain in overall productivity. There are several reasons for this:

a. Few men can maintain such a frantic pace for prolonged periods without getting excessively fatigued:
b. Productivity of the roofing crew depends as much on pace as it does on coordination and it is very difficult to achieve a sustained coordinated fast pace in a crew.

The problem of frantic pace in some roofing crews is made much more serious by the fact that these crews are often composed of young inexperienced workers. A number of older roofers have indicated that they used to work for small marginal contractors because they were "too inexperienced and too young to know better." If you couple the prevalence of high accident rates with young workers, with job inexperience, and with high pressure or excessively paced crews; then you have a tragic compounding of high accident causal factors.

Interview Theme II

The foreman is seen as a key to safety on the job. Question 47 of the interview asks the worker to indicate whom he thinks has a primary responsibility for safety on the job. The choices are (1) the roofer himself, (2) the foreman, (3) the company, (4) the union, and (5) other (with the roofer to supply the name of the other factor). The response so far to this question has been that the roofers, by a ratio of over two to one, indicated that the foreman has more primary responsibility for safety on the job than

the roofer himself. This almost universal theme is expressed in such comments as: "The job is as safe as the foreman allows it to be." Or "The foreman orders the men to perform unsafe acts, and the men fear for their jobs if they refuse."

Comment:

We think this is a very significant finding and believe that the foreman has a much greater effect on safety in construction work than in most other occupations. Some of the reasons why the supervision is more critical in regard to safety in a construction job are as follows:

- Every construction job is different.
- The work crews tend to vary considerably from job to job and even from day to day.
- Consequently, the foreman is constantly giving directions and making decisions on what is to be done next.
- The foreman tends to be the permanent employee of the contractor, and the worker is a more temporary employee, and he may have come that morning from the union hall. Thus, the foreman exercises more direct power over the worker than in a factory situation.
- In roofing, the foreman is a key member of the work crew and he sets the work pace.

For all these reasons the foreman is obviously an important element in obtaining safety on a construction job. We feel that this area needs to be explored and researched in regard to safety of construction occupations. There is a possibility that certification of foremen might be effective in reducing industrial accidents. This has long been the practice in the hazardous occupation of mining.

Interview Theme III

The men indicate that they almost always learned safe practices and habits from older, more experienced workers or from their own trial and error experience. The roofers do not view the apprenticeship program or other company sponsored programs as effective sources for learning safe practices. The workers often comment that the apprenticeship program "is nice" but does not have a lot to do with "how to actually learn to do roofing work." Other workers have been noted to say that "the apprenticeship program is almost worthless" or "I never learned much on the apprenticeship program."

Comment:

Many of these comments may be reactions from workers who did not participate in the apprenticeship program. The ap-

prenticeship or union or contractor association representatives indicate that the program is very successful, especially in comparison to the days prior to the California apprenticeship legislation. They cite as evidence of success the number of apprentices who remain in the trade and who become foremen or organizers of contracting firms. They believe that the apprenticeship program provides a man with the necessary technical knowledge to become a good roofer, hence, as they see it, a "safer" worker. The California roofing apprenticeship program has been widely copied by other states. The program obviously has some outstanding technical features.

However, both contractor and union leaders admit that there are some serious safety problems associated with apprentices. Probably the most important safety problem is that apprentices go to work on a roofing job without any orientation except that provided by their roofing foreman and prior to their first apprenticeship class. Thus, no opportunity is provided to develop much knowledge or skill level prior to being exposed to hazardous situations. The results are apparently disastrous. An influential union vice president and a number of business representatives have indicated that the apprentice is a man most likely to have an accident on the job, especially the first few days and weeks. (Our accident data indicate apprenticeship accident rates are about three times greater than those of journeymen.)

The instructors in the apprenticeship program are very sensitive to this criticism about safety training and they have worked very hard in the last few years to try to provide more safety emphasis to the apprenticeship curriculum. For example, in the first few months they stress safety rules and the reasoning behind these safety rules. They have also prepared quite an extensive battery of slides and movies of actual hazardous situations on roofing sites. They use these to impress upon the new workers the hazards of the job and the safe practices that are necessary to cope with these hazards.

A typical problem associated with apprentices is that in too many companies, the apprentices are given the "dirty work." Thus, all too often the apprentices, without any previous experience, are being constantly harrassed to "hurry up" as they carry buckets of hot tar back and forth between the supply point and the point of application. All too often they are the ones who are climbing up and down the ladders. Often, they must lift and carry heavy material without knowledge of the proper way to lift, handle material, or climb a ladder. Couple this lack of knowledge with a natural anxiety of a new job, working from an elevation and being assigned tasks with more

exposure to falls and burns than other workers in the crew. The result is a very high accident rate for apprentices.

As could be expected, the turnover of apprentices is very high. The turnover is reportedly in excess of 50% in the first three months of the three-year apprenticeship program. A fear of heights is sufficient to discourage many men during the first hour on the job. Unless apprentices have been given training and practice on how to properly lift and handle roofing material, they no doubt suffer excessive fatigue their first few days on the job, especially if they are not in good physical condition. In addition, they are under considerable work pace pressure to keep materials supplied to the work crew, and some suffer a considerable amount of verbal abuse from their foreman to maintain the pace. All these factors create considerable physical and psychological stress on the new worker.

Interview Theme IV

The roofers indicate that roofing equipment and tools usually are poorly maintained by the companies. Such comments as the following are very typical: "We have a dozen kettles, and not one of them has a temperature gauge that works." "You should see the stuff we have to work with."

Comment:

Our movies and slides show many actual examples of this particular complaint voiced by the roofers in the interviews. There is absolutely no doubt in many cases that poor housekeeping constitutes a serious safety hazard at many work sites. Sometimes the hazards are so obvious that it is hard to imagine that the worker would tolerate working with such environmental hazards. Such examples include ladders with broken rungs or kettles heavily coated with asphalt, and heavily frayed ropes on lift pulleys.

Interview Theme V

The roofers indicate a general lack of self-esteem or pride in their craft or trade. Some of the comments from the roofers are "this is a lost trade . . . nobody cares," or "everyone hates to see the roofers, on a job . . . dirty . . . smelly . . ." or "roofers usually look like hell. They wear old dirty and smelly clothing." This theme of low self esteem and lack of pride in the craft is further augmented by two other questions in the interview. The men, for example, consistently reject recommending roofing as a career for their sons. The men also give a consistently negative response to the question, "if you had to do it all over again, would you be a roofer?"

Comment:

An attempt to test a potential link between lack of self-esteem to accident occurrence is part of the present study. It does seem that this lack of self-esteem is consistent with the apparent disregard of personal appearance and the inherent lack of concern about poor housekeeping and poor maintenance of equipment at so many roofing sites. We have noticed that many companies that have excellent safety records also stress good housekeeping, and a number of general foremen from these companies stress "the importance of quality work" or "need to remind the workers of the importance of doing a good job," or "I constantly harp at the roofers on quality and safety."

Another closely related theme that we are investigating is based on the comments of some of the workers with outstanding safety records who have made comments such as "a good man has to be able to tell a foreman to go to h--- when the foreman orders him to do something unsafe," or comments such as "I have to tell many foremen to go to h--- when they tell me something stupid."

Interview Theme VI

Most of the workers feel a need for an independent safety inspector or someone with authority to shut down jobs for violation of safety regulations.

Comment:

It has become a union bargaining demand to have a safety inspector who is an employee of the union and whose wages are paid by a surcharge on all wages paid in the same manner that a check-off system for union dues is collected. Surprisingly, many contractors do not seem to reject the idea of a joint safety inspector but rather reject the idea that this inspector would be an employee of the union and not a joint employee of the contractors. A reduction in insurance premiums provides strong economic incentive for the idea.

We have been actively discussing this idea with both union leaders and contractors in terms of trying out the idea on a demonstration basis. A very carefully controlled experiment would need to be designed to realistically separate the true effects of such an inspector versus other factors. Many contractors frankly admit that they cannot possibly have first-hand knowledge of what is going on at the site and thereby they must rely on the quality of their foreman. Many contractors admit that they hear of many incidents on the job which they personally claim that they would never allow if they had been able

to catch the foreman or the men involved in the act first-hand. Unfortunately, too many contractors have the attitude that "if the men are so stupid to do things unsafely, then the men have to bear the consequences." Or "I can't spend all my time running around to all the sites, straightening out all the problems and stay in business."

Interview Theme VII

Roofers express a general disregard for the importance of safety clothing and equipment.

Comment:

Both contractors and workers tend to downgrade or even sneer at obvious safety motivated procedures or devices. Most contractors and the men tend to down-grade the effectiveness of safety railings or clothing or guarding openings on the roof. One almost never sees men wearing gloves on the site unless it is a cold day. Moreover, men without shirts of any kind are typical on a hot day, and if shirts are worn, they will almost never wear a long-sleeve shirt. This is true especially of the young men. Also, we have yet to see any helmets worn on the work sites even though both the helmets and the shirts potentially could significantly reduce the fatigue related to heat stress on the roof.

Preliminary Safety Research Needs

1. *Need for Standard Work Practices Document for Roofing*

It is difficult to separate equipment, clothing and procedural safety standards because they interact. Even relatively safe equipment can be used improperly and unsafely. Consequently, a standard work practices document for roofing should be referenced and integrated with equipment and clothing standards.

Many current state and federal safety regulations relating to roofing work involve obviously sensible and needed rules - tying down ladders, not carrying material up a ladder, weight limits on material, etc.

The recently published procedural standards relating to mandatory perimeter guarding of even one-story buildings are very much resented by contractors. Many knowledgeable roofers claim that the proposed guard rail rules could actually increase worker exposure to falls at the edge of the roof. The lack of detailed accident data and lack of knowledge concerning existing customary work practices prevent a meaningful discussion about the various safety claims and counterclaims of proposed procedural regulations and current practice. The effectiveness of many procedures is also related to specific kinds of roof construction.

Documenting and analyzing current procedures relative to types of roof, especially in regard to hazardous exposure, would help resolve much of the current controversy. It is possible that some advantageous safe and productive procedures are being overlooked or that certain procedures are not being sufficiently emphasized. Obviously, proposed procedures that both increase productivity and safety are the ones that are most likely to gain quick acceptance.

There are a very large number of roofing contractors in the United States, and many safety oriented contractors have already found productive and safe procedures that reduce their accidents. A compilation of the best and safest procedures already in use in the U.S.A. would provide a valuable service to the industry without precluding synthesis of even better procedures and rules.

2. *Need for Roofing Equipment Safety Standards*

The lack of detailed accident data precludes estimating the number of accidents associated with various pieces of equipment or various work procedures. However, our direct observation of equipment in use, and our interviews with men who have experienced accidents or who have seen accidents, suggest that roofing equipment changes could significantly lessen the number of accidents. "Engineering out" safety hazards is often the cheapest and most reliable means of eliminating certain types of accidents. It is much easier and cheaper to spot non-standard equipment than to catch crews following non-standard procedures or verify that each worker possesses adequate skills and knowledge of safe procedures.

The first problem of developing engineering safety standards is to assess the need, i.e. the number of accidents associated with various pieces of equipment and procedures now. Then potential engineering remedial concepts can be evaluated for cost effectiveness. The most promising measures then need to be built and demonstrated to prove their effectiveness.

The following partial list, not necessarily in order of importance, will indicate some obvious safety issues about roofing equipment:

a. *Felt Machines.* This machine which resembles the appearance of a manual lawn mower or common yard fertilizer spreader is the source of hotly contested safety issues. A box on the machine contains hot asphalt, and as the machine is pulled or pushed, it spreads hot asphalt on the roof while a felt roll, also mounted on the machine, is unrolled over the freshly applied asphalt. The machine replaces the old fashioned method of mopping on large roofs. Of course, even on large roofs, openings or other rooftop geometric irregularities require mopping and direct manual application of roofing paper. The safety questions related to this machine are as follows:

- Do more accidents occur with backward pulling machines per man-hour of operation than with forward pushing machines or forward moving powered felt machines?
- Are backward pulling machines associated with higher productivity as claimed, and if so, what are the relative accident rates per "square" (100 square feet) laid?
- Should there be a limit on the amount of force required to push or pull the machine? A man can obviously pull a heavier load than he can push with this machine. Does an excessive pull on the machine contribute to back sprain and backward falls, either on the roof or through skylights or over the edge of roofs?

b. *Roofing Kettles.* Asphalt is delivered to a roofing site in solid chunks and is melted in the kettle to an ideal temperature of 450°F and then usually pumped to the roof. The kettle is usually mounted on a two-wheel trailer chassis and towed by the contractor's truck to the site. If the hot asphalt is pumped very far from the kettle, it cools rapidly. Consequently, there is a tendency for crews to heat the asphalt to a higher temperature in the kettle to compensate for the heat loss. This is a very undesirable practice, because the asphalt begins to vaporize intensely above 450°F and the vapor can reach a flash point, depending on the material and conditions, at about 550°F.

The vapors of the hot asphalt at any temperature are unpleasant and polluting. Contractors in Southern California are receiving intense air pollution regulatory pressure. This pressure has caused the roofing contractors to band together and make contributions toward development of a non-polluting kettle. For the first time, contractors and union personnel are working together at a national level on the kettle air pollution problem.

A non-polluting kettle design may eliminate some of the safety problems, but at the present a number of equipment safety design problems need to be explored:

- Can the line heat loss be reduced and thus reduce the tendency to overheat asphalt in the kettle?
- Can an inherently splashless asphalt loading method be devised? Now kettle tenders are asked to chop up the 100-lb. asphalt chunks with an ax and gently lower the small chunks directly to the boiling surface of asphalt so that the final splash from the solid material will be minimized.

Of course, the shortcut method is to drop the entire chunk into the kettle. Are loading small asphalt pellets a practical alternative?

- Should kettles be constantly tended for safety reasons as is now required?

  Small non-union roofing crews, doing recover work in residential areas, will sometimes leave kettles unattended. Kettle tenders on small or medium jobs are not fully utilized productively by kettle tending, thus the temptation to utilize the kettle tender on other tasks is always present. What are the possibilities of automatic loading devices and automatic controls to regulate temperature as well as automatic warning devices for problems?
- Should kettles be required to be cleaned and painted with fireproof paint?

c. *Roofing Pails.* Should there, or could there, be a special design of roofing pails used to carry hot asphalt that would be inherently splashless?

d. *Rooftop Material Handling Tractors.* More and more roofing contractors are using small utility tractors to pull trailers of roofing material around on large commercial roofing jobs. The tractors were originally designed for home gardening, lawn care, or snow removal, and they are powerful and convenient. However, many men feel these machines are dangerous on a roof. Some machines are capable of speeds up to 30 mph.
   - Do these tractors create a hazard on the roof?
   - Should automatic or panic shut-off switches, speed governors, back-up or forward operation warning devices, etc., be required?

The foregoing list of roofing equipment and related safety issues is not meant to be a complete list. These are examples of some of the natural equipment safety questions that have emerged out of our site visits and interviews. We believe that "engineering out" equipment and tool related safety hazards can have a very high cost effectiveness ratio for reducing accidents in the roofing industry.

Equipment investment per man is low and the safer equipment we envision would not likely cost that much more than existing equipment. The problem is that potential equipment safety features need to be explored and evaluated relative to the actual number of accidents prevented or whose severity is reduced.

3. *Need for Detailed Accident Data*

All of the above research needs involve a fundamental need to

develop a detailed accident data base. An analyst needs to "normalize" the raw accident frequencies into accident rates and probability levels associated with each safety issue.

Some examples of data needs include:

a. Accident rates for different parts of the country reflecting different practices and different types of construction.
b. Sheet metal and shingling work needs to be separated from hot composition roofing.
c. Accident rates for differing kinds of roofing (sloped versus flat, hot asphalt versus hot coal tar pitch, gravel top versus cap sheet, etc.)
d. Accident rates for different kinds of buildings: high rise, residential, one or two story commercial office buildings and warehouses, low-rise apartment building construction.
e. Accident rates associated with various kinds of accidents, equipment, procedures, and related safety inspection rules:
   - falls from edge of roof versus through roof openings related to type of activity and equipment such as forward versus backward felt machines, carrying material, presence of guard rails, etc.
   - burns relating to part of body and worker activity and procedures
   - strains related to activities, etc.

FIGURE 1. Worker chopping asphalt.

FIGURE 2. Worker getting ready to "drop" asphalt into a roofing kettle.

f. Accident rates related to contractor: size, foreman experience, work pace or production rates, unique contractor and worker practices or rules (especially for low accident rates).
g. Accident rates related to employee: age, experience, apprentice versus journeyman, training.

All of the above data will tend to substantiate or disprove the competing claims and counterclaims about safety needs in roofing work. These data can best be gathered by surveying existing accident data, conducting field studies and setting up a voluntary detailed accident collection system with a group of contractors. In other words, more precise problem definition and documentation are required to develop the most cost-effective counter-measures.

BEHAVIORAL TOXICOLOGY WORKSHOP DEMONSTRATION

Mercury Exposure Behavioral Test Battery
University of Michigan

Demonstration: Quantitative measures of human tremor, electromyogram, psycho-motor tests, static fatigue, eye-hand coordination.

Equipment: Bioamplifiers, ten pound weight, force transducer to sense movement (tremor), surface electrodes only (electromyogram), panel with buttons, and pencil stylus with a maze of holes.

Degree of Intervention: Non-invasive.

References: Chaffin, Dinman, Miller, Smith, Zontine. *An Evaluation of the Effects of Chronic Mercury Exposure on EMGs and Psychomotor Functions* Final Report on NIOSH contract HSM-099-71-62, University of Michigan, January 73.

Risk to Subject: There is a possibility of electrical charge to the subject from the electrodes in the EMG, but this is minimized by fuses in electrodes. These fuses break the flow below perception level. There is a possibility of fatigue from holding the ten pound weight for five minutes in the static fatigue test.

Demonstration Team: Don Chaffin, James Miller, James Foulke, Shashikant Kelkar.

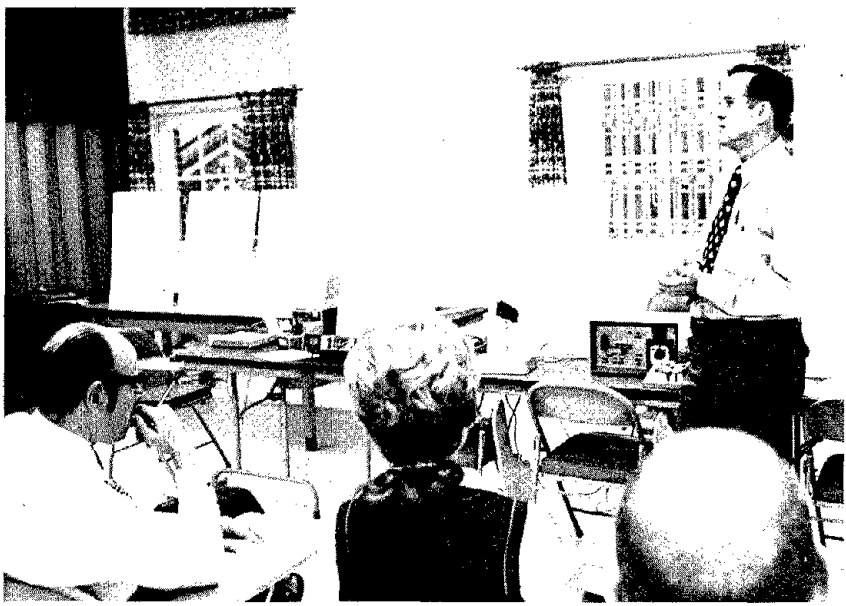

FIGURE 1. Jim Foulkes, University of Michigan, lectures to workshop participants on behavioral performance tests used in NIOSH mercury study.

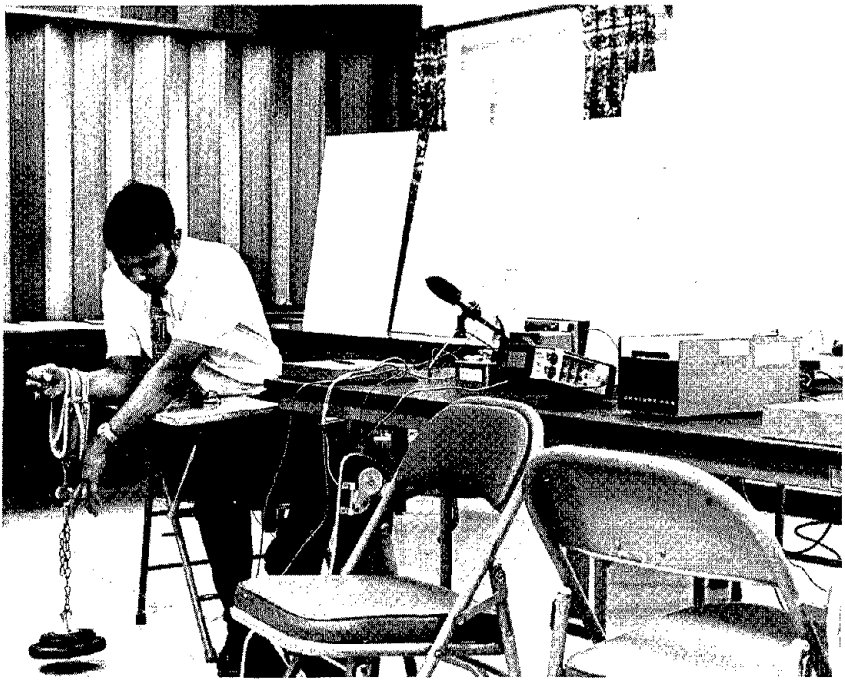

FIGURE 2. Shashikant Kelkar is shown holding 15-pound weight and tremor measuring device used in chlor-alkali study.

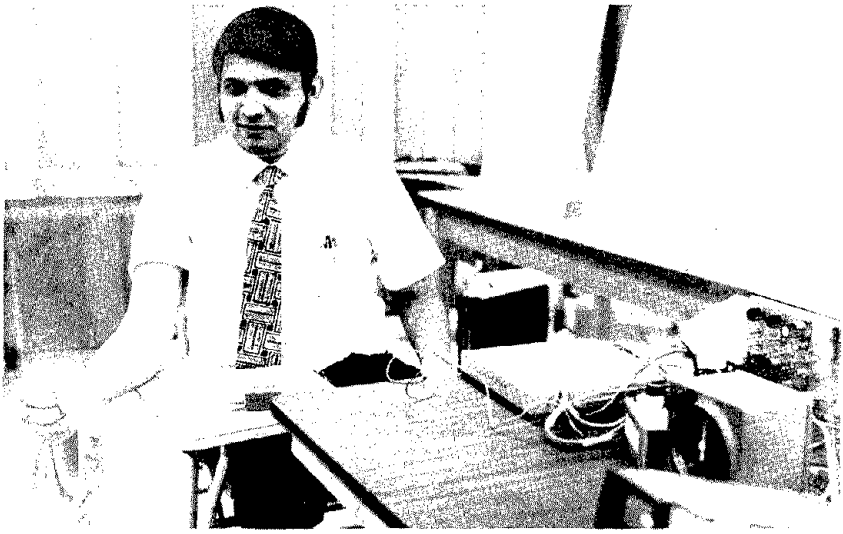

FIGURE 3. Close-up of subject showing EMG surface electrode attached to the skin over the biceps brachii.

BEHAVIORAL TOXICOLOGY WORKSHOP DEMONSTRATION

Lead Exposure Behavioral Test Battery
University of Louisville

Demonstration: A battery of twelve performance tasks involving arithmetic computations, reaction time to a warning light, target identification, memory tasks, and screening for hearing defects.

Equipment: An operator panel, twenty by fourteen inches in size.

Degree of Intervention: Completely non-invasive.

References: (1) Morgan and Alluisi "Synthetic Work: Methodology for Assessment of Human Performance," *Perceptual and Motor Skills* (1972) 35, pg. 835-845 and (2) Jennings, Chile, and West "Methodology in the Measurement of Complex Human Performance: Two Dimensional Compensatory Tracking" *FAA Office of Aviation Medicine* Report (1972) AM-71-21.

Risk to Subject: None.

Demonstration Team: Ben B. Morgan, John Repko, Mike Lyddan, Karl Rothrock, Don Corson.

FIGURE 1. Dr. John Repko, University of Louisville, lectures to participants on multiple-behavioral testing used in NIOSH lead study.

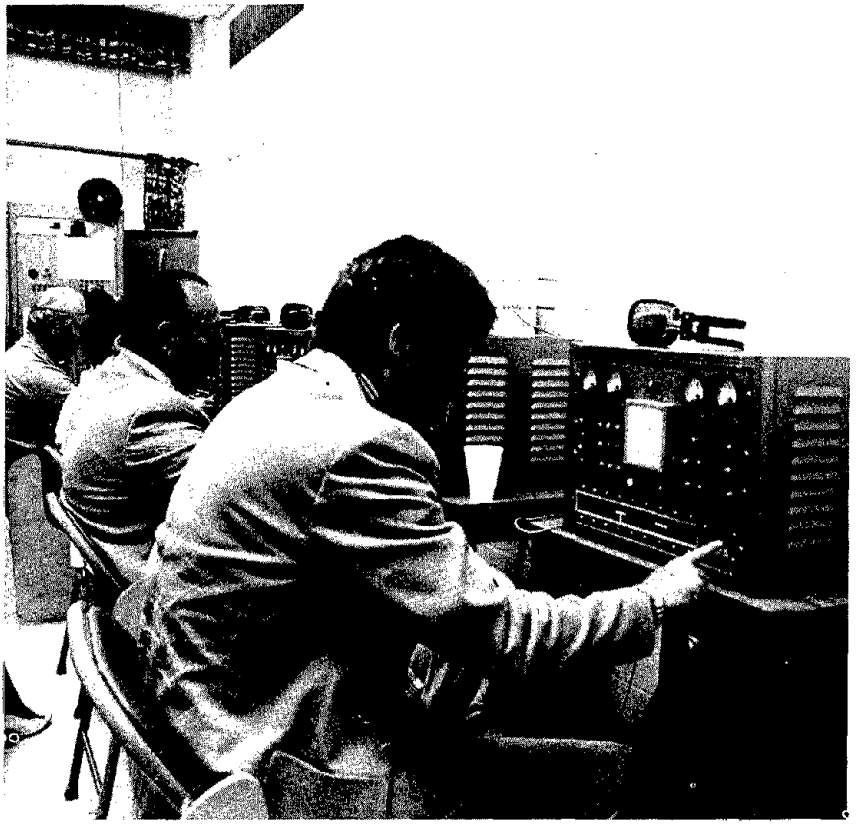

FIGURE 2. Several participants are performing at behavioral test panels.

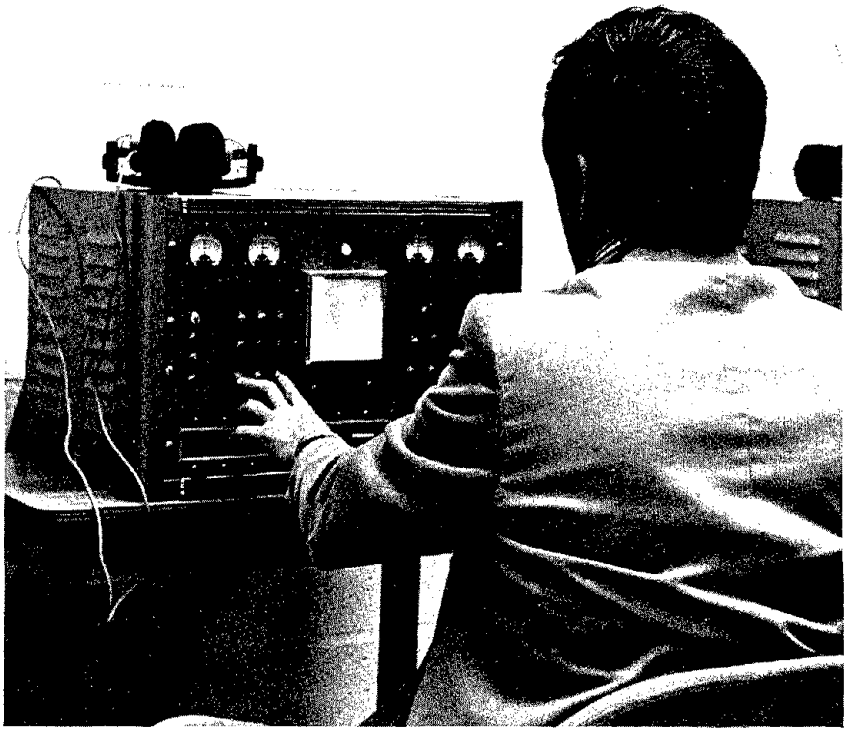

FIGURE 3. Dr. George Winneke, Dusseldorf, observes "metric histoforms" as part of a target identification task.

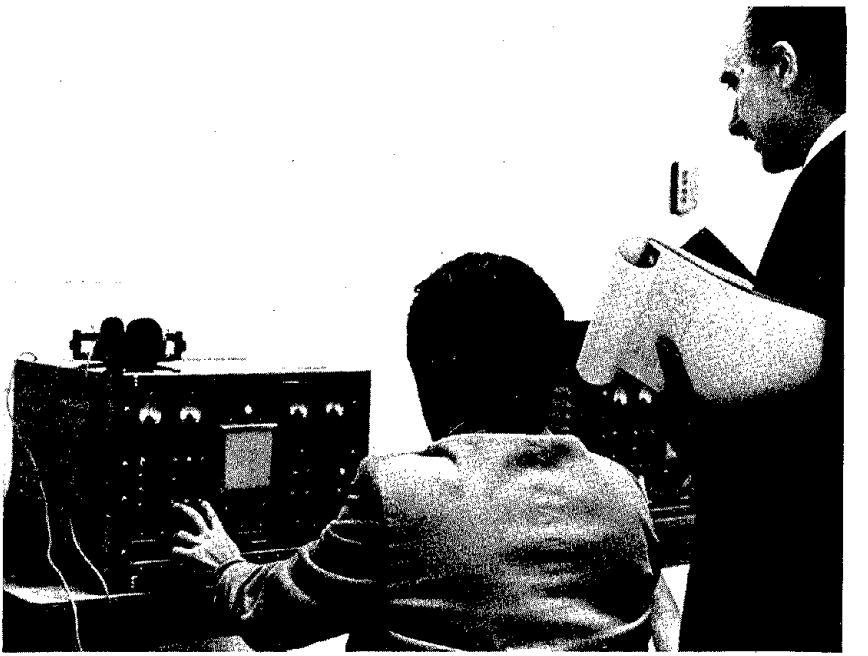

FIGURE 4. Dr. M. Salvini, Pavia, Italy, observes performance of Dr. G. Winneke during a watch-keeping (vigilance) task.

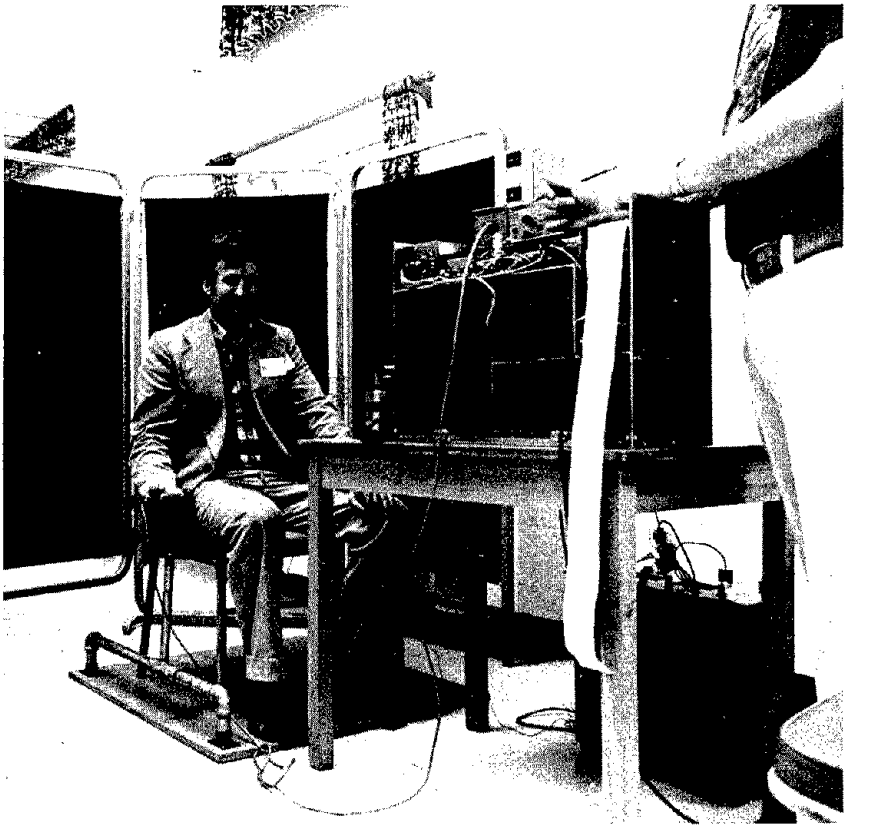

FIGURE 5. Dr. G. Winneke performs on task for assessing muscular strength, endurance and recovery.

BEHAVIORAL TOXICOLOGY WORKSHOP DEMONSTRATION

Pesticide Exposure Behavioral Test Battery
University of Iowa

Demonstration: Medical diagnosis techniques using electromyograms (EMG) and nerve conduction velocities. There will also be movies showing reaction time testing and tape recordings of verbal standard psychometric tests.

Equipment: Electrodes, a Grass S-8C stimulator, and an oscilloscope.

Degree of Intervention: There will be electrical stimulation, but to members of the team, not to participants.

Reference: J. Goodgold, M.D. and A. Eberstien, Ph.D. *Electrodiagnosis of Neuromuscular Disease* (1972) Williams and Williams Co., Boston.

Risk to Subject: None, except in the case of equipment malfunction. If the equipment shorts out there is the possibility of skin burns under electrodes.

Demonstration Team: David L. Mick, Harvey S. Levin, Robert L. Rodnitzky, Jun Kimura.

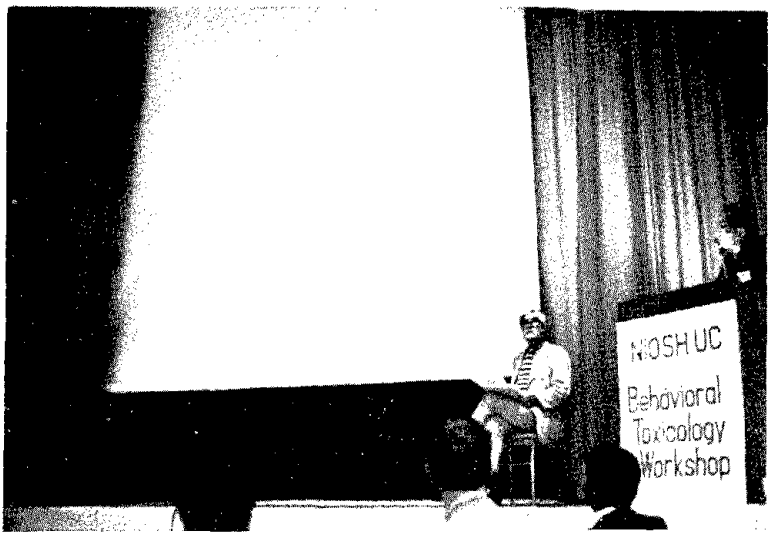

FIGURE 1. Dr. Jun Kimura describes to participants electromyographic techniques used in the Iowa study of pesticide exposed workers.

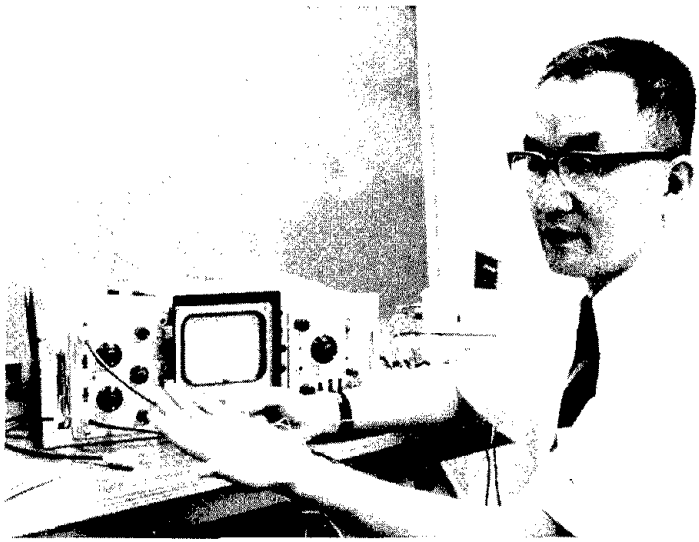

FIGURE 2. Dr. J. Kimura demonstrates to workshop participants nerve conduction velocity measurement and muscle action potential recording techniques.

BEHAVIORAL TOXICOLOGY WORKSHOP DEMONSTRATION

Alcohol Behavioral Test Battery
U.S. Department of Transportation

Demonstration: Breath testers and psychomotor performance testers.

Equipment: Compensatory tracking testers, "quickie" reaction time testers, and alcohol sensing devices.

Degree of Intervention: Listerine can be used in the breath tester. Also breath jars (containing alcohol) can be used. The subject blows into the breath jar which is attached to the breath analyzer. The subject does not come into contact with the alcohol. Other testing is completely non-invasive.

References: (1) Dunley and Associates, Inc., Darien Conn. *Basic Training Program for Breath Examiner Specialists* May 1971 prepared for U.S. Department of Transportation, National Highway Traffic Safety Administration under contract FH-11-7540 prepared by Dunely and Associates, Inc., Darien, Conn., (2) Sussman and Abernathy "Laboratory Evaluation of Alcohol Safety Interlock Systems" Vol. 1 Summary Report.

Risk to Subject: The breath tester involves exhaling with force. This could have adverse effects on individuals with *severe* coronary conditions or *severe* emphysema.

Demonstration Team: Charles Abernathy.

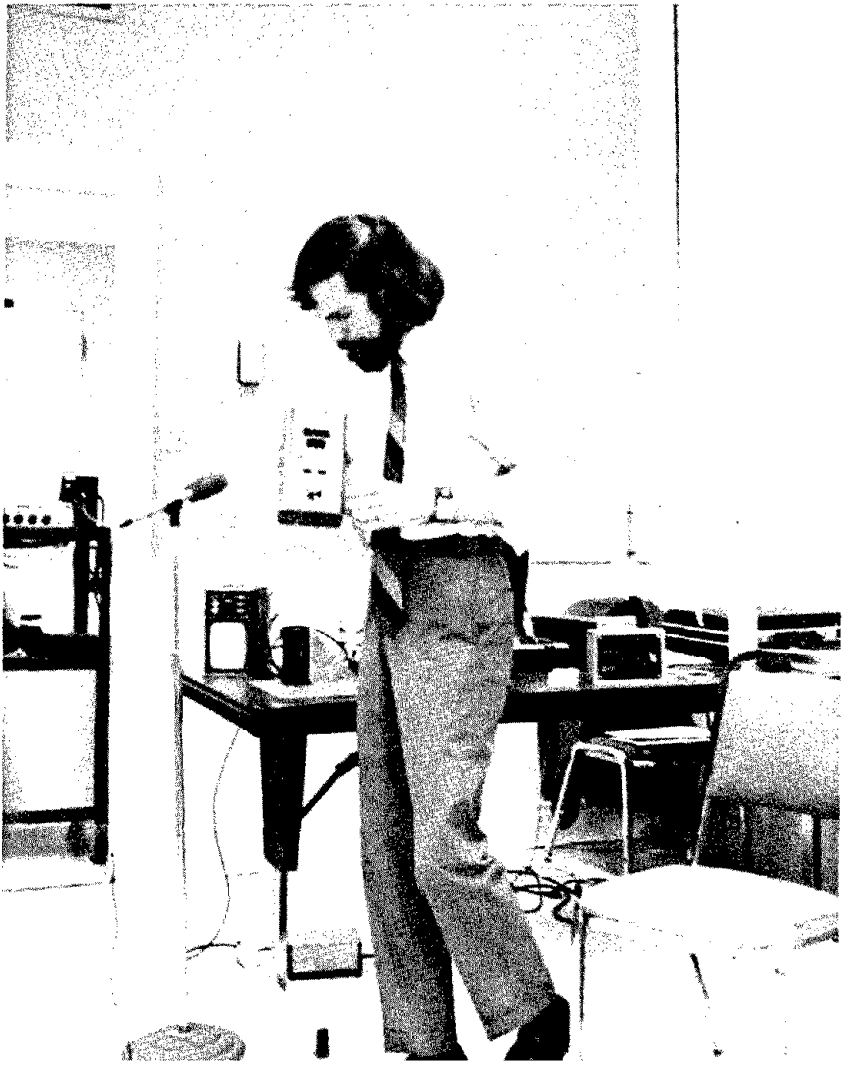

FIGURE 1. Dr. Charles Abernathy demonstrates use of alcohol breath analysis measurement techniques.

BEHAVIORAL TOXICOLOGY WORKSHOP DEMONSTRATION

Carbon Monoxide Exposure Behavioral Test Battery
National Institute for Occupational Safety and Health

Demonstration: Behavioral performance tests utilized in the NIOSH carbon monoxide study. The tests consist of eight-choice reaction time, time estimation, complex arithmetic, time-sharing tests, visual discrimination, eye-hand coordination and critical flicker frequency. (These procedures and tests have been reviewed and approved by the NIOSH Human Subjects Review Board.)

Equipment: Reaction timer, a tape recorder, computer, eye-hand coordination tester, headphones and a signal generator.

Degree of Intervention: Completely non-invasive.

References: Annals of the New York Academy of Sciences Vol. 174, Article 1, pgs. 1-430 with special regard to the chapters by R. R. Beard, A. M. Ray, P. Mikulka, and T. G. Hanks.

Risk to Subject: None.

Demonstration Team: Barry L. Johnson, Robert Struble, H. Harvey Cohen, W. Kent Anger, Bruce Gutnik, James Setzer.

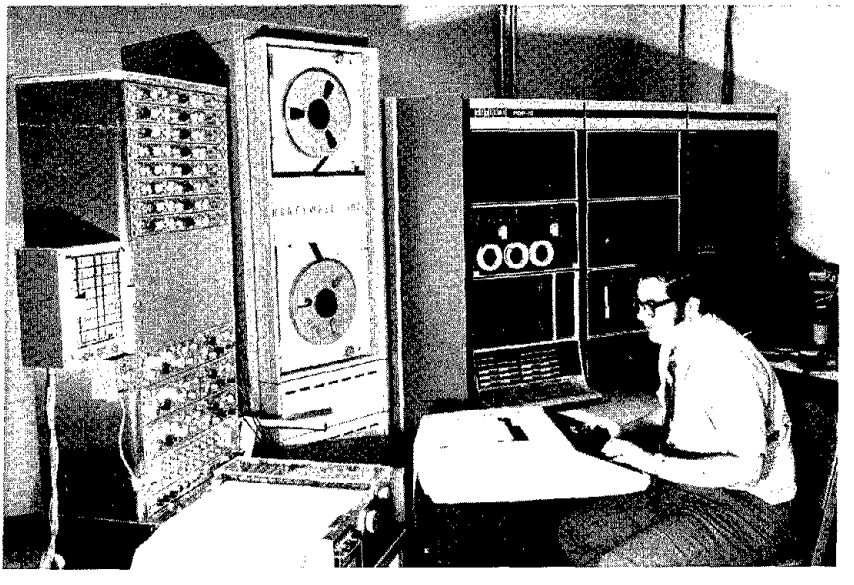

FIGURE 1. Dr. Barry Johnson demonstrates data analysis techniques used in a recent NIOSH carbon monoxide study.

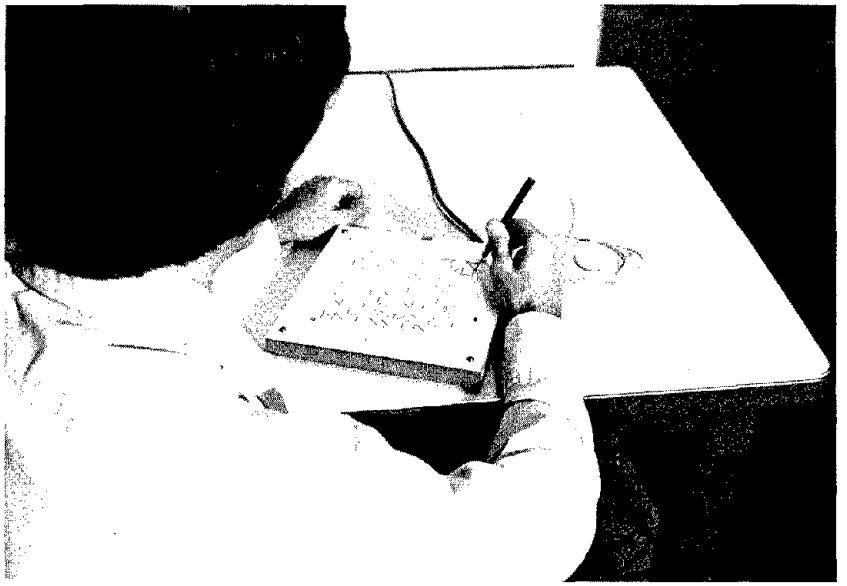

FIGURE 2. Robert Struble practices eye-hand coordination using a NIOSH modified, University of Michigan tester.

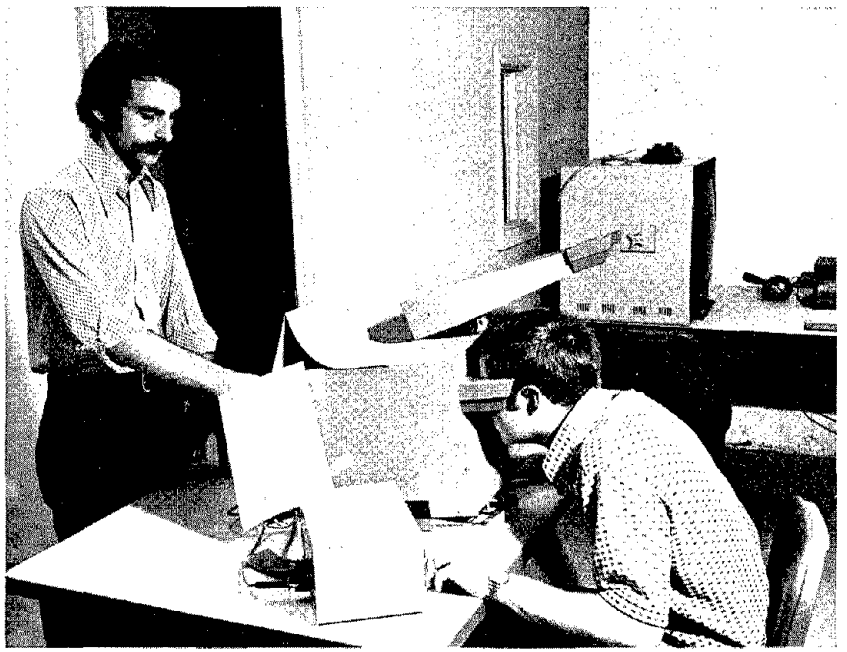

FIGURE 3. Dr. H. Harvey Cohen administers Bender Visual Perception test to W. Kent Anger during workshop demonstration.

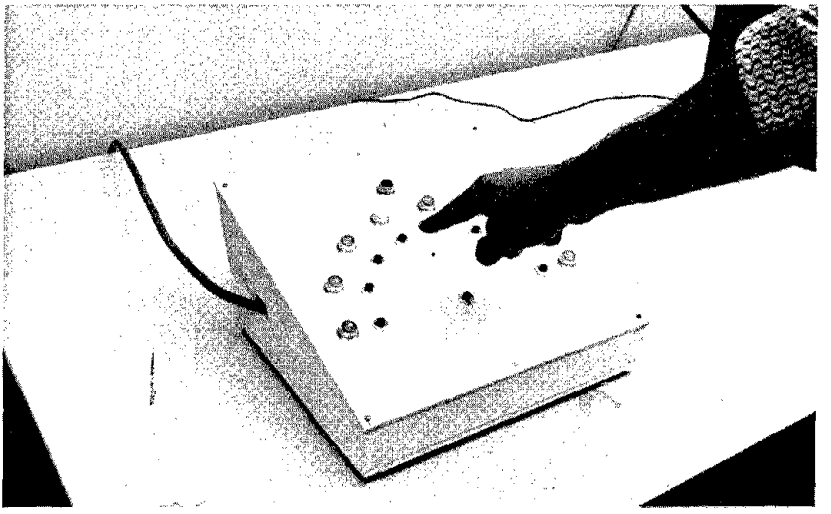

FIGURE 4. Subject responding on an 8-choice reaction time task.

FIGURE 5. Robert Struble responds by depressing hand-held switches during a visual binary choice task and at the same time maintains an interval production task with his foot.

BEHAVIORAL TOXICOLOGY WORKSHOP DEMONSTRATION

Visual Function Testing
Matthew Newman, M.D.

Demonstration: Visual tests of perimetry, dark-adaptation, and color vision.

Equipment: Commercially available optical instruments for measuring perimetry (perimeter), dark-adaptation, and color vision.

Degree of Intervention: Techniques are completely non-invasive visual tests.

References: (1) Harrison, T. R. (Ed.) Principles of Internal Medicine, 1966: McGraw-Hill, N.Y. (2) Graham, C.H. (Ed). Vision and Visual Perception, 1965: Wiley, N.Y.

Risk to Subject: None.

Demonstration Team: Matthew Newman.

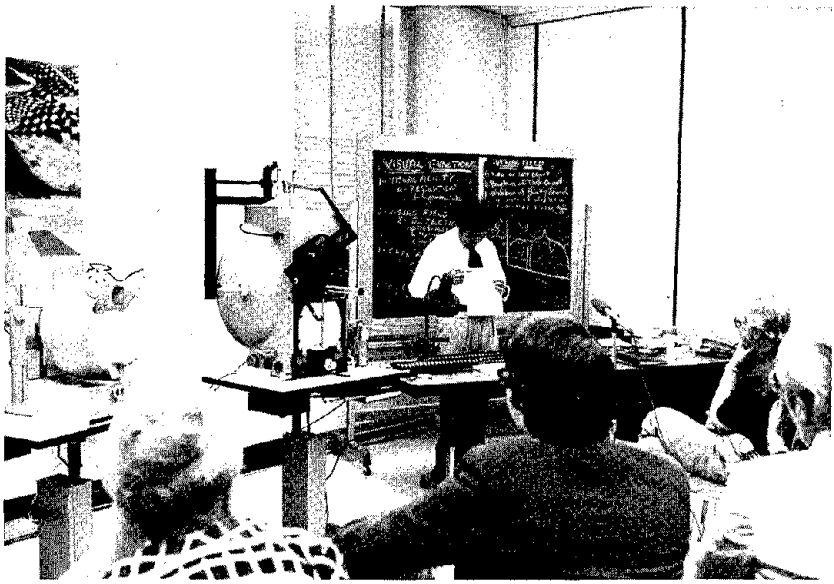

FIGURE 1. Dr. Matthew Newman, an opthalmologist, lectures to workshop participants on use of visual function tests in behavioral toxicology research.

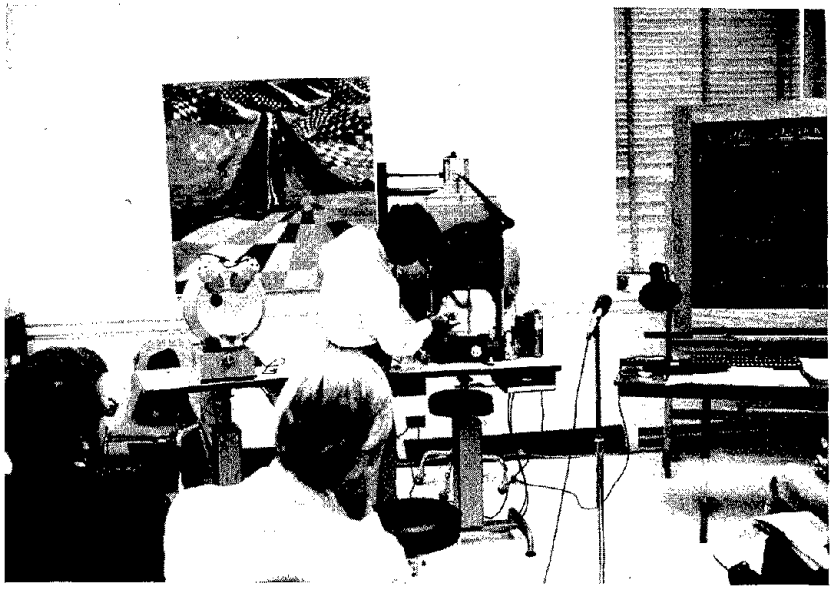

FIGURE 2. Dr. Newman shows participants how subjects' performance is scored during use of the Goldman perimeter for visual field analysis.

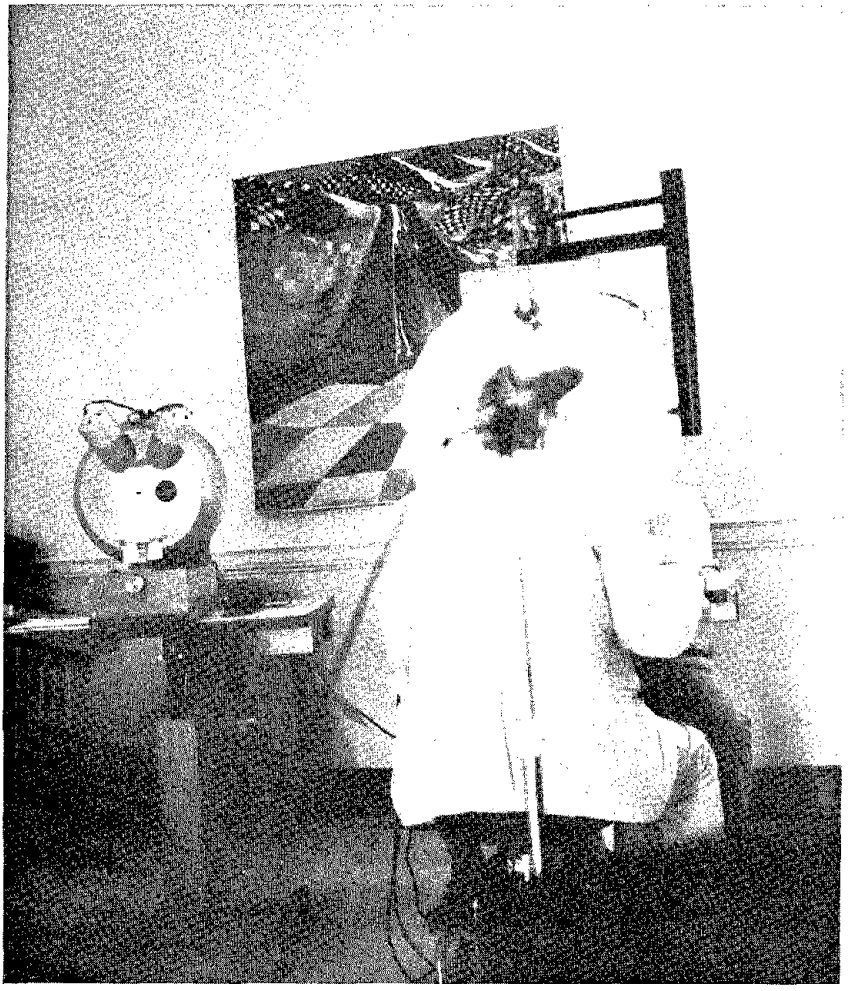

FIGURE 3. Subject is being tested with the Goldman perimeter.

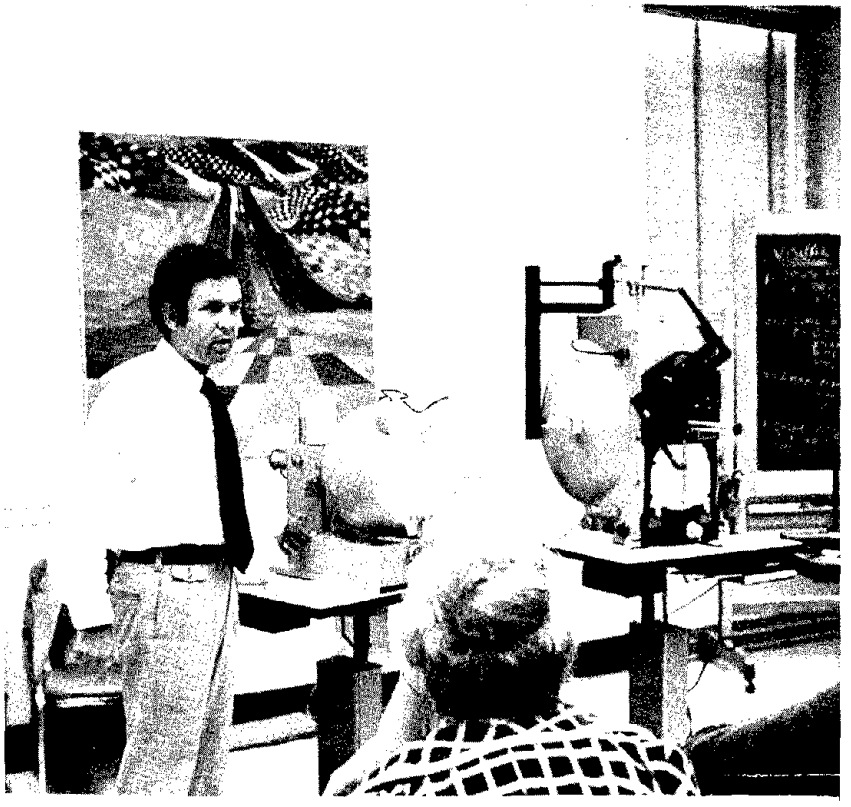

FIGURE 4. Usefulness of dark adaptation measures in behavioral toxicology research is discussed by Dr. Newman.

BEHAVIORAL TOXICOLOGY WORKSHOP DEMONSTRATION

Complex Coordination and Short Term Memory
NASA/J. W. Microelectronics/Georgia Institute of Technology

Demonstration: Psychomotor tests of complex coordination and short-term memory.

Equipment: Custom made instruments each with separate control console and display/response panel.

Degree of Intervention: Techniques are completely non-invasive psychomotor tests.

References: (1) Scow, J. O'Connor, W. F., and Pendergrass, G. E. Complex coordination performance and time of useful consciousness. Paper presented at Aerospace Medical Assoc. Meeting, Miami, Florida, April 1966. (2) Maraman, G. V. "The effects of alcohol on three levels of complex human behavior." Thesis submitted in partial fulfillment of the requirements for the Degree of Doctor of Philosophy at the Medical College of Virginia, Health Sciences Center, Virginia Commonwealth University, 1970.

Tasks have been used by a number of human performance laboratories, including NASA (Langley Research Center), Department of Transportation, Duke University, NIOSH.

Risk to Subject: None.

Demonstration Team: John Samos (NASA), Randy Chambers (Georgia Institute of Technology), Jeff Wright (J. W. Microelectronics).

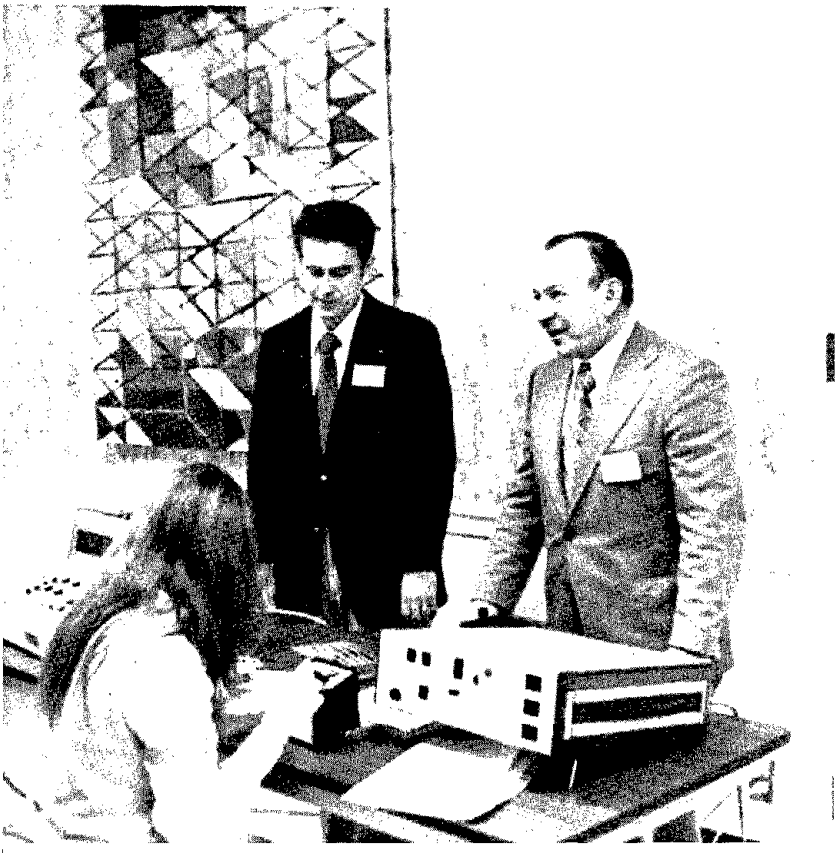

FIGURE 1. Mr. John Samos, at right (NASA), and Dr. Randy Chambers, at left (Georgia Institute of Technology), discuss short-term memory device with a participant.

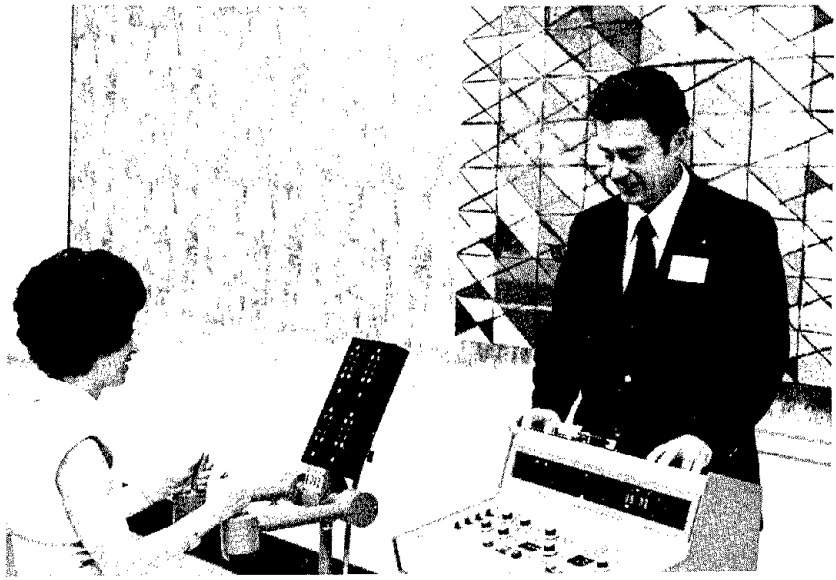

FIGURE 2. Dr. Randy Chambers observes performance of participant on "EPIC", a multiple-limb coordination test device.

BEHAVIORAL TOXICOLOGY WORKSHOP DEMONSTRATION

Space Motion Analyzer
General Electric Technology Center

Demonstration: Spatial motion monitoring including the recording of data and essentially real time evaluation of hand tremor data.

The data are obtained from the subject by having the person grasp a small manipulandum and describe a motion in space or hold the manipulandum as stable as possible for thirty seconds.

Equipment: An electro-mechanical device to measure and record both voluntary and involuntary movements (hand-arm tremor). The analysis consists of spectro-analysis or a computer analysis of gross movements.

Degree of Intervention: Non-invasive.

References: There is no open literature available; the demonstration is from research done for NASA.

Risk to Subject: None.

Demonstration Team: Stacey Hunt.

FIGURE 1. Dr. Stacey Hunt explains the use and application of the space motion analyzer.

BEHAVIORAL TOXICOLOGY WORKSHOP DEMONSTRATION

Tremor Analysis
U.S. Navy Medical Research Center

Demonstration: Measurement of finger tremor.

Equipment: A tabletop tremor device with a lever that measures finger tremor, oscilloscope and paper chart recorder.

Degree of Intervention: Non-invasive.

References: (1) Bachrach, Thorne, and Conda "Measurements of Tremor in the Makai Range - 520 feet Saturation Dive" Aerospace Medicine, 1971, Vol. 42, pg. 856-860. (2) Bachrach, Bennett "Tremor in Diving" Aerospace Medicine - in press.

Risk to Subject: None.

Demonstration Team: George Tresansky, Arthur Findling.

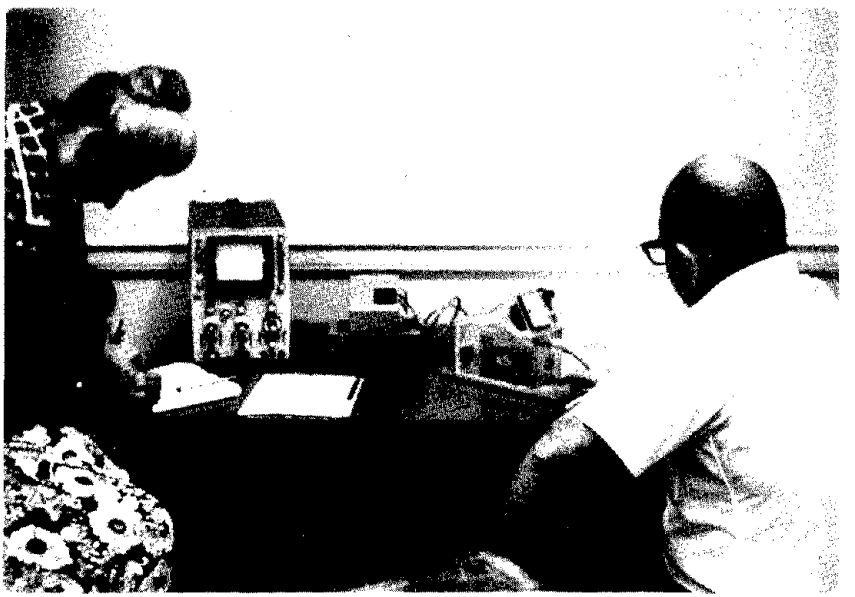

FIGURE 1. Dr. Jack E. Peterson, Medical College of Wisconsin performs on tremor analysis device as Dr. A. Jusic, a neurologist with the Institute for Medical Research and Occupational Health, Yugoslav Academy of Sciences, records performance data.

BEHAVIORAL TOXICOLOGY WORKSHOP DEMONSTRATION

Anesthetics Behavioral Test Battery
Northwestern University Medical School

Demonstration: Tasks used to measure differences in reaction time in cognitive and motor performance.

Equipment: A tachistoscope and audiovisual equipment (a tape recorder, oscilloscope, and polygraph.)

Degree of Intervention: Non-invasive.

References: (1) Salvini, Binaschi, and Riva "Evaluation of Psycho-physiological Functions in Humans Exposed to Trichloroethylene" British Journal of Industrial Medicine (1971) Vol. 28, pg. 293-5, (2) Rolfe "Secondary Task as a Measure of Mental Load" Chapter in Measurement of Man at Work by Singleton, Fox and Whitfield (1970), (3) November 15, 1972 progress report by the demonstrators under contract 8SM99-72-48.

Risk to Subject: None.

Demonstration Team: David Bruce, Mary Jane Bach.

FIGURE 1. Volunteer seated at audiovisual test. The tape recorder is sending auditory signals to the earphones and visual signals to the two channel oscilloscope. Changes in either modality are noted by the subject depressing the appropriate button on the response panel.

BEHAVIORAL TOXICOLOGY OVERVIEW

SESSION VII

Chairman

DR. CHARLES XINTARAS
National Institute for Occupational Safety and Health

EARLY DEVELOPMENT OF BEHAVIORAL TOXICOLOGY IN THE U.S.

Rodney R. Beard, M.D.

Interest in Behavioral Toxicology in the United States began in the latter part of the 1950's, principally because we learned that the Russians were setting more stringent standards than we for air quality in industry. Published comments on these differences such as those of Elkins[1] and of Smyth[2] do not begin to convey the passion of the responses of several American leaders of industrial hygiene and toxicology. There were cries of outrage at the thought that anyone should be so brash as to think they could do better than we. It was learned that the exacting Russian standards, some of them over ten times as strict as ours, were based on what seemed to be peculiar ideas, such as protection from odors — not only noxious odors, but any odors and protection from physiological responses — not only from pathological responses, but from any response at all. Finally, it was said that some of the standards were based on studies done by "Pavlovian methods".

None of us who were concerned about industrial health appeared to be aware that the study of the influence of toxic agents upon the behavior of animals was already well established in the United States. Such work had gone on for years in the pharmacology laboratories. When the first volume of *Annual Reviews of Pharmacology* was published in 1960, it had a long report by Dews and Morse on Behavioral Pharmacology. However, nothing had yet been done with any of the industrial solvents or other industrial materials. Only drugs had been tested by this technique which showed such great promise of useful applications.

The first operant conditioning studies of a toxic material from industry in the U.S. appear to have been started about 1960 at the University of Rochester. Elliott Maynard and Harold Hodge had become interested in behavioral methods. They sent one of their staff, Robert Armstrong, to work with Peter Dews at Harvard. Dews, who had come under the influence of B. F. Skinner and C. B. Forster, had worked out some very satisfactory methods for the study of drugs using pigeons as experimental animals. Armstrong became familiar with these methods and introduced their use at Rochester. In the spring of 1961, Armstrong and others[3] reported to the American Industrial Hygiene Association that they had observed severe disturbances of behavior associated with exposures to mercury vapor. The concentration was high — 17 mg

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per cubic meter, as compared with a Threshold Limit Value of 0.1 mg/m^3 — and the birds developed tremors, but the effects were reversed when the mercury exposure was discontinued, and the conditioned reflex returned.

Behavioral studies were begun about February 1960, by M. E. Goldberg and others in the laboratory of Henry Smyth, Jr., at the Mellon Institute in Pittsburgh. In March 1962, they published a report[4] of the suppression of an aversive conditioned reflex in rats following inhalation of a solvent, monomethyl ether of ethylene glycol at several times its TLV. The dose was insufficient to impair the motor responses of the animals. At the same time, it was found that ethanol interfered with the motor response before there was any impairment of the conditioned reflex. This article gave the first animal manifestation paralleling the human encephalopathy attributed to the solvent 24 years ago[11].

During this period, Charles Xintaras who had earlier completed undergraduate studies in the biochemical sciences at Harvard came to the University of Cincinnati's Institute for Industrial Health for doctoral studies[10]. He was primarily interested in electrical activity of the brain associated with exposures to ozone, carbon monoxide, and other materials and used operant behavior procedures to control animal behavior during inhalation and electroencephalographic studies.

Goldberg, Smyth and their Mellon Institute colleagues[5] went on to try shock-avoidance-trained rats as subjects for experiments with nine other industrial solvent vapors. The results were varied. Most significantly, in no instance was an effect observed with a solvent concentration lower than the established Threshold Limit Value. Consequently, doubts grew that behavioral methods would lead to any refinement of occupational safety precautions. Since then, Goldberg has become a distinguished investigator in the field of psychopharmacology, but the Mellon Institute did not pursue behavioral toxicology any further.

Beliles and others at Rochester[6], trained rats in the same shock-avoidance and shock-escape programs used by Goldberg and Smyth, and exposed them to mercury vapor in a 17 mg/m^3 concentration two hours per day. They saw significant performance decrement after 15 days, and also a marked increase in fighting behavior. They followed this by another experiment with pigeons[7], but this time with a concentration approximating the Threshold Limit Value of 0.1 mg/m^3. Although the birds were exposed six hours a day for twenty weeks, there was no perceptible change of behavior and no signs of mercury poisoning.

My own interest in behavioral toxicology was reluctantly developed beginning in the spring of 1961. I had gone on a sab-

batical to study occupational medicine with Vigliani in Milan. There was no interest in behavioral studies there. The reactions of the Italians to the Russian standards for air quality were similar to those in the United States — incredulity about the validity of the observations, certainty that the standards were unattainable and impractical. In planning a tour of major centers of occupational health work, I felt no inclination to visit Russia. I would even have passed up Czechoslovakia if I had not felt that Professor Vigliani would have been disappointed.

I came to Prague prepared to be polite about air quality standards and tolerant of behavioral studies; after a visit with Milan Horvath in Professor Teisinger's Institute, I felt some doubts of my superiority. His systematic approach to various aspects of animal and human behavior was impressive. The experiments were thoughtfully planned, adequate numbers of animals were used, and the statistical treatment of the observations followed good practice as I knew it. An effort was made to relate the laboratory observations of lower animals with observations made of workers in their workplaces.

My feeling that behavioral studies were close to witchcraft took another severe blow when I visited the laboratory of Etienne Grandjean in Zurich. There, in addition to interesting studies of open field behavior, he and his colleagues had shown that the effect of a toxic agent may be not to suppress a conditioned reflex, but rather to prolong the period required for its extinction.

Soon after my return to the United States, I learned of an episode in which the use of an excellent new insect repellent was held up for years because of uncertainty about its neurotoxicity. I thought at once how valuable behavioral studies might be in such cases. At the same time, Professor Ted Hatch of Pittsburgh was telling industrial hygienists that they should be concerned with transient functional effects of industrial poisons, not only with the prevention of manifest disease. At this time, I found Karl Pribram, of Stanford's psychology and psychiatry departments, understanding and receptive to the idea that behavioral methods might be used for the detection of effects of poisons on the central nervous system. Moreover, he recently had devised a machine which would give a sequence of stimuli and reinforcements, the sequence being modified according to the kinds of responses which the subject made. With this device, monkeys and men had been trained to recognize complex patterns, to store them in memory, and make the appropriate behavioral response. It was a promising tool for the investigation of brain functions which could be adapted for our use.

Pribram soon introduced Halmuth Schaefer, an ingenious and

experienced experimeter in behavioral psychology, and together we worked out plans for a sequence of experiments to test the idea that operant behavior schedules could be used to detect toxic effects in the central nervous system. The Army Surgeon General gave us a contract, and by 1962 our laboratory was established in the Department of Preventive Medicine, and we were under way.

Our decision to work with carbon monoxide was secondary; it was chosen more because it would be easy to work with than because of any special interest. However, it was a fateful decision, for the dearth of information upon which to base community air quality standards for carbon monoxide led to great interest in our work.

Before we had any definite results, Schaefer had left us for a better job. He was succeeded by George Wertheim, who was responsible for a very fruitful sequence of studies in rats, in which successively more sophisticated behavioral programs were explored. These led to the experiments on time discrimination in humans, to which so much attention has been given[8]. He also carried out studies on visual functions in man[9].

When Wertheim decided to pursue his ambition to be a physician, Netta Grandstaff joined us. She has planned and executed additional studies of timing-behavior, problem-solving, and visual performance, reported elsewhere in this meeting, and has introduced investigations of evoked potentials and other electrophysiological phenomena.

As you have seen during this week, as a consequence of these several beginnings, a number of capable workers have been inspired to enter the field. New laboratories are developing, several of them equipped very elegantly, and handsomely staffed. We are beginning to agree on some basic guidelines of methodology, and there is a gratifying eagerness among us to design experiments which are comparable from one laboratory to another. Although some influential toxicologists have yet to be convinced, there is growing evidence that behavioral studies will be very important in the quest for that knowledge which we must have as the foundation for the protection of the health of American workers.

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USE OF CONDITIONED REFLEX METHODS IN SOVIET BEHAVIORAL TOXICOLOGY RESEARCH

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Attending this behavioral toxicology workshop on occupational hazards is by itself a serious occupational hazard for all American participants: every day of the workshop you have been exposed to English with all the varieties of European accents, and now, this last day of the workshop, again I am contributing to this hazard. If some sensitive method of behavioral toxicology were applied to any one of you, I am sure the effects of this hazard would be as clear and dramatic as those of a most deadly industrial toxin.

I am a psychologist, not a toxicologist, and I have practically no experience with any toxins except one, alcohol, with which I do have some experience. No, it is not the kind of experience you suspect. Rather, for the last year at the Highway Safety Research Institute of the University of Michigan, I have been involved in a large-scale experimental project on the effects on driving of alcoholic intoxication. Beyond that experience, my knowledge of behavioral toxicology is limited.

However, by a lucky coincidence, it happens that I have worked in the field of conditioned reflexes (CR) in two leading laboratories of Eastern Europe, that of Professor Konorski in Warsaw, and that of Professor Sokolov in Moscow. Now, the CR methodology has been widely applied in the Soviet Union for the detection of the behavioral effects of industrial toxins. And, as I understand it, the application of this methodology is considered here to be the major factor responsible for the discrepancy between the much lower MACs of the Soviet Union and the much higher MACs of the United States. These methods may be sensitive enough to detect the behavioral effects which are undetectable with the methods which are used most commonly in the United States.

I would like to share with you what I know about the CR methodology, its applicability to behavior toxicology, and its actual applications in the Soviet Union. However, rather than present you a complete review of relevant Soviet studies, I shall concentrate on the methods based on Pavlovian physiology that either have been used successfully or that could be used to detect the behavioral effects of industrial toxins. I shall illustrate their application with selected examples, and will venture some possible reasons for their stronger detective power.

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CONDITIONED REFLEX METHODS

Theoretical premises for usefulness of CR methods in detection of toxic effects.

As far as I could get acquainted with the field of behavioral toxicology in the United States, the methods of detecting effects of toxins in this country can be grossly classified into two general categories, biochemical methods, and methods based on observations of the spontaneous behavior of the experimental animal or human subject together with specially devised psychological tests. A good sample of the latter was demonstrated here during the workshop.

Why can the CR methods be more sensitive to toxic effects than those mentioned above? There are three possible explanations.

(1) One, is the very nature of the nervous system in general, and the cerebral cortex, the conditioned reflex site, in particular. It has been widely recognized by all the eminent students of the nervous system that it is the system which integrates the functions of all of the other bodily systems (cf. very symptomatic titles of some major studies: "The Integrative Action of the Nervous System", by C. S. Sherrington, or "The Integrative Action of the Brain", by J. Konorski). The *integrative action* of the nervous system was particularly emphasized by Ivan Petrovich Pavlov. That being so, if any one of the bodily organs and systems is affected by a toxin the effect is most likely to show in the activity of the affected system and in the nervous system, but at the same time may not show at all in other bodily systems and organs. This may be a possible explanation of the fact that some effects of toxins may not be detected by some biochemical methods, but may show up when tested by CR methods; a toxin in fact may not affect the organ tested by a biochemical method, but at the same time may affect some other system. Either way the effects will be reflected in the activity of the nervous system.

In addition to this integrative nature of the nervous system, as a system most recently developed in phylogenesis, it is the most vulnerable of all the bodily systems to adverse agents (e.g., it is the first system to die when the oxygen supply is stopped). And of all the parts of the brain, the most vulnerable is the cerebral cortex which is the phylogenetically newest part, and it is the site of conditioned reflexes, a fact strongly emphasized by Pavlov. This *vulnerability* of the nervous system, and of the cerebral cortex in particular, may also explain the fact that a very low level of intoxication may not show effects in other bodily systems or organs, but may show effects in the activity of the nervous system, and of

the cerebral cortex in particular, i.e., in conditioned reflex activity.

The *integrative nature* and *vulnerability* of the nervous system in general and the cerebral cortex in particular may explain a possible higher sensitivity to toxins of the CR methods compared to some biochemical methods. But, it cannot explain why the CR methods should be more sensitive than other behavioral methods since not only conditioned reflexes, but spontaneous behavior, and performance on psychological tests are all controlled by the cerebral cortex. One must look, then, for another possible explanation of different sensitivity of the CR methods and other behavioral methods.

(2) The second reason for a possible greater sensitivity of CR methods is their *analytical* and *quantitative* nature. These methods allow for the analysis of behavior into separate measurable dimensions. Thus, a small effect which might escape detection when gross spontaneous behavior is observed, is more likely to be detected by these more refined observations. That may explain a possible superiority of CR methods over methods based on the observation of spontaneous behavior, but it does not explain a possible superiority of CR methods over psychological test methods which might be as analytic and as well quantified as the CR methods. To account for a difference in sensitivity between CR methods and psychological test methods, we must look for a third possible factor.

(3) There is ample evidence, stemming out of both psychological and neurophysiological experiments, for an amazingly high extent of *compensation* in complex forms of behavior. When some behavioral mechanism is impaired - be it by the way of intoxication, neurosurgical lesion, or some other way - another mechanism is very likely to take over, i.e., to compensate for the impaired one, and thus permit the overall behavior to be virtually unimpaired. Besides abundant evidence to this effect gathered in the literature, we also observed strong compensation in the behavior of drunk drivers in the study I mentioned before. Drunk drivers, with their motor skills heavily impaired by alcohol, tended to compensate for that impairment by driving more defensively. That is, they were still able to drive in a reasonably safe manner by compensating for an impaired motor skill dimension of their driving performance with a relatively much less impaired decision-making component of their driving performance.

Now, the great majority of psychological tests (and spontaneous behavior even more so) requires rather complex performance involving many and various elementary skills. A loss in one of those skills can to a large extent be compensated for by a gain in another. That is, even well quantified measures of the overall performance might not detect a toxic effect if the toxin affects only some specific

component of the tested behavior. Only *investigating relatively basic, elementary components of complex behavior*, and this is exactly the case with conditioned reflexes, is one likely to detect small toxin-induced impairments. It is that difference in the complexity level of the behavior involved in CR methods and of psychological test methods that may be responsible for the possible difference in sensitivity to toxic effects.

Thus we end up with the real possibility, although so far on purely theoretical speculative grounds, of a basic superiority of CR methods over the other two classes of methods for detecting toxic effects, namely biochemical methods and those behavioral methods which are based on observations of spontaneous behavior or which use psychological tests. The possible advantage results from: (1) CR methods investigate directly the functional state of the cerebral cortex the organ most likely to be affected due to its *integrative nature* and *high vulnerability*, (2) CR methods are more *analytical* and *quantitative* in nature, and (3) the CR methods investigate the *basic, elementary functions of the cerebral cortex*, thus leaving little room for *compensation.*

CR categorization.

Usually the conditioned reflexes (CR) are categorized into two classes: (1) classical CR (known also as respondent behavior), and (2) instrumental CR (known also as operant behavior).

(1) In developing a classical CR, first and indifferent stimulus (S) is applied, and immediately after an unconditioned stimulus (US) follows to which the animal responds with an unconditioned response (UR) specific to the applied US. The US in that sequence is referred to as reinforcement. The CR is established when the originally indifferent S evokes the UR without the US being applied. The previously indifferent S is now a conditioned stimulus (CS). The essential property of the classical CR is that it is qualitatively the same as the UR used to develop it.

(2) In developing an instrumental CR, first an indifferent S is applied, and immediately after the animal is guided or shaped by a variety of means to produce a definite, selected, usually motor response. The S-R sequence is then reinforced by a reward (most commonly food). The CR is established when the originally indifferent S evokes the selected, motor response (provided the animal is in a motivational state making the reward appropriate, e.g., is hungry when the reward is food). The indifferent S has become a CS, and the response to it is a CR. The essential feature of instrumental CR is that the response has no specific or necessary relation to the reinforcement, contrary to the classical CR. Thus, with the same reinforcement any desired motor response can be established as a CR.

In formation of both classical and instrumental CRs a variety of URs may be used. Most commonly, however, two categories of URs are employed, alimentary and defensive; and consequently two respective kinds of CRs are usually studied.

Finally, with respect to CR categorization, two other classes of CRs are commonly distinguished: (1) positive, or excitatory CRs, and (2) negative, or inhibitory CRs. Excitatory CRs are those manifested in overt behavior, and inhibitory CRs are manifested as refrainings from overt activity. An example of the latter would be a lack of salivation in response to an orange light by a dog with which a red light had always, and an orange light had never, been reinforced with food. The evidence is overwhelming that a lack of overt response to a negative CS is not the result of just a passive state of the cerebral cortex, but of a functional state as active as that which underlies positive CRs although of a different type. There is also abundant evidence to indicate that the cerebral processes underlying inhibitory CRs are more complex, more delicate, and more vulnerable to distortion than are the cerebral processes underlying excitatory CRs.

As the rat has been the laboratory animal in American psychology, so was the dog in Pavlov's laboratory, and thereafter in subsequent Soviet studies on CRs. However, in the late 1940s the need for expanding CR studies to other animals led in the Soviet Union to the development of special methods and devices suitable for studying CRs in other species. In 1951 L. I. Kotl'arevskii published a paper describing a procedure and device for studying instrumental CRs in small animals (such as rats, guinea pigs and the like), which consequently became the standard procedure for studying CRs in small animals in the Soviet Union. For the purposes of behavioral toxicology small animals are more convenient than dogs, therefore there it is not surprising that Kotl'arevskii's method became very popular among Soviet behavioral toxicologists.

CR measures used in Soviet behavioral toxicology and sample experimental results.

What CR methods have been or may be successfully used for detecting toxic effects?

Of all the possible measures of CR activity the following four have been most commonly used by Soviet behavioral toxicologists for detecting toxic effects: (1) formation rate, (2) strength, (3) durability, (4) phasic phenomena.

(1) *Formation rate.* It has been established in basic studies on CRs that agents adversely affecting the cerebral cortex make the rate of formation of CRs decrease. Since the neural mechanism of inhibiting CRs is considered more delicate and vulnerable than

that of excitatory CRs, it is the formation of inhibitory CRs which slows down first. Hence, it has been assumed that toxic effects might be revealed as a decreased rate of formation of CRs, particularly the inhibitory ones.

(2) *Strength.* There are two measures considered related to the strength of CRs, response magnitude and response latency. Of course, the notion of strength as understood here applies only to excitatory CRs. A decrease of response magnitude and an increase of response latency are known to be symptoms of a deteriorating functional state of the cerebral cortex, and they were assumed, to be potential measures of toxic effects.

(3) *Durability.* This property is measured by how long CRs, both positive and negative, can persevere without reinforcement. Short durability is considered a symptom of some malfunction in the cerebral cortex, and consequently has been viewed as a potentially sensitive symptom of toxic effects.

(4) *Phasic phenomena* (states). Normally with a well established CR, a stronger S evokes a stronger response, and a weaker S a weaker response. However, when the animal is in a less than optimal functional state, particularly in a state of pronounced fatigue, lack of sleep and the like, this relationship is abolished and replaced systematically by others which result from deterioration of the functional state of the cerebral cortex. First the *equivalence phase* (or state) develops in which both strong and weak Ss evoke CR of the same strength. The second is called the *paradoxical phase.* This is when a weaker S evokes a stronger response than a stronger S. In the third phase, the *ultraparadoxical state,* weak Ss can still evoke the CR while strong Ss cannot. The last phase is that of deep sleep when the animal does not respond to any CS. As with the other measures, it was hoped that the phasic states would develop as early effects of toxins.

In a recent paper (1972) summarizing some applications of CR methods and of the specific measures listed above, S. M. Pavlenko (a well known student of toxic effects on behavior, a research physiologist of the Erisman State Scientific-Research Sanitary Institute in Moscow, a leading institute in the field of behavioral toxicology in the Soviet Union) stated that the theoretical expectations regarding the value of these methods were fully justified:

"The use of CR methods has proved very fruitful, not only for studying the nature of the effects various toxic substances produce on higher nervous activity, but also for detecting the threshold of this effect . . . As a result, the CR method is widely used in experimental toxicological studies for establishing the MPC for harmful substances in the environment . . . The published data and our own studies of the effects of over 30 industrial poisons . . . has shown

that this method is sensitive and that it has frequently detected effects of small amounts of poison undetectable by other methods."

The most sensitive indicators, for even very small amounts of toxins, proved to be:

(a) durability of inhibitory CRs: inhibitory CRs (e.g., differential inhibition) were quickly disinhibited when the animal was intoxicated, i.e., Ss similar to the CS, but never reinforced and evoking no CR before intoxication, evoked the CR after intoxication,

(b) latency of positive CRs: this measure was found to increase with exposure,

(c) phasic phenomena: equivalence and then paradoxical phases developed when the animal was exposed to toxic substances.

Here are a few examples of toxicological studies in which CR methods were used. S. M. Pavlenko used the CR methods successfully for detecting effects due to various pollutants in water, food, and also in the air of industrial establishments. O. P. Shalamberidze (1960) studied the effects of lead sulfide with CR methods. V. N. Kurnosov (1960) used CR methods for establishing the MAC of mercury vapors. N. F. Izmerov (1960) did the same for MAC of gasoline vapors. Yu. V. Novikov (1957) used the CR methods for establishing the MAC of benzene in atmospheric air.

In all the studies cited rats were used as the laboratory animals, and the procedure and apparatus of Kotl'arevskii were employed. These few examples do not exhaust, of course, Soviet behavioral toxicology based on CR methods. They are merely meant to show that the theoretical considerations on which the potential applicability of CR methods to the field of behavioral toxicology was based were subjected successfully to experimental verification.

Let me now turn briefly to the orienting reflex (OR) which is another very promising method for detecting toxic effects.

ORIENTING REFLEX METHODS

Orienting Reflex.

First, let me start with some general remarks about the OR. It was first observed by Pavlov in the form known to most experimenters as the "demonstration effect." When one of Pavlov's collaborators succeeded in establishing a conditioned reflex in his dog and invited Pavlov to the laboratory room to demonstrate it, the conditioned reflex disappeared. Instead of salivating in response to the CS the dog turned to the "intruder" (Pavlov) and "paid no attention" to the CS. After observing this effect

repeatedly Pavlov subjected it to systematic investigation and eventually concluded that the behavior is a very specific unconditioned reflex. According to Pavlov, the unconditioned stimulus for it is "novelty" (any change in the usual environment of the animal); the reaction is a directing of the sense organs towards the source of the new stimulus (turning head, etc.) and it involves an inhibition of the ongoing conditioned activity. Following Pavlov's study, the OR became the subject of intensive study by many Soviet investigators; the most eminent of them is E. N. Sokolov in Moscow. I was very fortunate to have had an opportunity to do research on the OR in his laboratory.

As a result of this intensive research a considerable body of knowledge on OR has been gathered. It is now generally recognized that there are four classes of components in the OR: (1) motor (turning the head and the body so as to set the appropriate sense organs to receive the stimulus), (2) vegetative (vaso-motor reflexes of a very complex nature and galvano-skin reflex), (3) sensory (an increase of sensitivity of the receptors), (4) central (inhibition of the ongoing conditioned activity and changes in EEG).

The interesting property of the OR is that to evoke it, the stimulus need not be very strong; it is sufficient that it be novel. In fact, there are experimental data which show that stimuli within the threshold range of intensity evoke a more pronounced OR than stimuli just above this range. All this makes the OR a very good prospect as a tool for detecting the effects of very slight concentrations of toxic substances.

So much for theoretical expectations. How do they stand experimental verification?

Sample experimental results of OR application to the detection of toxic effects.

It is remarkable that two components of the OR have been used as indicators of toxic effects by Soviet behavioral toxicologists probably without realizing that what they used had anything in common with OR. One is the interaction of different sensory modalities; the other is EEG changes.

The sensory interaction used was the increase of visual sensitivity to light as a result of weak olfactory stimulation. In the field of behavioral toxicology it was used for the first time in 1957 by K. A. Bushtueva. She noticed that a threshold level concentration of sulfuric acid aerosol in the atmospheric air raised visual sensitivity to light, and she used the phenomenon as an indicator of the effect of that substance. This olfactory-visual sensory interaction has also been used successfully by other investigators to detect effects of other toxic substances: sulfur dioxide (F. I. Dubrovskaya,

1957), methanol (Chao Chen-Si, 1960), methyl, ethyl, and amyl acetates (V. A. Gofmekler, 1960).

Desynchronization of the EEG resulting from exposure to toxic substances was observed by K. A. Bushtueva and others (K. A. Bushtueva, 1960; K. A. Bushtueva, E. G. Polezhaev, A. D. Semenenko, 1960). Bushtueva observed EEG desynchronization with low concentration SO_2 exposure; S. M. Pavlenko (1972) observed the same effect with sulphur vapors.

Extrapolating from these few examples of successful use of two OR components for detecting toxic effects one may expect a more extended use of methods based on OR at least worth closer investigation.

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METHODOLOGY IN BEHAVIORAL TOXICOLOGY RESEARCH

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Dr. Ekel has pointed to some of the methodological details which must be considered whenever experimental work is evaluated. How many subjects were used? How were the data analyzed? Did the experimental design have proper controls, etc.? In addition to questions of that sort, and they are important, there are certain broader issues which should be considered when evaluating the Soviet literature, or for that matter, when evaluating our own literature. These issues concern the validity of the methodology, i.e., the degree to which the methods used provide measurements of the phenomena of interest. I am in one sense describing some of the questions that I think must be asked about Soviet toxicology, but at the same time I am using this opportunity to express some of my concerns about the field of behavioral toxicology in general.

There appear to be three general purposes for wanting to employ behavioral and neurophysiological methods. First, these methods may be more relevant and more sensitive to situations involving low concentration, long-term exposures than are those methods which are applied to acute exposures. Second, these measures may be useful as a means for predicting or anticipating irreversible tissue damage and, therefore, they may permit earlier diagnosis of impending damage, as well as less drastic investigative techniques. Third, these methods may describe effects on performance which can be generalized to work and home situations. In order to interpret what research is available, it will be helpful to understand the investigator's orientation to these three purposes.

If the investigator is interested in the first purpose, he will be much concerned with the processes which underlie the measures that he takes. He will, of course, be interested in the reliability (i.e., consistency) of his measures and with the sensitivity of the methods. But his interests in reliability and sensitivity are in regard to the reflection of behavioral processes. If instead, he is interested in the second aim, he will still be interested in reliability and sensitivity, but in this case it will be with respect to how well his measures correlate with more acute effects, effects which reflect different processes than those that underlie the behavioral measures. And if the investigator's interests are in work performance, he will be concerned with how reliably and with what sensitivity the methods will predict a decrement in "real world" activities. Without an awareness of these distinctions in purpose, it is possible that a

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method may be rejected because it does not meet one objective when it may be quite suitable for another. It is important, then, in evaluating the Soviet literature, as well as our own, to know the purpose intended of the methods used and then to agree or disagree with the investigator's conclusions about his findings in terms of *his* purpose.

Different purposes, as described, make different demands on the researcher and lead him to want different kinds of behavioral methods. If he is interested in the work situation, he may wish a group of measures each of which are components of work requirements. For example, he may observe that some jobs require quick reactions, or involve long monitoring periods, or very precise movements. If the substance being investigated permits the use of human subjects, then he may want a battery of representative task *components* or he may wish tasks which are simulations to some degree of actual jobs. The issue of the validity of the methods as predictors of job performance is the basic question of industrial psychology. However, most of the substances of interest will require animals as subjects and that poses questions about the validity of the predictions. Questions about the generalizability of animal findings to humans are not new, of course, but in this case they are especially difficult because the human skilled performance to be predicted is far different in kind from the animal phenomena that can be studied. The criteria must not only be correlated with the animal findings, they must also serve to predict actual job performance. In this sense the research task is unlike that of the physiologist whose relationships between animal and human phenomena are much more direct.

The investigator who is concerned with behavioral measures as a means for predicting acute effects would seem to have the easiest task. On the face of it behavioral measures should show effects well before the occurrence of grosser phenomena. Unfortunately, in the past this expectation has sometimes led researchers to use very gross and poorly controlled forms of behavioral methods, often with little interest or understanding of the more basic meanings implied by the methods. The techniques have often been thought of crudely as reflecting the response of the central nervous system, and although that is certainly true of behavioral methods, it is also true that poorly controlled methods are unreliable in the results that they produce. As a consequence investigators who approach the behavioral methods this way become discouraged in their use of them. Discussions among such people are frequently concerned with which *test* is more sensitive and not more reliable. The problem, however, seems to lie with the investigators rather than with the methods.

In the last analysis all purposes for the use of behavioral methods must rely upon them as methods which provide measures of the activities of underlying processes. Psychology is no different than the other sciences from which toxicology borrows. Its methods are intended as a means for understanding the processes upon which behavior depends. The use of those methods as *tests* for unvalidated purposes, rather than as tools for the study of specific processes generally leads to disappointment.

Fortunately, behavioral methods are available with which specific processes may be evaluated with regard to their responses to chemicals. These methods appear to take a wide variety of forms when viewed by the layman. But to the psychologist who starts by naming the process in which he is interested, they are not really large in number. He has not only developed rather specific, reasonably standardized techniques, but he has developed a large body of information about those techniques. He can generally state the major variables on which the measures depend and provide data from which tests can be developed. A failure to use the information that is available merely places the user in possession of unreliability on the one hand, and an unknown validity on the other. Yet, it does appear that toxicology in this country has not fully appreciated that. Is that also the case in the Soviet Union?

In summary, in evaluating the Soviet literature it will be important to evaluate the work that has been done in terms of the purposes of the investigators. Those purposes will differ. The differences will be reflected by the choice and deployment of the methods used. As in the United States, Russian behavioral methods are strongly dominated by animal conditioning procedures and electrophysiological indices. Do those procedures dominate behavioral toxicology, and if so are they used with care and precision, and interpreted in terms of behavioral and neurophysiological concepts, or are they used simply as tests of the higher functions of the nervous system and, possibly, used imprecisely? Questions of these sorts are among those which will have to be asked in evaluating Soviet behavioral toxicology along with those other more detailed questions which concern the mechanics of good experimentation.

TOOLS FOR THE ASSESSMENT OF BEHAVIORAL TOXICITY

Bernard Weiss, Ph.D.

Behavioral Toxicology is viewed as particularly applicable to the detection of sub-clinical intoxication. For some substances, heavy metals being among them, the detection of disorders in their early stages is important because, even where reversibility is not in question, the course of recovery may be slow and tedious. The detection of sub-clinical intoxication, however, by its ambiguous nature and imprecisely defined variables, confronts us with problems that we have rarely been forced to deal with before.

In the first place, we are compelled to try to measure a variety of covert variables. Exposed persons may reflect exposure by symptoms of depression, irritability and similar responses of a non-public nature. In the second place, even when one attempts to look for objective signs, there may be no clear-cut signals of where to look. Suppose one decides that sensory systems should be investigated for signs of toxicity. Should one employ clinical tests or the most precise laboratory techniques currently available? Which systems and which functions should be weighted more heavily? For well known classes of chemicals, clues may reside in previous clinical experience. For new chemicals, especially those with unique structures, such clues may be unavailable. What do we do next?

Let me turn first to the measurement of what we might call subjective responses. Some of my best friends are clinical psychologists. I tease them a lot. They possess, however, tools and perspectives to offer to which those of us concerned with behavioral toxicology should pay attention. Many psychologists have devoted themselves to the measurement of subjective variables for a very long time. In the context of psychotherapy for example, one can find an enormous literature dealing with outcome in which the emphasis has been placed on the client's evaluation. Although the techniques employed here are mostly concerned with the measurement of relatively stable behavior patterns, what psychologists call "traits", they offer at least a beginning to a program of measurement development that specialists in occupational health can examine with profit. As a bare minimum, the quantitative pro-

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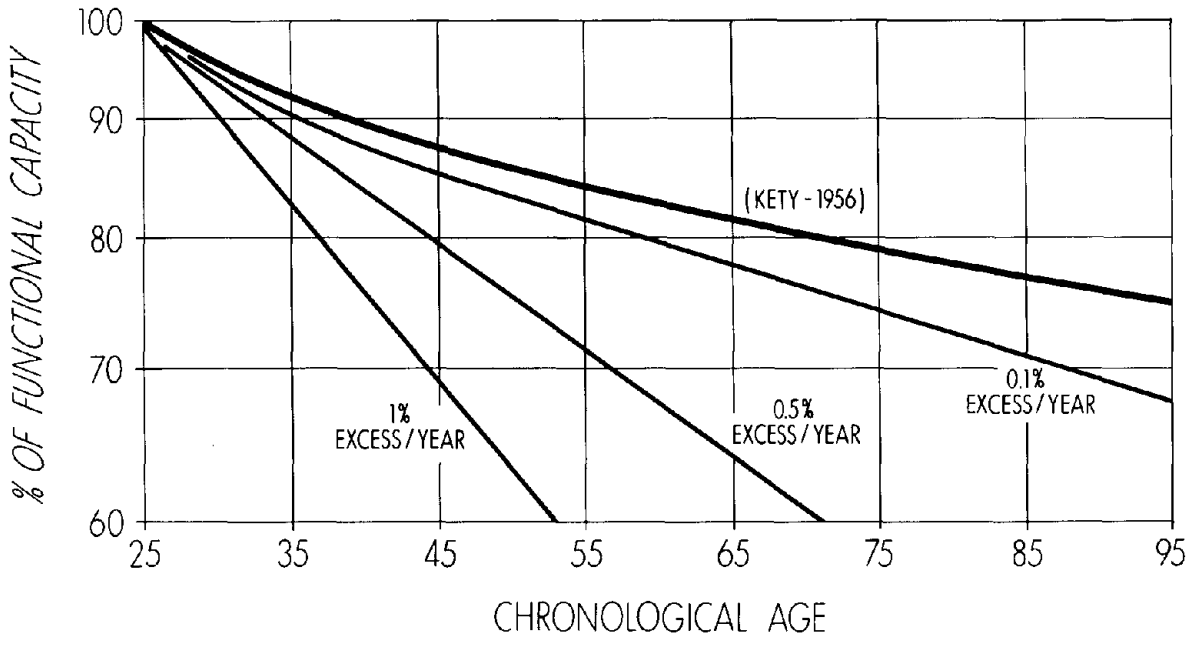

FIGURE 1. Extrapolations from function proposed by Kety to depict brain aging, assuming additional decrements of 0.1, 0.5 and 1% per year. Age 25 is assumed as the baseline.

cedures and techniques for devising tests, that is to say, behavior samples, for specific purposes, are available.

A second aspect of outcome research is devoted to how the client's behavior changes as a function of some therapeutic procedure. This can range from relatively simple behavioral data, such as whether or not the individual's employment patterns remain stable, to the more complex question of the maintenance of social relationships. In most well regulated industrial environments, where workers' health is monitored by professionals, data are available on medical status and may often include subjective complaints. Skilled interviewers, however, who can make use of standardized observations to determine the extent to which a person's customary behavior has changed, are not employed. Instead, the occupational health specialist usually makes contact with only those patients and those complaints where an effect has attained a level beyond some aversive threshold. I strongly doubt that the next step that might be taken, for example, measuring the behavior of the worker in a social context, such as the home, or obtaining data from other family members, has been undertaken in connection with industrial contaminants.

The above catalog of possibilities is not a recital of complaints of current practices. I want to make it clear that a direct application of the methods used by psychologists in other contexts holds only dubious promise. The appropriate applications require a rather delicate tuning of the potential of such methods in the context of occupational safety and health. They hold the promise, however, of yielding much better data than has been available in the past.

Three other sources also provide clues about where to look for subtle dysfunctions in behavior and performance that might arise through exposure to chemicals. Psychopharmacology is perhaps the most closely related of these areas. Quite early in the current history of psychopharmacology, it became quite clear that, in order to evaluate certain components of drug action in humans, it would be necessary to sample a wide variety of behaviors. These behaviors range from performance on a variety of diagnostic tests to rating scales by psychiatric personnel, to studies of social behavior in groups, to self-ratings, and, of course, to a variety of objectively measurable performances. Much of this early literature is summarized in Uhr and Miller[1]. A more recent summary can be found in Efron[2].

Another discipline within which to search for clues to appropriate measurement techniques is neuropsychology, as related to the assessment of brain damage. Such assessments have been the concern of psychologists for many years and although, again, the

emphasis has been on more clear-cut dysfunction than that produced by low level exposure to an occupational contaminant, the approaches have much in common. Their commonality stems from the fact that neuropsychology is often called upon to determine whether or not a deficit exists and, sometimes, to try to localize it anatomically. Recent books by Small[3] and by Russell, Neuringer and Goldstein[4], treat many of these problems in detail.

A third important source of information for the assessment of behavioral toxicity is the literature on aging. I say this because of the possibility that certain agents acting over a long period of time may produce, not outwardly manifested signs of intoxication, or even dysfunction, but instead, may simply promote an acceleration in the processes we call aging. Psychologists and other investigators who have dealt with these processes have discovered an enormous number of changes taking place through the lifespan. For example, changes in sensory function such as dark adaptation and hearing, changes in discriminative capacity, such as the ability to recognize rapid speech, differences in speed of motor response, and a host of others.

The special role that I have assigned to aging can be illuminated by two figures that also are relevant to the presentation at this meeting by Lehrer.

Stimulated by his studies on cerebral oxygen consumption, several years ago Kety[5] tried to organize the then existing literature on brain aging. He concluded that, from the late teens onward, there is perhaps a 25% decline in neuronal cell density, oxygen uptake and blood flow, during the next 50 years. The heavy line in Figure 1 is essentially a transformation of Kety's graph. Weiss and Simon[6] calculated the additional decrements in neuronal cell density, relying on Kety's figures, produced by accelerations in cell loss of .1, .5 and 1% per year. You will note from the graph that even the minutely small acceleration of .1% per year produces a large difference at the end of 50 years, a working lifetime, one might say. Figure 2 transforms the data in another way. It plots chronological age versus what we have called brain age, and you can note there that with an additional loss of 0.1% per year at the age of 65 the brain by these criteria would be 80 years old.

I am not presenting these charts as a model of brain aging, nor as an argument that, even if such phenomena occurred, they would be significant in the life of the individual. I consider them important heuristically. They point to the necessity of longitudinal studies encompassing a lifetime if we are dealing with an agent whose effects are subtle, either because of some intrinsic properties or because of the doses to which persons are exposed. Agents that produce a gradual, undetectable erosion of central nervous

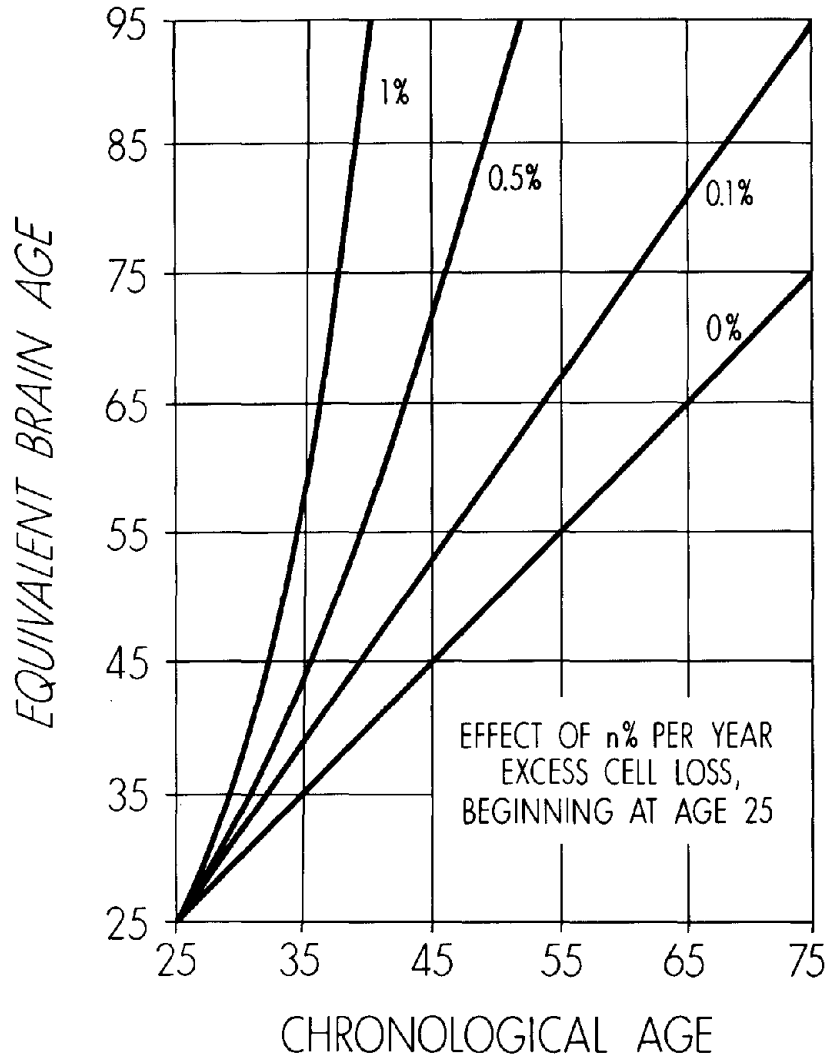

FIGURE 2. Equivalent brain age given accelerations of 0.1, 0.5 and 1% per year in cell loss from age 25 to specified chronological age.

system could pose a not inconsiderable danger to the exposed population, and the animal data provided by Spyker at this conference are cogent experimental support for the kind of model that we have proposed.

ABSTRACT

Behavioral toxicology, in the context of occupational health, is seen as a tool for the detection of sub-clinical intoxication. Psychological research provides three bodies of information that are

relevant to this purpose. 1) The assessment of client response to psychotherapy, including behavior modification; 2) psychopharmacology, especially because of its simultaneous interest in behavioral measurement and side effects; 3) aging research, which is particularly relevant because many toxic processes may involve an acceleration in the rate at which function declines.

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MEASUREMENT OF MINIMAL BRAIN DYSFUNCTION

Gerard M. Lehrer, M.D.

As a clinical neurologist and neurochemist concerned with the effects of disease on brain function, I am specifically interested in the actions of environmental contaminants on brain development and brain function. The research of our Division of Neurochemistry is essentially concerned with factors which regulate differentiation in the brain and brain development in general and how these are affected by, for example, lead or tetrahydrocannabinol.

FUNCTIONAL RESERVE

Neuron Pools

How can we devise tests for measuring minimal changes in brain function? As most neurologists who see many patients will tell you, we are often amazed at the amount of brain damage that can be tolerated with little or no clinical effect. Severe reductions in brain substance might be seen in an individual with remarkable functional intactness. The problems in evaluating *minimal brain dysfunction* are encountered not only in the evaluation of effects of environmental toxins on the brain but also in the evaluation of drug treatment for such conditions as demyelinating diseases. In these latter cases we are dealing with a disease state in which there may be considerable damage to the central nervous system and yet routine neurologic examination may reveal no abnormalities. It is well documented that the pool of neurons available to an individual decreases throughout life because of normal wear and tear. Whether this decrease is due to normal life functions or whether toxic factors in the environment accelerate this loss is not entirely clear. However, if there were toxic factors in the environment which accelerate this depletion of the neuron pool how could we devise means of detecting such depletions?

It is clear that some people can function satisfactorily with depleted neuron pools. I recall one patient who had severe arrested hydrocephalus, probably developed early in life, and to the point where his functional brain substance was reduced to less than half of normal. In other words, he had enormous ventricles and one could therefore assume that central nervous system substance was depleted considerably. Yet, this man, in his early forties, was a consulting engineer, had an IQ of about 150 and was indistinguishable from normals. The pathological finding was discovered incidentally.

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Furthermore, consider people like Pablo Casals who still played the cello beautifully at the age of 80. By this age his neuron pool was unquestionably depleted from what it was when he was 30 years old. Yet he could deftly modulate the pressure of the bow and had the necessary speed, manual dexterity and coordination to produce the virtuoso tone.

What measures can one devise that will indicate whether a toxin in the environment has a long-term effect? We are faced with this same problem, of course, in the evaluation of long-term effects of drugs, whether these be deleterious or not. What is the responsibility of the scientist and what is the responsibility of government in finding means of optimizing brain function of the general population?

We probably need the largest possible neuron pool for some tasks; i.e., it is likely to be of greater advantage to have more neurons than less. What tests can we devise that will provide a reasonable measure of brain functional capacity? Even the best neurologic examination as we know it today cannot do this. Among others, Dr. Wallace Tourtellotte and his collaborators have shown that it is possible to devise a quantitative neurologic examination that can be performed by paramedical personnel. The cost of testing is, no doubt, a factor in industrial medicine and one can employ a paramedical person at considerably lower salary than an M.D. Even if one could measure a "10%" decrease in performance with the finest neurologic examinations, and you have seen some examples of this at the workshop, does this signify a 10% decrease, a 50% or a 90% decrease in brain functional reserve capacity?

We must face such questions constantly in the day-to-day clinical practice of neurology. For example, patients who have multiple sclerosis in remission often perform quite well on neurologic examinations, even quantitative examinations. Some of these patients have died due to accident or intercurrent disease and have come to autopsy. They might have sclerotic plaques distributed throughout their brains; yet these people performed normally.

All I have said so far, should emphasize the fact that the normal human brain has an enormous functional reserve. It is apparent that there is great redundancy in the neuron pool and brain pathways. Consequently, a random destruction of half or more of the neurons may not interfere with normal function.

Brain Sensitivity

The brain is most sensitive during development to environmental changes, be they toxic, drug induced or altered metabolic conditions. At a time when the *neuron* must synthesize an enormous amount of RNA and protein for growth of cell extensions and the *oligodendrocyte* must synthesize protein and lipids for myelinogenesis, reserve capacity is taxed most severely.

I can give you one example from our laboratory. Inbred mice which are genetically homogeneous were given 0.8% of lead as lead carbonate in their diet. This produces a body burden of lead quite comparable to that seen in industrial exposure and probably near that experienced by a large number of children playing in the streets or eating paint off the walls in slum dwellings, i.e., blood levels of 60-100 μg per 100 ml and corresponding brain levels. Pair feeding is necessary because the food consumption is somewhat lower in animals which are getting lead. In body growth alone, lead-fed animals show a significant lag, when compared to their pair-fed controls. The animals were given lead through their mothers, through the lactation period up to the 30th day postnatally and then put on a normal diet. Gross brain weight at 30 days, 42 days, 60 days, and 90 days was significantly reduced, and animals that received 0.8% lead never caught up to their controls even though fed a normal diet after the 30th day. The undernourished (pair-fed) control animals, when put on a normal diet at 30 days caught up with comparable normal, ad lib fed animals by 60 days postnatally, whereas the lead animals never caught up. Of course, in industrial medicine we are not concerned usually with individuals during the period of brain development, but certainly there are processes which occur in the nervous system throughout life which involve establishment of new synaptic connections as well as repair and maintenance of axonal and synaptic beds; and even the myelin sheath must be maintained by the oligodendrocyte, an enormously metabolically active cell that is undoubtedly affected by environmental toxins.

DETECTION OF MINIMAL EFFECTS

As a neurologist, how can I detect minimal effects on brain function? Certainly, one could subject the patient to a quantitative neurological examination or even measure the performance of intellectual or manual tasks under carefully controlled conditions as was so beautifully illustrated by Dr. Stewart. Unfortunately, even under such circumstances, it may still be necessary for brain function to decrease markedly before a significant decrease in performance could be measured. Because of the enormous reserve capacity of the normal brain, it seems reasonable to apply a principle which has long been used in materials testing; i.e. to measure performance after application of a known stress. There are two types of stress which are particularly applicable in the measurement of brain function: Physical stress such as is imposed by elevation of the body temperature and chemical stress which might be imposed by relative anoxia or decrease in blood pH or by the action of a well standardized drug known to interfere with brain function such as barbiturate or ethanol. Both procedures have already been suggested and applied

in clinical neurology. However, precise quantitative standardization is not yet available.

PROVOCATIVE TESTS

Sodium Amytal

The use of sodium amytal as a provocative test in the evaluation of organic brain disease was suggested by Weinstein and Kahn in 1955[3]. Although this method was standardized clinically and psychologically, no quantitative performance measures were used. The efficacy of the method is perhaps best illustrated by a clinical example: a 28 year old man with known rheumatic heart disease and atrial fibrillation was admitted to the Neurologic service at the Mount Sinai Hospital in New York with sudden onset of a right hemiplegia, right hemisensory syndrome, and global aphasia. The presumptive diagnosis was left middle cerebral artery branch embolism. The patient's symptoms cleared rapidly over the course of two weeks and four weeks after his acute attack neurologic examination revealed no abnormalities and there was no evidence of aphasia. A dose of 100 to 400 mg of sodium amytal, administered intravenously to a normal individual, will usually produce only minimal drowsiness and some nystagmus. Neurologic examination usually reveals no gross deficits and the patient is able to perform most mental tasks reasonably accurately, if somewhat more slowly than usual. In our patient his hemiplegia and global aphasia were completely reproduced under the stress of this drug action. After the drug was eliminated all neurologic abnormalities were again reversed. Thus, the *compensated lesion* which was undoubtedly present in this man's brain, was *unmasked* by the drug by the overall reduction in brain function.

Body Temperature Elevation

The second example is the controlled elevation of body temperature, now used in some centers for the evaluation of patients with multiple sclerosis. A patient with multiple sclerosis in remission, who may be presumed to have considerable diminution of brain substance, may be essentially normal neurologically. If the body temperature is raised by two or three degrees, there is a sudden appearance of marked deficits in neurologic function. Thus, scotomas which previously were not present now become very obvious or a small scotoma might grow to almost complete anopsia. Namerow has shown that more quantitative measures of neurologic function such as sensory evoked potentials may demonstrate dysfunction with even minimal elevations in temperature[2]. An electrical stimulus is delivered to the supraorbital branch of the trigeminal nerve. The evoked potential in the opposite parietal cortex may show marked

prolongation of the stimulus-response interval, i.e., the interval between the delivery of a stimulus at the periphery and the appearance of the sensory evoked potential in the cortex may be significantly delayed in the presence of minimal lesions with elevations in body temperature of as little as 0.2°C.

Blood pH

In another form of metabolic stress, increase in size of a scotoma may be demonstrated after lowering blood pH by as little as 0.1-0.2 pH units by infusion of ammonium chloride. This effect can be reversed by raising blood pH by infusion of sodium carbonate[1].

SUMMARY

I have illustrated how central nervous system damage that normally would be undetectable can be revealed by changing metabolic conditions. I believe that techniques which assess nervous system function after application of graded, specific and standardized stresses produced either by administration of drugs or by elevation of the body temperature provide the most likely means of detecting subliminal deterioration of nervous system function. Actually, the tests to be used should suit the modality most likely to be affected by the industrial toxin and should yield quantitative results wherever possible. Thus, if neuromuscular function were to be assessed (as influenced, for example, by anticholinesterase insecticides) then the provocative test might include the use of such short acting cholinesterase inhibitors as edrophonium in conjunction with electromyographic measurements such as the Jolly Test. The application of this technology will require rigorous standardization both in normal subjects and under conditions of industrial exposure. However, I believe that the investment of time and effort is both justified and necessary if accurate assessment of minimal impairment of nervous system function is to be achieved. Without such provocative techniques, I fear that considerably greater than minimal damage to the nervous system must occur before the earliest functional changes can be detected.

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OCCUPATIONAL SAFETY AND HEALTH RESEARCH

P. G. Rentos, Ph.D.

It is a distinct pleasure to address such a distinguished group of scientists, both from this country and from abroad. The unit of NIOSH that I represent is the Office of Extramural Activities. The major responsibility of the office is the awarding of grants in support of research and demonstration projects in the many areas of occupational safety and health. I'd like to discuss the activities of our office in some detail. But before I get into this I wish to share with you some of my thoughts and impressions — in a very general way — concerning the topics developed during the past few days.

First of all, I believe Dr. Xintaras and Professor deGroot — and the other members of the planning committee should be congratulated for planning and developing a very effective forum for these discussions. These matters take a lot of time and their efforts have certainly been productive.

EVALUATION

Of course, all of the material presented has some relevance to occupational safety and health. For some of it, as for example, the work described in the area of odor perception, the implications from the point of view of worker health are not as clearly evident. However, in fairness to our colleagues who are engaged in the research just cited, the point that odors frequently signal danger is well taken. It is not inconceivable that by attempting to either mask or completely remove an unpleasant odor one may, in fact, be inviting a more serious situation.

Although the work presented has occupational safety and health relevance, not all was approached from the behavioral toxicology point of view. In fact, an inordinate amount of time was spent in discussing acute and chronic effects with, in some cases little or no treatment of behavioral factors. Perhaps the state of our knowledge in these areas does need further elucidation but it seems important that we also begin to think in terms of how this knowledge can be translated into prevention and control measures.

Behavioral/neurological equipment and techniques appeared more sophisticated than did approaches in relating findings to working populations. For those of us in NIOSH this has been called "The Year of the Criteria Document." The standard format

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for the development of such documents calls for a critical evaluation of available scientific data including behavioral effects. Thus, it behooves those of us who are in the process of providing information of this kind not only to indicate, for example, what subclinical sign or sympton was observed, but to illustrate in some meaningful way that what has been shown has utility in averting some serious occupational health injury.

The behavioral toxicology information described, although significant, was in most instances in its early stages of development and consequently too premature for use in establishing satisfactory exposure standards. In addition, the number of chemicals discussed were relatively few when one considers that the 1973 edition of the NIOSH *Toxic Substances List* will contain 11,000 different entries. Obviously, not all will be found in industry and only a small percentage may be amenable to behavioral/neurological measurement techniques. But it does point to the fact that although important achievements in behavioral toxicology have been demonstrated during the past few days, much more work needs to be accomplished. The work is important and you obviously have our support.

RESEARCH AND DEMONSTRATION GRANTS

As I alluded to in my opening remarks, my special interest for being here is to talk to you about the grants program of the Institute as it relates to our demonstration and research support.

We are presently involved in a variety of activities ranging from behavioral and motivational factors to acute and chronic disease. Our funds are made possible through two pieces of recent Federal Legislation: the "Federal Coal Mine Health and Safety Act of 1969"; and the "Occupational Safety and Health Act of 1970." At this time we are working with a 1.6 million dollar research and demonstration grants budget, most of which has been allocated in support of 61 project grants in 12 broad scientific categories. To qualify for review and funding consideration, the plan must originate from a non-profit institution and be received at the Division of Research Grants, National Institutes of Health, prior to one of three deadlines: October 1, February 1, and June 1. Both solicited and unsolicited proposals are received and reviewed.

The review process, we believe, is quite thorough. In fact, each proposal receives three separate reviews before it may qualify for support.

The initial and most critical assessment is for scientific merit. This evaluation is conducted by a group of highly competent individuals outside of NIOSH who meet three times a year for that purpose. Proposals are either approved, disapproved or de-

ferred for additional information or site visit. Approved proposals are given priority scores from 100 (highest) to 500 (lowest).

Occurring somewhat before or concurrent with this evaluation, proposals undergo interest and relevance review by qualified in-house program consultants. Through this independent review process the highest degree of objectivity is attained.

Proposals with favorable outcome from these procedures are subjected to one additional consideration: program balance. This review is conducted by technical personnel of the Office of Extramural Activities who, in recommending to the Institute Director that an award be made, take into account existing research priorities and short and long term goals of NIOSH.

Funds for new or renewal research and demonstration projects, although severely limited because of recent budgetary constraints, are nevertheless available but highly competitive. But as mentioned earlier several factors are considered prior to making an award; in addition, the funding picture frequently changes permitting awards to be made from an existing backlog of approved applications as funds become available.

NIOSH/UC BEHAVIORAL TOXICOLOGY WORKSHOP

Phillip L. Polakoff, M.D.

I want to take this opportunity to thank you for taking the risk of having me on the program. As a preface to my remarks, it is deemed necessary to make this declaration: they will be biased, that they will represent my personal philosophy on occupational health as it exists today and that they are offered in the context of constructive criticism and are in no way meant as a content assassination of any one presentation.

The question that was first and foremost in my mind when I started this workshop on Monday was, whether or not the researchers in attendance would be able to provide fellow co-workers, workers of America and myself with any new relevant ideas on research methodology or on actual ways to test the behavioral manifestations to exposures to particular toxic substances in the work environment. This question had and still has the highest priority in my way of thinking. In the last year, I have been engaged as an investigator for the U.S. Public Health Service in occupational health hazards. I have travelled some 150,000 miles. Almost goes like the lyrics of the song, "This Land is Your Land, This Land is Mine." Well, it is true, I have dealt with exposure to vapor fumes in the lumber industry in Oregon, to occupational health problems in federal prisons throughout the country, to the plight of the auto worker on the assembly line in Flint, Michigan, to the migrant workers in New York State, to the miners in Arkansas and on and on. It has been a very productive and exciting year.

With only one night to summarize the week's proceedings, I have tried to focus and limit my critique to several key issues. The critique will be in the form of constructive criticism, so I hope that none of you take it too close to heart on an individual basis, but rather as a way of working closer together in the future as professionals. The three areas presented for discussion are: (1) The strict logistics of the seminar as it existed this past week, (2) The definition of behavioral toxicology as defined to myself throughout the week, and (3) The practical reality of where behavioral toxicology is going.

LOGISTICS OF THE SEMINAR

In relationship to the logistics of the seminar, which is the most mundane of the three areas of discussion, there are several

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points that I think are very salient and should be brought forth for future workshops.

Paper Presentations

There was not enough dialogue. I think that it is a very unfortunate position to be in when they choose discussants to discuss what you might want to discuss after the presentations. I know that I had quite a few things on my mind which were germane to the issues at hand, but I had to hope that someone else would choose similar issues and bring them up. In many cases they were not brought up and thus were lost.

The sessions were too long. I don't think any of us had the capacity to stay and hear ten lectures on any issue. There was much repetition of information.

There were no papers available for distribution throughout the week. For some of our foreign visitors, I am sure that they would have appreciated having papers to take back to their own countries for immediate dissemination when the issues were fresh in their minds, and not to wait until a later time when they are preoccupied with other scientific investigations.

WORKSHOPS

The value of workshops is questionable. On the positive side there were some good workshops that did show some new techniques that I might be able to relate to in my journeys throughout this country and overseas. But, many of the workshops were just too esoteric and didn't seem to offer very much as far as practical applicability. I have had extensive scientific training, but, hopefully, in the future my major efforts will be in demystifying science and making the scientist and the worker relate in a more cohesive manner.

DEFINITION OF BEHAVIORAL TOXICOLOGY

I do not know how many of you are as confused by the definition of behavioral toxicology as I am now and have been all week. This creates a problem because it was never appropriately or clearly stated from the outset which definition of behavioral toxicology we were going to use as our frame of reference. Maybe this is my own naivete that I don't have a definition, maybe I should have come better prepared. But, I think that there were other people in a similar predicament as myself during this conference. Do we deal with electromyographics studies? Do we deal with visual perceptive tests or do we deal with the psychological manifestations of toxic exposures? In the field I have been mandated by Section 20 (a) (6) of the Occupational Safety and Health

Act to detect whether a substance is present in the environment and, if present, is present in a concentration that is toxic to a worker. Now, with regard to the definition of behavioral toxicology, I am confused on how to use this "scientific discipline" in investigating whether a worker has had a toxic exposure at a given time. Do I look at behavioral manifestations through the eyes of the industrial psychiatrist or psychologist, or do I look at issues as a neurologist would, utilizing EMG's and other provocative tests which all require sufficient technical manpower.

I think that you all should be cognizant that there are five physicians like myself in the country who are mandated by the law to cover the 4.1 million work-places. This is as grave a situation as the one which George Perkel pointed to concerning the lack of scientifically-oriented union people. We only have five people to do all the hazard evaluations in the entire United States and we are not fulfilling the mandate that has been given to us. So, I do have a question, to reiterate, on the definition of behavioral toxicology now and hopefully what it will be in the future.

PRACTICAL REALITIES

I was somewhat concerned by the lack of positive results that were presented. I understand the complexities of the issues which the people were trying to address themselves to. I don't know if positive results are the most important thing. I don't know what the ultimate end point of many of the studies that were presented is supposed to be. I don't know if they fulfilled all the criteria that Dr. Beard suggested are important to scientific studies — these being — that they be thoughtfully planned, that they have sufficient numbers and that there be proper statistical analysis performed on the collected data. I tend to doubt that all the papers presented at this seminar met these criteria. Also, the question that was raised by Mr. Perkel is also very relevant, the development of the experimental design in future research projects. I myself would like to see the workers of America represented or being consulted in the design of future studies. It would also be desirable to consult with the various segments of the academic community. It seems like a significant number of the papers were approached in a very individualistic manner. This breeds personal bias, which is intrinsic to any study when there is only one or two points of view present from the start.

The last point that I would like to talk about is the feasibility of continuing seminars like this. Most definitely it has a place. Most definitely it's pointed in the right direction. Most definitely there should be future workshops on this order with, hopefully, some of the suggestions that I have mentioned here incorporated into future

seminars. As Dr. Rentos mentioned, only fourteen substances were talked about. Many of these substances have been the substances that have had the greatest amount of research done in the past. It seems, in part, appropriate to work on a substance that we know about to define our research methodology, but, there are 11,000 toxic substances on the research toxic substance list now and according to Dr. Stewart in his presentation, there are between 500 and 600,000 substances existing in our ambient atmosphere. Somehow we will have to get rolling at a faster clip. NIOSH, according to one figure, represents .006 percent of the national budget representing 80,000,000 workers. We have the second largest mandate, second to EPA. They have to deal with the entire environment, that is, 210,000,000 people. We have to care for 80,000,000 people. We are budgeted $2.6 million for research, equivalent to my best calculation to one wing of an F-111 fighter jet. That is pretty low when you think of one wing of one fighter jet for all the research in occupational health for this country.

For my own personal needs, I need from you desperately, quicker and more easily administered screening tests that I can take out in the field and deal with the workers in their own work environment. I cannot expect the workers to come into a fancy exposure chamber in a university center and be experimented on in an unnatural surrounding. Also, we have to define now whether to continue to try to make a diagnosis or do we try to make cures for existing conditions. We do not know the effects of toxic substances such as lead, mercury and carbon monoxide on individuals. We do have a good handle on the problems even if not in their entirety; so, do we continue to do studies exposing people to 500 parts of CO when we know it has toxic effects or do we go on to new horizons with new toxic substances and new research methodologies.

OCCUPATIONAL HAZARDS AND WORKER PROTECTION

George Perkel

There are very few unions in the United States that employ trained scientists to deal with these problems. This means that there is quite a gap in communications between the scientific community and the people who represent the workers in the plant. I look forward to this workshop as one means of helping to bridge that gap. Especially in learning more about and developing more techniques for early detection of occupational hazards.

As I said earlier there is a problem of communication between the scientific community and ourselves. Even where we have people with some capability for keeping up with literature and attending meetings such as this we have the problem of transmitting the information to the local level to the people who actually work in the plants. So there is a great need for not only communicating but developing the kinds of tests and techniques that can be applied at the plant level.

OCCUPATIONAL SAFETY AND HEALTH ACT

I would like to give you a little background about what seems to be happening from my point of view in American industry that makes the subject of this discussion so important to us. The adoption of the Occupational Safety and Health Act of 1970 represented what seems to me and to many in the union movement to have been a water shed in American history. For the first time the Federal Government recognized an obligation to assure that every work place is safe. Until that time the states presumably had that obligation but, as anyone who has had any experience with trying to live under the state regulations for occupational health and safety would agree, that obligation was simply not fulfilled. Most state laws were dead letters in this field. Qualified personnel were generally not available and not used to protect working conditions in the plants. With the enactment of this law we looked forward finally to the fulfillment of the promise that safety and health of workers would become a top priority item in the government's responsibility. In fact, we were informed by the Secretary of Labor at the time that he regarded this area of his jurisdiction as top priority.

WORKER AWARENESS

Whether it has developed along this line or not I will leave to a later point in my talk. But, at least as far as workers are

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concerned and their interest in health and safety, the adoption of this law has meant a new era. Workers are growing more alert to the health problems that are associated with their work. They are becoming more sensitive to the effects of environmental insults. The days when people worked in noisy places and accepted the noise and regarded it as a part of life that they couldn't do anything about are over. People are beginning to understand that they are not required to expose themselves to environmental insults of that type in order to make a living. So, there is a great hunger on the part of the people who work in the plants for information, for techniques for protecting themselves. It has been our experience that management has not done an adequate job in providing conditions which are safe and healthful. Of course, management does have as one of its goals to operate safely and not cause disease but in the competition of various factors for management's attention we find that health and safety tend to get displaced by other factors and they tend to have a pretty low priority when it comes to allocating the necessary funds and attention to create the conditions which are necessary for safe work.

One of the big problems is that people who work find that they have all sorts of things happen to them at work. They become nauseated, feel drowsy, dizzy, develop coughs and a whole range of symptoms which disturbs them. It is very difficult for them to find out what connection if any there is between their work and their symptoms and what can be done to prevent them from suffering these health effects. Usually the company doctor will minimize any such symptoms. If it is something that can happen to people outside the plant then it will frequently be diagnosed as something that they got outside the plant. We very badly need early means of detecting the effects of exposure to health hazards.

HEALTH STANDARDS

We are familiar with the fact that industrial toxicology, hygiene and medicine have developed certain approaches in protecting workers from adverse health effects. There are standards established under the TLV concept and maximum allowable concentration concept but it is obvious that these are grossly inadequate. Some 11,000 toxic substances will appear on the 1973 NIOSH list and we have TLVs for only about 400 of them. Even allowing for the fact that not all of these substances are in industrial use there is obviously quite a gap between the coverage of the TLVs and the toxic substances that people are exposed to.

In those cases where I've had some personal experience in trying to evaluate standards or TLVs it seems to me that they are

in the nature of informed guesses rather than scientific determinations. Certainly the amount of resources that our society expends to conduct the research necessary for determining more scientifically based standards is ludicrously inadequate. OSHA has been in operation over two years now and it has so far adopted only one permanent standard for health hazards, viz., asbestos; the existing TLVs have been used as standards for the other airborne contaminants. NIOSH has completed only a handful of criteria documents for revision or development of new standards.

Effective enforcement of standards for air contaminants requires environmental monitoring. Workers practically never see anybody come in to monitor the air. Nor is there extensive use of fixed monitoring systems. There are about 40 or 50 industrial hygienists employed by OSHA to monitor the environment in the nation's workplaces. They cover over 4 million establishments with 60 million workers. So we have an army of 40 industrial hygienists for the task of monitoring the environment!

Still another obstacle to be overcome is the limitations in available analytical techniques. I have had some experience trying to improve the cotton dust standard and even after several years of work there is still no operable sampler that has proven adequate to the task of measuring the contaminant. Then too there are many unanswered questions on sampling methods, where and when, and the frequency of sampling. It has seemed to me in observing the efforts to characterize the environment that much depends on who is doing the characterization.

When we enter the field of toxicological assessment through means other than environmental monitoring, I think we would all agree that it is in its infancy. It certainly has not had any impact on the standards that have been established to date. In fact, biological monitoring, which has been studied for much longer than behavioral toxicology, has had virtually no effect on existing standards. I don't know of any standards that have a requirement for biological monitoring. Hopefully, future OSHA standards will do so. Surely these standards should make use of additional techniques for developing criteria including biological, neurological and behavioral toxicology.

RECOMMENDATIONS

Obviously, with all these limitations, the task of developing and applying effective standards requires a high degree of professionalism. Unfortunately there is a gap between the highly professional scientific research that is necessary and the laymen who is the potential beneficiary of this work. We need to find new ways by which the findings of the professionals can be transmitted

and simplified so that they can be applied on the floor of the workplace by people without the advanced preparation that is required for the research.

It would be most desirable if techniques could be developed to enable work crews or their representatives, with only short training, to find answers to the questions the workers raise, such as why am I tired all the time in the plant? Why am I getting dizzy? Why are these various behavioral effects being experienced when the boss says there is nothing wrong - that conditions are within the TLVs - or whatever he may say. The worker experiences vague signs of adverse effects from his environment and nobody can tell him whether these effects presage pathology, or whether there is something he needs protection against.

There are vast unmet needs for knowledge in this field. Workers and their union representatives are demanding to know what hazards they are exposed to in the workplace. They need practical means for detecting whether the level of their exposure is potentially harmful. They need to know what to look for in their behavior and in any other manifestation that may constitute an early warning. They look to the scientific community - and particularly to those working in behavioral toxicology - for assistance in developing the means by which workers can learn to protect themselves against the many hazards confronting them on the job.

BEHAVIORAL TOXICOLOGY METHODS FOR ASSESSING CHRONIC EXPOSURES

Anna Maria Seppäläinen, M.D.

Not a very long time ago physicians used to think that with every disease there was associated a notable pathological change in the organism and the moment of truth was on the autopsy table.

It is a long way from that kind of thinking to "Behavioral Toxicology." The way is real long also if you think only in terms of behavioral tests, such as reaction time, eye-hand coordination, etc. When you are dealing with toxicology, at least with toxicology in chronic exposures, you should consider other methods also.

Psychological measures are very good in short exposures and have been useful in showing effects at low concentrations. However, if you are dealing with a chronic exposure and this usually is the case when we are dealing with workers, who also experience exposure to several different substances and other environmental factors, you need additional sensitive methods to show changes in the functions of the central nervous system. Neurology is a good way of doing it but neurological medical examinations lack the necessary and sensitive methods. When you look to neurophysiology for help, you have access to more sensitive measures.

It seems to me that in this area of research we should be developing more correlations between psychophysiological and neurophysiological measures.

We have had very wonderful papers at this meeting in each of the branches I have noted above, but there hasn't been too much in common with these papers. It would have been helpful if more time was available for discussions and for learning more about each others' methods. There was some possibility of doing this in the workshop demonstrations. However, the demonstrations didn't help all of us because there was only a limited number of people assigned to each demonstration.

It was a very good idea to bring together psychologists, neurophysiologists, pharmacologists, industrial hygienists, all of us, to try and solve some problems in behavioral toxicology but a major task of the future is to bring us together for discussions of our problems and the workers' problems. The problems of a worker who feels that he is not in good shape and thinks something is wrong with him. What is wrong is very difficult to determine if you only take one measure and especially if you don't know what his behavioral pattern was before exposure.

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In the future I think we should have a more leisurely workshop, more time to sit together and discuss and to try and find common ways of doing our work and solving our problems, especially in solving the problems of multiple exposures at the work-site.

APPROACHES IN BEHAVIORAL TOXICOLOGY RESEARCH

Victor G. Laties

I was glad to see Moskowitz using concurrent performances in his work on ethyl alcohol, as did Morgan in one part of his study of the behavioral effects of exposure to lead. Man frequently does two things at a time but usually not as well as he does the same two things separately. There is some evidence at the animal level that concurrent performances are more sensitive to environmental change (Catania[1]). This is not always true for humans, as Weiss and I discovered when we tried to increase alcohol's effect on a timing behavior task by requiring that our subjects do other things concurrently (Laties and Weiss[2]). But it is true under some circumstances, as Moskowitz has discovered. This is a very important fact and, hopefully, this investigator and others will follow it up by exploring its ramifications fully. What, for instance, is it about the added task that leads alcohol to have a greater effect on the primary performance? Seeking the answer to this type of question would help us come up with rules that would have some generality. Behavioral pharmacologists have found this technique of carefully teasing apart the behavior under study, all the while watching how drug action is modified, the best way to characterize drug effects (Kelleher and Morse[3], Laties and Weiss[4]). Incidentally, there almost certainly will be circumstances where adding a second task increases the probability that the overall performance will be more impervious to pharmacologic or toxicologic insult. This direction of analysis should not be ignored, for it often is easier to solve a problem by modifying the environment than by modifying either the individual or his intake of a drug or toxin. For example, I can envision monotonous assembly line jobs that would be rendered less prone to disruption by a drug through the addition of another set of performance opportunities.

This research approach, making a detailed analysis of a performance in an effort to gain insight into which aspects are most involved in the way the drug is exerting its action, is rarely followed early in the history of an applied area. More often an investigator examines performance on a large number of tasks, each of which

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hopefully represents a distinct psychological function. Casting one's net widely in this fashion may pay off in picking up a quick idea of what types of behavior are most easily changed by the agent under investigation. But the approach is difficult to justify once this is known. Such preliminary studies should be - and very likely will be - rapidly followed by more sophisticated in-depth studies of particular dimensions.

Behavior offers a great challenge to the toxicologist. Lehrer offered several examples of how humans can remain functionally intact despite great neurological damage. However, there is the impression abroad that behavior is an exquisitely sensitive indicator of CNS damage. While this may be true of some aspects of behavior and some types of damage it is probably a false generalization. I think it would be fairer to say that most aspects of behavior are remarkably robust in the face of any type of insult, whether pharmacologic, toxicologic or simply mechanical. Remember that the striking fact about the effects of alcohol on driving behavior is that most drinking drivers do *not* get into trouble.

Two ancillary points should be made. The lack of a behavioral effect does not guarantee that no damage of any kind has been done; what Lehrer called "an enormous functional reserve" may itself have been diminished, with consequences manifested only at some future time, and then only if the system has been subjected to a higher load. And, of course, one cannot argue from the lack of a particular behavioral effect that no effect on any behavior has been or can be produced.

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OCCUPATIONAL HAZARDS AND THE PREGNANT WORKER

Joan M. Spyker, Ph.D.

A special group of workers for whom exposure to hazardous substances can have tragic, far-reaching effects is *pregnant women.* Exposure to radiation and to many drugs and environmental contaminants is known to cause greater risk to the unborn than to adults. That is, prenatal exposure often is more likely to produce toxic effects than adult exposure. Consequently, although a pregnant worker exposed to low-levels of "occupational" poisons may not experience health problems herself, her child may indeed be affected.

Many substances to which a worker is exposed on-the-job may be teratogenic, i.e., capable of producing abnormalities in offspring. Teratogens may have a special affinity for developing brain centers and produce effects that show up as behavioral problems, sometimes not until later life. Particularly at low doses, exposure to chemicals *in utero* can cause behavioral changes in the absence of any clinical signs.

Subtle changes in the functioning of the brain and nervous system produced by low-level exposures to a wide variety of substances are the subject of a fascinating new field of job health research known as *behavioral toxicology.* These subtle changes are important of themselves and also, perhaps more importantly, as **an** *early warning* of more severe problems that could develop months or even years later. A key concept is that toxic effects may be manifest as subtle disturbances of behavior long before any classical symptoms of poisoning become apparent.

In this paper I will present background and some examples from research to show 1) why the pregnant worker should be given special consideration when assessing occupational hazards, and 2) why I think that detection of behavioral deviations in children of mothers exposed to a hazardous substance while pregnant may be one of the most sensitive indicators of toxicity.

FETUS AT RISK

Two previously widespread beliefs - the idea of a "placental barrier" that protects the fetus, and the assumption that the immature organism has the same capacities as the adult to metabolize and detoxify noxious substances - have recently been dispelled. It is now accepted that the function of the barrier is limited, and that

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molecules of almost all substances can cross the placenta, either by simple diffusion or by some type of active transport system. Consequently, nearly every chemical entering the pregnant woman ultimately will be found in the fetus (Fig. 1). Furthermore for a

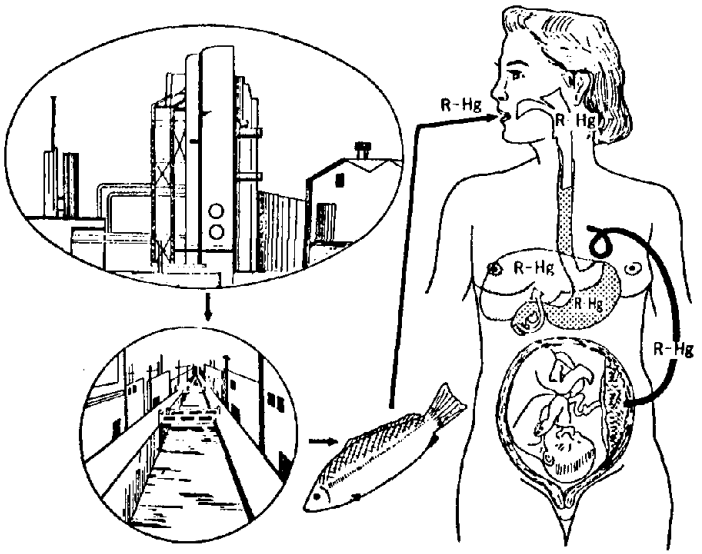

FIGURE 1. Nearly every chemical to which the pregnant woman is exposed will ultimately reach the fetus. Figure schematically illustrates the etiology of congenital methylmercury (R-Hg) poisoning (Reproduced from Takeuchi and Matsumoto, 1968). The etiological sequence was shown to be: mercury discharge from industrial waste into water → concentration in fish → mercury in pregnant woman → concentration in fetus → neurological symptoms in child. Although in this illustration the teratogen is entering the pregnant woman via ingestion, maternal inhalation and skin absorption are also routes by which toxic substances may reach the developing baby.

variety of drugs and environmental chemicals, the fetus and newborn have not yet developed the mechanisms to detoxify and excrete noxious substances (Hagerman and Villee, 1960; Moya and Smith, 1965).

Perhaps nowhere is the vulnerability of the developing organism more dramatically evident than in the thousands of congenital malformations and severe functional deficits resulting from *in utero* exposure to certain drugs, radiation, industrial wastes, and other chemicals in our environment (Hicks *et al.*, 1959; Lenz, 1962; Taussig, 1962; Rugh and Wohlfromm, 1966; Murakami, 1971).

Thus, for many substances recently studied, the unborn organism appears to be at much greater risk to toxic effects than the adult. Although it has not been studied, this is probably true

for many occupationally hazardous substances as well. However, it is unlikely that prenatal exposure to "poisons" at levels currently found in the occupational environment will result in clinically recognizable birth defects. The problem is, that in the absence of congenital anomalies or overt functional impairment, subclinical damage may exist.

STATUS OF TESTING

Following the thalidomide tragedy of the 1960's - which revealed for the first time that *a chemical having few or no side effects in the pregnant woman may disastrously affect the human fetus* - there has been increased concern by the public, the medical profession, the drug industry, and governmental regulatory agencies about the effects of chemical substances on the unborn child.

Testing of new drugs in pregnant experimental animals has been required in the United States since 1962. However, this requirement for drugs should be extended to encompass every potentially toxic substance introduced into our environment. Included in this category are chemicals to which women in the child-bearing age may be occupationally exposed.

There are two other important considerations in evaluating the effects of chemicals on the fetus which are now being virtually neglected. With few exceptions, testing of prenatally exposed animals has been restricted to assessment of morphological anomalies or grossly recognizable functional defects. *Behavioral testing* to detect subtle functional deficits should be included in the protocol. Furthermore, most evaluation is done at the time of birth and/or within a month or two thereafter. *Long-term studies* of prenatally exposed individuals are necessary to discover delayed effects from interference during development.

BEHAVIORAL TERATOLOGY AND TOXICOLOGY

Behavior is at least as susceptible to teratogenic influence as other developing systems. However, unlike the overt birth defects of conventional teratology (i.e., the study of abnormal development), subtle behavioral abnormalities are not readily evident and may be revealed only by special tests during postnatal life. Particularly at low doses of teratogens, behavioral deviations may be observed in the absence of gross functional impairment or congenital malformations.

The emerging science of Behavioral Teratology encompasses the behavioral implications of insult (e.g., exposure to toxic chemicals) during prenatal and early postnatal development. Figure 2 illustrates the areas of responsibility and overlap in behavioral teratology; the principles of teratology as well as of behavioral

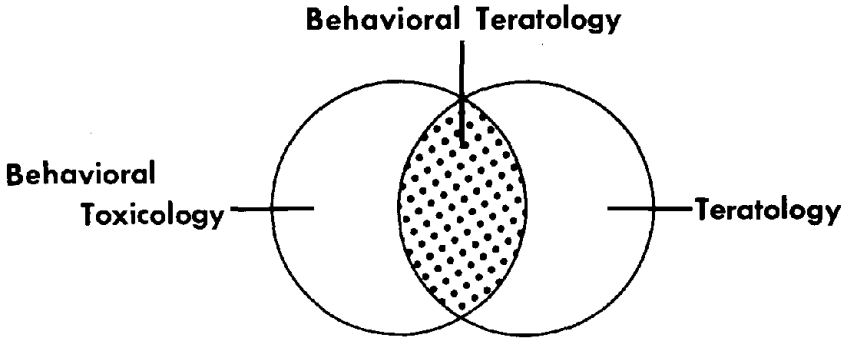

FIGURE 2. Schematic representation of the areas of responsibility and overlap in behavioral teratology; the principles of teratology (the study of abnormal development) as well as of behavioral toxicology must be considered (Spyker, 1973).

toxicology must be taken into account. The underlying hypothesis is that teratogens have special affinities for particular developing fetal brain centers and that alterations in their development become manifest as alterations in behavior - *some of which may not emerge until the individual is much older.* A morphological or biochemical lesion may remain dormant and not manifest itself until later in life as a behavioral disorder or functional impairment (Nair and Du Bois, 1968). Subtle delayed effects are difficult to detect; evaluation over a lifetime in a controlled environment is necessary.

DELAYED EFFECTS

We are now beginning to realize that a defect in a developmental process may not become evident for years. Thus, a baby prenatally exposed to a toxic chemical may be apparently normal at birth but may exhibit behavioral problems as he matures and develop more serious problems when he reaches adulthood.

Attempts to determine if there are delayed effects from prenatal exposure to toxic substances necessitates long-term evaluation. Most developmental research uses the "cross-sectional" method, which involves studying several age periods concurrently with the assumption that antecedent events would have been held constant. This method cannot address those questions concerned with long-term changes.

To determine long-term or delayed effects of a particular prenatal influence on biological or behavioral functions requires "longitudinal" research. This involves following specific individuals for life, controlling or monitoring genetic background, controlling or monitoring prenatal and postnatal environmental experiences, and periodically assaying behavioral functions (Fig. 3).

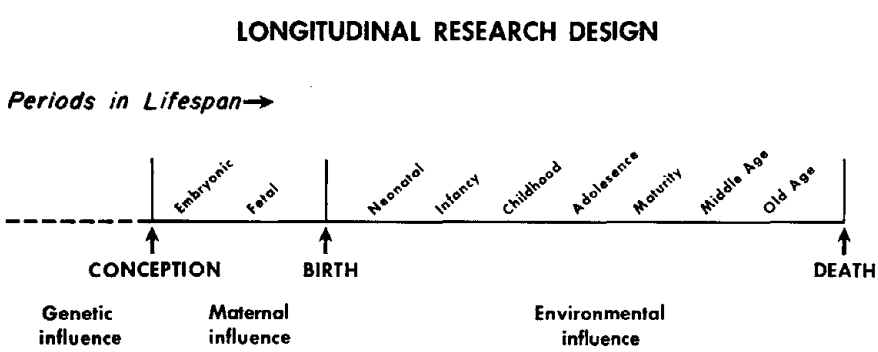

FIGURE 3. Longitudinal research designs must be employed to detect long-term or delayed effects of prenatal insult. This involves following specific individuals for life, controlling or monitoring genetic background, controlling or monitoring prenatal and postnatal influences, and assaying biological and behavioral functions at each major stage of the subjects' lifespan (Spyker, 1973).

Since changes may be seen at one developmental stage and not at another, it is important to assay functions at each major stage of the subject's lifespan. Furthermore, since one function may be affected and not another, a battery of tests should be applied.

MATURATIONAL AND BEHAVIORAL EVALUATION

Since it is extremely difficult to predict *a priori* what types of behavioral changes will be seen following a particular prenatal exposure, it is essential to utilize a battery of maturational and behavioral measures to assess the effects of chemicals at any period in the life span. Experimental animal tests to evaluate the effect of prenatal exposure to toxic substances frequently include evaluation of the following:

1) Morphological and physical characteristics (e.g., examination for congenital defects at birth; notation of sex, age of eye opening and pilage; routine appraisal of physical appearance, ptosis of eyelid, abnormal postural changes, etc.).
2) Growth (weight at birth, weaning, and other age periods; frequently used as best index of general health).
3) Specific responses, reflexes, and sensory-motor capacities (e.g., righting reflex, tactual placing reflex, corneal reflex, grasping, orienting, etc. - especially during first few weeks of life to assess maturation).
4) Activity levels (e.g., spontaneous activity in home cage at various ages, open-field performance, measures of exhaustion).
5) Neuromuscular ability (e.g., tests of strength, speed, endurance, agility; locomotor performance; gait evaluation; vary degrees of difficulty).

6) Learning measures (vary complexity from simple classical conditioning to highly complex conceptual problems).
7) Measures of emotionality (to assess role of autonomic nervous system: techniques may be parallel to evaluating role of personality variables in man).
8) Motivational factors (ex: differential responses to positive and aversive stimulation).
9) Sexual development, sex role, reproductive efficiency, maternal behavior.
10) Psychopathological behavior related to CNS functions (ex: convulsive disorders).
11) Physiological rhythms (e.g., maturation of homeothermic temperature regulation, circadian rhythm). (Adapted from Werboff, 1970).

MECHANISMS OF BEHAVIORAL EFFECTS

Following are possible mechanisms by which a chemical administered to a pregnant mammal may affect the behavior of her progeny:

- Direct, Prenatal - the drug may alter the germ plasm or it may cross the placental barrier and alter developing embryological structures.
- Direct, Postnatal - the drug may persist in the maternal organism and be secreted in the mother's milk to the newborn.
- Indirect, Prenatal - the drug may create conditions in the mother which alter the surrounding and sustaining internal environment of the fetus (e.g., anoxia, toxemia, or eclampsia may produce morphological and behavioral changes in the progeny).
- Indirect, Postnatal - the drug may alter the mother's ability to care for her offspring so that rearing conditions are not conducive to survival, adaptation, or normal development.

ANIMAL vs. HUMAN STUDIES

Although the ultimate data must come from human "subjects", animal studies represent the only ethical, legal and practical means to obtain reliable and valid data relative to behavioral teratology-toxicology. Furthermore, requirements for obtaining information on subtle but delayed effects of prenatal exposure to occupational hazards almost precludes the study of man.

Advantages of developmental studies in animals (versus humans) include the following: 1) Shortened life-span - permits the complete study of developmental effects from conception to death in a relatively brief period; 2) Controlled environmental conditions - possible to manipulate the testing conditions of the "on-the-job environment" and to control gestational conditions of the female to evaluate their interaction; 3) Altered subjects - can create special conditions within animals (ex: CNS lesions) to determine mechanisms underlying chemical-behavior effects; 4) Selection of subjects - possible to choose a given species and strain that possesses a unique biological or behavioral system comparable to man in order to study a given process; and 5) Determined genetic background - possible to create homogeneous populations through selective breeding, mutations, inbreeding and crossbreeding to determine the influence of genotype.

EVIDENCE FROM RESEARCH

My co-workers and I have carried out a series of long-term developmental, behavioral, biochemical and morphological studies of mice from mothers exposed to methylmercury at different stages of gestation (Spyker, 1971; Spyker and Sparber, 1971; Spyker and Smithberg, 1971, 1972; Spyker, 1972; Spyker *et al.*, 1972; Spyker and Fernandes, 1973; Spyker and Chang, 1973; Spyker, 1973).

Our objective was to determine if there were subtle and long-range effects from prenatal exposure to a neurotoxic substance. Healthy, pregnant mice were exposed to low-levels of methylmercury. (All forms of mercury, which is widely used in industry, are capable of converting naturally to methylmercury. In the methylated form, mercury readily crosses blood-brain and placental "barriers".) After the young were born, 372 offispring that appeared normal were selected for the long-term study.

Close evaluation of these "normal" offspring throughout their three-year life spans produced some startling results. The major findings from this research are schematically illustrated in Figure 4 in the order in which they were detected.

In summary, in the absence of any overt signs, offspring from treated mothers responded differently from controls when tested for subtle behavioral deviations at various stages throughout postnatal development. These early indications of trouble were indeed forewarnings of later, more severe developments: neuromuscular and learning deficits, infections, postural problems, immuno-deficiencies, generalized debilitation, and early aging. Evidence from this research suggests that evaluation of *subtle* and *long-term* consequences of *prenatal exposure* is essential for a thorough assessment of the impact of occupational hazards on human health.

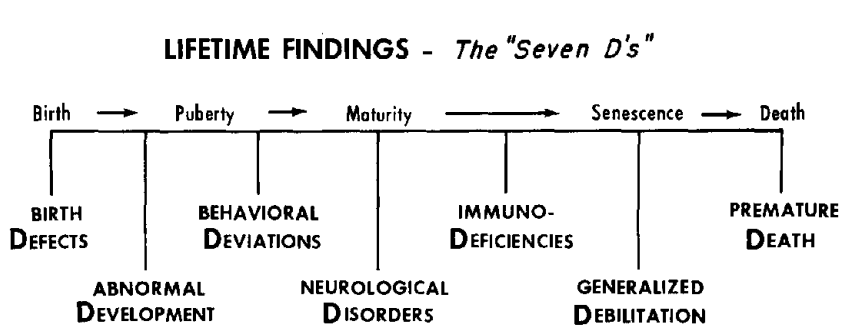

FIGURE 4. Schematic of seven major ways animals exposed to methylmercury **in utero** were found to be significantly different from controls as they grew older. Except for congenital malformations, none of these deviations from normal would have been detected without the use of a longitudinal research design. All 372 offspring evaluated postnatally were apparently normal at birth (Spyker, 1973).

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METHODOLOGICAL PROBLEMS IN BEHAVIORAL TOXICOLOGY RESEARCH

Ben B. Morgan, Ph.D. and John Repko, Ph.D.

In anticipation of future workshops in the area of Behavioral Toxicology, it will be of benefit to those involved to spend more time discussing a number of methodological problems that are always present in an emerging science. Specifically, discussions are needed concerning the following:

(a) *The standardization of behavioral tests.* At this early stage I think that the "shotgun" approach is probably the best. However, as sensitive tasks are developed and identified, these tasks should be standardized and developed for use by different researchers investigating different toxicological agents. Not only will this improve comparability among studies and agents, it will also increase the comparability among the various standards of exposure developed by NIOSH. I suggest, therefore, that consideration be given to ways in which this standardization can be more quickly achieved.

(b) *The validation of behavioral tests.* Tasks which are standardized for general use in Behavioral Toxicology research should also be validated against real-work tasks. Attention should be directed toward the need for and problems of validation; particular emphasis should also be directed toward the selection of appropriate criteria against which tasks can be validated. Not only should validation be discussed in future meetings, it should be supported by future research funding.

(c) *The development of an information data bank.* It could be of value to the researchers in the area, and particularly useful to NIOSH in its effort to develop standards, to establish a central storage of behavioral-toxicology data. Consideration could even be given to the development of a large computer network (such as the one operated by the Defense Advanced Research Projects Association) into which individual researchers could "plug" their computers. This would make available to interested researchers, raw data collected by other researchers at other locations. It would also make standardization of tasks and analysis techniques much easier.

(d) *The dissemination of findings.* It is our belief that research in Behavioral Toxicology is for the ultimate benefit of the worker exposed to potentially toxic substances at his workplace. Thus, it is imperative that the results of behavioral-toxicology studies be communicated to both labor and management. The individual

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researcher's responsibility in this regard should be emphasized. Special attention should also be given to ways in which to hasten the application of findings to the workplace.

We have one final suggestion. We think it would be very beneficial to review and evaluate the behavioral tests which have been employed in human behavioral-toxicology research. This perhaps would be similar to what Warren Teichner is doing for the Russian literature but would deal primarily with publications in English.

The purposes of this review would be to look at all the *behavioral* methodologies which have been employed in behavioral toxicology research involving humans (and workers in particular) to evaluate the available tasks with respect to their validity, reliability, portability (for field testing), and sensitivity, and to recommend which tests should be standardized for common use in future behavioral toxicology research. We believe that such a review would make a very interesting presentation for next year's Workshop and, of course, would provide valuable guidance to every researcher in the area who is trying to develop his own behavioral test battery.

WORKSHOP SUMMARY

SESSION VIII

Chairman

DR. CHARLES XINTARAS

National Institute for Occupational Safety and Health

FUTURE OF BEHAVIORAL TOXICOLOGY

Ralph Wands

The last speaker of any program, particularly one as intensive and extensive as this, is a worker at high risk. At the risk of redundancy, at the risk of having his audience walk out on him and go to lunch and several other risks. Last night when Charles and I were relieving the stress on our central nervous system I threatened to make a one word speech. This morning I am even more tempted to do so following the very excellent comments from this panel. I am very tempted just simply to say, Amen.

All of the remarks this morning are things which I am sure all of us have had on our minds and in our hearts as we have dealt with this problem of developing applicable behavioral toxicology methods, applicable to our charge. You will recall when Senator Taft spoke at the very beginning of the conference he told us that the basic purpose of the occupational safety and health act was to provide a mechanism, whereby we could protect the safety and the health of all workers. This is the framework or ball park within which we operated this week. We went on to find that our purpose really was twofold:

(1) To exchange information on the methodology of behavioral toxicology;
(2) To explore the applicability of this methodology to the process of setting standards that will protect the worker.

Dr. Xintaras and Professor deGroot have brought together a great diversity of people having a wide variety of backgrounds, nationalities, and professional specialties. The only point in common with all of us was our concern for the development of behavioral toxicology for the protection of people from adverse effects of their work environment.

During the course of this week we have learned about so many different approaches and techniques to behavioral toxicology that I am reminded of the story of the four blind men asked to identify an object unknown to them, an elephant. One found a snake that possessed an odor of peanuts, another identified a braided rope in his hand, someone else indicated a wall, and the fourth blind man suggested the trunk of a tree. How we deal with this problem is a real perplexity to all of us.

During this workshop we have had our eyes and our ears open and I hope our minds have also been opened by our encounters with each other. We have now links of communication among ourselves and even an understanding of some of the individual jargon of

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each of our own professional approaches. I hope that this will continue and I would encourage Dr. Xintaras to issue a list of attendees with addresses so that we can at least keep in touch with each other in the ensuing months.

I was particularly pleased to hear Mr. Perkel's report of the concern of the worker about his own safety and health. We need the help and cooperation of the workers in this business of developing and using standards for protection of safety and health. The key to this is continued increase in our communication and our understanding of each others problems and viewpoints. All of us are acutely aware of the shortcomings of our own abilities and of the profession's abilities. To deal with these problems, we are desperately aware of the needs of simple financial support. We cannot help but feel that we have been shorted by society in our dedication to this common cause of safety and health.

On the first day following Senator Taft's introduction, we heard Dr. Stokinger speak, who is chairman, as you all know, of the Threshold Limits Value Committee. He gave us some very specific goals to achieve this week in order for behavioral toxicology to be used in standard setting. Many of those goals have been reiterated this morning, but let me very quickly summarize the points that Dr. Stokinger mentioned. We need larger numbers of experimental subjects. We must reduce the variability in our response data. We must identify the significance of our measurements to the problem of the worker. Along with this he put his finger on one of the biggest stumbling blocks we have when we deal with behavioral testing and that is the motivation of our experimental subject.

In the panel on solvents, Dr. Stewart gave us a history of the progression of industrial health standards and reminded us that we must take the results of our laboratories into the workplace. We heard about the effects of solvents on our workers, notably, carbon disulfide and the chlorinated hydrocarbons which produce both acute changes in the behavior of our employees and also those more subtle effects that are difficult to separate from the problems of aging.

For example, Dr. Lilis described the changes in the retina associated with 10 or 20 years of exposure to carbon disulfide. Dr. Seppäläinen reported central nervous system functional changes and Dr. Hänninen spoke of psychologic alterations such as neuroticism and introversion. Dr. Salvini and Dr. Stewart presented some details of the uptake, retention, excretion and effects of chlorinated hydrocarbons. Such data are valuable in setting standards for occupational exposures.

The pesticide panel, especially the Iowa group, clearly showed

the difficulties of finding small changes in a retrospective epidemiological study. Even with positive findings these become difficult to interpret for lack of measurement of the exposure. Nevertheless we must find ways to cope with this problem since the effects can be quite serious. For example, the association reported by Dr. Jusic between myasthenia gravis and exposure to organo-phosphate insecticides. Drs. Kay and Kinoshita added another complication to our task by pointing out the problem of interaction of the numerous ingredients in our exposure pattern in our workplace.

On Tuesday, Dr. Bingham urged that the behaviorist should have knowledge of the information available on the compounds from other fields of study, such as biochemistry, toxicology, pharmacology. Dr. Chaffin and the Michigan group gave us an example of behavioral testing and its correlation with body burdens of a contaminant, in this case mercury. Dr. Morgan reported on behavioral effects of workers exposed to inorganic lead. This is a large step toward standard setting. They reported a fact of which we were all intuitively aware, that is the high degree of human individual variability. This variability exists in fact and in hard data, as well as in the subjective area of how we look at our workers and how they look at each other.

Dr. Lehrer gave us a suggestion in his discussion for making our tests more sensitive to these differences in people so that we might then detect the effects of chemicals at lower exposures. He suggested that we "load" our test subjects to reduce their capacity for reserve performance. In the panel on worker exposure to metals, we got our first glimpse through Drs. Chaffin, Morgan, Seppäläinen, Barrett and Cooper of the critical role of the neurological scientists in understanding the mechanisms of behavioral changes. Dr. Wedig presented information suggesting that urinary mercury at 500 mg/liter had no effect on employees.

Dr. Spyker also reminded us that there is no such thing as a placental barrier protecting the fetus from environmental contaminants. With the increasing number of women in our work population, we must have a very special concern for the protection of these women.

In the section on drugs and alcohol we saw that ethyl alcohol has very little effect on simple tasks. But, when we have a complex task to perform, ethyl alcohol is most devastating. Dr. Moskowitz showed a marked decrease in performance of complex tasks with increasing intake of alcohol. His data indicated that absorption of marihuana also decreases performance but differently from ethyl alcohol. Dr. LeBlanc gave us a very interesting visual conceptualization of interactions between materials when he showed his diagram resembling the spinnaker of a sailing ship in a full gale.

Following all of these formal discussions, we spent two days and nights, looking at and also using behavioral toxicology equipment. Dr. Xintaras went to great effort and NIOSH great expense to bring all of these demonstrations to one place so that we might become directly familiar with this hardware. We saw instruments that were in the very early stages of development and others that have been used for a long while by a wide variety of scientists with varying degrees of success and significance. There were two things in common to most of these with perhaps one or two notable exceptions, such as Dr. Kimura's nerve conduction demonstration. We know very very little about the underlying mechanisms of physiology, neurology, and biochemistry that affect these test procedures. We do not yet have any great understanding of the relationship between such measurements as fine tremor or tracking ability and the workers actual performance on the job. This correlation has yet to be made.

There was one aspect of the demonstrations of which we all laughed and joked and yet for which we have great concern. This was the requirement of having to sign the release form before we could pick up the stylus and do some tracking or before we could look into the window of the tachistoscope. This has real significance to our work. Anytime that we deliberately stress an individual for experimental purposes, either chemically, physically, or psychologically we are taking risks. There is a risk of impressing an excessive stress on some particular sensitive individual in our test population. There is also the risk of mechanical failure of our equipment which may produce an undesirable effect. Even the electrical stylus which we might use for tracking could get a short in it and give us a little problem, or the valve on the gas cylinder with which we are filling our exposure chambers might go astray or our analytical method might not be reliable. These mechanical failures are things which are real and against which we must protect ourselves.

These problems of risks to our test people are things which we must also protect them against. We have both a moral and a legal responsibility for protecting these experimental subjects. I would urge that we develop an up-to-date set of guidelines for the safe conduct of behavioral toxicology studies. These might well be patterned on the famous Helsinki agreements and principles of ethics. I think that they should be directly applied to our area of behavioral toxicology. This becomes especially important when we consider that our intention is to discover ways and means of detecting early changes that are predictive of significant injuries. As Shakespeare wrote, "Aye, there's the rub." What constitutes significant injury? We don't know. Man needs to find some way

of relating early effects like tremor to an ultimate disease, such as Parkinsonism. Is fine tremor really predictive of excessive manganese exposure for example or is it an irreversible precursor?

Man is a highly variable species, man is a changing species-continually in a dynamic state of change. Man is also a highly adaptive species. We need to separate from his ability to adapt, our ability to overstress his capacity to adapt. We need to be sure of the significance of our tests. We need to have a dose-response relationship. For this we must fall back upon the animal toxicology studies and we need to correlate them with the limited range of human test data that have been developed. Epidemiology, especially retrospective studies, can produce reams of negative data. We need more prospective studies that include measures of exposure. These can be correlated with animal data and the total knowledge can then be useful for setting standards.

We have learned this week that:

(1) There is a high variability of response in man.

(2) We need to be alert for serendipity in our test procedures so that we might pick up some unusual response, for example, the degreasers flush noted by the Wisconsin group.

(3) We need to know and we need to recognize that neurology and the related sciences are important in understanding the basic mechanisms of behavioral changes. Other sciences are equally important and we need close cooperation among all of the scientists. Particularly I would call attention to biochemistry, pharmacology and toxicology.

(4) We need to increase the sensitivity of our tests. We have learned that these might be increased by increased complexity of our tests but this then puts us in the awkward position of trying to define exactly what it is we are measuring.

(5) Animal studies are necessary precursors for human experimentation.

(6) In at least some cases, if not all, the placenta is no barrier preventing exposure of a fetus. This leads to a special concern for occupational exposure of women.

The future of *behavioral toxicology* is whatever we choose to make it. We can resolve the various problems we have identified this week on the lack of comparability of our test data. We can resolve the problem of the physiological significance of our test data. We can resolve the problems of the applicability of our test data to the protection of human health. We can move our laboratory

results into the workplace and set standards which protect all workers. We have made an excellent beginning, let us not stop now.

I would propose that we make this the First Annual NIOSH Workshop on Behavioral Toxicology and that we meet perhaps next year with our European colleagues where we can have a similar exchange.

FIGURE 1. Participants gathered on final day of workshop to critique the workshop activities. Seated at the center table are Dr. Charles Xintaras (NIOSH), Professor Ido deGroot (University of Cincinnati) and Ms. Sherry Selevan, Executive Secretary (University of Cincinnati).

FIGURE 2. Mr. Ralph Wands, National Academy of Sciences, summarized the workshop activities and discussed the future of behavioral toxicology research. Seated next to Mr. Wands are Dr. George Ekel, Mr. George Perkel and Dr. Anna Maria Seppäläinen.

SUBJECT INDEX

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NAME INDEX